Screening and Mechanism based Study for Use of Natural Products for Treating Obesity

THESIS

Submitted in partial fulfillment

of the requirements for the degree of

DOCTOR OF PHILOSOPHY

by

SHAIFALI GURJAR

Under the Supervision of

Prof. Suman Kapur

BITS Pilani

Pilani I Dubai I Goa I Hyderabad

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE, PILANI

2013

BIRLA INSTITUTE OF TECHNOLOGY AND SCIENCE
PILANI (RAJASTHAN)

BITS Pilani
Pilani | Dubai | Goa | Hyderabad

Certificate

This is to certify that the thesis entitled-"*Screening and Mechanism based Study for Use of Natural Products for Treating Obesity*" and submitted by Mrs. Shaifali Gurjar, ID No. 2005PHXF412P for award of Ph.D. degree of the Institute embodies original work done by him/her under my supervision.

Signature of the Supervisor: ..... Suman Kapur

Name in capital letters: Dr. Suman Kapur

Designation: .....PROFESSOR.....

Date: 23.5.13

Dedicated to Daddy, Mummy, Jai and

My little angel Anusha

Acknowledgements

The culmination of this thesis is a result of the research, prayers, blessings, guidance and friendship from a great number of people whom I have been associated with, since so many years. It is a pleasure to convey my gratitude to all of them in my humble acknowledgment.

I would first like to express my sincere gratitude to my supervisor, Prof. Suman Kapur, who has ploughed through preliminary versions of my thesis, making critical suggestions and posing challenging questions. It was her keen eye on the logistics and planning due to which this thesis has taken its present shape. It is a privilege to have found her as my mentor and to be trained under her watchful guidance. Her scientific acumen and intuition has made her as a constant oasis of ideas, and working with her has been inspirational for my growth as a student, and a researcher.

This work is a result of an active support from Dr. Susheel Yadav, veterinary in-charge, at BITS animal facility for his help during animal experiments. I thank Vishal bhaiyaji for taking care of my experimental mice and providing help which enabled smooth working in animal house. A special mention here for Dr. Archana Sharma, MD Pathology for taking out her valuable time to study

histology slides which helped immensely in the progress of this work.

I was delighted to interact with Dr. Jitender Panwar by attending his classes and having him as a member of my doctoral research committee (DRC). Prof. S. K Verma, deserve special thanks as DRC member and advisors at BITS, Pilani. I thank Dr. Shibashish Chaudhary and other faculty members of Biological Sciences Group for their wonderful courses and their help. Special thanks to Dr. Prabhat Nath Jha for his valuable time whenever needed. I thank Prof R. N Saha who has put in considerable time and effort in facilitating the needs of research at BITS. I thank the members of the DAC - Dr. Lalita Gupta and Dr. Uma Dubey who have put in considerable time and effort, not only to judge the overall result, but to comment in detail on the text. Thanks are also due to the instruction division of BITS which provided timely relief that enabled me to concentrate on my thesis without the distraction of other obligations within the institute. I would have been disappointed in my doctoral years at BITS had I not received BITS Junior research fellowship.

I would like to record my gratitude to my senior Dr. Manav Kapoor for his advice, and guidance from the very early stage of this research as well as giving me extraordinary experiences through the initial stages of my work. Above all

and the most needed, he provided me unflinching encouragement and support in various ways. Within our group at Genomics I also have to thank Dr. Shashwat, who taught me a lot about discipline and how to get on in life with people. They are both an integral part of my doctoral work with specific contribution in more than one ways difficult to put down here.

I cherished the prayers and support between me and Shipra. This friendship has withstood the blows of time and emerged even stronger. I would treasure all the precious moments that we have shared throughout my life. She was always available to chat with me, cheer me up and wipe away my tears during troubled times. I want to thank for all her invaluable contribution in all phases of my life especially during the writing phase of my thesis. Your encouragement is priceless during the last phase of my thesis which gave me the strength to withstand the most difficult time of my life. This thesis would have never been completed without your supportive talks.

All my lab buddies at the Genomics Laboratory made it a welcoming place to work. In particular, I would like to thank Kuldeep and Mithilesh for their friendship and help in the past three years. All other folks including Anuradha, Satish and Akshat deserve worthy mention for the fun and interactions in the lab. I would also like to thank

Bhagwati and Shakil for efficient management of our lab. My batchmates at Biological Sciences Group for their friendship and helping hand especially Pradeep. Special thanks to my colleagues including Amit, Gagandeep, Garima, Navin, Arpit, Purva, Zarna, Parshant and Emil. They never made me felt that my lab has been shifted to Hyderabad campus and made everything available when needed.

My friends are my life line and I would like to thank Shaily and Neelu for being a part of my life through thick and thin and for their constant encouragement through all these years. A special mention here for Garima, Vibhu Deepali and Kiran for being the caring neighbours and friends at my stay at 21A/1F/7. You all never made me feel the absence of my family and took very good care of Anusha. Had you all not been there, it would have been very difficult for me to stay with my daughter in Pilani alone. Vimla Didi for keeping me alive all these years with her timely support at times of hunger. Papuji and Shashi Bhaiya for their timely support at time of mobile and internet recharges. Off course Airtel Network, Rajasthan Roadways, Haryana transport and Pradeepji's taxi which were indispensable part of my stay at Pilani.

In the end, I express my heartfelt gratitude to my family. My Parents although sometimes mystified by the subject

matter of my work, have rarely questioned its value and have provided peaceful sanctuary over the last years to allow intensive writing. My Father, in the first place is the person who showed me the joy of intellectual pursuit ever since I was a child and always encouraged me to push through my limits to get the best in life. I love u Daddy. My Mother, is truly my idol for endurance, has sincerely raised me with her caring and gentle love. Her phone calls, twice, everyday kept me alive in my hardships. Inspite of her health, mummy you were always ready to come with me to Pilani to take care of my daughter. I love u Mummy. My brother's Naunihal and Yuvraj have been very good at bringing me back to senses and helping me make through anxious times in my life sometimes by hearing me and other times by scolding and laughing at my foolish things. No matter you are both younger to me but have been my role models and have always been the best counselors. A special thanks to my in-laws and my brother-in-law in making of this thesis till end.

I am grateful to God for giving me an adorable daughter "Anusha" who always filled my senses with happiness and infused new life in me merely by seeing and holding her. Anusha you have been the biggest reason and support every second in the past two years for making me compile my thesis inspite of all odds. I can never ask God for anything more but you being with me always.

Words fail me to express my appreciation for my husband Jai whose dedication, love and persistent confidence in me, has taken the load off my shoulder. I owe him for so many things that are difficult to even think off. I have no words to thank you for your patience wait for me all these years. This combination of saintly, intelligent, passionate and ambitious nature is difficult to stumble upon yet I was the lucky one to have found him. Without him I wouldn't have seen this day. Therefore, I would also thank my parents for making him as my life partner.

Most of all, thanks to God for staying with me, through all these years. You have made my life more bountiful. May your name be exalted, honored, and glorified.

(Shaifali Gurjar)

Abstract

Obesity, defined as excessive accumulation of fat in adipose tissue, has high prevalence, and carries a several fold higher concomitant health risks for almost all non-communicable diseases. This makes it a particularly relevant worldwide public health challenge. Preventive or therapeutic strategies to control human obesity should target the abnormalities associated with obesity. Almost all approved medications for long term use in the treatment of obesity result in harmful side effects. Due to the adverse side effects associated with many of the anti-obesity drugs, more recent drug trials have focused on screening herbal medicines that have been reported to treat obesity and that generally are expected to have minimal side effects. Recent research has suggested that the gut microbiota is a potential nutritional and pharmacological target in the management of obesity and obesity related disorders. The present study was undertaken with an aim to investigate the effect of herbal powders on high fat diet induced obesity and to test the role of microbiota, if any, in reducing the visceral fat deposition. In India, Triphala and its constituents have been traditionally administered as therapeutic agents for promoting digestion and satiety.

The present study investigated the effects of triphala and its constituents (*T. bellirica* [bibhitaki/bahed], *T. chebula* [haritaki/harad], and *E. officinalis* [amalaki/amla]) on the dietary induction of obesity (diet-induced obesity [DIO]), and other symptoms of visceral obesity syndrome, in mice fed a high-fat diet (HFD). The effect of these herbal powders was also studied in relation to change in composition of gut microbiota. First step towards the present study was to estimate the toxicological effect of Amla, Harad and Bahed in normal Swiss albino mice. There were no observed clinical signs of toxicity or any other adverse effects.

Effect of triphala and its constituents on treating obesity was studied by two different approaches. In the first approach, treatment with the herbal powders was given simultaneously with the high fat diet to the experimental mice. Swiss-albino mice were fed high-fat diet (HFD) for 10wks along with treatment with triphala or its individual components and the effect on body weight, daily energy intake, fasting

plasma glucose, serum lipid profile, oxidative stress parameters (TBARS), anti-oxidant levels (Catalase and TSOD) and change in composition, if any, of gut microbiota were evaluated. In the second approach, the mice were first made obese by feeding high fat diet for 4-weeks. Treatment was then started with powders obtained from triphala and its individual components and the effect on body weight, daily energy intake, fasting plasma glucose, serum lipid profile, oxidative stress parameters (TBARS), anti-oxidant levels (Catalase and TSOD) and change in composition, if any, of gut microbiota were evaluated. Mice used were at 7 weeks of age. Mice were divided into six weight-matched groups of seven mice each: normal diet (ND), high-fat diet (HFD), triphala (HFD+T), amla (HFD+A), harad (HFD+H), and bahed (HFD+B). Energy intake was evaluated daily for the entire experimental duration (10 weeks) and the body weight of each mouse was measured every third day during the course of the experiment. Fasting blood samples were taken at 2, 4, 8 and 10 weeks duration. Fasting plasma concentrations of glucose and cholesterol, triglycerides (TG), LDL, HDL, and alanine transaminase (ALT) were estimated using commercial kits. Oxidative stress parameters and anti-oxidant levels were also estimated along with the above clinical parameters. At the completion of each experiment, fecal pellet from each mouse was collected for analysis of gut microbes. Formalin fixed and hematoxylin-eosin stained tissue sections of liver, intestine and adipose tissue were examined for the presence of fat deposits and other abnormalities.

Results showed that mice fed HFD for a 10-week period, supplemented with herbal preparation(s) of triphala or its constituents, presented with a significant reductions in body weight ($P < 0.0001$), energy intake, and body fat accumulation ($P < 0.001$), as compared with mice in the HFD group. Herbal treatment significantly improved the lipid profiles of the mice by lowering serum total cholesterol (Total-C), TG, and low-density lipoprotein cholesterol (LDL-C) and increasing levels of high-density lipoprotein cholesterol (HDL-C) as compared to the mice in the HFD group. The herbal treatment also attenuated glucose levels, oral glucose tolerance as measured by the oral glucose tolerance test (OGTT), and levels of ALT. Similar trend was observed in case of TBARS, TSOD and

Catalase levels. The treatment was effective in lowering the TBARS levels and improving the TSOD and Catalase levels. The results were similar in both the experiments (preventive and curative). The treatment also reversed the pathological changes in liver, intestine and decreased the relative weight of visceral adipose fat pads. Out of all the herbal powders chosen for the study, triphala proved out to be the most effective followed by amla and then harad. Bahed did not show any significant effect with all the parameters and thus did not prove to be effective individually, when used alone.

Fecal pellet culture showed the presence of more bacteroides in the treated groups (triphala > amla> harad> bahed), which was evident from the color change observed in culture of recovered bacteria on Esculin agar media plates. PCR-based analysis of gut microbiota for presence of bacteroides and firmicutes also revealed a similar pattern. PCR analysis showed an improved Bacteroides/Firmicutes ratio in the treated groups (triphala > amla> harad> bahed) as compared to the HFD group. Similar results were obtained when the mice were first made obese and then treated with the herbal powders.

In conclusion, the present findings suggest that triphala and its constituents can counter the effects of an environment (i.e., high dietary intake of fats) and have the potential for use as antiobesity agents with desirable lipid-profile modulating properties, mediated through both by improving the anti-oxidant levels and manipulation of the gut-microbiota.

Contents

Page No.

List of Tables

List of Figures:

List of Abbreviations/symbols

WHO-World Health Organisation	TBARS- Thiobarbituric Acid Reactive Species
BMI- Body Mass Index	CRP- C Reactive Protein
NFHS- National Family Health Survey	LDL- Low Density Lipopolysaccharide
T2DM- Type 2 Diabetes Mellitus	TG- Triglyceride
NNMB- National Nutrition Monitoring Bureau	FFA- Free Fatty Acid
ROS- Reactive Oxygen Species	HFD- High Fat Diet
OS- Oxidative Stress	DIO- Diet Induced Obesity
CAT- Catalase	GMP- Good Manufacturing Practice
TSOD- Total Superoxide Dismutase	SDA- Sabouraud's Dextrose Agar
USP- US pharmacopeia	DW- Distilled Water
TAMC- Total Aerobic Microbial Count	SCD- Soyabean Casein Digest Broth
TYMC- Total Combined Yeast and Moulds Count	TSB- Trypticase Soya Broth
CFU- Colony forming Units	ND- Standard mice chow (normal diet)
IAEC- Institute Animal Ethics Committee	PCR- Polymerase Chain Reaction
HFD+ T- High Fat Diet + Triphala	FER- Food Efficiency Ratio
HFD+ A- High Fat Diet + Amla	VLDL- Very Low Density Lipopolysaccharide
HFD+ H- High Fat Diet + Harad	SGPT- Serum Glutamic Pyruvate Transaminase
HFD+ B- High Fat Diet + Bahed	SGOT- Serum Glutamic Oxaloacetic Transaminase
ALT- Alanine Transaminase	OGTT- Oral Glucose Tolerance Test
AST- Aspartate Transaminase	λ- Lambda
CTAB- Cetyl Trimethylammonium Bromide	β- Beta
BW- Body Weight	SEM- Standard Error of Mean
TB- *Terminalia bellerica*	EO- *Emblica officinalis*
TC- *Terminalia chebula*	H_2O_2- Hydrogen peroxide
GST- Glutathione S- transferase	GSH- Glutathione

Chapter 1

Introduction

Chapter 1

Introduction

1.1 Defining the problem of obesity

Obesity is defined as the excessive accumulation of fat in adipose tissue, to an extent that health may be impaired. Obesity is now a global public health problem, with about 315 million people world-wide estimated to fall into the WHO-defined obesity categories with a Body Mass Index (BMI) $\geq$ 30 [Caterson I, 2003]. A person with a BMI $\geq$ 25 is considered overweight. The prevalence of overweight and obesity is increasing at an alarming rate in the developed and the developing countries throughout the world [Abelson P, 2004; Haslam D, 2005]. Epidemiologic studies indicate that overweight and obesity are important risk factors for diabetes, cardiovascular diseases, cancer and premature death. [Haslam D, 2005]. Obesity also contributes to shorter lifespan, depression and decreased quality of life [Roos C et al, 2007; Roth J, 2004]. Overweight and obesity are now dramatically on the rise in low-and middle-income countries. The latest obesity statistics released for India shows that 75% of Indian women and 58% of Indian men are obese. In India, urbanization and modernization has been shown to be associated with increased incidence of obesity. The 2005-2006 NFHS data showed that the combined prevalence was 12.6% in women and 9.3% in men (NFHS-3, 2005–06). The NNMB data for adults showed a moderate increase in the combined prevalence between 2000-2001 and 2005-2006 among men (5.7% to 7.8%, increased by 0.4% a year) and women (8.2% to 10.9%, increased by 0.5% a year) in the rural population (NNMB Technical Report No: 24. 2007). Women of high socioeconomic class have a higher incidence of obesity of 10.4% as opposed to an incidence of 0.9% in women from low socioeconomic class. With people moving into urban centers and increasing wealth, concerns about an obesity epidemic in India are ever growing [Agrawal P, 2002; Yajnik C S, 2007; Misra A, 2001] and demand immediate attention.

The high prevalence of overweight and obesity, combined with their concomitant health risks, makes it particularly a relevant worldwide public health challenge.

Chapter 1

Global projections estimate 1.12 billion individuals to be obese by the year 2030 [Kelly T, 2008] and this rapid growth of obesity has occurred in both adults and children [Wang Y, 2008].

The fundamental cause of obesity and overweight is a lack of energy balance between calories consumed and expended. Increases in overweight and obesity are attributable to a global shift in diet towards increased intake of energy-dense foods and a trend towards decreased physical activity. Obesity is a major contributor to morbidity and mortality across the world, surpassing drinking and smoking in its negative effects on health. This will have negative effects on life expectancies of generations born after the rise of the obesity epidemic [Sturm, 2002]. Obesity results from complex interactions between genes and environmental factors such as diet, food components, lifestyle and can be viewed as an energy storage disorder in which weight gain results from an energy imbalance (i.e. energy input exceeding output), with most of the excess calories stored as triglycerides in adipose tissue [Hill J, 1998]. The evidence for a genetic component in obesity is strong [Friedman J, 2004; Lyon H, 2005]. The evidence include differences in prevalence between ethnic groups [Knowler W,1990; Zimmet P,1990], higher fat concordance in monozygotic compared to dizygotic twins [Stunkard A,1986; Börjeson M, 1976] and 30-70% BMI heritability between individuals [Hebebrand J,2003; Farooqi I,2005; Bell C,2005 ; Schousboe K,2003] . There are several theories explaining the genetics of obesity but there is no current consensus in the area as a consequence of the complex nature of obesity susceptibility [Walley A, 2009]. Common obesity is polygenic with no simple Mendelian inheritance pattern [Walley A, 2009]. The theories available overlap to a great extent, but differ in their views of key tissue involved. First, as mentioned previously, obesity has been viewed mainly as a disease of energy balance due to an excess energy intake or decreased energy expenditure. A great deal of attention has been focused on the mechanisms controlling feeding behavior, food intake and adipose mass [Smith J, 2006]. Second, obesity is also seen as a disorder of the adipocyte as it has a mechanism of fat storage and mobilization. With growing understanding of adipose tissue metabolism the traditional view of adipose tissue has switched from being a passive energy

"reservoir" with insulatory attributes to a complex, highly active and essential metabolic and endocrine organ, churning out an assortment of hormones and other signals regulating the whole-body physiology (*e.g.* food intake, body weight and brain activity) [Macia L et al, 2006]. It has been recognized as having an independent endocrine role that can result in an inflammatory response with increased risk of T2DM and cardiovascular disease, leading to increased morbidity and early mortality [Korner J, 2009]. Adipose tissue affects energy homeostasis and cardiovascular health by releasing adipokines that regulate energy expenditure, food intake, insulin sensitivity and inflammation [Korner J, 2009]. Third view which explains obesity as a neurobehavioral disorder has emerged with the control of appetite and food intake involved in obesity pathogenesis [Walley A, 2009; Korner J, 2009; O'Rahilly S, 2008]. Obesity is strongly associated with insulin resistance, i.e. there is suppressed or delayed responses to insulin in insulin sensitive tissues. Hormones, cytokines and metabolic fuels from the adipocyte can diminish insulin action. Large adipocytes in obese subjects are resistant to insulin suppression of lipolysis, particularly in visceral fat. This all results in elevated levels of fatty acids and glycerol, which exacerbate insulin resistance in skeletal muscle and liver [Boden G.1997].

Obesity per se may also induce systemic oxidative stress and that increased oxidative stress in accumulated fat is, at least in part, the underlying cause of dysregulation of adipocytokines and development of metabolic syndrome. As an early instigator of obesity-associated metabolic syndrome, increased oxidative stress in accumulated fat has become an important target for the development of new therapies. In patients suffering from T2DM, oxidative stress impairs glucose uptake in muscle and fat and decreases insulin secretion from pancreatic β islet cells. Increased oxidative stress also underlies the pathophysiology of hypertension and atherosclerosis by directly affecting vascular endothelial cells [Furukawa S, 2004].

Recent findings suggest that obesity results from an imbalance between energy intake and expenditure, associated with chronic low-grade inflammation. Gut microbes are considered to contribute to body weight regulation and related disorders by influencing metabolic and immune host functions. The gut microbiota

as a whole improves the host's ability to extract and store energy from the diet leading to body weight gain, while specific commensal microbes seems to exert beneficial effects on bile salts, lipoprotein and chronic metabolism. The gut microbiota and some prebiotic also regulate immune functions, protecting the host from infections and chronic inflammation. In contrast, dysbiosis and endotoxaemia may be inflammatory factors responsible for developing insulin resistance and body weight gain. In light of the link between the gut microbiota, metabolism and immunity, the use of dietary strategies to modulate microbiota composition is likely to be effective in controlling metabolic disorders. Although so far only a few preclinical and clinical trials have demonstrated the effects of specific gut microbes, the findings indicate that advances in this field could be of value in the struggle against obesity and its associated-metabolic disorders.

Preventive or therapeutic strategies to control most of human obesity should target the abnormalities associated with obesity. Pharmacologic options include sibutramine, orlistat, phentermine, diethylpropion, and fluoxetine or bupropion. Phentermine and diethylpropion have potential for abuse and are only approved for short-term use. Approved medications for long term use in the treatment of obesity are sibutramine and orlistat, however, these agents should be used with caution in patients with a history of cardiovascular disorders [Mahan LK, 2008] due to harmful side effects associated with these drugs. Due to the adverse side effects associated with many of the anti-obesity drugs, more recent drug trials have focused on screening the herbal medicines that have been reported to treat obesity and that generally have minimal side effects. Anti-obesity foods and food ingredients may avert the condition, possibly leading to the prevention of lifestyle-related diseases, if they are effective in reducing the visceral fat mass. The present study has been undertaken with an aim to investigate the effect of herbal powders on high fat diet induced obesity and to test the role of microbiota, if any, in reducing the visceral fat deposition.

1.2 Gap in existing research

As obesity has considerable implication for human health and clinical morbidities, it is only appropriate to investigate safe therapeutic approaches to treat obesity and

associated diseases. It is necessary to treat obese individuals by both lifestyle interventions and/or pharmacological therapy. Pharmacologic treatment and surgical interventions used in some circumstances are not always appropriate [Hardeman W, 2000]. Unfortunately, drug treatment of obesity despite short-term benefits, is often associated with rebound weight gain after the cessation of drug use, side effects from the medication, and the potential for drug abuse [Abdullah M, 2003].

The general public uses many other methods for weight loss including herbs, vitamins, nutritional supplements, and meal replacement preparations. Complementary and alternative therapies have long been used in the Eastern world but recently these therapies are being used increasingly worldwide [Hasani-Ranjbar S, 2008]. When conventional/allopathic medicine fails to treat chronic diseases and conditions such as obesity efficaciously and without adverse events, many people seek unconventional therapies including herbal medicine [Liu JP, 2004].

Ayurveda has been used since ancient time with minimal side effects. Ayurvedic formulations are mostly crude herbal preparations. Also there is a very high probability that along with the crude formulations the plant associated microbiota is also being ingested or these formulations once ingested may assist in restoring the ratio between firmicutes and bacteriodetes and overall weight condition of the individual. This hypothesis has never been tested for its health implications. **The aim of this thesis is thus a step forward in this regard where we will study the effect of various ayurvedic herbal preparations with their effect on obesity and the link, if any with the associated gut microbiota.**

1.3 Objective of the proposed research

The aim of this thesis is to study the effects of herbal formulations on visceral obesity related biomarkers in a high fat diet induced obesity mouse model. The broader objective of this work is to obtain new insights into the efficacy of these herbal formulations on visceral adiposity and identifying there effect, if any, on the gut-microbiota. In brief the two main objectives of this thesis are as follows-

1. Screening of standard ayurvedic formulations for associated microbiota.

2. Study the effect of these ayurvedic formulations on High Fat Diet induced mice (Swiss Albino) model for obesity with respect to expression of various biomarkers for obesity.

1.4 Approach taken

There are plenty of medicinal plants which are being used in treating obesity. A survey was done for the commonly used herbal medicines available in the market. Among such medicines, Triphala and its constituents (Harad, Bahed and Amla) were found to be one of the key ingredients of anti-obesity medicines. Till date, there is no clinical evidence on how these conventional medicines work. To study obesity, the high fat diet induced animal models are best suited for such studies. The Swiss albino male mice were chosen for this study. The effects of these herbal powders on anthropometric, clinical and oxidative stress markers were studied. Also the effect on gut microbiota was studied.

1.5 Plants chosen for the present study

The plant powders of Triphala, *Emblica officinalis* (Amla/Amlaki), *Terminalia chebula* (Haritaki/Harad) and *Terminalia bellirica* (Bibhitaki/Bahed) were chosen for this study because Triphala and its constituents are the most common ingredients of many anti-obesity herbal medicines

1.6 Significance of the study

Several therapeutic remedies have been suggested for treating obesity and related metabolic disorders. Despite the urgent need for safe and efficient therapeutics and the potential size of the market for anti-obesity drugs, the current status for the development of such drugs is still unsatisfactory. Compliance with conventional weight management programs, which often include increasing energy expenditure via physical activity, is low. It is not surprising to see the marketing of many new dietary slimming aids aimed at satisfying the need for palatable (as well as safe, effective and therapeutic) options. In accord with this approach there are numerous investigations on the effectiveness of medicinal plants as natural supplements to

reduce body weight. The study is relevant in all parts of world, as obesity has been recognized as a worldwide epidemic especially in developed and developing nations. The aim of this thesis is to assess all the anthropometric and clinical parameters with use of the herbal formulations for reducing obesity and related metabolic disorders. Till date, there is very little clinical data available on the efficacy of triphala and its constituents with reference to visceral adiposity and gut microbiota.

1.7 Outline of the thesis

Chapters 2 summarize the prevalence, incidence, disease burden and etiology of both overweight and obesity. Chapter 3 focuses on the first aim and provides a detailed review of all studies on efficacy and safety of effective herbal medicines in management of obesity in humans and animals. The methodology has been described in chapter 4. Effect of herbal formulations on anthropometric, clinical and oxidative stress markers for obesity has been described in chapter 5. Chapter 6 describes the effect of these herbal formulations on tissue histology and gut microbiota. Finally, Chapter 7, the last section of the thesis provides conclusion of this work along with specific contribution and future scope of the study.

Chapter 2

Review of Literature I

Chapter 2

Review of Literature I

2.1 Defining Obesity

Obesity is a medical condition in which excess body fat has accumulated to the extent that it may have an adverse effect on health, leading to reduced life expectancy and/or increased health problems. Obesity, particularly which is caused by visceral fat accumulation, is a serious risk factor for so-called lifestyle-related disease such as diabetes, coronary heart disease, hypertension and cancer [Keun-Young KIM et al, 2008]. Obesity is influenced by genetic and environmental factors and particularly by changes in diet and physical activity. It is now classically characterized by a cluster of several metabolic disorders. Most of them are related to the glucose homeostasis and to the development of cardiovascular diseases (**Fig. 2.01**) [Alberti KG, 2005; Matarese G, 2007.]

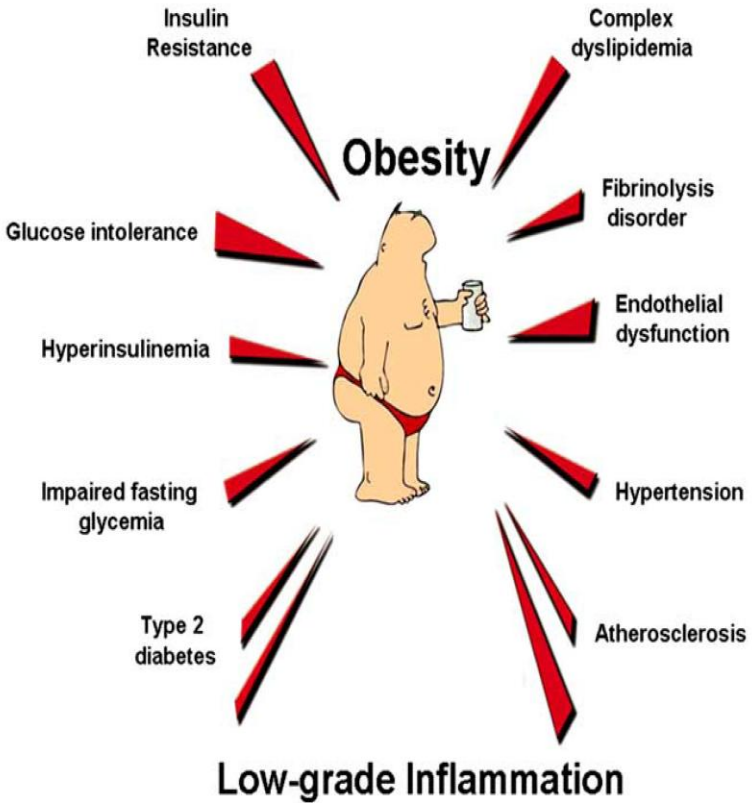


Figure 2.01: Obesity and associated metabolic disorders [Patrice D Cani, 2009]

During the past decade, it became clear that a low-grade inflammation contributes to the development of the pathologies associated with obesity [Heilbronn LK, 2008]. Unequivocal experimental, clinical or epidemiological evidence have causally linked inflammation, or the inflammatory signalling responses to the development of theses metabolic disorders associated with obesity. The analysis of the nutritional disorders associated with obesity reveals that the adverse health consequences of weight gain and obesity are especially prominent following prolonged periods of positive energy balance and is mostly associated with a high-fat diet ingestion.

2.2 Prevalence of Obesity

It is only a decade ago that the problem of obesity was seen as a minor issue which concerned only a few endocrinologists intrigued by the manifestations of the condition. Their main concern was to attempt to identify the genetic component of the obesity arising in a few children and adults who put on substantial amounts of weight. Clinical management was also known to be frustrating for both patients and doctors; the major improvements achieved on weight loss were universally recognized but the frequent inability of patients to achieve and maintain effective weight loss led to clinical frustration and the temptation to blame the individual for a lack of will power. Now, however, overweight and obesity have a completely different perspective. Although the epidemic of obesity really started to increase markedly in the 1980s it is only since 1997 that WHO and many national governments have recognized the importance of obesity as a major public health problem affecting both the developed and the developing world (Table 2.01) [Misra A, 2008].

Table 2.01: Prevalence of obesity in developing countries [Misra A, 2008]

Author and year	Country/region and urban/rural area	Age (yr)	Sample (n)		Cutoffs of BMI (kg/m²) or waist circumference (cm)	Prevalence of obesity (%)	
			Male	Female		Male	Female
Shapo *et al.*, 2003 (205)	Albania (Urban)[a]	>25	535	585	BMI ≥ 30	22.8	35.6
Monteiro *et al.*, 2001 (206)	Brazil[a]	>20	1971	2588	BMI ≥ 30	4.4	12.6
Zaman *et al.*, 2001 (207)	Bangladesh (rural)	>18	238	272	WC ≥ 94 (M), ≥80 (F)	2.9	16.8
Fezeu *et al.*, 2006. (36)	Cameroon (urban)	>25	1301	1530	WC ≥ 94 (M), ≥80 (F)	18.0	67.0
Du *et al.*, 2002 (208)	China[a]	18–49	2796	2936	BMI ≥ 25	15.3	17.1
Gu *et al.*, 2005 (40)	China[a]	35–74	15540[b]		BMI ≥ 25	26.9	31.1
Jadue *et al.*, 1999 (209)	Chile[a]	24–64	1020	2100	BMI ≥ 30	15.7	23.1
Fan *et al.*, 2008 (210)	Shanghai, China (urban)	20–88	1524	2379	WC ≥ 90 (M), ≥80 (F)	35.4[b]	
					BMI ≥ 25	43.3[b]	
Shi *et al.*, 2008 (211)	Jiangsu, China[a]	>20	2849[b]		WC ≥ 90 (M), ≥80 (F)	19.5	38.2
Sabanayagam *et al.*, 2007 (212)	Chinese adults, Singapore (urban)	40–81	402	540	BMI ≥ 25	33.0	34.0
Pang *et al.*, 2008 (213)	China (rural)	>35	45925[b]		BMI ≥ 25	17.6	27.3
Zhang *et al.*, 2008 (214)	China (rural)	>35	29970[b]		BMI ≥ 25	15.1	22.1
					BMI ≥ 30	1.2	2.2
Galal, 2002 (215)	Egypt[a]		1974	2909	BMI ≥ 30	20.0	45.2
Dhurandhar and Kulkarni, 1992 (216)	Western India (urban)	>15 y	791	791	BMI ≥ 30	4.8	7.8
Gupta *et al.*, 2003 (217)	North India (urban)	≥20	532	559	WC ≥ 102 (M), ≥88 (F)	21.8	44.0
Misra *et al.*, 2001 (176)	North India (urban)[c]	>18	170	362	BMI ≥ 25	13.3	15.6
Gupta *et al.*, 2004 (51)	North India (urban)[d]	Mean: 43.2 (M) 44.7 (F)	226	232	BMI ≥ 30,	20.8	32.3
					WC ≥ 102 (M), ≥88 (F)	34.5	55.6
Prabhakaran *et al.*, 2005 (218)	North India~(industrial population)	20–59	2935[b]		BMI ≥ 25	35.0[b]	
					WC ≥ 90 (M), ≥80 (F)	43.0[b]	
Misra *et al.*, 2005 (113)	North India (urban)	38.9	640[b]		WC ≥ 90 (M), ≥80 (F)	10.1	25.9
Gupta *et al.*, 2004 (219)	North India (urban)	>20	960	840	WC ≥ 102 (M), ≥88 (F)	25.6	44.0
Deepa *et al.*, 2007 (220)	South India (urban)	>20	2350		BMI ≥ 25	43.2	47.4
					WC ≥ 90 (M), ≥80 (F)	56.2	35.1
Gupta *et al.*, 2007 (221)	North India (urban)	>20	532	559	BMI ≥ 25	37.8	50.3
Park *et al.*, 2006 (222)	Korea[a]	20–80	6824[b]		WC ≥ 90 (M), ≥85 (F)	19.4	22.5
Oh *et al.*, 2004 (35)	Korea (urban)	30–80	269	505	WC ≥ 90 (M), ≥80 (F)	33.1	25.7
Grabauskas *et al.*, 2003 (223)	Lithuania[a]	20–64	4337	5440	BMI ≥ 30	16.2	16.0
Benjelloun, 2002 (224)	Morocco[a]	>20	6875	7153	BMI ≥ 25	25.4	45.0
					BMI ≥ 30	4.3	16.0
Hodge *et al.*, 1996 (225)	Mauritius[a]						
1987			5021[b]		BMI ≥ 25	26.1	37.9
1992			5111[b]		BMI ≥ 25	35.7	47.7
Al-Lawati *et al.*, 2003 (38)	Oman (urban)	20–99	1419[b]		WC ≥ 102 (M), ≥88 (F)	4.7	44.3
Dodani *et al.*, 2004 (226)	Pakistan (urban)	Mean 41.07	1147[b]		BMI ≥ 25	52.2[b]	
					BMI ≥ 30	18.5[b]	
Jacoby *et al.*, 2003 (227)	Peru (urban)	>18	1163	1159	BMI ≥ 30	16.0	22.7
Jahns *et al.*, 2003 (228)	Russian Federation[a]	19–55			BMI ≥ 30		
1992			17150[b]		BMI ≥ 30	7.1	21.6
2000			9006[b]			10.3	19.1
Bovet *et al.*, 2006 (229)	Republic of Seychelles[a]	25–64	1255[b]		BMI ≥ 30	15.0	35.2
					BMI ≥ 25		68.3
South Africa Department of Health, 1999 (230)	South Africa[a]	>15	5671	8156	BMI ≥ 30	9.1	29.3
Hodge *et al.*, 1994 (231)	Samoa[a]	25–74	797	989	BMI ≥ 30	48.7	68.0
Bourne *et al.*, 2002 (232)	South Africa[a]	>15	4006	5897	BMI ≥ 25	25.4	58.5
					BMI ≥ 30	6.0	31.8
Wijewardene *et al.*, 2005 (233)	Sri Lanka[a]	30–65	2692	3355	BMI ≥ 25	20.3	36.5
Kosulwat, 2002 (234)	Thailand[a]	Adults	NS: 1991		BMI ≥ 25	7.7	15.7
			NS: 1996		BMI ≥ 25	13.2	25.0
Florez *et al.*, 2005 (37)	Venezuala[a]	>20	3108[b]		WC ≥ 102 (M), ≥88 (F)	42.9[b]	

Data are given according to alphabetical order of countries. NS, National survey; M, male; F, female, WC, waist circumference.

[a] Representative of sample total population in the area.

[b] Overall including male and female.

[c] Data from urban slum population of New Delhi, north India.

[d] Data from Punjabi Bhatia community in north India.

WHO regional offices have also been involved in taking a series of initiatives to cope with the epidemic in their regions. The WHO European Office and the European Ministries of Health have gone on to agree in late November 2006 a charter for tackling obesity. This, of course, depends not only on establishing the basis of the epidemic, but also the most effective preventive and management measures. The serial increasing trends in obesity prevalence that were evident in most Western countries, especially the USA, from about the beginning of the 1980s affected not only adults, but also children. The documentation in adults was much easier as the use of BMI was readily accepted, but the paediatric world, whilst interested in unusual genetic problems associated with obesity, only accepted that this was a public health problem at the beginning of this Millennium when the International Obesity Task Force had established a reasonable scheme for linking the children's cut-offs for BMI (which changes by age and differentially by sex) with the accepted adult cut-off points of 25 and 30 [Cole TJ et al, 2000]. Since then the obesity epidemic has been documented in adults and children and shown that this seems to be rising relentlessly**,** with 20% of all adults in most European countries already obese and with higher rates often found in the Southern, Central and Eastern European nations.

2.2.1 Global scenario

Overweight and obesity are the fifth leading risk for global deaths. Overweight and obesity are linked to more deaths worldwide than underweight. For example, 65% of the world's population live in countries where overweight and obesity kill more people than underweight (this includes all high-income and most middle-income countries).The high prevalence of overweight and obesity, combined with their concomitant health risks, makes it a particularly relevant worldwide public health challenge [Kelly T et al, 2008]. At least 2.8 million adults die each year as a result of being overweight or obese. In addition, 44% of the diabetes burden, 23% of the ischemic heart disease burden and between 7% and 41% of certain cancer burdens are attributable to overweight and obesity. Some WHO global estimates from 2008 follow:

- 1.5 billion Adults, 20 and older, were overweight.

- Of these 1.5 billion overweight adults, over 200 million men and nearly 300 million women were obese.
- Overall, more than one in ten of the world's adult population was obese.

In 2010, around 43 million children under five were overweight. Once considered a high-income country problem, overweight and obesity are now on the rise in low- and middle-income countries, particularly in urban settings. Close to 35 million overweight children are living in developing countries and 8 million in developed countries. **Figure 2.02** shows the estimated age-standardized prevalence of overweight (upper) and obesity (lower) in 2005 among adults 20 years and older for all countries in the world.

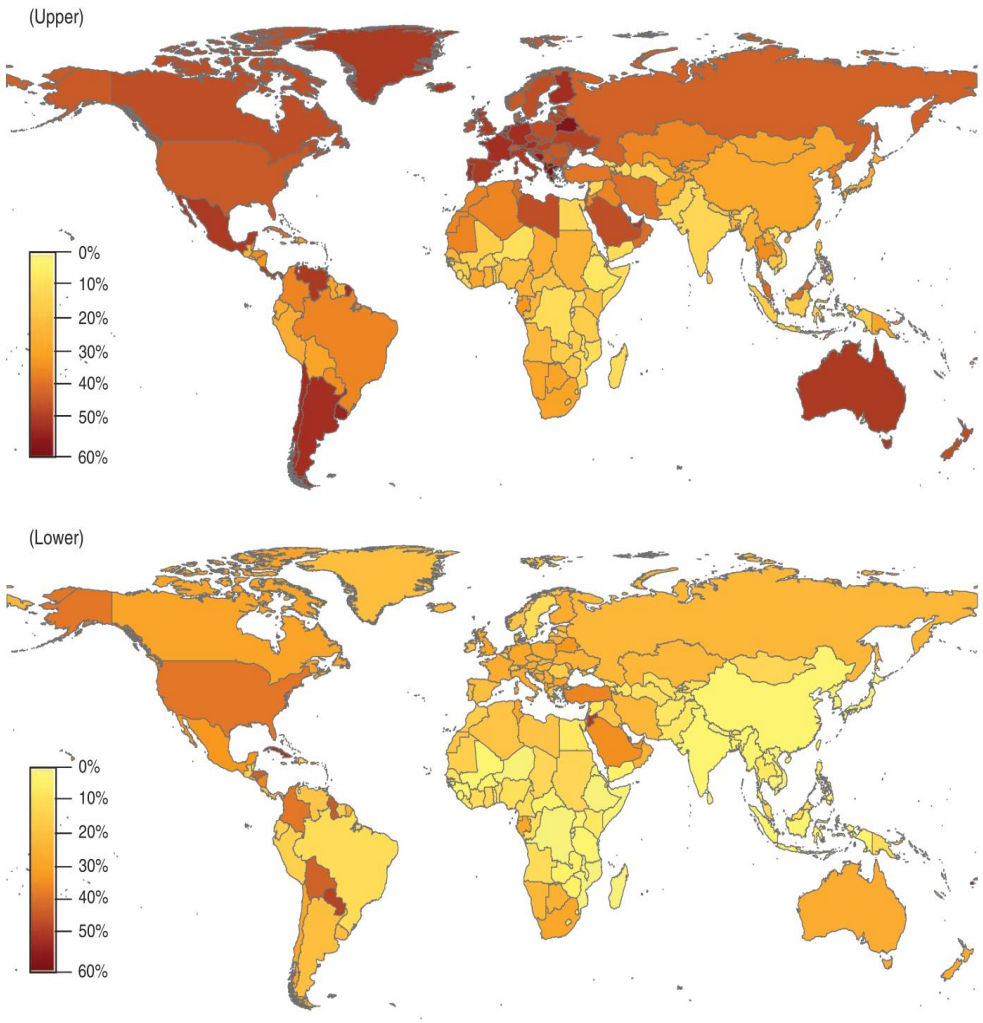


Figure 2.02: Worldwide prevalence of overweight (upper) and obesity (lower) in adults 20 years and older by country in 2005 [Kelly T et al, 2008].

2.2.2 Indian Scenario

Obesity epidemic is a rapidly escalating epidemic all over the world [Prentice A, 2001]. In the developed countries the epidemic attracts much attention, but there is little realization of a similar and perhaps more serious epidemic in the developing countries. Countries like India have controlled the problem of severe under-nutrition to a substantial extent, but are now facing a rising epidemic of obesity [Yajnik CS, 2004].

In India, the second most populous country in the world and where under-nutrition has been the major public health concern over the past several decades, little attention has been paid to obesity until recently. The emerging evidences suggest an increase in obesity in children and adults [Gupta AK, 1990; Monga S, 2004]. Several recent studies reported an increase in overweight in adults in urban areas over the past two decades [Gopinath N, 1994; Gupta R, 2003; Misra A et al, 2001; Mohan V, 2001; Ramachandran A et al, 2001; Ramachandran A, 1997; Ramachandran A, 2000].

India looks as though it has perhaps the greatest amplification of risk as weight increases. Indians have a marked tendency to develop abdominal obesity which Yudkin and his colleagues have ascribed to a variety of environmental factors including the cytokine in-flow from chronic gut infestation and infection in an environment with extremely poor standards of clean water and sanitation [Chan JCN, 2002]. In general, prevalence of overweight and obesity were higher in urban areas and in higher-socioeconomic populations. The NFHS-3 2005–2006 data showed that combined prevalence (BMI $\geq$ 25) was 9.3% and 12.6% among men and women aged 15–49 years respectively. On the other hand, the NNMB 2005–2006 data show that in the rural areas of nine states across the country, the combined prevalence was 7.8% and 10.9% among adult men and women aged 18–60 years respectively. These two nationwide surveys showed that the overweight–obesity- combined prevalence in urban areas was higher than in rural areas, which was consistent with findings of other smaller, local surveys (Table 2.02 & Figure 2.03) [Wang Y et al, 2009].

Table 2.02: The prevalence of overweight, obesity and central obesity among adults in India.

Author/published year (reference)	Study year (data collection)	National/regional	SES	Age group (years)	Sample size	Overweight (%)	Classification of overweight	Obesity (%)	Classification of obesity	Central obesity (%)	Classification of central obesity
Nationwide											
Singh *et al.* 2007 (47)	1993–1996	U, 5 metro cities	NA	≥25	T = 6 940 M = 3 507 F = 3 433	T = 40.3 M = 38.3 F = 42.4	BMI ≥ 25	T = 6.8 M = 6.2 F = 7.3	BMI ≥ 30	NA	NA
Singh *et al.* 1998 (28)	1994–1996	Nationwide, U (5 cities)	All	25–64	F = 3 212	NA	NA	NA	NA	F = 55.0	WHR > 0.85
IIPS and Macro International 2000 (19)	1998–1999	NFHS-2, national, U R		15–49	F = 77 119	10.6	BMI ≥ 25	2.2	BMI ≥ 30		
Ramachandran *et al.* 2001 (15)	2000	Nationwide (6 cities)	All	≥20	T = 11 216 M = 5 288 F = 5 928	T = 30.8	BMI ≥ 25	NA	NA	T = 50.3	WHR ≥ 0.90/M WHR ≥ 0.85/F
NNMB 2002 (21)	2000–2001	Nationwide, 9 states, R	All	18–60	M = 11 074 F = 17 318	M = 5.7 F = 8.2	BMI ≥ 25	M = 0.4 F = 1.2	BMI ≥ 30	NA	NA
Reddy *et al.* 2006 (29)	2002–2003	Nationwide, 10 industries, U	All	20–69	T = 19 973 M = 11 898 F = 8 075	T = 51.3 M = 50.9 F = 51.9 T = 30.9 M = 28.6 F = 34.3	BMI ≥ 23 BMI ≥ 25	NA	NA	M = 30.9 F = 32.8 M = 18.2 F = 23.3	WC > 90 cm/M WC > 85 cm/F WC > 94 cm/M WC > 88 cm/F
IIPS and Macro International, 2007 (18)	2005–2006	NFHS-3, national, U R	All	15–49	M = 65 742 F = 111 781	M = 9.3 F = 12.6	BMI ≥ 25	M = 1.3 F = 2.8	BMI ≥ 30		
NNMB 2007 (22)	2005–2006	Nationwide, 9 states, R	All	18–60	M = 14 039 F = 18 603	M = 7.8 F = 10.9	BMI ≥ 25	M = 0.8% F = 1.8%	BMI ≥ 30	NA	NA
Regional											
Gopinath *et al.* 1994 (11)	1985–1987	N, U	All	25–64	T = 13 414 M = 6 143 F = 7 171	T = 27.6 M = 21.3 F = 33.4	BMI>25	NA	NA	NA	NA
Ramachandran *et al.* 1997 (16)	1988–1989	S, U	All	≥20	T = 900	T = 22.0 M = 10.0 F = 33.0	BMI ≥ 27/M BMI ≥ 25/F	NA	NA	NA	NA
Dhurandhar and Kulkarni 1992 (58)	1989–1990	W, U	All	15–76	T = 1 784 M = 791 F = 993	T = 40.9† M = 36.9 F = 44.1	BMI ≥ 25	NA	NA	NA	NA
Ramachandran *et al.* 2000 (17)	1990, 1995	S, U	All	≥20	T = 2 463 M = 1 196 F = 1 267	T = 24.6† M = 19.6 F = 29.4	BMI ≥ 25	NA	NA	T = 33.9 M = 40.6 F = 27.6	WHR > 0.90/M WHR > 0.85/F

Table 2.02: Continued……

Author/published year (reference)	Study year (data collection)	National/regional	SES	Age group (years)	Sample size	Overweight (%)	Classification of overweight	Obesity (%)	Classification of obesity	Central obesity (%)	Classification of central obesity
Gupta *et al.* 2003 (59)	1991	W, U	All	≥20	T = 1 800 M = 960 F = 840	T = 27.2† M = 24.5 F = 30.2	BMI ≥ 25	NA	NA	T = 32.1† M = 21.8 F = 44.0	WC > 102 cm/M WC > 88 cm/F
Shukla *et al.* 2002 (48)	1991–1994	W, U	All	≥35	T = 99 598 M = 40 071 F = 59 527	T = 25.5† M = 19.2 F = 29.7	BMI ≥ 25	T = 5.0† M = 2.2 F = 7.0	BMI ≥ 30	NA	NA
Reddy *et al.* 2003 (60)	1991–1995	N, U R	All	35–64	M: U = 1 456 R = 1 070 F: U = 1 594 R = 1 417	M: U = 35.1 R = 7.7 F: U = 47.6 R = 11.3	BMI ≥ 25	M: U = 7.1 R = 0.7 F: U = 16.4 R = 2.2	BMI ≥ 30	M: U = 71.8 R = 44.9 F: U = 39.5 R = 35.7	WHR ≥ 0.95/M WHR ≥ 0.85/F
Gupta *et al.* 1997 (30)	1992–1993	W, R	L	≥20	T = 3 148 M = 1 982 F = 1 166	NA	NA	T = 5.7 M = 5.2 F = 6.3	BMI ≥ 27	T = 4.4 (22/503) M = 4.3 (17/399) F = 4.8 (5/104)	WHR ≥ 0.95 (data available only in part of participants)
Khongsdier 2005 (61)	1993–1994	E, R	L	18–59	M = 575	M = 11.65	BMI ≥ 23	NA	NA	NA	NA
Singh *et al.* 1998 (31)	1993–1995	N, U	All	25–64	T = 1 806 M = 904 F = 902	T = 39.0† M = 31.7† F = 46.2†	BMI > 25	T = 11.9† M = 10.5† F = 13.3†	BMI > 27	T = 47.9† M = 40.9† F = 55.9†	WHR > 0.88/M WHR > 0.85/F
		N, R	All	25–64	T = 1 769 M = 894 F = 875	T = 16.2† M = 15.7† F = 16.7†	BMI > 25	T = 5.1† M = 5.0† F = 5.3†	BMI > 27	T = 19.1† M = 23.7† F = 14.3†	WHR > 0.88/M WHR > 0.85/F
Ramachandran *et al.* 1997 (16)	1994–1995	S, U	All	≥20	T = 2 183 M = 1 081 F = 1 102	T = 18.1 M = 8.0 F = 28.0	BMI ≥ 27/M BMI ≥ 25/F	NA	NA	NA	NA
Gupta *et al.* 2003 (12)	1995	W, U	L	≥20	T = 2 212 M = 1 415 F = 797	T = 20.4† M = 20.7 F = 19.9	BMI ≥ 25	NA	NA	T = 60.3† M = 54.7 F = 70.1	WHR > 0.90/M WHR > 0.80/F
Prabhakaran *et al.* 2005 (62)	1995–1998	N, industry	NA	20–59	M = 2 122	35 58.5	BMI ≥ 25 BMI ≥ 23	3.3	BMI ≥ 30	7.2 43.7	WC > 102 cm WC > 90 cm
Beegom *et al.* 1995 (63)	(1995)	S, U	All	25–64	T = 1 497 M = 737	NA	NA	NA	NA	T = 56.0 M = 54.5	WHR > 0.88

Table 2.02: Continued……..

Author/published year (reference)	Study year (data collection)	National/regional	SES	Age group (years)	Sample size	Overweight (%)	Classification of overweight	Obesity (%)	Classification of obesity	Central obesity (%)	Classification of central obesity
Mohan *et al.* 2001 (14)	1996	S, U	All	≥20	T = 1 262	M = 38.0[H] M = 13.4[L] F = 33.1[H] F = 24.2[L]	BMI > 25	NA	NA	M = 53.4[H] F = 41.6[H] M = 30.8[L] F = 16.9[L]	WHR > 0.90/M WHR > 0.85/F
Misra *et al.* 2001 (13)	1998	N, U	All	>18	T = 532 M = 170 F = 362	T = 13.9 M = 13.3 F = 15.6	BMI > 25	T = 30.7 M = 10.6 F = 40.2	%BF > 25/M %BF > 30/F	T = 37.8 M = 9.4 F = 51.1	WHR > 0.95/M WHR > 0.80/F
Reddy 1998 (33)	(1998)	S, U R	All	18–75	T = 1 119 M = 456 F = 663	T = 8.6[†] M = 6.6 F = 10.0	BMI ≥ 25	NA	NA	NA	NA
Sidhu and Tatla 2002 (64)	1998–1999	N, U	UM	≥20	F = 1 000	F = 45.3	BMI ≥ 25	F = 25.3	BMI ≥ 30	NA	NA
Griffiths and Bentley 2001 (54)	1998–1999	S, U R	All	15–49	F = 4 032	T = 12.2 U = 37.0 R = 8.0	BMI ≥ 25	T = 2.2%	BMI ≥ 30	NA	NA
Gupta *et al.* 2003 (12)	2002	W, U	L	≥20	T = 1 123 M = 550 F = 573	T = 36.3[†] M = 33.0 F = 39.4	BMI ≥ 25	NA	NA	T = 62.0[†D] M = 54.4[D] F = 69.2[D]	WHR > 0.90/M WHR > 0.80/F
Anand *et al.* 2007 (49)	2003–2004	N, U	L	15–64	T = 2 561	M = 15.9 F = 21.6	BMI ≥ 25	M = 2.1 F = 5.6	BMI ≥ 30		
Hazarika *et al.* 2004 (65)	(2004)	E, R, Assam	L	≥30	T = 3 180 M = 1 441 F = 1 739	T = 6.9	BMI ≥ 25	T = 0.9	BMI > 30	T = 60.8	WHR < 0.9
Deshmukh *et al.* 2006 (66)	2004	W, R	NA	≥18	T = 2 700 M = 1 059 F = 1 641	T = 11.0 M = 11.6 F = 10.6	BMI ≥ 23	T = 5.1 M = 5.1 F = 5.2	BMI ≥ 25	Definition 1: M = 7.6 F = 8.7 Definition 2: M = 21.5 F = 30.5	Definition 1: WC > 90 cm/M WC > 80 cm/F Definition 2: WHR > 0.9/M WHR > 0.8/F

When the original studies did not report the survey year, the publication year was listed in '()'.
[†]Calculated by researcher based on reported data.
A, prevalence of obesity for 1988–1989; B, prevalence of obesity for 1994–1995; BF, body fat; BMI, body mass index (kg m^{-2}); E, east region; F, female; H, high- and medium-income group; L, low-income group; M, male; N, north region; NA, not available; NFHS, National Family Health Survey; NFHS-2, NFHS 1998–1999; NFHS-3, NFHS 2005–2006; R, rural area; S, south region; SES, socioeconomic status; T, total; U, urban area; UML, upper middle-income group; W, west region; WC, waist circumference; WHR, waist-to-hip ratio.

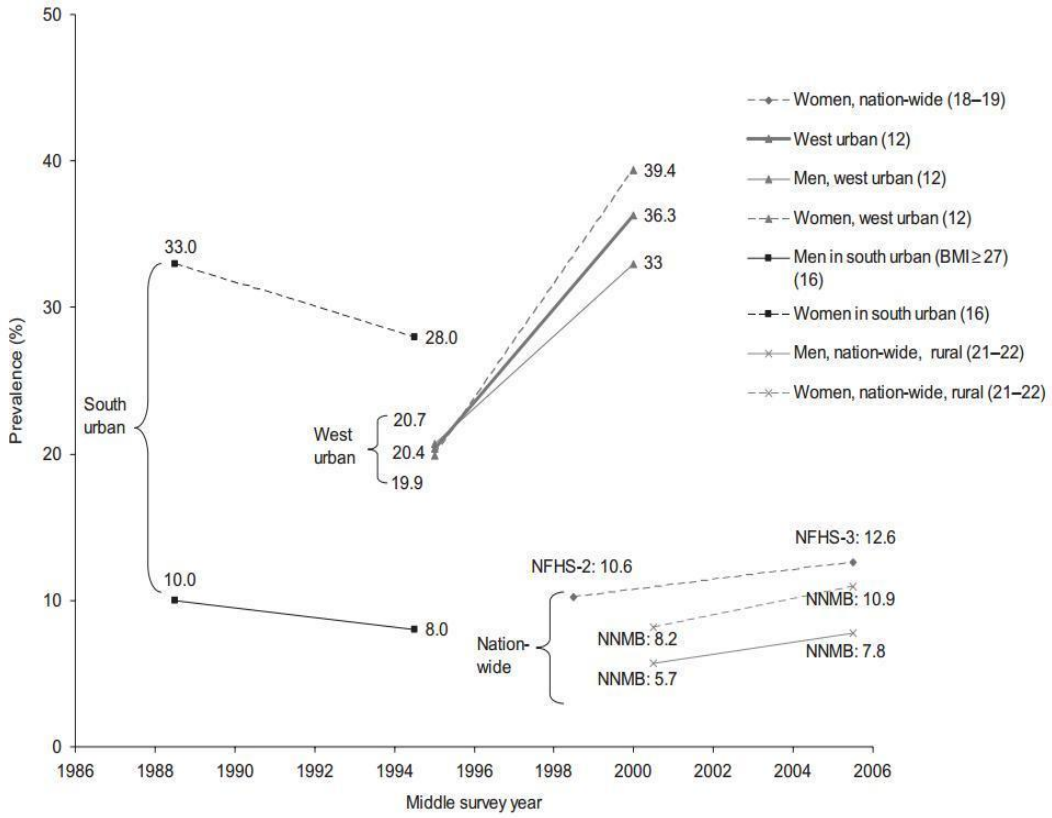


Figure 2.03: Time trends in the prevalence of overweight and obesity in India between the 1980s and 2000s: based on nationwide or regional surveys. Overweight and obesity, BMI □ 25, unless noted for other definition. Middle survey year, the mid-point of the survey period. Solid lines denote for men and dashed lines for women, while thick solid lines denote for overall prevalence. The NFHS-3 found that the prevalence in men was 9.3. BMI, body mass index; NFHS, National Family Health Survey; NFHS-2, NFHS 1998–1999; NFHS-3, NFHS 2005–2006; NNMB, National Nutrition Monitoring Bureau [Wang Y et al, 2009].

2.2.3 Future projection of Obesity

Overweight and obesity are significant and increasing public health challenges in both economically developed and developing regions of the world, with 33.0% of the world's adult population (1.3 billion people) overweight or obese in 2005. Moreover, if recent trends continue, by 2030 up to 57.8% of the world's adult population (3.3 billion people) could be either over-weight or obese. The prevalence of overweight and obesity was higher in economically developed countries

compared with economically developing countries, 35.2 vs 19.6% and 20.3 vs 6.7%, respectively, in 2005 [Kelly T et al, 2008]. Although overweight and obesity is more common in economically developed countries, the much larger population of developing countries results in a considerably larger absolute number of individuals affected. Moreover, compared with developed regions of the world, developing regions are projected to have a much larger proportional increase in the number of overweight and obese individuals between 2005 and 2030 (Table 2.03). Growth in population size, population aging, urbanization and changes in lifestyle including increases in total calorie intake and reductions in physical activity, all contribute to an epidemic of overweight and obesity in developing regions.

Table 2.03: Projected absolute burden of overweight and obesity in 2030 (in millions) [Kelly T et al, 2008].

World regions	*Constant prevalence*[a]		*Assume past trends*[b]	
	Prevalence	*Number*	*Prevalence*	*Number*
Overweight				
Established market economies	36.3	270.6	30.0	223.5
Former socialist economies	37.6	90.4	28.6	68.8
Middle eastern crescent	22.2	142.4	22.6	145.2
Latin America and the Caribbean	32.3	162.0	43.6	218.6
India	12.9	134.8	27.8	290.7
China	26.6	298.4	59.7	669.2
Other Asia and islands	21.7	155.7	49.6	355.3
Sub-Saharan Africa	14.9	96.2	28.7	185.8
Total	23.9	1350.4	38.1	2157.0
Obesity				
Established market economies	22.1	164.7	36.2	270.1
Former socialist economies	21.5	51.6	37.0	89.0
Middle eastern crescent	14.6	93.5	24.1	154.5
Latin America and the Caribbean	19.6	98.0	38.3	191.8
India	4.0	42.2	5.0	52.1
China	4.2	47.1	12.6	141.2
Other Asia and islands	4.7	33.4	14.5	104.0
Sub-Saharan Africa	6.6	42.4	17.5	113.1
Total	10.1	573.0	19.7	1115.8

[a]Projections are based only on current prevalence and population growth and demographic shifts (age distribution and urbanization). [b]Projections are based on regional secular trends in prevalence of overweight or obesity estimated from past data and population growth and demographic shifts (age distribution and urbanization).

The global burden of overweight and obesity in 2030 has been projected under two distinct assumptions:

(1) The age-specific prevalence of overweight and obesity in each world region by gender and urbanization would remain stable, and

(2) The age-specific prevalence of overweight and obesity in each world region by gender and urbanization would continuously increase based on recent secular trends.

Existing data suggest that the prevalence of overweight and obesity is increasing in many developed and developing countries and these changes are likely to continue over the next few decades [Prentice A, 2006] The challenge of weight reduction combined with its costliness makes primary prevention of overweight and obesity a more feasible and cost-effective alternative for curbing the obesity epidemic, particularly in areas where healthcare resources are limited. A reduction in the global burden of overweight and obesity will translate into worldwide decreases in diabetes, cardiovascular disease, cancer, all-cause mortality and other associated complications [Kelly T et al, 2008].

2.3 Types of Obesity

Obesity cannot be seen anymore as a homogeneous phenotype, yet a commonly agreed-on classification of the obesity phenotypes does not exist. Nonetheless, four different types of human obesity can be recognized [Bouchard C, 1991] on the basis of the topography of the adipose tissue and its association with a variety of metabolic characteristics. These four types of obesities are outlined in Table 2.04.

Table 2.04: Types of Obesity [Bouchard C, 1991]

Type I	**Excess body mass or percentage of fat**
Type II	**Excess subcutaneous truncal-abdominal fat (android)**
Type III	**Excess abdominal visceral fat**
Type IV	**Excess gluteofemoral fat (gynoid)**

Type I is characterized by excess total body fat without any particular concentration of fat in a given area of the body. The other types have to do with excessive

accumulation of fat in some areas of the body, that is, they are based on the anatomical distribution of body fat. Type II is defined as excess subcutaneous fat on the trunk, particularly in the abdominal area, and is equivalent to the so-called android or male type of deposited fat. Type III is characterized by an excessive amount of fat in the abdominal visceral area (abdominal visceral obesity), and type IV is defined as gluteofemoral obesity and is observed primarily in women (gynoid obesity). Thus, excess fat can be stored primarily in the truncal-abdominal area or in the gluteal and femoral area; in other words, for a given excess body fat, one could be either type II (android), type IV (gynoid), or type III obese. This implies, for example, that a body fat content of 30% or 50 kg may exhibit different anatomical distribution characteristics.

2.3.1 A Multifactorial Phenotype

Strictly speaking, therefore, one should talk about the obesities rather than the obesity. Figure 2.04 describes a simple model designed to integrate the various classes of affecters of obesity and their interactions [Bouchard C, 1987; Bouchard C, l990; Bouchard C, 1991].

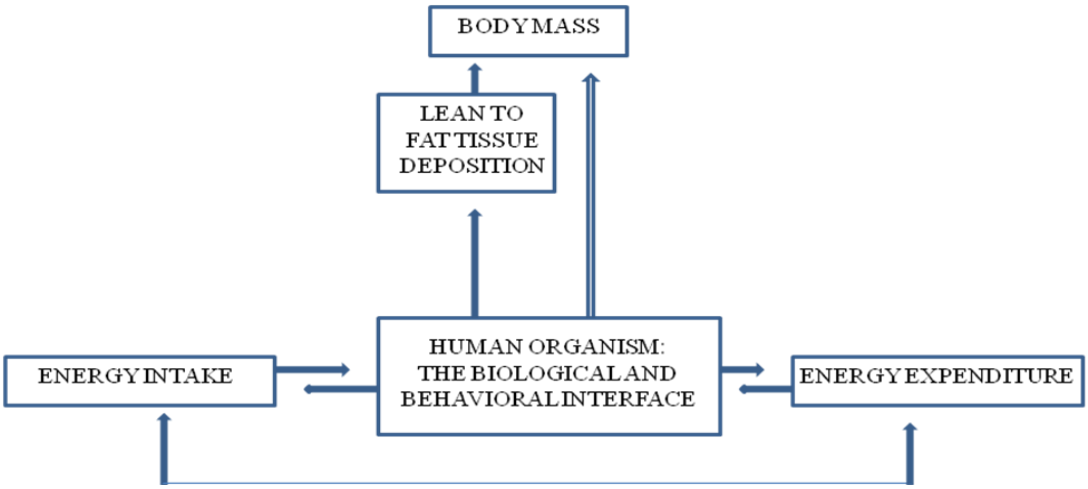


Figure 2.04: A paradigm of the major affecters of body mass or body mass changes with variations in energy balance [Bouchard C, 1991].

Each affecter constitutes a family of components so that the total picture is a complex one. Energy intake includes the total caloric intake, the macronutrient composition of the intake, the palatability of the diet and its content of various

amino acids and other molecules as they influence the metabolic outcomes of the food ingested, appetite, and satiety. Energy expenditures include basal and resting metabolic rates, thermic effect of food, energy cost of exercise and work, level of habitual physical activity, temperature-induced thermogenesis, stress-induced thermogenesis, and other components. The next component is probably the most complex. It is defined as the interface between energy (nutrient) intake and expenditure and results from the fact that there are characteristics of the human body that have an impact on the outcome of a given set of energy intake and expenditure conditions. Variations in the biological-behavioral interface can be caused by inherited or acquired conditions. For instance, there could be inherited variants in enzymes of the liver (or other tissues) that may influence the efficiency of lipid metabolism in the carrier individuals and affect substrate use under certain dietary conditions. This in turn may have an impact on energy balance and influence the body-fat phenotype. Furthermore, on the basis of some of the most recent data, it appears that a critical determinant of total body fat is the proneness to store the surplus of ingested energy as triacylglycerides in adipose tissue or as lean tissue [Bouchard C, l980].

2.3.2 What is a normal BMI?

The WHO defined overweight and obesity as abdominal or excessive fat accumulation that may impair health. The WHO [1995] accepted the body mass index (BMI) as the appropriate method for crudely assessing degrees of underweight and overweight. BMI is a simple index of weight-for-height that is commonly used to classify overweight and obesity in adults. It is defined as a person's weight in kilograms divided by the square of his height in meters (kg/m^2). The WHO definition is:

- A BMI greater than or equal to 25 is overweight
- A BMI greater than or equal to 30 is obesity.

BMI provides the most useful population-level measure of overweight and obesity as it is the same for both sexes and for all ages of adults. However, it should be considered a rough guide because it may not correspond to the same degree of

fatness in different individuals. The WHO considered 18.5 limit for distinguishing normal from underweight following a series of earlier international analyses of the capacity for work and propensity to infections which came with progressively lower BMIs [James WPT, 1988].

2.3.3 Major Determinants of Obesity

The most commonly recognized determinants or correlates of excess body weight-for-height or excessive body fat content are listed in Table 2.05.

Table 2.05: Determinants and correlates of excess body weight or fat [Bouchard, 1991]

Age	More prevalent in adults and middle-aged individuals.
Gender	Females have more fat.
Positive energy balance	An absolute requirement over a relatively long period.
Amount of energy intake	Overfeeding leads to gain in weight and fat mass.
Composition of intake	High-fat intake may be a contributing factor.
Physical activity level	Low or decreasing level of activity.
Resting metabolic rate	A low value with respect to body mass and fat free mass is correlated with weight gain.
Thermic effect of food	Low for energy intake in some obesity cases.
Lipid oxidation	A high respiratory quotient is correlated with body fat and weight gain.
Ratio of fat to lean tissue	A high fat mass-to-fat free mass ratio is correlated with excess weight or weight gain.
Adipose-tissue lipoprotein lipase activity	High in obese individuals and remains high (perhaps even increases) with weight loss.
Variety of social and behavioral factors	Obesity is associated with socioeconomic status, familial conditions, network of friends, pattern of leisure activities, television time, smoking habits, alcohol intake, etc.
Undetermined genetic characteristics	These affect energy balance particularly via the energy expenditure components, the deposition of the energy surplus as fat or as lean tissue, and the relative proportion of lipids and carbohydrates oxidized.

Of the correlates identified in Table 2.06, composition of the diet, resting metabolic rate, thermic response to food, proportion of lipid oxidation, and proneness to store energy in the form of fat or lean tissue are of particular interest. A high-fat diet is becoming increasingly recognized as a factor that enhances the risk of being in positive energy balance [Romieu I, l988; Dreon DM et al, 1988]. Resting metabolic rate is correlated with weight gain over time, thus with positive energy balance [Ravussin E, et al. l988]. A depressed thermic response to food is observed in a subgroup of obese individuals but not in all of them [Jequier E, 1985]. The respiratory-exchange ratio, an estimate of the relative proportion of lipid and carbohydrate oxidized, is correlated with weight gain over time [Zurlo F, 1990]. Furthermore, the proneness to store excess food energy as fat or lean tissue was correlated with weight gain in an overfeeding experiment [Bouchard C, l980]. Our own studies of cohorts of large eaters and small eaters indicate that for comparable body-weight levels, the small eaters have a higher ratio of fat mass to fat free mass [George V et al, l989; George V, et al. 1991].

2.4 Causes of the epidemic: the roots of the problem

The fundamental cause of obesity and overweight is an energy imbalance between calories consumed and calories expended. Globally, there has been:

- An increased intake of energy-dense foods that are high in fat, salt and sugars but low in vitamins, minerals and other micronutrients; and
- A decrease in physical activity due to the increasingly sedentary nature of many forms of work, changing modes of transportation, and increasing urbanization.

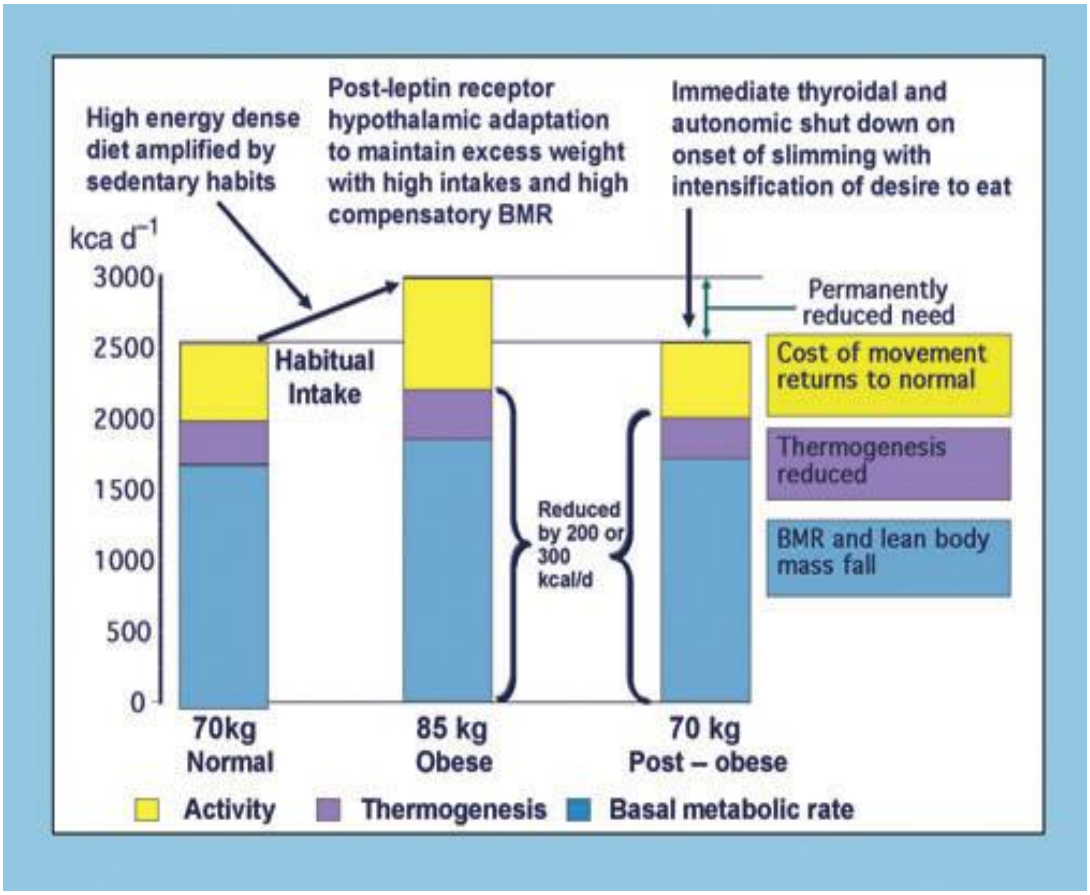


Figure 2.05: The sequence of energetic and physiological adaptations to weight gain and subsequent slimming explaining the frequency of weight re-gain [James WPT, 2008].

Changes in dietary and physical activity patterns are often the result of environmental and societal changes associated with development and lack of supportive policies in sectors such as health, agriculture, transport, urban planning, environment, food processing, distribution, marketing and education. This epidemic is assuming serious proportions in cities and is affecting young adults and children. The strongest risk factor for obesity is urbanization [Fall CHD, 2001]. Obesity is at least three times more common in cities than in villages, although it is increasing rapidly even in villages because traditional villages are also becoming urbanized in their habits (urbanization in situ, also termed rubanization). Another related risk factor is higher socio-economic status. This situation is opposite to that in developed countries where lower socio-economic groups are more affected (‘reversal’ of socio-

economic gradient). In simple mechanistic terms weight gain occurs when energy intake by an individual exceeds energy expenditure over a period of time. Changing patterns of food intake (both in quality and quantity) and physical activity contribute to the positive energy balance. Genetic as well as non-genetic determinants affect an individual's response to energy intake as well as physical activity, and therefore influence the balance between the two factors [Prentice A, 2001]. It is possible that a 'thrifty genotype' may have helped man survive famine conditions by successfully depositing fat. However, in the current situation of excess food and reduced activity this genotype may lead to obesity. Like most other polygenic conditions, the contribution of genetic factors to obesity is not clear at the population level. However, a number of rare syndromes of extreme obesity have been related to specific mutations in genes [Barsh GS, 2000]. Studies in twins also favour a role for genetic factors in the etiology of obesity [Bouchard A et al. 1990; Sims EAH, 1990]. It is possible that like other chronic polygenic disorders (diabetes and hypertension); the expression of obesity is influenced by environmental conditions. Recent interest has focused on the possible role of early life environment in the pathogenesis of obesity [Yajnik C.S, 2004].

The recent constitution of the Developmental Origins of Health and Disease (DOHaD) [Kuzawa C, 2007; Silveira PP et al, 2007; Sinclair KD et al, 2007] society has drawn great interest to study the public health challenges faced by countries under rapid economic and nutrition transitions, particularly in those developing countries that have experienced economic development and improvement in people's living standard, and as a result, a shift from under- to over nutrition problems. Obesity increases the risk of many other chronic diseases [WHO, 2000]. The prevalence of obesity has been increasing globally, and its impact on public health is marked in both developed and developing countries [WHO, 2000; Wang Y, 2005; Wang Y, 2006].

2.5 Common health consequences of overweight and obesity:

Raised BMI is a major risk factor for noncommunicable diseases such as:

- Cardiovascular diseases (mainly heart disease and stroke), which were the leading cause of death in 2008.

- Diabetes.
- Musculoskeletal disorders (especially osteoarthritis - a highly disabling degenerative disease of the joints).
- Some cancers (endometrial, breast, and colon).

Childhood obesity is associated with a higher chance of obesity, premature death and disability in adulthood. In addition to increased future risks, obese children experience breathing difficulties, increased risk of fractures, hypertension, and early markers of cardiovascular disease, insulin resistance and psychological effects.

2.5.1 Facing a double burden of disease

Many low- and middle-income countries are now facing a "double burden" of disease.

- While they continue to deal with the problems of infectious disease and under-nutrition, they are experiencing a rapid upsurge in noncommunicable disease risk factors such as obesity and overweight, particularly in urban and semi-urban settings.

- It is not uncommon to find under-nutrition and obesity existing side-by-side within the same country, the same community and the same household.

Children in low- and middle-income countries are more vulnerable to inadequate pre-natal, infant and young child nutrition. At the same time, they are exposed to high-fat, high-sugar, high-salt, energy-dense, micronutrient-poor foods, which tend to be lower in cost. These dietary patterns in conjunction with low levels of physical activity result in sharp increases in childhood obesity while under nutrition issues remain unsolved.

2.6 Prevention of the disease: Overweight and Obesity:

Overweight and obesity, as well as their related noncommunicable diseases, are largely preventable. To cope with the huge burden of chronic disease, supportive environments and communities are fundamental in shaping people’s choices,

making healthier choice of foods and regular physical activity the easiest choice, and therefore preventing obesity.

At the individual level, people can:

- Limit energy intake from total fats;
- Increase consumption of fruit and vegetables, as well as legumes, whole grains and nuts;
- Limit the intake of sugars;
- Engage in regular physical activity;
- Achieve energy balance and a healthy weight.

Individual responsibility can only have its full effect where people have access to a healthy lifestyle. Therefore, at the societal level it is important to:

- Support individuals in following the recommendations above, through sustained political commitment and the collaboration of many public and private stakeholders.
- Make regular physical activity and healthier dietary patterns affordable and easily accessible to all - especially the poorest individuals in the society.

The food industry can play a significant role in promoting healthy diets by:

- Reducing the fat, sugar and salt content of processed foods.
- Ensuring that healthy and nutritious choices are available and affordable to all consumers.
- Practicing responsible marketing.
- Ensuring the availability of healthy food choices and supporting regular physical activity practice in the workplace.

Obesity now, is also, the concern of most, if not all, governments who now readily accept its complexity and major public health impact. Their main concern is now rapidly moving towards prevention and not simply treating the consequences. The medical profession, however, is working out in clinical and experimental terms what the principal mechanisms underlying weight gain and how to determine the complex intestinal / neurological control of energy balance and the mechanism of biological resistance to weight loss in obese patients [James WPT, 2008].

Chapter 3

Review of Literature II

Review of Literature II

3.1 Ayurveda

Ayurveda, which translates as the ‘‘Science of Life,’’ is a comprehensive, holistic health care system that originated in the ancient Vedic times of India. Ayurveda primary emphasis is on prevention of disease and preservation and promotion of health; this system also provides treatment for disease. According to Ayurveda, the human body is composed of five Mahabhutas (basic elements that have the properties of space, air, fire, water, and earth) that combine to form Vata, Pitta, and Kapha, the three psychophysiologic principles known as Doshas (Table 3.01). The body is regulated by the three Doshas and the three qualities of mind known as the Gunas (Sattva, Rajas, and Tamas).

Table 3.01: Combinations of Mahabhutas That Form the Doshas [Sharma H, 2011]

Mahabhutas	*Doshas*
Space Air	Vata
Fire Water	Pitta
Water Earth	Kapha

The Doshas regulate various functions in the body. Vata governs functions associated with movement and communication (e.g. blood flow, nerve conduction, intestinal motility, etc.) Pitta governs functions associated with metabolism, digestion, and transformation (e.g. appetite, endocrine functions, etc.) Kapha governs the structure and cohesion of the body (e.g. strength, stability, weight, etc.) (Sharma H et al, 2007]. The Gunas are mental qualities. Sattva is the creative influence associated with intelligence, purity, and balance. Rajas is the spur to activity. Tamas is the influence of inertia, which results in stability. (Sharma H, 1998]. In the body there are also seven Dhatus, which are fundamental principles

that upport the various bodily tissues; these are Rasa (plasma), Rakta (blood), Mamsa (muscle), Meda (fat), Asthi (bone), Majja (bone marrow), and Shukra (sperm or ovum). There are three metabolic waste products known as Malas, which are Mutra (urine), Purisha (feces), and Sweda (sweat). Energy flow and communication take place through various channels of organization known as Srotas. The spectrum of health and disease depends on the functioning of these various constituents. A state of equilibrium in their functioning results in health and disequilibrium leads to disease.

Prakriti is the individual's psychophysiologic constitution and is determined at the time of birth by the individual's Dosha proportions. Each individual has a certain ratio of Vata, Pitta, and Kapha that is unique to him/her. Individuals with a Vata-predominant Prakriti have a light, thin build; perform activity quickly; have a tendency toward dry skin and constipation; have an aversion to cold weather; have a tendency to worry; and sleep lightly. Individuals with a Pitta-predominant Prakriti are moderate in build; perform activity with medium speed; have an aversion to hot weather; have sharp hunger and digestion; prefer cold foods and drinks; have a tendency toward irritability and short temper; and are excellent speakers. Individuals with a Kapha-predominant Prakriti have a solid build and great strength and endurance; are slow and methodical in activity; have oily, smooth skin; have a steady and tranquil personality; sleep long and heavily; have slow digestion and mild hunger; and have a tendency toward greed. [Sharma H, 2011].

Current research in the fields of genomics and pharmacogenomics is revealing the possibility of utilizing Prakriti to correlate phenotypes with genotypes in the human population. This would have a significant impact on the field of personalized, predictive medicine [Ghodke Y, 2009; Prasher B, 2008; Patwardhan B, 2008; Patwardhan B, 2005].

3.2 Ayurvedic concept of obesity:

Ayurveda identifies eight different types of bodies that are prone to diseases. Out of these, an obese body is described as the one afflicted with the most diseases and troubles. The Ayurvedic approach is the perfect answer to overweight because

Ayurveda does not recommend any weight loss pills or fast weight loss programs. Ayurveda emphasize on a holistic solution that allows people to lose excess weight and keep it off, while also improving their overall health [Sharma H, 2011].

According to Ayurveda both overweight and underweight are due to the imbalance in three doshas: vata, pitta, and kapha [Sharma H, 2011] This can happen irrespective of how much or how little food you eat. Improper digestion or improper metabolism due to excessive Kapha and ama is at the root of both overweigh and underweight. When Kapha is imbalanced, the heavy characteristics of Earth and water elements become exaggerated. However, obesity can be found in people with the other two doshas also. For example, people with vata and pitta doshas can suffer from poor digestion if their digestive fire is too low. As a result, food isn't processed properly, and that, in turn, can result in weight gain or obesity. For people with vata dosha the problem generally is that of underweight rather than overweight. They cannot properly absorb nutrients. However, they can become overweight when imbalanced if they attempt to eat sweet, oily foods in an attempt to calm their vata imbalance. The Ayurvedic approach to achieving your ideal weight is based on taking a realistic look at your body type and revamping the entire digestive & metabolic processes. Nevertheless, low calorie diet and regular exercise are at the very basis for weight management [Sharma H, 2011].

3.2.1 Prameha and Obesity

Ayurveda describes a set of complex clinical disorders, collectively called Prameha, that are characterized by frequent abnormal micturition. [Manyam BV, 2004). The generalized causes of Prameha include long periods of physical inactivity, laziness, sleeping for long hours, and consumption of dairy products, aquatic and marshy animals, sugar/jaggery preparations (jaggery is an unrefined form of cane sugar), fresh grains, and similar foods that increase Kapha. Prameha, though a Tridoshaja Vyadhi (a disease involving all three of the Doshas [i.e. Vata, Pitta, and Kapha]), is basically a disease with Kapha predominance. Also affected are the Dhatus, Vasa (fat), Udak (fluid), Lasika (lymph), and Ojas (the subtlest material substance in the body; the essence of the body; Ojas maintains the body's immunity and vitality). Low Agni (digestive and metabolic process) also leads to accumulation of Ama

(buildup of toxins from improperly digested food and metabolic products). All these factors combine to produce the disorders known as Prameha. Prameha may be hereditary or acquired. It may have Kapha, Pitta, or Vata as the predominant Dosha in the disease process. Patients with Prameha may be obese or asthenic [Shastri A, 2003]. There are 20 subtypes of Prameha resulting from the interaction of the three Doshas and ten Dushyas (disturbed functioning of the principles that support the various bodily tissues). These subtypes include ten Kapha-predominant types, six Pitta-predominant types, and four Vata-predominant types [Shukla VD, 2002]. The clinical conditions associated with these subtypes of Prameha have much in common with disorders described in allopathic medicine that are associated with obesity, metabolic syndrome, and diabetes mellitus [Shastri KN, 2004]. The etiology of Prameha is discussed in Sushruta Samhita, which identifies two types of Prameha: Sahaja, which is hereditary, and Apathyanimittaja which is acquired. Charaka Samhita mentions Jatah Pramehi due to Bija Dosha, meaning Prameha that arises due to genetic factors [Gupta KA, 2007].

The description of Apathyanimittaja Prameha in Sushruta Samhita is very similar to that of type 2 diabetes (also known as non-insulin-dependent diabetes mellitus or adult onset diabetes). The types of food and drink likely to precipitate this disease have been enumerated in all the classical Ayurvedic texts. [Gupta KA, 2007].

These are briefly listed below, along with lifestyle factors and psychologic factors that lead to the onset of Prameha:

1. Dietary factors: Excessive intake of yogurt, meat of aquatic animals, milk, new (not aged) grains, foods/drinks containing sugar and jaggery (an unrefined form of cane sugar), cold foods, sweet foods, sour foods, unctuous (oily) foods, liquid foods, foods that are heavy to digest, and slimy foods.
2. Lifestyle factors: Sedentary lifestyle, excessive sitting, excessive sleeping, sleeping during the daytime, lack of exercise, and laziness.
3. Psychologic factors: Disturbance in mental health caused by extremes of psyche such as Vishada (depression) and bipolar disorder.

3.2.2 Doshic Classification of Prameha:

Prameha has been classified according to the predominant Dosha in the disease process. Ayurveda describes three groups of basic clinical distinctiveness, which are Kaphaja, Pittaja, and Vataja Prameha. [Shastri A, 2003; Shukla VD, 2002; Gupta KA, 2007]. Ayurveda has identified a progression of Prameha through several stages. In the initial stage, Kapha is in excess, which vitiates Meda (fat) and Kleda (body fluid), thereby precipitating Kaphaja Prameha. Further progression results in Kshaya (loss) of Kapha. Pitta then predominates, which vitiates the blood (Rakta), precipitating Pittaja Prameha. Further progression results in loss of Pitta. This leads to vitiation of Vata, which drags vital substances/vital essence out of the body through the urine, precipitating Vataja Prameha [Gupta KA, 1951]. Ayurveda also specifies that any of these three types of Prameha can be precipitated directly, depending upon genetic predisposition and improper diet and lifestyle.

Correlating Prameha with obesity, metabolic syndrome, and diabetes mellitus, the early manifestation of the disease process in these conditions, with carbohydrate, lipid, and protein metabolism disturbances accompanied by glycosuria, proteinuria, etc., correlate with Kaphaja Prameha, which can be easily controlled and cured. The inflammatory, hepatic, and gallbladder complications and lipid and blood abnormalities are much more in line with the description of Pittaja Prameha, which can be managed. The advanced stage of disease, with metabolic disturbances associated with loss of immunity, correlates with T2DM that has progressed into insulin-dependent diabetes, and correlates with the hereditary form of type 1 diabetes, which both correlate with Vataja Prameha. Both of these are incurable as described in Ayurveda.

3.2.3 Sthaulya (obesity) and Prameha:

The striking relationship between Prameha and Sthaulya (obesity) has been discussed in Ayurvedic literature, where Prameha is said to be one of the complications of obesity. [Shastri A, 2003]. As a result of physical inactivity and excessive intake of sweet substances, there is formation of Ama, which is a buildup of toxins from improperly digested food and metabolic products. The buildup of Ama leads to additional formation of Meda (fat). This refers to an increase in

adipose tissue in the body, resulting in the individual becoming overweight. It reflects the current understanding of the peculiar metabolic state in obese individuals, wherein carbohydrate is largely converted to fatty acids. The multifactorial involvement of Meda (fat), Kapha, Vata, and Agni (digestive metabolic activity) is a common pathophysiologic phenomenon of both Prameha and obesity. Hyperinsulinemia and insulin resistance are pervasive features of obesity, increasing with weight gain and diminishing with weight loss. Insulin resistance is linked more closely to intra-abdominal fat than to fat in other locations. [Gopalani A, 2007; Ascaso JF et al. 2003]. A pathway through which obesity causes insulin resistance has been discovered in mice, in the form of an adipose tissue–derived hormone named resistin an important link between obesity and diabetes. [Steppan CM et al, 2001].

Charaka Samhita has firmly established the relationship between obesity and Prameha [Shukla VD, 2002]. The role of Meda (fat/adipose tissue) is of great importance in the pathogenesis of Prameha. Its role is not only as Dushya (disturbed functioning of the Dhatus), but something more than that. According to Charaka Samhita, Bahudrava Shleshma (Kapha that contains too much liquid) joins and affects Meda, causing it to become Abaddha (unobstructed or fluid) in nature. This form of Meda has been described as acting on Mamsa (muscle tissue), thereby increasing the volume of body fluid. This has been described as Sharira-Kleda (body of fluid) in Ayurveda. Thus, excess water in the blood causes increased diuresis. This is how the Sharira-Kleda is converted into urine, as discussed in Charaka Samhita. This route of pathogenesis for Prameha is closely related to obesity.

3.3 Ayurvedic Approach to obesity:

In ayurvedic text, 'Charakacharya' has described eight 'nindya prakruties' (undesirable constitution) according to the body constitution. Among them he has also mentioned obesity. Obesity is described as 'Medoroga' in Ayurved. It is said that it is comparatively easy to help an underweight person, rather than an overweight person. The overweight problem can be due to an actual increase in the fat component (Meda Dhatu), or it can be due to malfunctioning. These, accordingly, will need different approaches. In very few cases it can be an offshoot

of other metabolic disorders. Body is made of 7- Dhatus {Rasa (Lymph), Rakta (Blood), Maans (Muscle), Meda(Fat), Asthi (Bones), Majja (Nervous System) Shukra (Reproductive System)}. But in Obese fellow Meda is excessively nourished and remaining other Dhatus get malnourished. Kapha gets accumulated in between. When Kapha increases in abnormal fashion, Fat metabolism gets hampered and person becomes Obese.

The prevalence of obesity is increasing worldwide [WHO, 1997] resulting in an association with major health problems such as type 2 diabetes, ischemic heart disease, stroke, and cancer. It is necessary to treat obese individuals by both lifestyle interventions and/or pharmacological therapy.

Pharmacologic treatment and surgical interventions used in some circumstances are not always Appropriate [Hardeman Wet al, 2000]. Unfortunately, drug treatment of obesity despite short-term benefits, is often associated with rebound weight gain after the cessation of drug use, side effects from the medication, and the potential for drug abuse [Abdollahi M, 2003]. Pharmacologic options include sibutramine, orlistat, phentermine, diethylpropion, and fluoxetine or bupropion. Phentermine and diethylpropion have potential for abuse and are only approved for short- term use. Approved medications for long term use in the treatment of obesity are sibutramine and orlistat, however, these agents should be used with caution in patients with a history of cardiovascular disorders [Mahan LK, 2008] . The general public uses many other methods for weight loss including herbs, vitamins, nutritional supplements, and meal replacement preparations. Rigorous scientific studies have not been carried out on these products, and in many cases safety and efficacy take a back seat to marketing. Complementary and alternative therapies have long been used in the Eastern world but recently these therapies are being used increasingly worldwide [Hasani-Ranjbar S, 2008]. When conventional medicine fails to treat chronic diseases and conditions such as obesity efficaciously and without adverse events, many people seek unconventional therapies including herbal medicine [Liu JP, 2004]. Although the number of randomized trials on complementary therapies has doubled every 5 years and the Cochrane library included 100 systematic reviews of unconventional

interventions [Liu JP, 2004], none of these studies specifically mentioned herbal therapy in obesity. Herbal medicines are defined as raw or refined products derived from plants or parts of plants (e.g. Leaves, stems, buds, flowers, roots, or tubers) used for the treatment of diseases. The synonyms of herbal medicines are herbal remedies, herbal medications, herbal products, herbal preparations, medicinal herbs, and phytopharmaceuticals, etc. Human and animal studies with or without specific dietary and exercise program have been outlined in Table 3.02 and 3.03 as weight loss programs. The route of administration of herbs in almost all studies was oral intake [Hasani-Ranjbar S, 2009].

Table 3.02: Human studies considering the anti-obesity effects of herbal medicine [Hasani-Ranjbar S, 2009].

Authors	Target	Herbs (scientific name)	Study	Dose/duration	Groups	Main outcome	Other relevant effects & complications
Ignjatovic *et al*[8] 2000	Healthy volunteers	Slimax: extract of several plants: Hordeum vulgare, Polygonatum multiflorum, Dimocarpus longan, Ligusticum sinense, Lilium brownie, and *Zingiber officinale*	RCT	6 wk	C: Placebo I: Compound	Sig. decrease in body wt. & waist & hip Cir. & BMI	Modification of lipid metabolism with sig. effect on the accumulation & the release of lipid from adipose tissue
Boozer *et al*[9] 2001	Over wt. (n = 35)	An herbal supplement: (*Ma Huang* & Guarana)	RCT (double-blind)	72 mg (ephedra) 240 mg (caffeine)/8 wk	C: Placebo (n = 24) I: Compound (n = 24)	Sig. decrease in body wt. & total body fat & sig. greater reduction in hip & waist Cir.	Greater reduction in serum TG, potentially treatment-related dropouts (23%) in the active group and none in the placebo group. Dry mouth, insomnia & headache were reported
Hoeger *et al*[10] 1998	Healthy	A natural dietary compound of chromium picolinate, inulin, capsicum, L-phenylalanine, and other lipotropic nutrients	RCT (double-blind)	4 wk	C: wt. loss program (n = 67) I: wt. loss program + compound (n = 56)	Sig. decrease in body fat percent, fat mass & FFM, but no sig. difference in body wt. BMI and energy intake	
Ziauddin *et al*[11] 2004	Hhyperlip-idemic (n = 30)	Terminalia arjuna Roxb	Before-after CT			Sig. improvement in obesity. Reduction in body wt. in some cases	Sig. decrease in serum total lipid levels. Sig. relief of palpitation, dyspnea, chest & joint pain. Reduction in BP in some cases
Abidov *et al*[12] 2006	Obese non-diabetic women (n = 32)	A compound of Aralia mandshurica (A) and Engelhardtia chrysolepis (E) extracts named ARALOX	RCT	450 mg (A) & 450 mg (E)/d	C: Diet + placebo I: Diet + compound	Decrease in total body wt. & fat wt.	Reduction in perilipin content in adipocytes and plasma TG. Stimulate activity of hormone sensitive lipase
Greenway *et al*[13] 2004	Human (obese & over wt.) healthy	Herbal supplement containing caffeine and ephedra	RCT (double-blind)	210 mg (e) & 72 mg (c)/12 wk	C: Placebo I: Compound	Sig. decrease in body wt. & the percentage of fat	No differences in lipid levels, or BP were shown. No serious adverse effect
Hioki *et al*[14] 2004	Obese women with IGT (n = 80)	Bofu-tsusho-san containing (Ephedrae Herba, Glycyrrhizae Radix, Forsythiae Fructus, Schizonepetae Spica &…)	RCT (double-blind)	Equivalent of (24 mg/ephedrine & 280 mg caffeine/24 wk)	C: wt. loss program I: wt. loss program + compound	Compared to baseline the I group lost significantly more body wt. & abdominal visceral fat & the placebo group lost sig. body wt. & had no sig. change in abdominal visceral fat	No decrease in RMR. Sig. improvement in insulin resistance compared to week 0. Loose bowel movements resulted in three withdrawals
Oben *et al*[15] 2008	Human (obese & over wt.)	A combination of *Cissus quadrangularis* (CQ) & *Irvingia gabonensis* (IG)	RCT (double-blind)	300 mg (CQ) & 500 mg (IG) per day/10 wk	C: Placebo I: CQ CQ + IG	Sig. decrease in body wt. & body fat percent & waist size in both I groups but the combination group (CQ + IG) resulted in larger reductions	Sig. decrease in Chol & LDL of plasma and fasting blood glucose levels
Chrubasik *et al*[16] 2008	Healthy (n = 80)	A combination of *Sambucus nigra* (S) and *Asparagus officinalis* (A)	Before-after CT	(S): 1 mg anthocyanin, 370 mg flavonol, 150 mg hydroxycinnamate (A): 19 mg saponin per day	-	Sig. decrease in mean of the wt.	Sig. improvement of BP, physical and emotional well-being and quality of life

Table 3.02: Continued……

Udani *et al*[17] 2007	Healthy (*n* = 25)	Proprietary fractionated white bean extract	RCT (double-blind)	2000 mg/14 wk	C: Placebo + wt. loss program I: Extract + wt. loss program	In both groups, decrease in body wt. & waist size from baseline was sig. but no sig value between groups	There were no adverse effect
Roongpisu-thipong *et al*[18] 2007	Obese women	Calcium hydroxycitrate in *Garcinia atroviridis*	RCT	2 mo	C: Diet I: Diet + extract	Sig. decrease in body wt. & greater reduction in BMI. Sig. decrease in the triceps skin fold thickness	
Kuriyan *et al*[19] 2007	Over wt. (*n* = 50)	Caralluma fimbriata	RCT	1 g/60 d	C: wt. loss program I: wt. loss program + extract	Sig. decrease in waist Cir. & hunger levels. Greater decrease in body wt., BMI, hip Cir., body fat & energy intake but not sig.	
Hackman *et al*[20] 2007	Obese & over wt. women (*n* = 41)	Multinutrient supplement containing ephedra (e) and caffeine (c)	RCT (double-blind)	40 mg (e) and 100 mg (c)/9 mo	C: Control supplement I: Multinutrient supplement	Sig. decrease in body wt. decrease in appetite	Sig. decline in serum chol, TG, glucose, fasting insulin & leptin levels & minor adverse effects like dry mouth, insomnia, nervousness and palpitation were reported
Garrison *et al*[21] 2006	Over wt. women	Proprietary extracts of Magnolia officinalis and phellodendron amurense	RCT	750 mg/6 wk	C: Placebo I: Extract	No sig. wt. gain for the I group but sig. wt. gain in C. groups	The I groups tended to have lower levels of cortisol in the evening
Coffey *et al*[22] 2004	Human (over wt. & obese) (*n* = 102)	Product containing ephedrine, caffeine & other ingredients.	RCT (double-blind)	12 wk	C: Placebo I: Compound	Additional wt. loss (1/5 kg) & greater reduction in BMI & waist Cir. No difference in body fat & fat mass percent was shown	No difference in pulse, diastolic & systolic BP & adverse events
Preuss *et al*[23] 2004	Obese (*n* = 60)	Hydroxycitric acid (HCA -SX) and a combination of HCA-SX and niacin-bound chromium (NBC) and Gymnema sylvestre extract (GSE)	RCT (double-blind)	HCA-SX: 4667 mg GSE: 400 mg NBC: 4 mg/8 wk	C: Placebo I1 = HCA-SX I2 = GSE + NBC + HCA-SX All groups had wt. loss program	5%-6% decrease in body wt. & BMI & sig. decrease in food intake in both I groups	Sig. decrease in serum lipids & leptin & increase in HDL & excretion of urinary fat metabolites in both I groups. There were mild adverse effects but not significant between groups
Udani *et al*[24] 2004	Obese (*n* = 24)	A proprietary fractionated white bean (Phaseolus vulgaris)	RCT (double-blind)	3000 mg/8 wk	C: Placebo I: Extract	Decrease of body wt. with 129% difference	Reduction of TG three times greater than C. group. No adverse effect was shown
Bhatt *et al*[25] 1995	Healthy (*n* = 58)	Guggulu (Medohar)	RCT	1/5, 3 g/30 d	C: wt. loss program I: wt. loss program + extract	Higher mean wt. reduction in I group. In I group, all patients > 90 kg lost wt. but 3 in C group did not lose wt.	
Oben *et al*[26] 2007	Over wt. & obese	*Cissus quadrangularis*	RCT (double-blind)	300, 1028 mg	C: Placebo I: Two extract formulation: CQR-300, CORE	Sig. decrease in body wt & body fat	Sig. decrease in serum lipids and glucose. Sig. increase in HDL-C plasma 5-HT and creatinine levels

Table 3.03: Animal studies on the anti-obesity effects of herbal medicine [[Hasani-Ranjbar S, 2009].

Authors	Target	Herbs (scientific name)	Dose/duration	Groups	Main outcome	Other relevant effects & complications
Wang *et al*[27] 2000	Rat (obese)	Haidonghua powder: Laminaria japonica Aresch & Benincasa hispida (Thunb.) Cogn. *etc*	(2.5 g/kg)	-	Sig. decrease in Lee's index & size of fat cells	Did not influence the function of thyroid gland & metabolism of water & salt
Jeon *et al*[28] 2003	Mouse	Rhus vemiciflua Stokes	8 wk	C: HFD I: HFD + extract	Sig. suppression of body wt. gain and lower wt. of subcutaneous adipose tissue	Lowered plasma TG
Alarcon-Aguilar *et al*[29] 2007	Mouse	Hibiscus sabdariffa	120 mg/kg 60 d	C: Healthy & obese (by MSG) + placebo I: Same groups + extract	Sig. decrease in body wt. gain in obese mice & increased liquid intake in both groups	No sig. change in TG & Chol levels. Increase in ALT levels was shown but was not sig.
Urías-Silvas *et al*[30] 2008	Mouse	Fructans extracted from Agave tequilana (TEQ) and Dasylirion spp (DAS)	10% supplement	C: STD I: STD + Raftilose/ DAS/TEQ	Sig. decrease in body wt. gain & food intake. The (TEQ) group had the lowest value	Lower serum glucose & Chol level but Sig. decrease in TG levels was shown in Raftilose group. Higher concentration of GLP-1 & it's precursor & proglucagon mRNA in I groups
Park *et al*[31] 2007	Rat	Platycodon grandiflorum	150 mg/kg 7 wk	C: NLD/HFD I: Same groups + extract	Sig. decrease in body wt & subcutaneous adipose tissue wt. & adipocytes size in I group	Sig. decrease in plasma TG & Chol concentrations, up-regulation of FABP mRNA expression induced by HFD
Jongwon *et al*[32] 2005	Rat (obese by HFD)	Allium victorialis var. platyphyllum leaves	100 mg/kg 2 wk	-	Considerable reduction of retroperitoneal, epididymal and total abdominal fat pad wt.	Sig. decrease in hyperlipidemia and increased lipid content in feces
Kobayashi *et al*[33] 2001	Rat	Evodiamine an alkaloid of a fruit: Evodia rutaecarpa	0/02%, 0/03% of the diet 12 wk	C: Control I: Extract	Sig. decrease in perirenal fat wt. & decrease of epididymal fat mass	Sig. decrease of lipid in liver & serum FFAs. Sig. increase of lipolytic activity in perirenal fat tissue & specific GDP binding in brown adipose tissue mitochondria as the biological index of heat production
Jin *et al*[34] 1994	Rat	Jiang-zhi jian-fei yao: the refined Rhubarb	Injected intragastrically		No sig. increase in body wt. but reduction of food intake. Decreased size of abdominal adipose cells	Prolongation of stomach evacuation time and acceleration of intestinal movements
Kim *et al*[35] 2008	Rat	Juniperus chinensis	1% supplement /79 d	C: NLD/HFD I: HFD + extract	Sig. decrease in body wt gain & visceral fat pad wt.	Sig. decrease in blood lipid, leptin & insulin levels. Sig. reversal of the down-regulation of genes implicated in adipogenesis & increased gene expressions & phosphorylations related to FABO
Shih *et al*[36] 2008	Mouse (obese by HFD)	Momordica charantia (bitter melon)	4 wk	C: Control I: Rosiglitazone/ extract	Sig. decrease in epididymal white adipose tissue wt. & visceral fat wt.	Sig. improvement in blood glucose, leptin, and FFA. Influenced PPARα/ PPARγ expression
Pang *et al*[37] 2008	Rat (obese by HFD)	Ilex paraguariensis			Sig. decrease in body wt. of visceral fat-pad wt.	Sig. decrease in blood and hepatic lipid, glucose, insulin and leptin levels. Reversed the down-regulation of genes implicated adipogenesis, thermogenesis & enhanced expression of uncoupling proteins in adipose tissue
Bruno *et al*[38] 2008	Mouse	Green tea	0%, 1%, 2% (wt.:wt.)/6 wk	C: Obese/lean I: Same groups + extract	Sig. decrease in body wt. of both I groups	In obese I group, sig. decrease in hepatic steatosis was observed dose dependently. Liver enzymes decreased. 30%-41% and 22%-33% lower serum ALT and AST activities were shown, respectively

Table 3.03: Continued……

Lee *et al*[39] 2008	Mouse	A combination of Morus alba, Melissa officinalis and Artemisia capillaries	12 wk	-	Sig. decrease in body wt. gain & adipose mass	Decreased serum levels of TG, Chol & inhibited hepatic lipid accumulation, and increased hepatic mRNA levels of enzymes responsible for FABO
Choi *et al*[40] 2008	Mouse (obese by HFD)	Cucurbita moschata	500 mg/kg 8 wk	_	Sig. suppression of body wt. & fat storage increase but amount of food intake was not affected	
Huang *et al* 2008[41]	Rat	Momordira charantia L. (Bitter melon)	5%	C: HFD I1: HFD + plant I2: HFD + thiazolidinedione	Sig. decrease in the number of large adipocytes in both I groups. Sig. decrease in adipose tissue mass in I_1 group compared to I_2 group	Sig. decrease in enzymes of adipose tissue implicating reduction of insulin resistance in I group as compared to C group
Lemaure *et al*[42] 2007	Rat (obese)	Cyperus rotundus L. tubers	45, 220 mg/kg 60 d	_	Sig. decrease in wt. gain without affecting food consumption	
Lei *et al*[43] 2007	Mouse	Pomegranate leaf	400/800 mg per kilogram 5 wk	C: HFD/NLD I: Same groups + extract	Sig. decrease in body wt. & energy intake and adipose pad wt. percents in I. group. Sig. decrease in appetite of obese mice on NLD was shown	Sig. decrease in serum TG, Chol, glucose levels & Chol/ HDL ratio, inhibition of intestinal fat absorption
Aoki *et al*[44] 2007	Mouse (obese by HFD)	Licorice flavonoids oil (LFO)	0/5%, 1%, 2% 8 wk	C: Placebo I: Extract	Sig. decrease of abdominal white adipose tissue & body wt. gain with 1% & 2% LFO groups, decrease of adipocyte size	Improvement of fatty degeneration of hepatocytes and changes in genes implicating regulation of lipid metabolism with 2% concentration
Oluyemi *et al*[45] 2007	Rat	Garcinia cambogia seed (bitter cola)	200, 400 mg/kg 5 wk	C: Placebo I: Extract	Sig. decrease in body wt.	Sig. decrease in TG pool of adipose tissue & liver but sig. increase of HDL & decreased LDL
Han *et al*[46] 2006	Mouse (obese by HFD)	Kochia scoparia	1%, 3%/3 d	-	Prevented the increases in body & parametrial adipose tissue wt.	Sig. increase the fecal content & fecal TG levels in day 3
Goyal *et al*[47] 2006	Mouse (obese gold thioglucose)	*Zingiber officinale*	250 mg/kg 8 wk	C: Placebo I: Extract	Sig. decrease in body wt.	Sig. decrease in serum Chol, TG, glucose, and insulin
Kishino *et al*[48] 2006	Rat and mouse	Salacia reticulata	0/5% 8 wk in mice 0/2% 35 d in rats	C: HFD I: HFD + plant	Sig. decrease in the body wt. and visceral fat mass increase	Sig. decrease in plasma TG, 4 h after ingestion; Sig. decrease in energy efficiency, plasma leptin and adiponectine levels
Jayaprakasam *et al*[49] 2006	Mouse	Cornelian cherry (cornus mas) (Purified anthocyanins (A) & ursolic acid (u)	1 g/kg (A), 500 mg/kg (u) 8 wk	C: HFD I: HFD + A/A + u	24% decrease in wt. gain in (A) group	Elevated insulin levels; Sig. decrease of liver TG in A + u group
Moreno *et al*[50] 2006	Rat	Arachis hypogaea nutshell	1% (wt:wt) /12 wk	C: HFD I: HFD + extract	Sig. decrease in body wt. gain and liver size	Increased fecal lipid excretion. Reduced TG content of liver and serum glucose and insulin
Galisteo *et al*[51] 2005	Rat (obese)	Plantago Ovata	3/5% 25 wk	C: STD I: STD + extract	Sig. decrease in body wt. gain	Sig. improvement of lipid profile, FFA & insulin & TNF-α & hypoadinectinemia
Zhao *et al*[52] 2005	Mouse (obese by hyperalimentation)	Phillyrin (Fructose forsythia)			Sig. decrease in wet wt. of fat & fat index & diameter of fat cells & lee index	Decrease in jejunum microvillus area, and serum levels of TG & Chol
Chen *et al*[53] 2005	Rat	Bitter melon (Momordica charantia)	0/75% or 7/5 g per kilogram 7 wk	C: LFD/HFD I: LFD/HFD + extract	Lower energy efficiency and visceral fat mass after 4 wk in I group	Reduced plasma glucose and hepatic TG but higher serum FFA after 4 wk; Higher plasma catecholamine after 7 wk in I group; Sig. decrease in hepatic TG & steatosis and sig. increase of serum epinephrine & FFA in HFD group of I

Table 3.03: Continued……

Han *et al*[54] 2005	Rat	Coleus forskohlii	50 g/kg	C: Sham operated/ ovariectomized + control diet I: Same groups + extract	Reduced body wt.& food intake & fat accumulation	
Han et al[55] 2005	Mouse	Chikusetsu saponins isolated from Panax japonicus rhizomes	1%, 3%/9 wk	C: HFD I: HFD + extract	Prevented body wt. gain & increase of parametrial adipose tissue wt.	Sig. increase of the fecal content & TG level in day 3; reduction of plasma TG 2 h after oral lipid intake & inhibition of pancreatic lipase activity
Han *et al*[56] 2005	Mouse	*Zingiber officinale* Roscoe	1%, 3%/8 wk	C: HFD I: HFD + plant	Sig. decrease in body wt. gain at 2-8 wk with 3% & in final parametrial adipose tissue wt. with 1% concentration	
Cha *et al*[57] 2004	Mouse	Acanthopanax senticosus	0/5 g per kilogram 12 wk	C: NLD/HFD I: NLD/HFD + extract	HFD group of I had lower wt. gain but no difference in food consumption was shown	In HFD group of I, lower serum LDL and restoration of liver TG at the same level as fed by LFD was shown; No alteration in carnitine status
Kim *et al*[58] 2005	Rat	Crude saponin of Korean red ginseng	200 mg/kg 3 wk, ip	C: NLD/HFD I: NLD/HFD + extract	Reduced body wt., food intake & fat content in HFD group of I similar to those fed with NLD	Reduction of hypothalamic NPY expression and serum leptin level in HFD group of I
Yun *et al*[59] 2004	Mouse	Wild Ginseng	250, 500 mg/kg	C: HFD I: HFD + extract	Sig. inhibition of body wt. gain dose dependently. Decrease of white & brown adipocytes diameters	Sig. inhibition of FBG, TG, and FFAs dose-dependently; insulin resistance improved
Junbao *et al*[60] 2004	Rat (obese)	Semen cassiae	6%	-	Sig. decrease in body wt. & lee index	Reduction of fasting serum TG, insulin & malondialdehyde
Kim *et al*[61] 2004	Rat	Adlay seed (CoixLachrymajobi var. mayuen)	50 mg/100 g of body wt.	C1: NLD C2: HFD + saline (sham group) I: HFD + plant	Sig. decrease in body wt. & food intake & epididymal and peritoneal fat & white adipose tissue size as compared to sham group	Increase of brown adipocytes as compared to NLD group but not significant
Kwon *et al*[62] 2003	Rodent	Dioscorea nipponica Makino	5%/8 wk	C: HFD I: HFD + plant	Sig. decrease in body wt. & adipose gain	Suppression of time dependent increase of serum TG level after lipid intake
Lu *et al*[63] 1999	Rat (obese by hyperalimentation)	Inspissation tea (Guangdong kudingcha)		C: Control I1: Extract I2: Fenfluramine	Stronger modulation on lymphocytes hypertrophy and quantity was shown in I1 group	Only fenfluramine showed sig. difference in small intestine villus model
Yoshikawa *et al*[64] 2002	Rat (obese)	Salacia reticulata	125 mg/kg 27 d		Suppression of body wt. and periuterine fat storage increase in female rats but no effect on male rats	
Xie *et al*[65] 2002	Mouse	Ginseng berry	150 mg/kg 12 d, ip	C: Diabetic/lean diabetic + placebo I: Same groups + extract	Sig. decrease in body wt. as compared to day 0 in diabetic group of I. wt. loss in lean mice was shown	Sig. increase in glucose tolerance in diabetic mice but no sig. decrease of FBG in lean mice.
Yamamoto *et al*[66] 2000	Rat	CT-Ⅱ, an extract from Nomame Herba	8 wk, 12 wk, 6 mo	C: Lean/obese + HFD I: Same groups + HFD + plant	Sig. inhibition of body wt. gain dose dependently without affecting food intake in lean rats after 12 wk. Sig. decrease in body wt. gain in obese mice after 24 wk	Sig. inhibition of TG elevation
Han *et al*[67] 1999	Mouse	Oolong tea	10 wk	C: HFD I: HFD + extract	No sig. difference in food intake but prevented obesity & liver induced by a HFD	Enhancement of noradrenalin induced lipolysis & inhibition of pancreatic lipase activity

Table 3.03: Continued……

Pusztai *et al*[68] 1998	Rat	Kidney bean (Phaseolus vulgaris)	130, 150, 280 g/kg 10-70 d	C: Lean/obese + LFD/HFD I: Same groups + extract	The growth was retarded dose-dependently lower body fat	Sig. decrease of body protein in lean I group. Sig. decrease in plasma insulin levels in obese I group. Sig. pancreatic growth after long term feeding in all I groups
Nagasawa *et al*[69] 1991	Mouse (obese)	Tree peony root (Paenia suffruticosa)	0/5% 30 wk	C: Control I: Extract	Sig. decrease in food intake and Lee index	Improvement in glucose tolerance. No sig. difference in serum FFA levels
Wang *et al*[70] 2008	Mouse	Parasitic loranthus from Loranthacea or Viscaceae	20 d	-	Sig. decrease in body wt. & food intake	High inhibitory ability on FAS-Loran thacea was nearly 400 fold stronger than that from the viscaceae
Hu *et al*[71] 2008	Mouse (female)	Escins extracted from Aesculus turbinata Blume (Hippocastanaceae)	2%/11 wk	I: HFD C: HFD + extract	Suppressed the increase in body & parametrial adipose tissue wt.	Suppressed the increase of liver TG content; increased TG in feces after fat ingestion
Ohkoshi *et al*[72] 2007	Mouse	Nelumbo nucifera Gaertn leaves (Nymphaceae)	50%	C: STD/HFD I: Same groups + extract	Sig. suppression of body wt. gain	Exhibition of lipolytic activity especially in visceral adipose tissue; β adrenergic receptor pathway was partly involved
Kang *et al*[73] 2004	Rat	PM-F2-OB composed of *Lycii Fructus*, *Rehmanniae Radix*, *Coicis Semen*, *Carthami Flos*, *Hoelen*, *Angelicae Radix*, *Nelumbinis Semen*, *Radix Dioscorea* and *Aurantii Fructus*	6 wk	C: STD/HFD I: Same groups + plant	No sig. difference in wt. change if STD was used but in HFD group of I resulted in sig. decrease in body wt. gain but showed no sig. difference in amount of food intake	Sig. decrease in serum Chol/ LDL and total lipids; reduction of kidney fat wt./FFA/PL & TG to levels equal or below the normal diet
Mary *et al*[74] 2003	Rabbit	Caps HT2 A herbal formulation	5 mg/kg (iv) 30 d 100/200/300/ 400/mg per kilogram orally	-	Sig. decrease in body wt.	Sig. increase in HDL after oral administration and decrease in atherogenic index in oral administration; Sig. increase of the release of LPL enzyme and sig. hypolipidemic effect in Ⅳ groups
Wu *et al*[75] 2005	Rat (diabetic by STZ)	Astragalus polysaccharide (APS) a component of Astragalus membranaceus roots	400 mg/kg (APS) 5 wk	-	Sig. decrease in body wt.	Sig. decrease in plasma glucose; improved insulin sensitivity
Xie *et al*[76] 2005	Mouse (Genetically obese)	Total, Ginsenosides in Chinese ginseng (TG CG), from leaves and the stem of Panax ginseng	100, 200 mg/kg (ip) 12 wk &150, 300 mg/kg (oral)/12 wk	C: Placebo I: Extract	Sig. decrease in body wt.	Sig. decrease in FBG in 200 mg/kg dose after injection Sig. decrease in FBG in 300 mg/kg dose
Palit *et al*[77] 1999	Mouse	Galega officinalis	10% (w/w) of the diet 28 d	C: Diabetic/NL I: Same groups + plant	Sig. decrease in body wt. in both I groups, sig. wt. loss in normal mice independent of a reduction in food intake but in diabetic mice wt. loss was with reduced food intake	Striking loss of body fat in both groups; Sig. decrease in serum glucose in both groups but Sig. decrease in serum insulin in diabetic mice
Oi *et al*[78] 1999	Rat	Garlic	8 g/kg of diet 28 d	C: HFD I: HFD + extract	Sig. decrease in body wt. & perirenal adipose tissue wt. & epididymal fat pad	Sig. decrease in plasma TG levels; sig. decrease in mitochondrial protein and (UCP) in brown adipose tissue, and in urinary noradrenaline and adrenaline excretion
Yoshida *et al*[79] 1995	Mouse (obese and lean)	Bofu-tsusho-san	1/4%, 4/7% of wt. of food 8 wk	-	Sig. decrease in body wt. & retroperitoneal white adipose tissue wt. and no change in food intake	Sig. increase in GDP binding dose dependently
He *et al*[80] 2008	Rat (obese by STZ & HFD	Yi-Qi-Yang-Yin-Ye	2, 4, 8 g/kg 4 wk	-	Body wt. decreased	Decrease in TG/Chol/ LDL/FFA/FBG/insulin; improvement of glucose tolerance

Table 3.03: Continued……

Jeong *et al*[81] 2008	Rat (fatty)	Gyeongshang angjeehwan: Liriope platyphylla F.T./Wang & T. Tang (Liliaceae), Platycodongrandiflorum A. DC. (Campanulaceae). Schisandrachinensis K. Koch (Magnoliaceae). Ephedra sinica Stapf (Ephedraceae)	8 wk	C: Placebo I: Compound	Sig. decrease in food intake & body wt. gain & abdominal fat	Sig. decrease in plasma leptin levels; decrease in circulating TG and inhibition of lipid accumulation in liver; increase of mRNA of genes responsible for FABO
Park *et al*[82] 2005	Rat (obese by diet)	Platycodon grandiflorum	150 mg/kg 7 wk	C: Convert to NLD/HFD I: Same groups + extract	Sig. decrease in wt. of body & adipose tissues in rats converted to NLD as compared to those remained on HFD	Sig. decrease in fat cell number & size in both I groups as compared to their state before intervention; decrease of FABP expression in HFD group of I
Akagiri *et al*[83] 2008	Mouse (obese by HFD)	Bofutsushosan (BOF)	1%/4 wk	C: Placebo I: Compound	The wt. of WAT and increase in size of adipocytes inhibited	Expression of UCP1 mRNA in WAT was found but not sig.
Kim *et al*[84] 2005	Mouse (diabetic)	Pine extract (bark and needle)	21 d	C: Control I: Extract	Sig. decrease in body wt.	Effectively suppressed the increase of postprandial blood glucose level by delaying absorption of diet
Attele *et al*[85] 2002	Mouse (obese diabetic)	Panax ginseng berry	150 mg/kg (ip) 12 d	C: Control I: Extract	Sig. loss of wt. with a sig. reduction in food intake & a very sig. increase in energy expenditure & body temperature	Sig. improvement in glucose tolerance & sig. reduction in serum insulin levels & plasma chol levels

MSG: Monosodium glutamate; FABO: Fatty acid β oxidation; STD: Standard diet; LFD: Low fat diet; NLD: Normal diet; HFD: High fat diet; FABP: Fatty acid binding protein; FFM: Fat free mass; sig.: Significant; AST: Aspartate transaminase; ALT: Alanine transaminase; C: Control; I: Intervention; FAS: Fatty acid synthetase; UCP: Uncoupling protein; GDP: Guanosine 5' diphosphate; FAS: Fatty acid synthetase; TG: Triglyceride; HDL: High density lipoprotein; LDL: Low density lipoprotein; FBG: Fasting blood glucose; ip: Intraperitoneal; iv: Intravenous. Caps HT2 is a herbal formulation containing methanolic extract of selected parts of plants: commiphora mukul; Allium Sativum; Plumbago indica/some carpus anacardium/Hemidesmus indicus/Terminalia arjuna/Tinospora cordifolia/Withania somnifera ocimum sanctum.

3.4 Triphala:

Triphala is an Ayurvedic [McIntyre A, 2005]. Herbal rasayana churna consisting of equal parts of three myrobalans, taken without seed: *Emblica officinalis* (Amalaki/Amla), *Terminalia chebula* (Haritaki/Harad), and *Terminalia bellirica* (Bibhitaki/Bahed). The fruits of *Terminalia belerica* Roxb. *Terminalia chebula* Ret., and *Emblica officinalis* Gaetn. are widely used in the Indian traditional system of medicine [Chopra et al, 1956; Kirtikar and Basu, 1991]. The popular ayurvedic formulation Triphala is used as an anthelmintic and purgative [Anonymous, 1952, 1976]. Triphala also forms a part of many other ayurvedic formulations. The word triphala means literally "three fruits". Triphala churna is a mild laxative, which cleanses and tonifies the gastro-intestinal tract. Triphala is known as a cleaning agent, including a blood cleanser. The herb also has a high nutritional value, including high levels of vitamin C [Tarwadi K, 2007]. Because of its high vitamin

content, Triphala is often used as a food supplement like vitamins are in Western countries. In fact, the benefits of this herb are so well known that a well known Indian saying goes like this: "You do not have a mother? Don't worry, as long as you have Triphala in your life!" In recent years, a number of research studies have found new uses for this herb, including treatment for various forms of cancer. It is also found to have high antioxidant qualities, and is even useful for treatment against noise and stress induced conditions [Srikumar R, 2005]

3.4.1 *Emblica officinalis* (amlaki/amla):

E. officinalis Gaertn., commonly known as amla, is a member of the small genus of Emblica (Euphorbiaceae). It grows in tropical and subtropical parts of China, India, Indonesia, and the Malay Peninsula. It is an important dietary source of vitamin C, minerals, and amino acids and also contains phenolic compounds, tannins, phyllembelic acid, phyllemblin, rutin, curcuminoides, and emblicol. Although amla fruits are reputed to contain high amounts of ascorbic acid (vitamin C), 445 mg/100g, [Tarwadi K, 2007] the specific contents are disputed, and the overall antioxidant strength of amla may derive instead from its high density of ellagitannins such as emblicanin A (37%), emblicanin B (33%), punigluconin (12%) and pedunculagin (14%).[Bhattacharya A,1999] It also contains punicafolin and phyllanemblinin A, B, C, D, E and F. [Zhang YJ, 2001]. The fruit also contains polyphenols: flavonoids, kaempferol, ellagic acid and gallic acid [Habib-ur-Rehman, 2007].

All parts of the plant are used for medicinal purposes. Especially, the fruit has been used in Ayurveda as a potent rasayana and in traditional medicines for the treatment of diarrhea, jaundice, and inflammation. In addition, the pulp of the fruit is smeared on the head to dispel headache and dizziness. Recently, amla extract has been tested for various pharmacological activities. The fruit extract was reported to have hypolipidemic [Anila, L, 2002), antidiabetic [Sabu MC, 2002], and anti-inflammatory activities [Asmawi MZ, 1993] and to inhibit retroviruses such as HIV-1 [El-Mekkawy S, 1995], tumor development [Jose JK, 2001], and gastric ulcer [Bandyopadhyay SK, 2000). Moreover, amla extract exhibits antioxidant properties

[Anila L, 2003], and it has been reported that the aqueous extract of amla is a potent inhibitor of lipid peroxide formation and scavenger of hydroxyl and superoxide radicals in vitro [Jose JK, 1995].In Ayurveda, the fruit of E. officinalis is used as a cardiotonic, cerebral and intestinal tonic [Aslokar et al, 1992], and it also is reported to have anticancer properties [Rajarama Rao and Siddiqui, 1964; Aslokar et al, 1992]. The fruit of E. officinalis is a rich source of vitamin C [Anonymous, 1952], a well-known antioxidant [Halliwell and Gutteridge, 1985a]. The crude extract of E. officinalis was reported to counteract the hepatotoxic and renotoxic effects of metals [Roy et al, 1991] due to antioxidant properties.

3.4.2 *Terminalia chebula* (haritaki/harad):

T. chebula (Combretaceae family) is widely grown in tropical regions as a shade and ornamental tree. It is also important in Ayurvedic medicines. It is used as an astringent, purgative and food supplement. It is reported to retard the effects of ageing and impart longevity, as well as boosting the immune system [Lemmens, 2003]. Moreover, T. chebula fruits have been studied for their antioxidant [Cheng HY, 2003], antimicrobial [Malekzadeh F, 2001] and anticancer activities [Lee et al, 1995; Saleem A, 2002]. Terminalia chebula is rich in tannin, which is a hydrolyzable pyrogallol. A total of 14 components of hydrolysable tannins, including gallic acid, chebulic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulagic acid, chebulinic acid, 1,2,3,4,6-penta-O-galloyl-α-D-glucose, 1,6-di-O-galloyl-D-glucose, casuarinin, 3,4,6-tri-O-galloyl-D-glucose and terchebulin, have been found in T. chebula fruits [Juang LJ, 2004]. The pericarp of T. chebula fruit were reported to be purgative [Chopra et al., 1956]. The fruit of T. chebula was traditionally used to cure asthma, urinary disorders, heart disease and it has cardiotonic activity [Reddy et al, 1990].Researchers have isolated a number of glycosides from Haritaki, including the triterpenes arjunglucoside I, arjungenin, and the chebulosides I and II. Other constituents include a coumarin conjugated with gallic acids called chebulin, as well as other phenolic compounds including ellagic acid, 2,4-chebulyl-β-D-glucopyranose, chebulinic acid, gallic acid, ethyl gallate, punicalagin, terflavin A, terchebin, luteolin, and tannic acid.[Saleem A, 2002]. Chebulic acid is a phenolic acid compound isolated from the ripe fruits [Lee

HS, 2006; Lee HS, 2010]. Luteic acid can be isolated from the bark [Nierenstein M, 1945]. It is also found to prevent microsomal lipid peroxidation [Anand et al., 1994].

3.4.3 *Terminalia bellerica* (bibhitaki/bahed):

T. belerica also referred to as, Beleric Myrobalan in English, Bibhitaki in Sanskrit, Locally known as Bahera in India, has been used for centuries in the Ayurveda, a holistic system of medicine originating from India. The dried fruit used for medicinal purposes [Indian Herbal Pharmacopoeia Revised New Edition 2002]. In Ayurveda the drug is classified as an expectorant. It is an integral part of Ayurvedic laxative formulation, Triphala used in treatment of common cold, pharyngitis and constipation[Amrithpal SS, 2011] . The bark is midly diuretic and is useful in anaemia and leucoderma. The fruits are astringent, acrid, Digestive, Anthelmintic, Aperient, Expectorant, Sweet, Anodyne, Stypic, Narcotic, Ophthalmic, Antipyretic, Antiemetic and Rejuvenating. Unripe fruit is an mild laxative and ripe fruit is an astringent. Seeds are used as aphrodisiac [Sabu MC, 2002]. Oil extract from the seed pulp is used in leucoderma and alopecia. Moderm investigations have proved the laxative activity of the oil [Amrithpal SS, 2011; Vaidyaratnam PS, 2004]. The vast study done on the plant proved that the plant has many important phytoconstituents like Glucoside (bellericanin) [Amrithpal S, 2006; The Ayurvedic Pharmacopoiea of India, 2001] , Gallo-tannic acid, Coloring matter, resins and a greenish yellow oil [Nadkarni KM, 2002] . Ellargic acid, gallic acid, lignans[termilignan and thanni lignan), 7-hydroxy 3'4' (methylene dioxy) flavone and anolignan B10 . Tannins, ellargic acid, ethyl gallate, galloyl glucose and chebulaginic acid, phenyllemblin, β-sitosterol, mannitol, glucose, fructose and rhamnose [Amrithpal SS, 2011; Indian Herbal Pharmacopoeia Revised New Edition 2002; The Ayurvedic Pharmacopoiea of India, 2001]. These compounds were found to be responsible for many of the pharmacological activities such as antimicrobial [Elizabeth KM, 2005], antioxidant [BadrulAlam, 2011], antidiarrhoeal [Bimlesh K, 2010], antidiabetic [Latha PCR, 2010], analgesic [Arif-Ullah Khan, 2010], immumomodulatory [Aurasorn S, 2008], antihypertensive [Arif-Ullah Khan , 2008], antisolmonella [Madani A et al ,2008], hepatoprotective [Sangeetha S,

2006], antispasmodic [Anwarul H, 2008] and bronchodilatory activities [Anwarul H, 2008].

3.5 Obesity and Oxidative stress:

Obesity is a chronic disease of multifactorial origin and can be defined as an increase in the accumulation of body fat. Adipose tissue is not only a triglyceride storage organ, but studies have shown the role of white adipose tissue as a producer of certain bioactive substances called adipokines. Among adipokines, we find some inflammatory functions, such as Interleukin-6 (IL-6); other adipokines entail the functions of regulating food intake, therefore exerting a direct effect on weight control. This is the case of leptin, which acts on the limbic system by stimulating dopamine uptake, creating a feeling of fullness. However, these adipokines induce the production of reactive oxygen species (ROS), generating a process known as oxidative stress (OS). Because adipose tissue is the organ that secretes adipokines and these in turn generate ROS, adipose tissue is considered an independent factor for the generation of systemic OS. There are several mechanisms by which obesity produces OS. The first of these is the mitochondrial and peroxisomal oxidation of fatty acids, which can produce ROS in oxidation reactions, while another mechanism is over-consumption of oxygen, which generates free radicals in the mitochondrial respiratory chain that is found coupled with oxidative phosphorylation in mitochondria. Lipid-rich diets are also capable of generating ROS because they can alter oxygen metabolism. Upon the increase of adipose tissue, the activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), was found to be significantly diminished and concentration of ROS such as thiobarbituric acud reactive species (TBARS) was found to increase [Furukawa S, 2004]..

Obesity per se may induce systemic oxidative stress and that increased oxidative stress in accumulated fat is, at least in part, the underlying cause of dysregulation of adipocytokines and development of metabolic syndrome (Fig: 3.01). As an early instigator of obesity-associated metabolic syndrome, increased oxidative stress in accumulated fat has become an important target for the development of new

therapies. In patients suffering from T2DM, oxidative stress impairs glucose uptake in muscle and fat and decreases insulin secretion from pancreatic β islet cells. Increased oxidative stress also underlies the pathophysiology of hypertension and atherosclerosis by directly affecting vascular endothelial cells [Furukawa S, 2004].

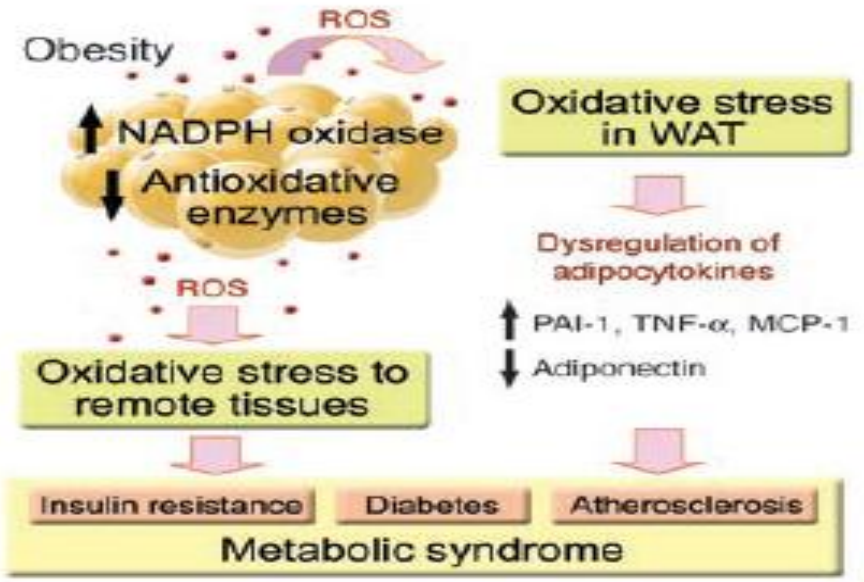


Figure 3.01: A working model illustrating how increased reactive oxygen species (ROS) production in accumulated fat contributes to metabolic syndrome [Furukawa S, 2004].

ROS occur under physiological conditions and in many diseases and cause direct or indirect damage in different organs; thus, it is known that oxidative stress (OS) is involved in pathological processes such as obesity, diabetes, cardiovascular disease, and atherogenic processes. It has been reported that obesity may induce systemic OS and, in turn, OS is associated with an irregular production of adipokines, which contributes to the development of the metabolic syndrome [Esposito K, 2006]. The sensitivity of CRP and other biomarkers of oxidative damage are higher in individuals with obesity and correlate directly with BMI and the percentage of body fat, LDL oxidation, and TG levels [Pihl E, 2006]; in contrast, antioxidant defense markers are lower according to the amount of body fat and central obesity [Chrysohoou C, 2007; Hartwich J, 2007]. A research showed that a diet high in fat and carbohydrates induces a significant increase in OS stress and inflammation in persons with obesity [Patel C, 2007].

3.5.1 Pathophysiology of OS: Peroxisomal fatty acid metabolism, in which H_2O_2 is formed as a byproduct, and despite that peroxisomes contain high catalase activity, they may cause OS under certain pathological conditions.

a) Cytochrome P450 microsomal reactions, which catalyze the metabolism of xenobiotic compounds by oxidoreducers, forming superoxide anion as a byproduct, which can cause OS.

b) Phagocyte cells, which attack invasive pathogens with a mixture of ROS and other oxidants. This is an immune response, but also damages surrounding tissues, producing inflammation.

c) The mitochondrial respiratory chain. It is considered that the mitochondria are the site within the cell where the largest amount of ROS are generated, causing defects in mitochondrial metabolism and diseases.

OS biomarkers, such as malondialdehyde (MDA) and F-2 isoprostanes (F2-IsoPs), are the products of the peroxidation of polyunsaturated fatty acids. One study showed that BMI was significantly related with the concentration of F2-IsoPs. In addition, dietary factors were analyzed, and it was observed that fruit consumption is inversely associated with the level of lipid peroxidation. This same study revealed that females demonstrated a higher peroxidation level compared with males, which may be caused by the higher percentage of fat possessed by females. We also found a positive relationship between lipid peroxidation level and plasma cholesterol concentration [Block G, 2002]. Another OS marker is the urinary levels of 8-iso Prostaglandin F2α (8-iso PGFα), which are positively related with obesity and insulin resistance [Keaney Jr, 2003] and negatively associated with plasma concentration of adiponectin.

3.5.2 Mechanisms of Formation of Free Radicals during Obesity

3.5.2.1 Adipose Tissue

Adipose tissue or body fat or fat depot or just fat is loose connective tissue composed of adipocytes. It is technically composed of roughly only 80% fat which in its solitary state exists in the liver and muscles. Adipose tissue is derived from

lipoblasts. Its main role is to store energy in the form of lipids, although it also cushions and insulates the body. Adipose tissue primarily consists of fat cells i.e. adipocytes producing a variety of biologically active molecules/mediators, collectively known as adipocytokines or adipokines, such as Plasminogen Activator Inhibitor–1 (PAI-1), Tumor Necrosis Factor-alpha (TNF-α), Resistin, Leptin, and Adiponectin. Dysregulated production of these adipocytokines is implicated in the pathogenesis of obesity-associated metabolic syndrome. Moreover, adipose tissue can affect other organ systems of the body and may lead to disease. Obesity or being overweight in humans and most animals does not depend on body weight but on the amount of body fat—to be specific, adipose tissue. Adipose tissue has switched from being a passive energy "reservoir" with insulatory attributes to a complex, highly active and essential metabolic and endocrine organ, churning out an assortment of hormones and other signals regulating the whole-body physiology (e.g. food intake, body weight and brain activity) [Macia L et al, 2006].

Human adipose tissue is divided into brown adipose tissue, which possesses multilocular adipocytes with abundant mitochondria that express high amounts of uncoupling protein 1 (UCP-1), which is responsible for the thermogenic activity of this tissue [Dulloo AG, 2010], and white adipose tissue, which is responsible for fat storage. Among the characteristics of white adipose tissue, we found that it consists of different cell types such as fibroblasts, preadipocytes, mature adipocytes, and macrophages. This tissue is very heterogeneous according to its visceral or subcutaneous location [Sánchez F, 2005]. In animals with obesity, there is a huge increase in white fat deposits due to the hyperplasia and hypertrophy of their adipocytes [Dulloo AG, 2010]. Hypertrophic-hyperplastic adipocytes exhibit a lower density of insulin receptors and a higher beta-3 adrenergic receptor, which facilitates the diapedesis of monocytes to visceral adipose stroma, initiating a proinflammatory cycle between adipo- and monocytes [Deng Y, 2010]. Adipose tissue is not only a triglyceride (TG)-storage tissue; studies in recent years have shown the role of white adipose tissue as a producer of certain substances with endocrine, paracrine, and autocrine action [Sikaris K, 2004]. These bioactive substances are denominated adipokines or

adipocytokines, among which are found plasminogen activator inhibitor-1 (PAI-1), tumor necrosis factor-alpha (TNF-α), resistin, leptin, and adiponectin [Deng Y, 2010]. These substances derive primarily from white adipose tissue and play a role in the homeostasis of various physiological processes (Figure 3.02).

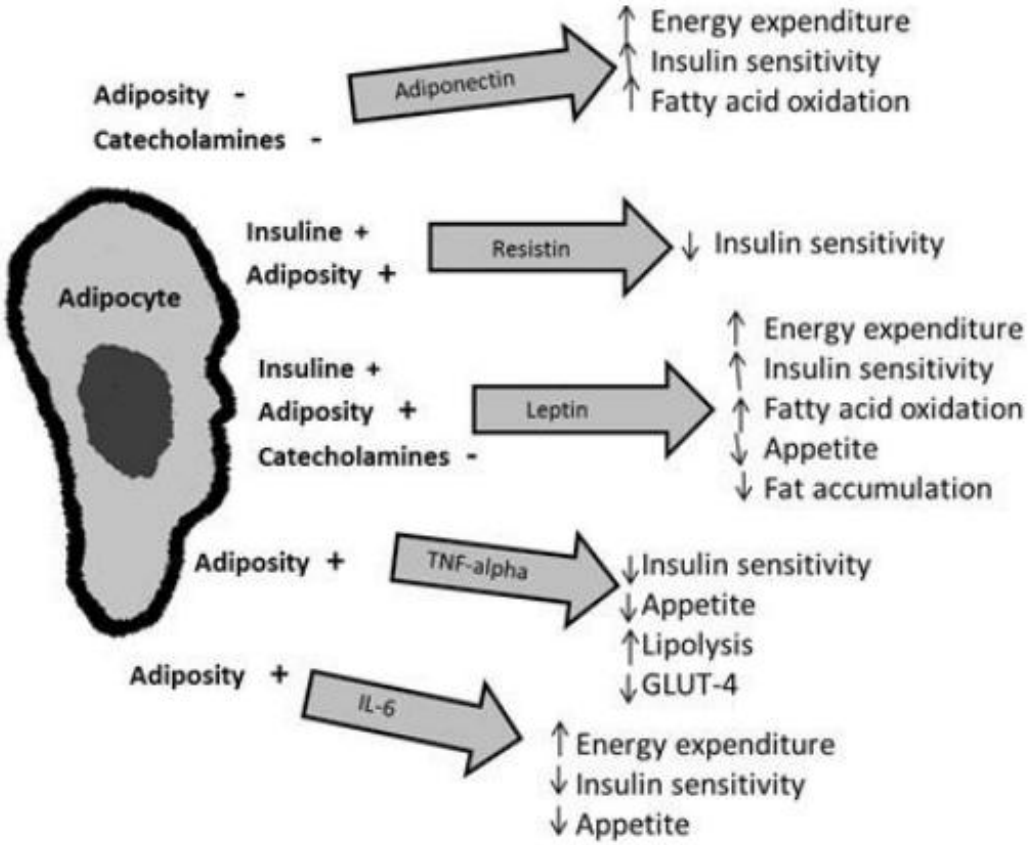


Figure 3.02: This figure depicts the **major adipokines and their roles**. Adipose tissue produces several adipokines that exert metabolic effects, both in central and in peripheral tissues. The production of these adipokines is regulated by insulin, cathecholamines, and adiposity [Deng Y, 2010].

The increase in obesity-associated OS is probably due to the presence of excessive adipose tissue itself, because adipocytes and preadipocytes have been identified as a source of proinflammatory cytokines, including TNF-α, IL-1, and IL-6; thus, obesity is considered a state of chronic inflammation. These cytokines are potent stimulators for the production of reactive oxygen and nitrogen by macrophages and monocytes; therefore, a rise in the concentration of

cytokines could be responsible for increased OS. TNF-α also inhibits the activity of PCR, increasing the interaction of electrons with oxygen to generate superoxide anion [Fonseca-Alaniz MH, 2007]. Adipose tissue also has the secretory capacity of angiotensin II, which stimulates Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase activity. NADPH oxidase comprises the major route for ROS production in adipocytes [Morrow J, 2003].

3.5.2.2 Fatty Acid Oxidation

Mitochondrial and peroxisomal oxidation of fatty acids are capable of producing free radicals in liver and, therefore, OS, which could result in mitochondrial DNA alterations in the oxidative phosphorylation that occurs in mitochondria, causing structural abnormalities and depletion of adenosine triphosphate (ATP). However, it is also possible that mitochondrial abnormalities are preexisting conditions that allow for overproduction of ROS [Duvnjak M, 2007].

3.5.2.3 Overconsumption of Oxygen

Obesity increases the mechanical load and myocardial metabolism; therefore, oxygen consumption is increased. One negative consequence of increased oxygen consumption is the production of ROS as superoxide, hydroxyl radical, and hydrogen peroxide derived from the increase in mitochondrial respiration and, of course, from the loss of electrons produced in the electron transport chain, resulting in the formation of superoxide radical [Amirkhizi F et al, 2007].

3.5.2.4 Accumulation of Cellular Damage

Excessive fat accumulation can cause cellular damage due to pressure effect from fat cells (i.e., non alcoholic steatohepatitis). Cellular damage in turn leads to high production of cytokines such as TNF-α, which generates ROS in the tissues, increasing the lipid peroxidation rate [Khan N, 2006].

3.5.2.5 Type of Diet

Another possible mechanism of ROS formation during obesity is through diet. Consumption of diets high in fat may alter oxygen metabolism. Fatty deposits are vulnerable to suffering oxidation reactions. If the production of these ROS exceeds

the antioxidant capacity of the cell, OS resulting in lipid peroxidation could contribute to the development of atherosclerosis [Khan N, 2006].

3.5.2.6 Role of Mitochondria in the Development of OS in Obesity

Mitochondria provide the energy required for nearly all cellular processes that ultimately permit the carrying out of physiological functions; additionally, they play a central role in cell death by the mechanism of apoptosis. Mitochondrial dysfunction has been implicated in a variety of diseases ranging from neurodegenerative diseases to diabetes and aging. Obesity takes place in disorders that affect mitochondrial metabolism, which favors ROS generation and the development of OS. On the other hand, another mechanism has been proposed that involves an effect of high triglyceride (TG) on the functioning of the mitochondrial respiratory chain, in which intracellular TG, which is also high, inhibits translocation of adenine nucleotides and promotes the generation of superoxide [Monteiro R, 2010].

3.5.3 Complications-generated Oxidative Stress in Obesity

Obesity and the consequent production of OS have been associated with the development of other pathologies (Table 3.04), the most straightforward of which is the metabolic syndrome.

Table 3.04: Diseases associated with obesity [Monteiro R, 2010]

Insulin resistance and diabetes
Systemic arterial hypertension
Ischemic heart diseases
Obstructive sleep apnea, asthma
Gout
Peripheral vascular disease
Psychology problems (social stigmatization)
Rheumatologic and orthopedics problems
Oncology problems
Liver failure

Another of the changes related with obesity is the development of non-alcoholic steatohepatitis, which appears as a result of the increased circulating FFAs that are released by adipose tissue in response to insulin resistance. The amount of internalized FFA in liver is not regulated; thus, it is proportional to the plasma, in addition it also increases lipogenesis in the body and enhances intracellular accumulation of TG [Monteiro R, 2010]. Excessive accumulation of fat (TG) in the liver is the first step in the development of non-alcoholic fatty liver disease, while the second step is inflammation and cirrhosis.

3.5.4 Obesity and Antioxidant Capacity

When obesity persists for a long time, antioxidant sources can be depleted, decreasing the activity of enzymes such as superoxide dismutase (SOD) and catalase (CAT) [Amirkhizi F, 2007]. The activity of SOD and glutathione peroxidase (GPx) in individuals with obesity is significantly lower compared with that in healthy persons, having implications for the development of obesity-related health problems [Ozata M, 2002]. A study in rats showed that the liver concentration of vitamin A having antioxidant activity was significantly lower in rats with obesity compared with those without obesity; the concentration of vitamin A in rats with obesity probably indicates the dilution of this fat-soluble vitamin in high liver lipid storage [Capel I, 1984]. In addition to vitamin A, levels of serum antioxidants, such as vitamin E, vitamin C, and β-carotene, as well as glutathione, are decreased in obesity [Vincent H, 2005]. In addition to this, ROS decrease the expression of adiponectin, suggesting that treatment with antioxidants or ROS inhibitors could restore the regulation of adipokines [Furukawa S, 2004]. Thus, supplementation with antioxidants would reduce the risk of complications related with obesity and OS [Higdon J, 200].

3.6 Gut microbiota:

Over the past years, numerous studies have deciphered key aspects of the mammalian host–gut microbiota relationship. The human intestine contains a diverse collection of microorganisms totalizing around trillions of bacterial cells,

harboring probably the most complex microbial ecosystems. It is now recognized that the gut microbiota plays an even more important role in maintaining human health than previously thought [Jia W, 2008]. Nowadays, the exact composition of the gut microbiota is unknown; however, continuing advances in genomic and information technologies are starting to unravel our microbial partners (the human microbiota), through the Human Microbiome Project [Hsiao WW, 2009; Turnbaugh PJ, 2007]. Recently conducted investigations have shown that 80–90% of the bacterial phylotypes are members of two phyla: the Bacteroidetes, gram negative (e.g. Bacteroides and Prevotella) and the Firmicutes, gram positive (e.g. Clostridium, Enterococcus, Lactobacillus, Ruminococcus), followed by the Actinobacteria (e.g. Bifidobacterium) and the Proteobacteria (e.g. Helicobacter, Escherichia) [Ley RE, 2005; Eckburg PB, 2005]. It is becoming evident that the gut microbiota provides us with essential genetic and metabolic attributes, sparing us from the need to evolve on our own. In the gut, for example, this includes nutrient and drug metabolism, epithelial cells proliferation, immune system and barrier function against enteric pathogens, synthesis and bioavailability of several vitamins [Jia W, 2008; Round JL, 2009; Neish AS, 2009]. Today more attention is paid to the role of interplay between gut microbiota and host energy-related metabolic functions.

Most research activities in this field have unveiled a glimpse into the mechanism of action and potential therapeutic role of nonpathogen bacteria (probiotics) on mucosal immunity, inflammatory bowel diseases, allergic diseases, etc. [Guarner F, 2005; Guarner F, 2003; Lara-Villoslada F, 2007; Hatakka K, 2008]. Nonetheless, most of the clinical studies have been designed to explore pathological situations rather than physiological or mild impaired health situation.

3.6.1 Gut microbiota and obesity: the dysbiosis concept

Recently, it has been proposed that alterations in the development or composition of the gut microbiota (known as dysbiosis) participate in the development of obesity. In accordance with this hypothesis, it has been shown; firstly in a rodent model, that obesity can be associated with an altered gut microbiota [Ley RE et al, 2005]. Hence, after the characterization of several thousands bacterial gene sequences from

the gut microbiota of genetically obese ob/ob mice and their lean counterparts, Ley et al. pointed out that ob/ob mice exhibited a 50% reduction in the abundance of Bacteroidetes and a proportional increase in Firmicutes. Similarly, the same group has also compared the distal gut microbiota of obese and lean human subjects and found that obese people had lower Bacteroidetes and more Firmicutes than did lean control subjects [Ley RE, 2006]. Interestingly, the authors observed that after weight loss (following a fat restricted or a carbohydrate restricted low-calorie diet), the ratio of Bacteroidetes to Firmicutes approached a lean type profile after 52weeks of diet-induced weight loss [Ley RE, 2006]. However, this study did not demonstrate that the relative change in bacterial strains profile lead to the different fates of bodyweight gain. More recently, Duncan et al. performed a similar study, and found data which do not support the hypothesis that the proportions of Bacteroidetes and Firmicutes are different between obese and lean subjects. The authors did not detect any differences between obese and non-obese individuals in terms of the proportion of Bacteroidetes measured in the fecal samples, and no significant changes of the percentage of Bacteroidetes occurred in feces from obese subjects upon weight loss [Duncan SH et al, 2008]. In accordance with this last study, Zhang et al. [Zhang H, 2009] found even more Bacteroidetes in the obese subjects than in normal-weight individuals. They provided evidence that a subgroup of Bacteroidetes (Prevotellaceae) was significantly enriched in the obese individuals. Moreover, the authors showed that surgical treatment for morbid obesity (gastric bypass) strongly altered the gutmicrobiota toward an increase in Gamma-proteobacteria (members of the family Enterobacteriaceae) and a proportional decrease in Firmicutes [Zhang H, 2009]. Thus, the connection between the relative abundance of Bacteroidetes in obese humans remains a matter of debate. Recently, a metagenomic study, investigating the gut microbiota, addressed how host genotype, environmental exposure, and host adiposity participate to the modulation of the gut microbiome. A total of 154 monozygotic or dizygotic twin pair individuals concordant for their lean or obese phenotype and their mothers have been taken into account in the study. The authors show that, even though there is no important overlap of microbiota among individuals, early changes in familial context influences the composition of microbiota [Turnbaugh PJ, 2009]. Despite such interfamilial/intrafamilial variations,

it exists a remarkably consistent core functions for gut microbes. All together, these data lend credence to the hypothesis that smaller changes or more specific modulation of the gut microbiota community (instead of those obtained at the wide phylum levels) are involved in the development of obesity.

3.7 Experimental models of obesity in the mice

A number of rodent's models of obesity have been established, due to great homology between the genomes of rodents and human beings. The most widely used models are High Fat Diet (HFD) induced obesity, ovariectomy induced obesity, ventro-medical hypothalamic nucleus lesion-induced obesity (induced chemically or surgically), genetically-induced obesity and viral-induce obesity [Butler and Cone, 2001; Buettner et al., 2007].

3.7.1 Diets induced obesity models

This is the simplest obesity-induction model, and possibly the one that most closely resembles the reality of obesity in humans. Diet-induced obesity (DIO) has a late onset and is developed after feeding mice with high-fat diet (HF diet). The use of high-fat diets to induce obesity, IR and hyperglycemia is well documented [Gajda et al., 2007]. Prolonged Exposure to HFD results in positive energy balance and obesity in certain rodents' models that can be considered an adequate model of human obesity [Gaiva et al., 2001; Lin et al., 2000].

The feeding of HFD (normal chow, cholesterol, casein, lard, dl-methionine, vitamins and mineral mix, yeast powder and sodium chloride) for 2 weeks produced insulin resistance and an increase in body weight (Obesity), mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia in rats [Srinivasan et al., 2005]. Depending on animal strain, gender, as well as composition and duration of feeding with high-fat diet, DIO mice developed increased adiposity that resulted in hyperleptinemia and peripheral leptin insensitivity at 8 weeks on HF diet [Van Heek et al., 1997; Buettner et al., 2007]. It is documented that high fat diet can produce rapid weight gain in rats [Saiki *et al.,* 2007; Wit et al., 2008]. The lipogenesis was upregulated by HFD in rats and leads to elevation of plasma lipid levels [Storlien *et al.,* 1991] which is characterized by elevated TG levels, LDL-C levels and decrease

in serum HDL-C [Luo *et al.,* 2009] in obese rats [Woods *et al.,* 2003]. Further, feeding of high fat diet produced hyperglycemia in rats [Ikemoto *et al.,* 1996]. Liver Steatosis and size of adipose tissue is increased by HFD administration [Adams *et al.,* 2005].

Most rodents tend to become obese on HFD but there can be variable responses in glucose tolerance, insulin resistance (IR), triglycerides (TG), and other parameters depending on the strain, gender [Levin et al., 1997; Rossmeisl et al., 2003] and source of dietary fat [Ikemoto et al., 1996; Wang et al., 2002b; Buettner et al., 2006]. High fat diet feeding for 8 day decreased the uncoupling protein-3, PPARγ-2 and fatty acid binding protein (aP2) mRNA level in adipose tissue but increased the parameter when animal were fed for 30 day [Margareto et al., 2001]. In mice, loss of sensitivity to systemic leptin occurred after 8 week, but not after 1 week on high fat diet [Lin et al., 2000]. High fat diet increased the lipoprotein lipase activity in visceral fat [Roberts et al., 2002] but reduced the activity of lipogenic enzyme and lipogenesis in retroperitoneal and inguinal fat depots [Gaiva et al., 2001]. In addition diet containing sucrose (fructose + glucose) or high in fructose are associated with increased weight gain, elevated circulating TG levels, and insulin resistance (IR) in humans and animal models [Daly et al., 1997; Basciano et al., 2005]. It seems that the fructose component of sucrose is largely responsible for the hypertriglyceridemia and IR produced by high sucrose diets [Thresher et al., 2000; Chicco et al., 2003]. The Sprague-Dawley and Wistar rat are established models of sucrose-induced IR and hypertriglyceridemia [Pagliassotti et al., 2000]. Both of these phenotypes can develop within two weeks when these animals are fed a diet containing 65% sucrose (by weight) relative to one with 65% corn starch [Pagliassotti et al., 1996]. Some inbred strains such as the C57BL/6 or AKR mouse are quite susceptible to obesity [Rossmeisl et al., 2003] while mice of the A/J and SWR/J strains tend to be resistant to obesity with VHFD [Surwit et al., 1995].

Chapter 4

Material and Methods

Chapter 4

Materials and Methods

4.1 Selection of Herbal plants

The plants [*Emblica officinalis* (amla/amlaki), *Terminalia chebula* (harad/haritaki), *Terminalia bellerica* (bahed/bibhitaki) and Triphala powder) were selected as these are the most common ingredients of the anti-obesity herbal medicines. Also, some anti-obesity herbal medicines are just comprised of Triphala powder.

4.1.1 Plant powder collection

The plant powders of Amla, Harad, Bahed and Triphala were procured from Sadhana Chemicals, Udaipur (GMP certified company).

4.1.2 Checking the contamination

The herbal powders procured were then screened for the microbes associated with these plant powders. The screening was done as per the guidelines of US Pharmacopeia. US pharmacopeia guidelines are globally accepted protocol for the screening microbial contamination in plant products.

4.1.2.1 US pharmacopeia (USP) protocol guidelines

USP guidelines provide tests for the quantitative estimation of bacteria and fungi that might be present in pharmaceutical articles of all kinds from raw materials to finished products. These tests are designed primarily to verify whether a sample complies with the established specification for microbial quality. The protocols used here for checking the contamination in the herbal powders were Total Aerobic Microbial Count (TAMC) and Total Combined Yeast and Molds Count (TYMC) using the through Pour-Plate Method.

4.1.2.2 Materials used

Amla powder, Harad powder, Bahed Powder, Triphala Powder, Peptone, Dextrose, Trypticase (animal peptone), Phytone (soya peptone), sodium chloride and Agar.

4.1.2.3 Media preparation

4.1.2.3.1 Composition of Media:

1) Sabouraud's Dextrose Agar (SDA):

Peptone = 10gm

Dextrose = 40gm

Agar = 15gm

Distilled water (DW) = 1000ml

pH = 5.6

2) Soyabean Casein Digest Broth (SCD)/Trypticase Soya Broth (TSB):

Trypticase (animal peptone) = 15gm

Phytone (Soya Peptone) = 5gm

Sodium chloride = 5gm

Agar = 15gm

Distilled Water = 1000ml

pH = 7.3

3)100ml of each SDA and SCD media were prepared and sterilized by autoclaving at15 lbs of pressure for 15 minutes

4) Conical flasks and petriplates that were used were also thoroughly washed and autoclaved.

4.1.2.3.2 Procedure

1) Sample preparation step: This step involves dissolving/ suspending 0.2gm of the test specimen (Amla, Harad, Bahed and Triphala powder) in 2ml TSB under sterile conditions.
2) Under sterile conditions (in Laminar Air Flow) pour plates were made using 1ml of the above suspended samples in duplicates using SCD and SDA media plates.
3) The plates were allowed to solidify at room temperature.
4) After the plates were solidified, SCD plates were incubated at 30-35°C for 3-5 days in inverted position. SDA plates were incubated at 20-25°C for 5-7 days in inverted position.

5) After completion of the incubation period, the plates were removed from the incubator and the colonies, if any, were counted.

4.1.2.3.3 Colony count method:

1. The counts recovered were averaged as number of Colony Forming Units (CFU) from the duplicate plates and the extent of contamination were obtained by multiplying by the dilution factor. Results are reported separately for TAMC and TYMC tests.
2. If no colonies were seen, the results were reported results as < 10 CFU/gram or ml of product for the pour-plate method.

4.2 Animal Experiments

All the animal experiments were performed in accordance with the protocol approved by the Institute Animal Ethics Committee (IAEC) of the Birla Institute of Technology and Science, Pilani, Rajasthan.

4.2.1 Selection of Mice:

The animals were obtained from Hisar Agriculture University, Haryana, India.

4.2.1.1 Strain, sex and age of mice:

Male and Female Swiss albino mice strain was used for the study. Seven weeks old mice (20gms) were used.

4.2.1.2 Caging and Housing of animals:

Mice were individually housed in standard cages and placed in a room where the temperature was kept at 21±2.0°C, the relative humidity at 50±5%, and the light on a 12-h light/dark cycle. All the mice consumed a commercial diet and tap water *ad libitum.*

4.2.2 Composition of Experimental Diets:

Standard mice chow (ND) was purchased from Pranav Agro Industries Ltd. The High Fat Diet (HFD) was prepared and standardized in the lab.

4.2.2.1 Preparation of high fat diet (HFD)

4.2.2.1.1 Materials used

Powdered standard mice-chow, saturated and unsaturated fat, cholesterol and Deoxy-cholic acid (Hi Media).

4.2.2.1.2 Procedure

The standard mice-chow was blended to homogeneity, mixed with vegetable fat, cholesterol and deoxy-cholic acid (Table 4.01). The mixture was then formed into equal sized pellets and stored at 4° C.

Table 4.01: Composition of experimental diets

Macronutrient composition	**ND**	**HFD**
Protein, % of energy	22.15	15.7
Carbohydrate, % of energy	73.8	52.4
Fat, % of energy	4.05	32
Total energy, Kcal/kg of diet	3620	5180.2

4.2.3 Preparation of Herbal formulation:

4.2.3.1 Stock preparation/ dose preparation:

The herbal powders of Amla, Harad, Bahed and Triphala were prepared freshly in normal saline solution at a concentration of 5gm/70Kg body weight (standard ayurvedic formulation dose).

4.2.4 Animal Experiment I

The experiment I was designed to check the toxic effects of these herbal formulations, if any. Also in this experiment mice of both sexes were used.

4.2.4.1 Experimental Design I (duration 8 weeks)

Mice were divided into 4 weight-matched groups: the normal diet (ND) group, Amla (ND + A) group, Harad (ND + H) group and Bahed (ND + B). Each group comprised of 6 mice (3M + 3F).

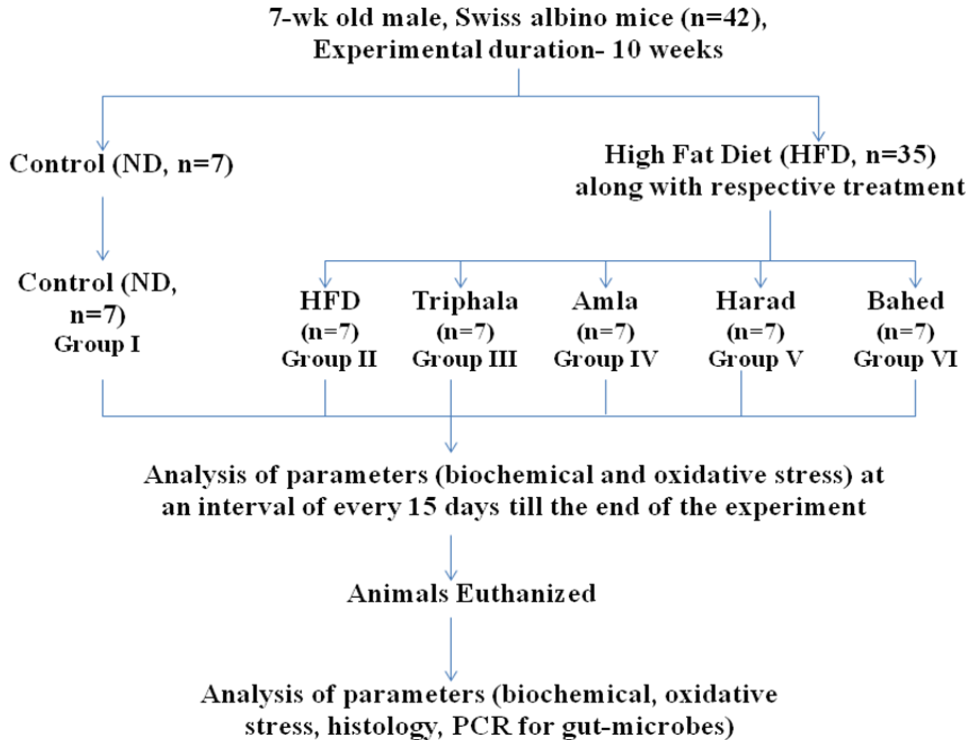
4.2.4.2 Dosing and feeding

All the animals were administered saline suspensions of the herbal powder orally as an oral gavage (using an 18-gauge, feeding needle). All mice were on ND and had free access to water. Their body weight was monitored every week, from 10:00 a.m. to 11:00 a.m., during the course of experiment.

4.2.5 Animal experiment II

Experiment II was designed to check the bioactivity of these herbal formulations. Mice were fed HFD simultaneously along with the herbal formulations to check whether these formulations could prevent the mice from the adverse effects of feeding HFD.

4.2.5.1 Experimental Design II (Figure 4.01)



4.2.5.2 Dosing and feeding

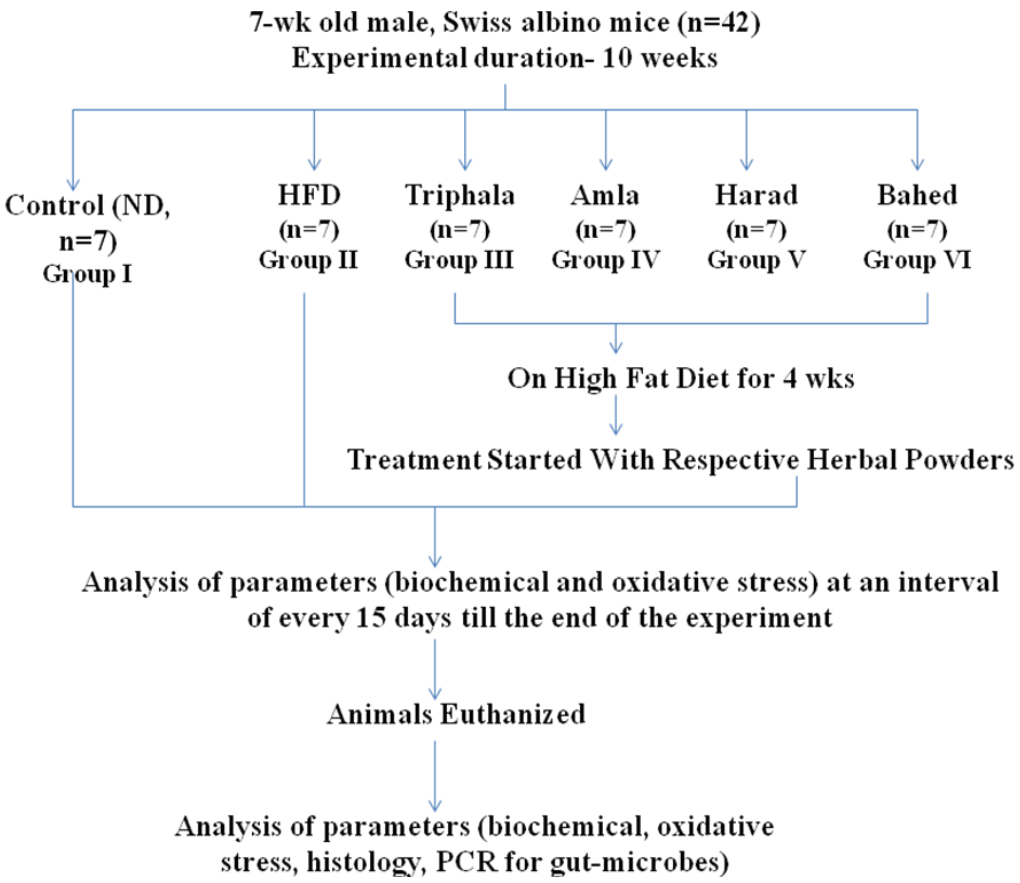
All the mice were divided into six weight-matched groups: the normal diet (ND) group, HFD group, Triphala (HFD+T) group, Amla (HFD + A) group, Harad (HFD + H) group and Bahed (HFD + B) group (Fig: 1). The groups i.e. HFD + T, HFD + A, HFD + H and HFD + B were given these herbal formulations at a concentration

of 5gm/70Kg body weight for 10 weeks. Formulation was freshly prepared before the experiment**.** Animals were orally administered the suspension as an oral gavage. The diet was given in the form of pellets for 10wk. One pre-weighed fresh 3.5–4.0 g food pellet was presented to mice in the home cage every day. Food and energy intake was measured every day for 10 weeks. Body weight of each animal was monitored every three days, between 10:00 a.m. to 11:00 a.m., during the course of the whole experiment.

4.2.6 Animal experiment III

Experiment III was designed to confirm the bio-efficacy of these herbal formulations. The mice were first fed HFD for 4 weeks and were made obese. After 4 weeks, HFD was stopped and herbal formulations were administered to check whether these formulations are effective in reversing the gain of weight in the HFD-fed obese mice.

4.2.6.1 Experimental Design III (Figure 4.02)



4.2.6.2 Dosing and feeding

Mice were divided into six weight-matched groups: the normal diet (ND) group, HFD group, Triphala (HFD+T) group, Amla (HFD + A) group, Harad (HFD + H) group and Bahed (HFD + B) group (Fig: 1). The groups i.e. HFD + T, HFD + A, HFD + H and HFD + B were given these herbal formulations at a concentration of 5gm/70Kg body weight for 10 weeks. Formulation was freshly prepared before the experiment**.** Animals were orally administered as described earlier. The diet was given in the form of pellets for 10wk. One pre-weighed fresh 3.5–4.0 g food pellet was presented to mice in the home cage every day. Food and energy intake was measured every day for 10 weeks. The mice's body weight was monitored every three days, from 10:00 a.m. to 11:00 a.m., during the course of experiment.

4.3 Anthropometric Measurements

Anthropometric measurements like height, weight, and ano-nasal length were recorded for each mouse according to standard techniques [Novelli et al, 2007; Bernardis et al, 1982]. Weight was recorded using weighing scale and height using scale.

4.3.1 Body Weight

Weight was recorded every three days using weighing scale.

4.3.2 Body Mass Index

Body mass Index was calculated as weight (g) divided by height squared (cm^2).

4.3.3 Lee Index

Lee index [$(\text{Body Wt})^{1/3}$ / ano-nasal length (cm) x 1000] was calculated before and after the treatment as an index of obesity.

4.3.4 Liver weight

The liver was isolated, freed from surrounding tissues and weighed.

4.3.5 Adipose tissue weight

The adipose tissue was isolated, freed from surrounding tissues and weighed individually.

4.4 Feed and Calorie Intake

4.4.1 Food Intake

Food intake was calculated as gm/day/animal. Feed intake was recorded daily using the weighing scale.

4.4.2 Energy Intake

Energy intake was calculated as kcal/day/animal. Calorie intake was recorded on a daily basis.

4.4.3 Food Efficiency ratio (FER)

FER was calculated as [body weight gain (g/d)/food intake (g/d)] and was calculated at the end of each experiment.

4.5 Clinical Test or Blood Parameter Estimation

Clinical investigations were performed on the plasma samples for glucose, lipid profile (cholesterol, TG, HDL, LDL and VLDL), Liver function test (SGPT, SGOT), Kidney function test (Urea and Creatinine), total protein and albumin.

4.5.1 Plasma Glucose levels

Plasma glucose levels were measured using commercially available kit (AutoZyme New Glucose, Accurex Biomedical Pvt. Ltd). This kit contains a reagent set for determination of glucose based on enzymatic method using Glucose oxidase (GOD) - Peroxidase (POD). Tests were performed in duplicate as per the manufacturer's instructions using a semi-auto biochemistry analyzer and absorbance was measured at 505 nm upon scanning from 500 - 550 nm range.

4.5.2 Plasma Cholesterol levels

Plasma cholesterol levels were measured using commercially available kit (AutoZyme New Cholesterol, Accurex Biomedical Pvt. Ltd). This kit contains a reagent set for determination of total cholesterol based on enzymatic method using Cholesterol esterase, Cholesterol oxidase and Peroxidase. Tests were performed in duplicate as per the manufacturer's instructions using a semi-auto biochemistry analyzer and absorbance was measured at 510 nm upon scanning from 505 - 530 nm range.

4.5.3 Plasma Triglyceride levels

Plasma triglyceride levels were measured using commercially available kit (AutoZyme New Triglycerides, Accurex Biomedical Pvt. Ltd). This kit contains a reagent set for determination of triglycerides, based on enzymatic method using Lipoprotein lipase, Glycerol kinase, Glycerol phosphate oxidase and Peroxidase. Tests were performed in duplicate as per the manufacturer's instructions using a semi-auto biochemistry analyzer and absorbance was measured at 510 nm upon scanning from 505-530 nm range.

4.5.4 Plasma High Density Lipoprotein Cholesterol (HDL Chol.) levels

Plasma HDL- Chol levels were measured using commercially available kit (Preccugent HDL- Cholesterol precipitating reagent method (PTA), Pinnacle Marketing Pvt. Ld. And AutoZyme New Cholesterol, Accurex Biomedical Pvt. Ltd). Preccugent HDL- Cholesterol precipitating reagent method (PTA) is intended for the separation and quantitative determination of HDL cholesterol and utilizes the well established precipitating properties of phosphotungstic acid to precipitate non HDL–Chol. The remaining cholesterol in the supernatant, HDL-Chol was then measured using commercially available kit for the estimation of Cholesterol.

4.5.5 Plasma Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) levels

The plasma LDL and VLDL levels were calculated from the Friedewald equation (Friedewald *et al.*, 1972).

LDL Level

Serum LDL levels (mg/dl) = Total cholesterol-(HDL level + VLDL level)

VLDL Level

Serum VLDL levels (mg/dl) = Triglyceride level/ 5

4.5.6 Plasma SGPT (ALT) levels

Serum SGPT levels (ALT) were estimated using commercially available kit (ACESURE, SGPT (ALT) Reagent Kit, Axis Diagnostics and Biotech Ltd, Gurgaon). SGPT (ALT), catalyses the transfer of amino group from L-alanine to 2-

Oxoglutarate to form pyruvate and L-Glutamate. The pyruvate thus formed reacts with 2, 4- dinitrophenylhydrazine to form a corresponding hydrazone, a brownish red colored complex in an alkaline medium. The absorbance was measured at 505 nm using a semi-auto biochemistry analyzer.

4.5.7 Plasma SGOT (AST) levels

Serum SGOT levels were estimated using commercially available kit (ACESURE, SGOT (AST) Reagent Kit, Axis Diagnostics and Biotech Ltd, Gurgaon). SGOT (AST), catalyses the transfer of amino group from aspartic acid to 2-Oxoglutarate to form oxaloacetate and L-Glutamate. The oxaloacetate thus formed reacts with 2, 4-dinitrophenylhydrazine to form a corresponding hydrazone, a brownish red colored complex in an alkaline medium. The absorbance was measured at 505 nm using a semi-auto biochemistry analyzer.

4.5.8 Plasma Albumin levels

Albumin levels in plasma were estimated using commercially available kit (AutoZyme: albumin, Accurex Biomedical Pvt. Ltd, Mumbai) which is based upon bromo-cresol green (BCG) dye binding method. The absorbance was measured against blank at 600 nm.

4.5.9 Plasma Total Protein levels

Total protein levels were measured using a commercially available kit (AutoZyme: total protein, Accurex Biomedical Pvt. Ltd, Mumbai) which is based upon Biuret method for total protein determination. The absorbance of specimen and standard was measured against blank at 546 nm using semi auto analyzer.

4.5.10 Plasma Urea levels

AutoZyme Urea kit (Accurex Biomedical Pvt. Ltd, Mumbai) was used to determine the plasma urea levels. This kit is based upon enzymatic method using Urease. Tests were performed in duplicate as per the manufacturer's instructions. The absorbance of assay mixture was measured against blank at 578 nm (570-620 nm.) using semi-auto analyzer.

4.5.11 Plasma Creatinine levels

Determination of creatinine was based on initial rate method using alkaline picrate. Test was performed using commercially available kit (AutoZyme: Creatinine, Accurex Biomedical Pvt. Ltd, Mumbai). The absorbance of assay mixture was measured at exactly 30 seconds after Standard/Specimen addition and then again at 90 seconds at 492 nm using semi auto analyzer.

4.5.12 Oral Glucose Tolerance Test (OGTT) Estimation

For OGTT estimation, blood was collected through tail vein at an interval of 0min, 30 min, 60 min and 120 min. Glucose was measured with the Accu-Chek active glucose monitor. Glucose was prepared at a concentration of 75mg/ml. Of this 100µl (7.5mg) was given orally to each mice after 20 minutes of the respective herbal treatment given.

4.6 Assay for estimating oxidative stress levels

Assays were performed on the plasma samples for Lipid peroxidation levels (TBARS levels) and levels of anti-oxidative enzymes (Catalase and TSOD levels).

4.6.1 Estimation of Thiobarbituric acid reactive species (TBARS)

Although lipid peroxidation of biological samples may be assessed by different chemical and physical methods, those based on the measurement of malondialdehyde (MDA) formed from the breakdown of endoperoxides during the last stages of the oxidation of a polyunsaturated fatty acid is the most widely used method. Among the various methods to evaluate malondialdehyde, which include direct spectrophotometry or high pressure liquid chromatography, the reaction with thiobarbiturlc acid (TBA) to form a colored adduct is a more rapid, inexpensive and sensitive technique. Two molecules of TBA react stoichiometrically with one molecule of MDA during the acidic-heating reaction to form a pink pigment known as TBA-Reactive Species which has an absorption maximum in acid solution at 532 to 535 nm (Valenzuela *et al*., 1990). The extinction coefficient of this pigment at 535 nm at pH 1.0 is 1.56×10^5 M^{-1} cm^{-1}.

4.6.1.1 Material used

Thiobarbituric Acid, Glacial Acetic acid (SRL, India), Tetra Methyl Propanaldehyde (TMP) (Fluka, USA), absolute ethanol (Merck, USA), Hydrochloric Acid (SRL, India), autoclaved triple distilled water.

4.6.1.2 Reagents and working solutions

1. Thiobarbituric Acid (29 mMoles/L) in Acetic acid (8.75 mMoles\L)
   12.57 ml Acetic Acid + 12.43 ml Distilled water + 0.104 gm TBA
2. 19 mM Tetra Methyl Propanaldehyde (TMP) used as a standard Malonaldehyde (MDA)
   Stock standard = 995.2µl Ethanol + 4.8 µl TMP
   10µM Working Standard 1 (WS 1) = 1.05µl of TMP stock standard + 2ml distilled water
   2.5 µM Working Standard 2 (WS 2) = 0.5ml of WS 1 + 1.5ml distilled water
3. 5M Hydrochloric Acid
   0.815 ml HCl + 9.185 ml Distilled water

4.6.1.3 Method:

1. 5 ml glass tubes for standard (S1, S2, S3…..) and test samples were arranged and labeled appropriately.
2. The respective volume of distilled water as shown in the Table 4.14 was added to the respective standard and sample tubes.
3. Followed by addition of WS2 (tetra methyl Propanaldehyde - TMP) in standard and blood plasma in sample tubes respectively as shown in the table 4.14.
4. 1ml of 29 mM Thiobarbituric Acid (TBA) solution was mixed with the contents of each tube and vortexed to ensure proper mixing. Tubes were sealed appropriately.
5. All the tubes were incubated in water bath at 100º C for 1 hour.
6. The tubes were cooled thereafter in running water and 25 µl of 5M HCl was added to each tube.
7. The absorbance for each standard/ test sample was measured at 530nm on Jasco UV-Vis spectrometer.

8. A standard graph of concentration of MDA versus absorbance observed at 530nm was prepared to calculate the concentration of the test samples from the graph.

Table 4.02: Preparation of standard/Samples for TBARS assay

Conc. Of MDA (nmol/ml)	**Standard/ sample**	**Volume of WS2/ sample** (µl)	**Distilled Water** (µl)	**Distilled Water** (ml)	**TBA Soln.** (ml)	**Incubation (1 hour at 100° C in water bath**	**5M HCl** (µl)
0.0	Blank	0.0	100 µl	1 ml	1ml		25 µl added after cooling of tubes
0.5	S1	20	80 µl				
1.0	S2	40	60 µl				
1.5	S3	60	40 µl				
2.0	S4	80	20 µl				
2.5	S5	100	0 µl				
	Test plasma	100	0 µl				

4.6.2 Estimation of Total Superoxide Dismutase Activity (TSOD)

A simple method for detecting TSOD was described by Kakkar *et al.*, 1984. The reduction of nitro blue tetrazolium (NBT) with NADH mediated by Phenazine methosulfate (PMS) under aerobic conditions is inhibited upon addition of superoxide dismutase. This observation indicated the involvement of superoxide aninon radical (O_2^-) in the reduction of NBT, the radical being generated in the re-oxidation of reduced PMS. The entire reaction is inhibited by addition of superoxide dismutase which catalyzes dismutation of O_2^- to form O_2 and H_2O_2.

4.6.2.1 Materials used

Potassium dihydrogen phosphate (KH_2PO_4), Disodium hydrogen phosphate (Na_2HPO_4), Reduced Nicotinamide Adenine Dinucleotide (NADH), Methylphenazonium Methyl Sulphate or Phenazine Methosulphate (PMS), Nitro Blue Tetrazolium (NBT), Dimethyl Formamide (DMF)

4.6.2.2 Reagents and working solutions

1. Stock Phosphate Buffer (PB) (0.1 M) pH 7.2: 0.1M KH_2PO_4 (3 parts) + 0.1M Na_2HPO_4 (7 parts) = 0.272g KH_2PO_4 + 0.356g Na_2HPO_4 (for 20 ml)

2. Working Phosphate buffer (50 mM) pH 7.2: 10 ml 0.1 M Phosphate buffer + 10 ml Distilled water.
3. NADH (Reduced Nicotinamide Adenine Dinucleotide)
   Stock I (7.14 mM): 10 mg in 2 ml of 50 mM Phosphate buffer
   Stock II (780µM): 546.2µl of stock I + 4453.8µl of 50 mM Phosphate buffer
4. Methylphenazonium Methyl Sulphate or Phenazine Methosulphate (PMS)
   Stock I (200mM): 61.4 mg in 1 ml Distilled water
   Stock II (186µM): 9.3µl of 200mM stock I + 9.9907 ml of Distilled water
5. Nitro Blue Tetrazolium (NBT)
   Stock I (12 mM): 10 mg in 1 ml 70% Dimethyl Formamide (DMF)
   Stock II (300µM): 250µl NBT stock I + 9.75 ml of Distilled water

4.6.2.3 Methodology:

The reagents were added sequentially as per the details given below in Table 4.15. Addition of NADH starts the reaction and 1 ml glacial acetic acid stops the reaction. The reaction mixture is stirred vigorously with 4ml of n-butanol after the reaction is stopped and is centrifuged briefly to separate out the butanol layer. Color intensity was measured at 560 nm against butanol by using a spectrophotometer. TSOD activity was calculated using the given formula below.

Table 4.03: Preparation of standard/Samples for TSOD assay

Sample	0.1M PB	Plasma	50 mM PB	DDW	200µM PMZ	300µM NBT	780µM NADH	Incubate for 90 sec at RT	Glacial Acetic Acid
Blank	10µl	-	1.2ml	1.19ml	100µl	300µl	200µl		1 ml
Test A	-	10µl							
Test B	-	10µl							
Test C	-	10µl							

Calculation:

Total SOD in plasma = (Reagent Blank-Test sample/Reagent blank)*4/3*1000*mg of protein = mU/min/mg of protein

4.6.3 Estimation of Catalase

A simple method for detecting Catalase was described by Aebi et *al.*, 1974. Catalase exerts a dual function: 1) Decomposition of H_2O_2 to give H_2O and O_2 (catalytic activity) and 2) oxidation of H donors, eg. Methanol, ethanol, formic acid, phenols with the consumptions of 1nmol of peroxide (peroxidic activity). In the ultraviolet range H_2O_2 shows a continous increase in absorption with decreasing wavelength. Thus, end decomposition of H_2O_2 can be directly followed by monitoring the decrease in absorbance (λA 240) of H_2O per unit time. This change in absorbance is directly proportional to the measure of catalase activity.

4.6.3.1 Materials used

Potassium dihydrogen phosphate (KH_2PO_4), Disodium hydrogen phosphate (Na_2HPO_4), Hydrogen peroxide.

4.6.3.2 Reagents and working solutions

1. Stock Phosphate Buffer (PB) (0.1 M) pH 7.2: 0.1 M KH_2PO_4 (3 parts) + 0.1 M Na_2HPO_4 (7parts) = 0.272g KH_2PO_4 + 0.356g Na_2HPO_4 (for 20ml).
2. Working Phosphate buffer (50mM) pH 7.2: 10ml 0.1 M Phosphate buffer + 10ml Distilled water.
3. H_2O_2 (60mM): 6µl for 1ml (freshly prepared just before the experiment)

4.6.3.3 Methodology

1. Take 280 µl of Phosphate buffer in a cuvette and add 10µl of sample.
2. Read the absorbance at λ240 prior to the addition of H_2O_2.
3. Add 10 µl of H2O2 into the cuvette and record the decrease in absorbance over a period of 3 minutes.
4. Calculate the change in absorbance/minute following the addition of the sample.

4.6.3.4 Calculations

$$\frac{\text{Change in Abs (OD)} \times 10^6}{40 \times \text{mg protein} \times 60} \times 300\mu l \text{ (total volume)} = \text{Catalase activity (nMoles / mg/sec)}$$

Extinction coefficient of H_2O_2 = 40.

4.7 Histological Analysis

4.7.1 Animal Dissection

At the end of each experiment, the mice were euthanised and part of liver and intestine were stored in 10% formalin for further histological analysis.

4.7.2 Tissue processing

The formalin-fixed segment of the liver and intestine were processed through a graded series of alcohol and then embedded in paraffin. Four-micrometer–thick sections of the processed tissue were cut from the paraffin block in a microtome. Sections were mounted on glass slides, stained with hematoxylin and eosin, and observed under a light microscope at 100X magnifications.

4.8 Fecal Pellet Culture

Fecal pellet culture was done to analyze the change in proportion of Bacteroides and Firmicutes if any in the gut. The standard culture for bacteroides [*B.fragilis* (MTCC No.: 1045)] was obtained from Microbial Type Culture Collection and Gene Bank (MTCC).

4.8.1 Fecal pellet collection

At the end of each experiment, before sacrificing the animals, fecal pellets of each mice were collected separately. The fecal pellets collected were then used for fecal fat analysis, anaerobic culture for Bacteroides and Firmicutes and DNA isolation.

4.8.2 Materials (Media) used

Thioglycolate media (for firmicutes), Bile Esculin Agar (for Bacteroides), Bacteriological Agar, glycerol, Anaerobic gaspack.

4.8.3 Methodology

1. Single fecal pellet from each mouse of each group was inoculated in 5ml Thioglycolate broth culture vial under sterile condition in Laminar Air

Flow. One negative control broth vial was kept in order to check any contamination in media.

2. All the vials after inoculation were incubated in anaerobic jar for 48 hrs at 37°C.
3. To maintain the anaerobic conditions, one anaerobic gaspack was kept inside the jar.
4. The jar was then tightly closed and was checked at regular intervals for any leakage of gas.
5. After the completion of incubation period, the culture vials were taken out from the anaerobic jar.
6. From the culture obtained, 1 ml culture was used for plating and 1 ml culture was used for making glycerol stocks.
7. The remaining culture was then pelleted in fresh 1.5 ml eppendorff tubes at 2000 rpm for 5 minutes. The bacterial pellet so formed was used for DNA isolation and was stored at -20°C till further use.
8. For plating, pour-plate method was used. Thioglycolate agar and Bile Esculin Agar was prepared and sterilized by autoclaving at15 lbs of pressure for 15 minutes.
9. Under aseptic conditions, 1 ml innoculum was plated in respective plates and then molten agar was poured.
10. The plates were allowed to solidify at room temperature and then kept for incubation in anaerobic jar for 48 hrs at 37°C.
11. After the completion of incubation period, the plates were taken out and photographs
    of all the plates were taken.

4.9 Fecal DNA Isolation

DNA isolation from fecal DNA was done to extract the bacterial DNA to further analyze the Bacteroides and Firmicutes**.** Broth culture was used for genomic DNA extraction using the CTAB procedure [Jones & Walker, 1963; Wilson, 1987]. All the tips and tubes used in DNA extraction were sterilized by autoclaving at 15 lbs of pressure for 15 minutes. The water used in DNA extraction was of Milli Q grade.

4.9.1 Protocol used for DNA extraction

Bacteria from a saturated liquid culture were pelleted and lysed using the detergent SDS. Proteins are removed by digestion with the nonspecific serine protease, proteinase K. cell wall debris, polysaccharides and remaining proteins are removed by selective precipitation with CTAB, and high molecular-weight DNA is recovered from the resulting supernatant by isopropanol precipitation.

4.9.2 Materials Required

Tris Cl, EDTA, Sodium Dodecyl Sulfate (SDS), Proteinase K, Sodium chloride (NaCl), CTAB, Chloroform, Isoamyl Alcohol, Tris-saturated Phenol, Isopropanol, Ethanol and 1.5ml sterile eppendorfs.

4.9.3 Reagent and Working Solutions

1. Tris EDTA (TE) Buffer
   0.1 M Tris-Cl
   0.5 M EDTA
2. 10 % SDS
3. 25mg/ml Proteinase K (stored at -20°C)
4. 50mg/ml RNase A (stored at -20°C)
5. 5 M NaCl
6. CTAB/NaCl Solution
   1% CTAB
   0.7 M NaCl
7. PCA
   Tris Saturated Phenol: Chloroform: Isoamyl Alcohol in 25:24:1 ratio
8. CA
   Chloroform: Isoamyl Alcohol in 24:1 ratio
9. Chilled Isopropanol
10. 70% Ethanol

4.9.4 Procedure

1. Grow bacterial strain to saturation.
2. Pellet down the culture at 2000rpm for 2 min in 1.5ml micro centrifuge tube.

3. Resuspend the pellet in 567 µl TE buffer, 30 µl of 10% SDS and 2.4 µl of Proteinase K (25mg/ml stock). Mix well and incubate for 1 hr at 37°C on dry bath.
4. Add 100 µl of 5M NaCl. Mix thoroughly.
5. Add 80 µl CTAB/NaCl. Mix well and keep for cold treatment for 15 min.
6. Incubate for 10 min at 65°C for 10 min in water bath.
7. Extract with an equal volume of chloroform/Isoamyl alcohol. Spin for 10 min at 5000 rpm in micro centrifuge.
8. Aqueous phase was transferred to an eppendorff tube. To this RNase A was added and incubated for 1 hr at 37°C.
9. Extract with equal volume of phenol/chloroform/Isoamyl alcohol. Spin at 5000 rpm for 10 min in micro centrifuge.
10. Transfer aqueous phase to a fresh tube. Repeat the CA step again to remove the residual phenol and transfer aqueous phase to a fresh eppendorff tube.
11. Precipitate DNA with 1/2 volume of chilled isopropanol.
12. The tube was gently swirled to precipitate the DNA till a thread like structure appeared, and was then kept at -20°C for overnight precipitation.
13. Precipitated DNA was centrifuged at 10,000 rpm for 20 minutes and the supernatant was discarded.
14. DNA pellet was washed with 70% chilled ethanol and centrifuged at 10,000 rpm for 10 minutes and again the supernatant was discarded taking care to retain the loose pellet. This step was repeated twice.
15. The pellet was air dried at room temperature and DNA was dissolved in 50 µl of sterile Milli Q grade water.
16. Dissolved DNA samples were stored at -20°C for further analysis and at -70°C for long term storage.

4.10 PCR Gut microbes

Table 4.04 Sequence of oligonucleotide primers (degenerate primer)

Assay	Primer name and sequence (5´-3´)	Approximate Size (bp) of product	Annealing temp (°C)	Reference
Bacteroidetes	F, GGARCATGTGGTTTAATTCGATGAT R, AGCTGACGACAACCATGCAG	126	60	X Guo, 2008
Firmicutes	F, GGAGYATGTGGTTTAATTCGAAGCA R, AGCTGACGACAACCATGCAC	126	60	X Guo, 2008

4.10.1 PCR conditions

The primer sets specific for Bacteroides and Firmicutes used are listed in Table 4.03. Oligonucleotide primers were ordered from Oscimum Biosolutions Hyderabad, India. Amplification and detection of DNA by real-time PCR were performed with the Eppendorf five color thermal cycler using optical grade 96-well plates. Triplicate samples were routinely used for the determination of DNA by real-time PCR, and the mean values were calculated. The PCR reaction was performed in a total volume of 10ul. Bacteroidetes, Firmicutes and all Bacteria were detected by using the Power SYBR PCR Master Mix (Sigma chemicals, with 100 nmol l) 1 of each of the forward and reverse primers and 2 ng DNA for each reaction. The conditions used were as follows:

PCR conditions for bacteriodes: 50C-2min, 95C-10min, 95C-15 sec, 60C-1min, 72C-20sec and 40 cycles

PCR conditions for firmicutes: 50C-2min, 95C-10min, 95C-15 sec, 58C-1min, 72C-20sec and 40 cycles

The ratio calculated in the excel sheet is in terms of CT values. The threshold cycle (CT) values and baseline settings were determined by automatic analysis settings. Data analysis was performed using Software supplied by Eppendorf.

4.11 Statistical Analysis

All results obtained are expressed as mean $\pm$ SEM of the seven mice in each group. Statistical analyses were conducted using GraphPad Prism 3 software (trial version downloaded free from internet). One-way ANOVA with Tukey's post hoc test was used for statistical analysis of BW gain, body fat and biochemical parameters. For analysis of BW and blood glucose over the treatment time period, repeated-measures ANOVA with Bonferroni's post hoc test was used. In all comparisons, the difference was considered to be statistically significant at $P < 0.05$.

Chapter 5

Results: Animal Experiments

Chapter 5

Results: Animal Experiments

5.1 Animal Experiment I: Estimation of the toxicological effect of Amla, Harad and Bahed in Normal Swiss Albino Mice:

Oral administration of the extracts was well-tolerated for the entire 8-weeks experimental duration. Body weight (Table 5.01) among the treated groups (Amla, Harad and Bahed) did not show any significant difference as compared to the control group (Figure 5.01). There were no observed clinical signs of toxicity or any other adverse effects. No significant differences were observed between the treated and the control group in plasma chemistry, viz. plasma glucose and serum triglycerides, cholesterol, creatinine, Albumin and ALT (Table 5.02).

5.1.1 Body weight chart: Body weight for all groups was recorded weekly. The Fig: 5.01 shows the body weight of ND group compared with groups treated with Amla, Harad and Bahed. Table 5.01 illustrates the comparative body weight showing initial and final body weight and body weight gain for all the groups.

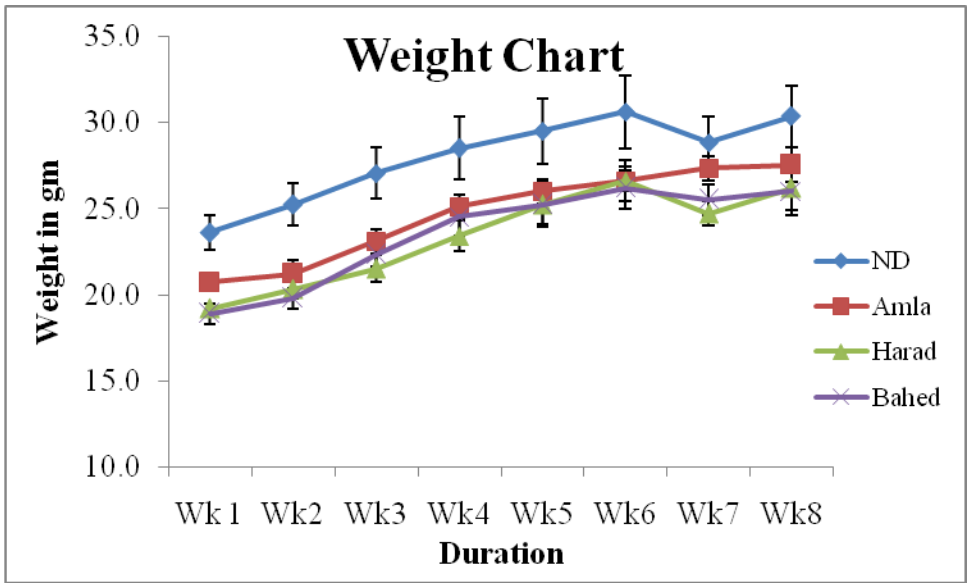


Fig: 5.01 Body Weight of Mice fed on Normal Diet for 8 weeks. Values are mean ± SEM; n=6. Means are not statistically different.

Table 5.01: Comparative table showing the Body weight in Mice Fed on Normal Diet and Herbal Powders for 8 Weeks

Groups	ND	Amla	Harad	Bahed
Initial body weight g	23.6 ± 1.0	20.7 ± 0.4	19.2 ± 0.3	18.9 ± 0.6
Final body weight g	30.3 ± 1.8	27.5 ± 1.0	26.2 ± 1.3	26.0 ± 1.4
Body weight gain g/8 wk	6.7 ± 1.6	6.8 ± 0.7	7.0 ± 0.9	7.1 ± 1.0

Values are mean ± SEM; n=6 in each group. All means do not show any statistically significant difference.

5.1.2 Clinical Parameters:

Table 5.02: Plasma Biochemistry of Mice Fed on Normal Diet and Herbal Powders for 8 Weeks

Group	Control	Amla	Harad	Bahed
Glucose	97.4±0.8	95.7±1.3	100.1±1·	93.2±2.8
Cholesterol	95.4± 1.3	92.6± 2.2	90.6± 1.3	98.7± 1.4
Triglyceride	64.2± 1.5	68.8±1.3	63.5±0.5	69.1±0.9
ALT	20.2±1.3	19.7±0.6	21.0±2.6	21.6±0.8
Albumin	3.22±0.21	3.4±0.1	3.1±0.1	3.1±0.1
Creatinine	0.2±0.2	0.22±0.04	0.15±0.05	0.2±0.05

Values are mean ± SEM; n=6 in each group. All means do not show any statistically significant difference

5.2 Animal Experiment II: Triphala and Its Constituents Prevent Visceral Adiposity in High Fat Diet-Induced Obesity in Mice

This experiment was designed to test the preventive aspect of Triphala, Amla, Harad and Bahed powders on HFD induced obesity in mice. To evaluate the effect of these herbal powders on visceral adiposity, anthropometric markers (BW, BMI, and Lee Index) were studied. Clinical parameters studied include estimation of glucose, OGTT, lipid profile, liver function test and oxidative stress markers.

5.2.1 Anthropometric Parameters:

5.2.1.1 Body weight chart: Body weight for all groups was recorded at an interval of 3 days (Figure 5.02). The Fig: 5.03 A shows the body weight of ND group compared with HFD group alone and ND, HFD group compared with Triphala, Amla, Harad and Bahed group individually (Fig: 5.03 B, C, D, E).

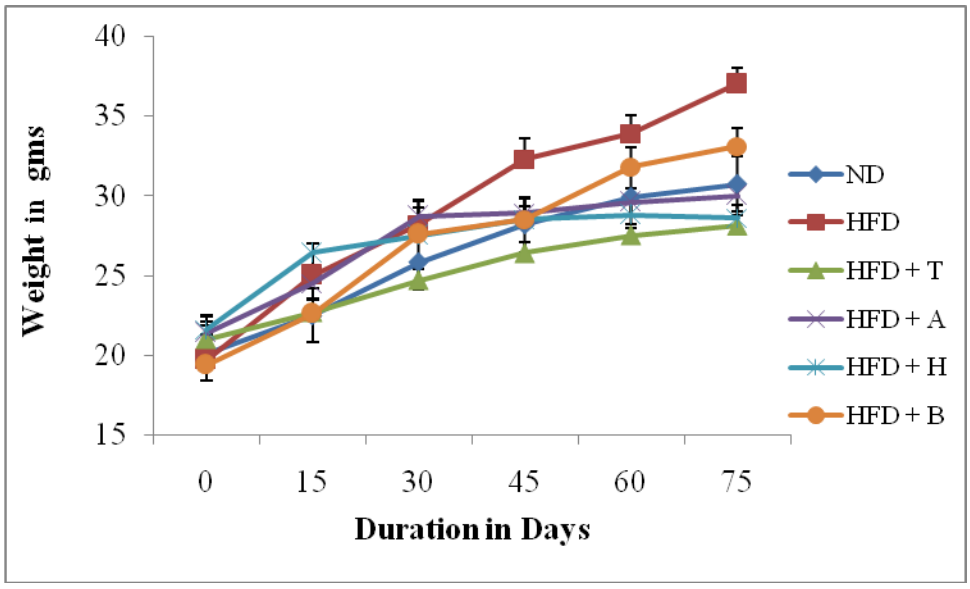


Fig: 5.02 Body Weight of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$)

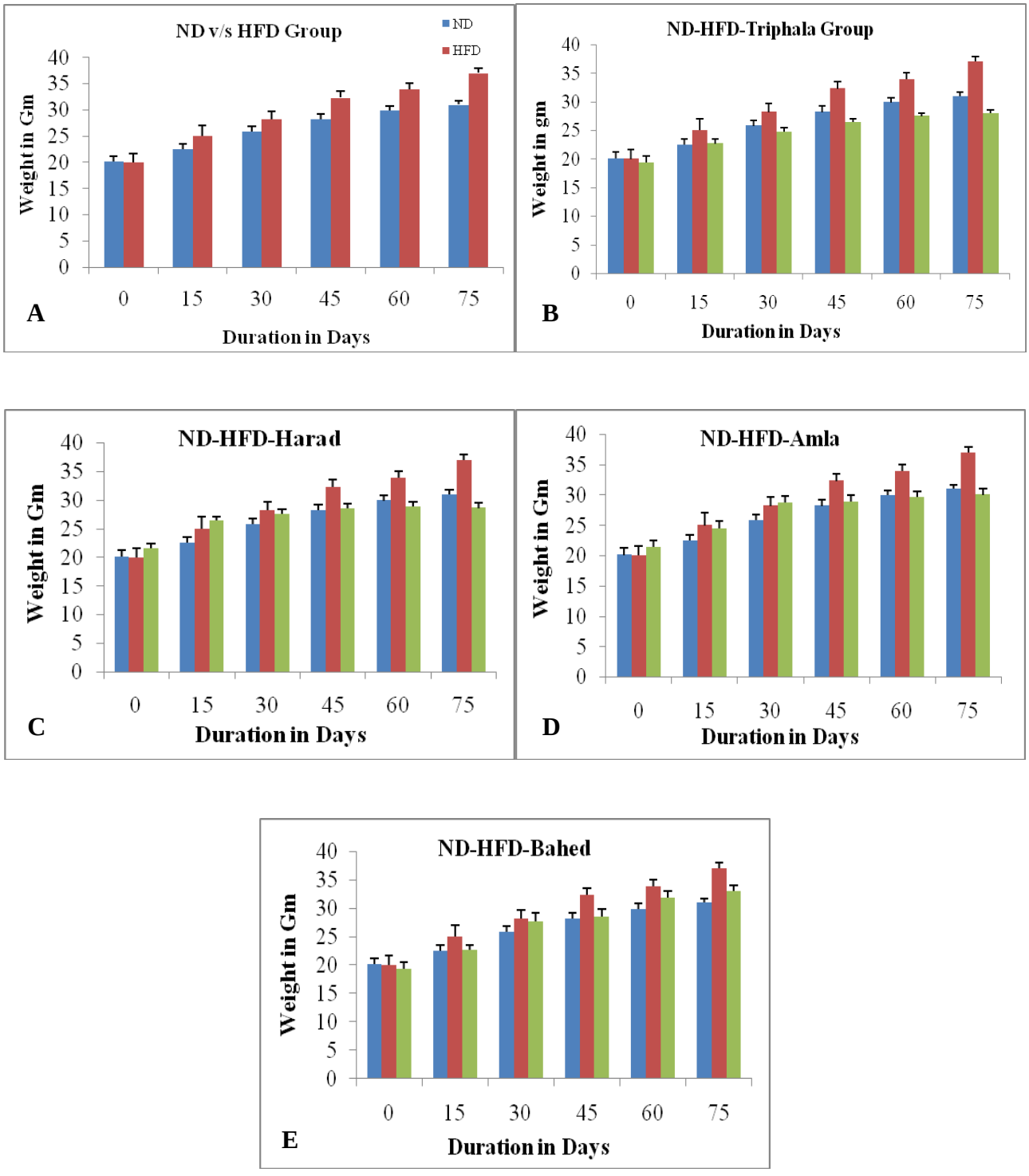


Fig: 5.03 Body Weight of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are significantly different ($p < 0.05$) except for the Bahed group.

It can be seen from Fig: 5.02 and 5.03, that the body weight of animals in ND, HFD, HFD+T, HFD+A, HFD+H and HFD+B groups began to diverge from day 15 and stayed different but comparable to ND group for the remaining experimental period ($p < 0.05$).

Table 5.03: Body Weight Gain in Mice Fed on the Experimental Diets for 10 Weeks

Groups	ND	HFD	HFD+T	HFD+A	HFD+H	HFD + B
0 Day	20.1± 1.1	20.0 ±1.6	19.4± 1.1	21.4± 1.0	21.6± 0.8	19.4 ±1.1
15 Day	22.5 ±0.9	25.0 ±2.0	22.7± 0.8	24.5± 1.3	26.4± 0.6	22.6± 0.9
30Day	25.8 ±1.0	28.2± 1.5	24.7 ±0.7	28.7± 1.1	27.5± 0.8	27.6± 1.6
45 Day	28.2 ±1.0	32.3± 1.3	26.4 ±0.6	28.9 ±1.0	28.5 ±0.8	28.5 ±1.3
60 Day	29.9± 0.9	33.9 ±1.1	27.5± 0.5	29.6± 1.0	28.8 ±0.8	31.8 ±1.3
75 day	31.0 ±0.7	37.0 ±1.0	28.0 ±0.6	30.0± 1.0	28.6± 0.8	33.0± 1.1
Body weight gain g/10 wk	**10.9 ±1.1**	**17.3 ±0.7**	**8.6 ±0.8****	**8.6 ±1.0****	**7.0 ±1.5****	**13.6 ±0.5***

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (** $p < 0.0001$, * $p < 0.05$)

The Table 5.03 gives the mean body weight values at an interval of 0, 15th, 30th, 45th, 60th and 75th day and mean body weight for the entire experimental duration.

5.2.1.2 Feed Intake

Food intake for all groups was recorded every day. Figure 5.04A shows the feed intake of ND group compared with HFD group alone and ND, HFD groups compared with Triphala, Amla, Harad and Bahed groups individually (Fig: 5.04 B, C, D, E).

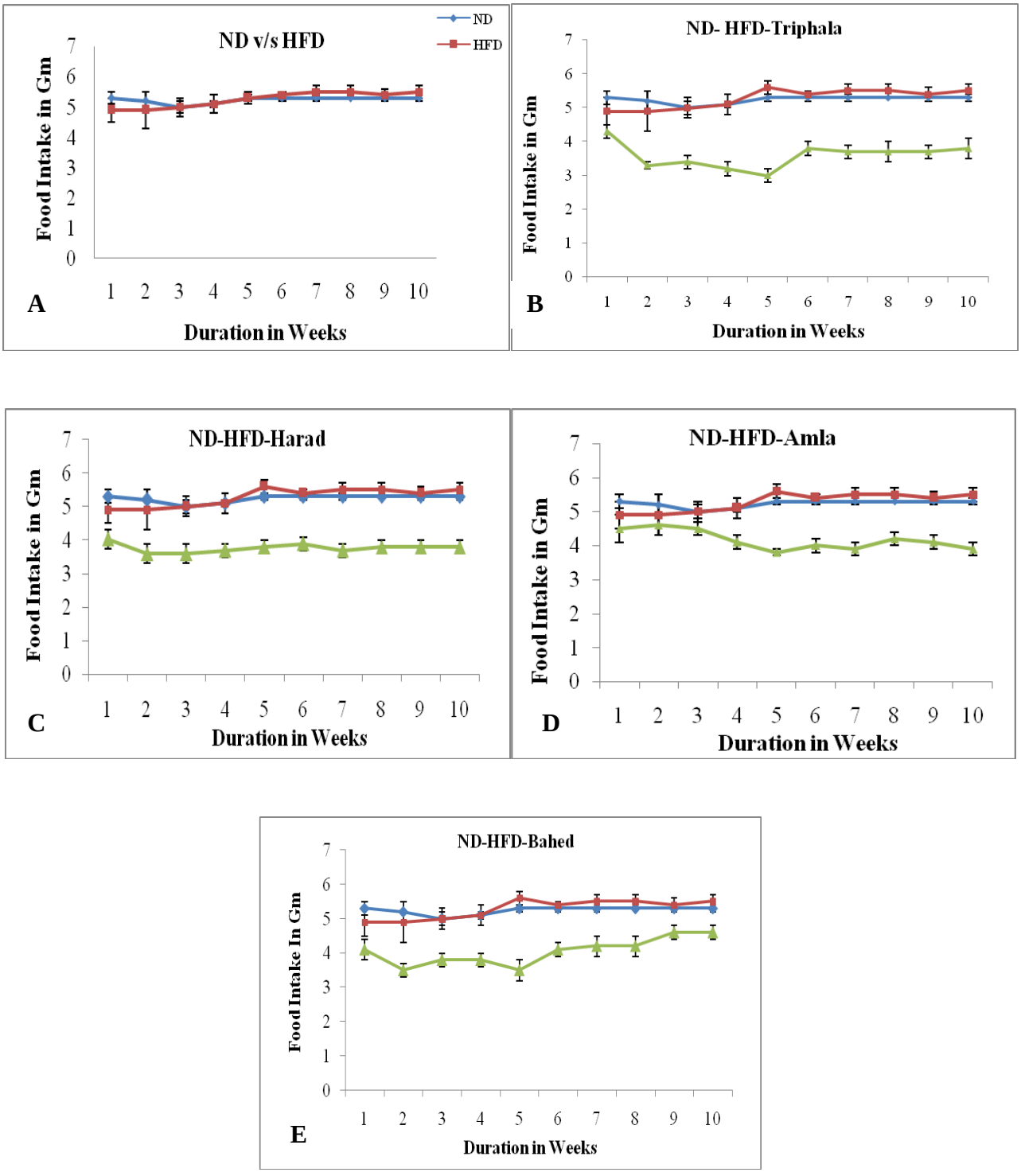


Fig: 5.04 Feed Intake of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

Table 5.04: Feed Intake in Mice Fed on the Experimental Diets for 10 Weeks

Groups / Weeks	ND	HFD	HFD+T	HFD+A	HFD+H	HFD + B
1 wk	5.3 ± 0.2	4.9 ± 0.4	4.3 ± 0.2	4.5 ± 0.4	4.0 ± 0.3	4.1 ± 0.3
2wk	5.2 ± 0.3	4.9 ± 0.6	3.3 ± 0.1	4.6 ± 0.3	3.6 ± 0.3	3.5 ± 0.2
3wk	5.0 ± 0.2	5.0 ± 0.3	3.4 ± 0.2	4.5 ± 0.2	3.6 ± 0.3	3.8 ± 0.2
4wk	5.1 ± 0.1	5.1 ± 0.3	3.2 ± 0.2	4.1 ± 0.2	3.7 ± 0.2	3.8 ± 0.2
5wk	5.3 ± 0.1	5.3 ± 0.2	3.0 ± 0.2	3.8 ± 0.1	3.8 ± 0.2	3.5 ± 0.3
6wk	5.3 ± 0.1	5.4 ± 0.1	3.8 ± 0.2	4.0 ± 0.2	3.9 ± 0.2	4.1 ± 0.2
7wk	5.3± 0.1	5.5 ± 0.2	3.7 ± 0.2	3.9 ± 0.2	3.7 ± 0.2	4.2 ± 0.3
8wk	5.3 ± 0.0	5.5 ± 0.2	3.7 ± 0.3	4.2 ± 0.2	3.8 ± 0.2	4.2 ± 0.3
9wk	5.3 ± 0.1	5.4 ± 0.2	3.7 ± 0.2	4.1 ± 0.2	3.8 ± 0.2	4.6 ± 0.2
10wk	5.3 ± 0.1	5.5 ± 0.2	3.8 ± 0.3	3.9 ± 0.2	3.8 ± 0.2	4.6 ± 0.2
Food intake g/d	**5.3 ± 0.1**	**5.3± 0.2**	**3.6 ± 0.1***	**4.0± 0.1***	**3.8± 0.1***	**4.2± 0.1**

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different ($p < 0.05$).

The Table 5.04 gives the weekly food intake values over the 10 week period and mean food intake for the entire experimental duration and there a little decrease in feed intake was observed in groups treated with Triphala, Amla, Harad and Bahed.

5.2.1.3 Energy Intake

Energy intake for all groups was recorded every day. Figure 5.05 shows the energy intake of ND group compared with HFD group alone 5.05A and ND, HFD group compared with Triphala, Amla, Harad and Bahed groups individually (5.05 B, C, D, E).

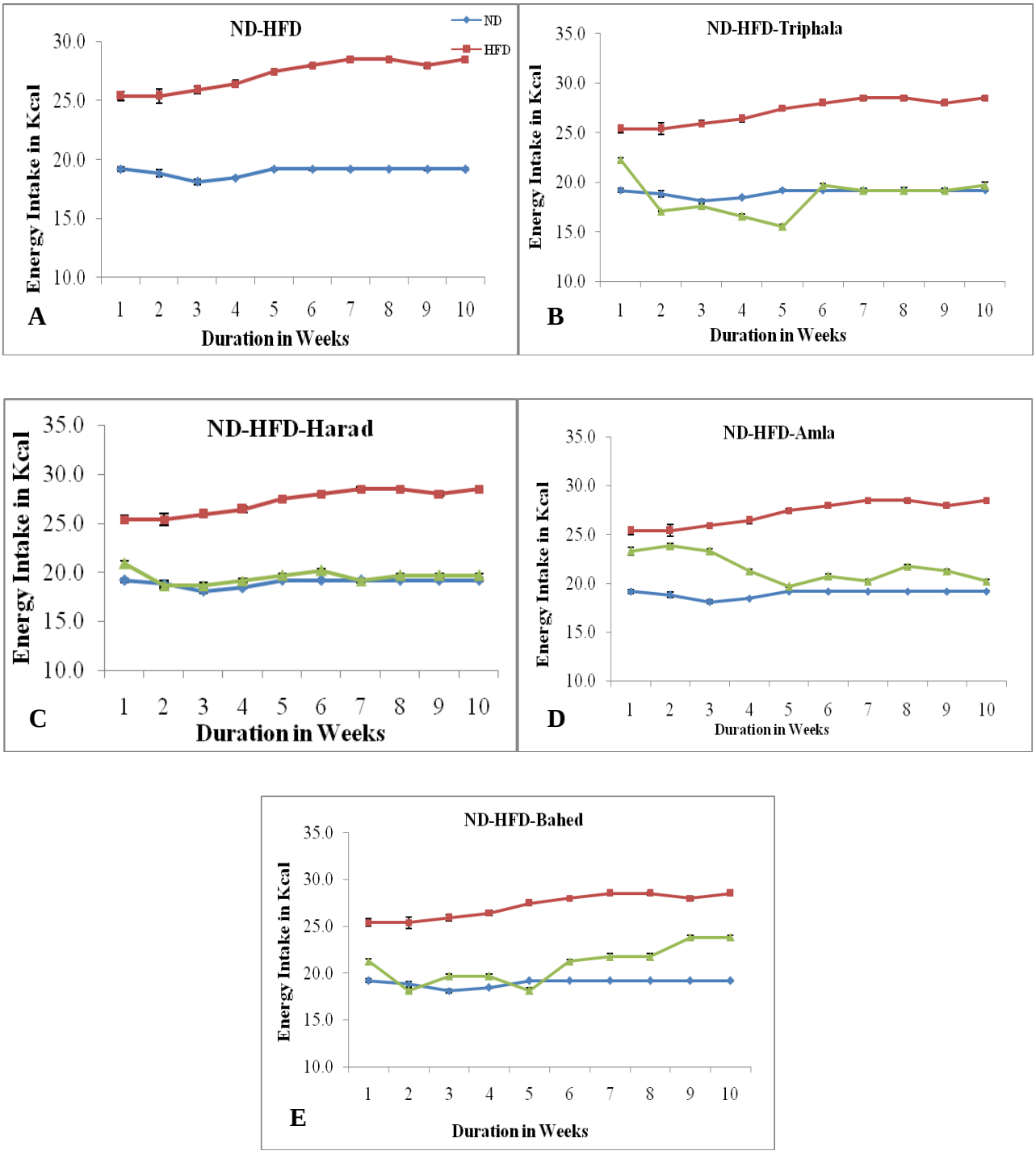


Fig: 5.05 Energy Intake of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

Table 5.05: Energy Intake in Mice Fed on the Experimental Diets for 10 Weeks

Groups / Weeks	ND	HFD	HFD+T	HFD+A	HFD+H	HFD + B
1 wk	19.2 ± 0.2	25.4 ± 0.4	22.3 ± 0.2	23.3 ± 0.4	20.9 ± 0.3	21.2 ± 0.3
2wk	18.8 ± 0.3	25.4 ± 0.6	17.1 ± 0.1	23.8 ± 0.3	18.6 ± 0.3	18.1 ± 0.2
3wk	18.1 ± 0.2	25.9 ± 0.3	17.6 ± 0.2	23.3 ± 0.2	18.6 ± 0.3	19.7 ± 0.2
4wk	18.5 ± 0.1	26.4 ± 0.3	16.6 ± 0.2	21.2 ± 0.2	19.2 ± 0.2	19.7 ± 0.2
5wk	19.2 ± 0.1	27.5 ± 0.2	15.5 ± 0.2	19.7 ± 0.1	19.7 ± 0.2	18.1 ± 0.3
6wk	19.2 ± 0.1	28.0 ± 0.1	19.7 ± 0.2	20.7 ± 0.2	20.2 ± 0.2	21.2 ± 0.2
7wk	19.2 ± 0.1	28.5 ± 0.2	19.2 ± 0.2	20.2 ± 0.2	19.2 ± 0.2	21.8 ± 0.3
8wk	19.2 ± 0.0	28.5 ± 0.2	19.2 ± 0.3	21.8 ± 0.2	19.7 ± 0.2	21.8 ± 0.3
9wk	19.2 ± 0.1	28.0 ± 0.2	19.2 ± 0.2	21.2 ± 0.2	19.7 ± 0.2	23.8 ± 0.2
10wk	19.2 ± 0.1	28.5 ± 0.2	19.7 ± 0.3	20.2 ± 0.2	19.7 ± 0.2	23.8 ± 0.2
Energy intake kcal/d	**19.2 ± 0.1**	**26.9± 0.2**	**18.6 ±0.1***	**21.8± 0.1***	**19.7 ± 0.1***	**20.7± 0.1**

Values are mean ± SEM; n=7 in each group. All means observed in treated groups are statistically significantly different (* $p < 0.05$)

The Table 5.05 gives the energy intake values at a weekly interval for the 10 weeks period and mean energy intake for the entire experimental duration. The energy intake in treated animal groups was comparable to the energy intake in the normal diet group.

5.2.1.4 BMI and Lee Index

Body weight for all groups was recorded at an interval of every 3 days. The mean BMI and Lee Index were calculated at the end of the entire experimental duration. The Fig: 5.06 shows the mean BMI and Fig: 5.07 shows Lee Index for all the groups.

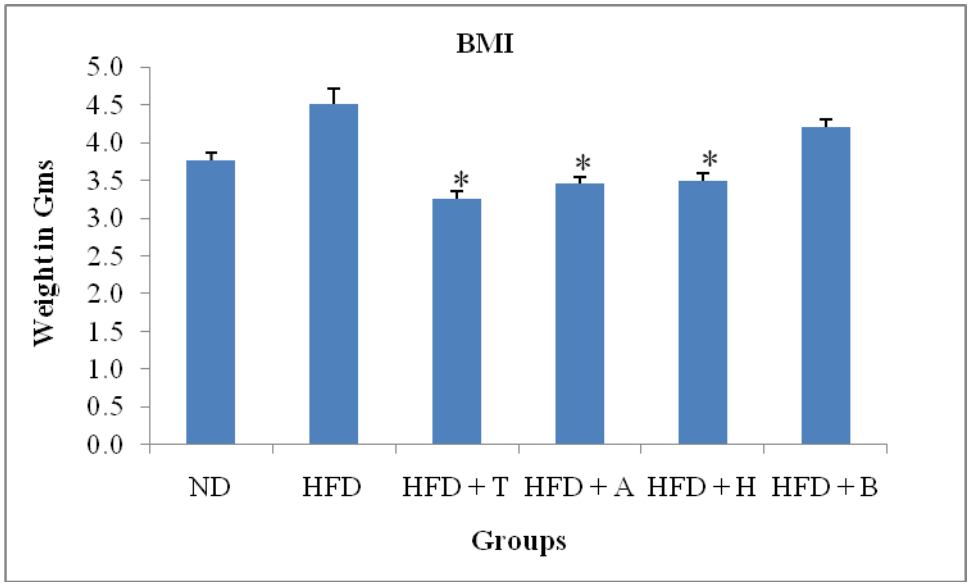


Fig: 5.06 Body Mass Index of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

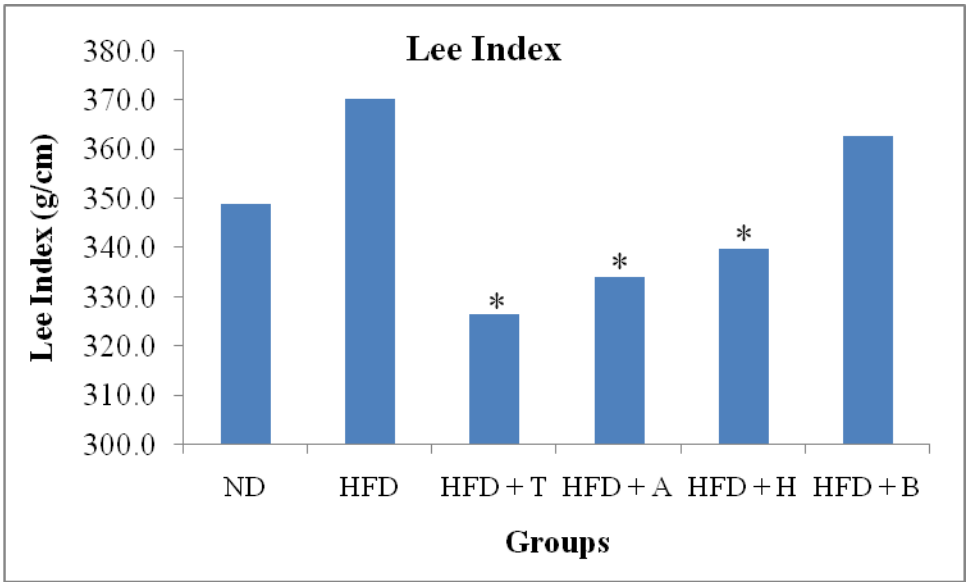


Fig: 5.07 Lee Index of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

5.2.1.5 Adipose Tissue (Fat pad) weight

Adipose tissue (fat pad) weight was taken at the end of the experiment after euthanisation of animals from all the groups. The fat pads were separated, washed in saline and weighed for all the mice (7 mice in each group). Figure 5.08 shows the mean fat pad weight for all the groups.

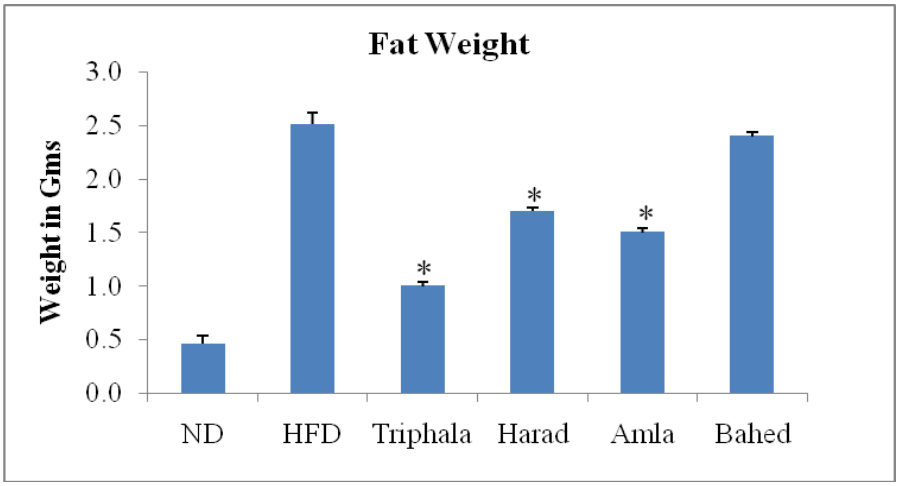


Fig: 5.08 Adipose Tissue Weight of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

5.2.1.6 Liver Weight

Liver weight was taken at the end of the experimental period after euthanisation of the animals from all the groups. The liver tissue was separated, washed in saline and weighed for all the mice (7 mice in each group). Figure 5.09 shows the mean liver weight for all the groups.

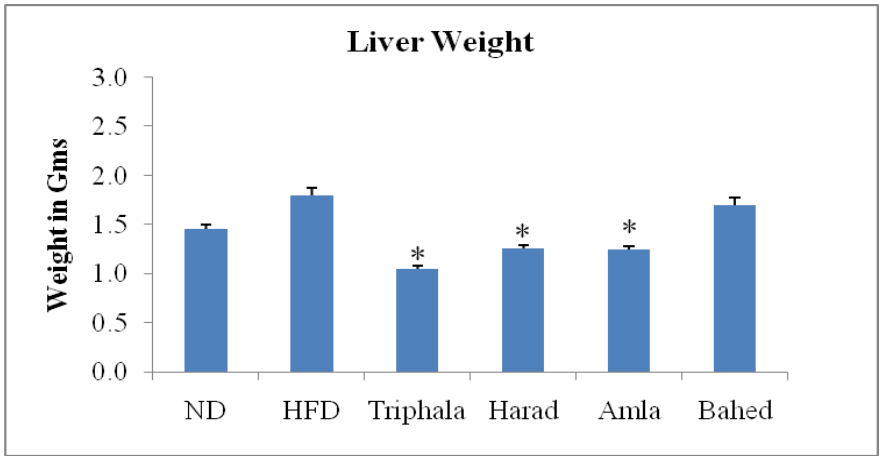


Fig: 5.09 Liver Weight of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

Table 5.06: Comparative table showing the Anthropometric Parameters in Mice Fed on the Experimental Diets for 10 Weeks

Group	ND	HFD	HFD+T	HFD+A	HFD+H	HFD + B
Initial body weight g	20.1 ± 1.1	20.0± 1.6	19.4± 1.1	21.4± 1.0	21.6± 0.8	19.4 ±1.0
Final body weight g	31.0 ±0.7	37.0± 1.0	28.0 ±0.4*	30.0± 1.0	28.6± 0.8	33.0± 1.1
Body weight gain g/10 wk	10.9 ±1.1	17.3 ±0.7	8.6 ±0.8**	8.6 ±1.0**	7.0 ±1.5**	13.6 ±0.5*
Food intake g/d	5.3 ± 0.1	5.3± 0.2	3.6 ± 0.1*	4.0± 0.1*	3.8± 0.1*	4.2± 0.1
Energy intake kcal/d	19.2 ± 0.1	26.9± 0.2	18.6 ±0.1*	21.8± 0.1*	19.7 ± 0.1*	20.7± 0.1
FER	2.1	3.3	2.4*	2.1*	1.9*	3.4
Lee Index g/cm	349.0	370.2	326.5**	334.1**	339.8**	367.8
Liver weight	1.5±0.04	1.8±0.08	1.0±0.08**	1.2±0.04**	1.3±0.04**	1.7±0.04
Weight of Fat pad	0.5±0.08	2.5±0.1	1.0±0.04**	1.5±0.04**	1.7±0.04**	2.4±0.04

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**$p < 0.0001$, * $p < 0.05$).

The body weight (BW) at 10-wk after treatment and the final body weight at the end of the experiment in the HFD group were 20% and 59% greater respectively, than the equivalent values observed in the ND group (Table 5.6). Treatment with the herbal powders significantly reduced the final body weight by 24.3% (Triphala), 19% (Amla), 22.7% (Harad) and 11% (Bahed) when compared with ND group and was 50.2%, 50.2%, 60%, and 21.3% respectively lower than the HFD group (Table 5.6). Food intake, energy intake and food efficiency ratio (FER) of mice in the HFD+T, HFD + A and HFD + H groups were significantly lower than the values observed for the HFD group, $p < 0.05$, as summarized in Table 5.6. The relative weight of the total adipose tissue was significantly higher (400% greater) in HFD fed mice than the value obtained for the ND mice, $p < 0.005$ and were significantly lowered 60%, 40%, 32%, 16% by supplementing HFD with the herbal powders, Triphala, Amla, Harad and Bahed respectively, $p < 0.0001$. The weight of the fatty liver was significantly higher (20% greater) in HFD fed mice than the values obtained for the mice in the ND group, $p < 0.005$. The enlargement of liver and accumulation of fat in the liver of mice fed HFD were considerably ameliorated by herbal treatment in all groups, namely HFD+T (44.4%), HFD + A (33.3%) and HFD + H (27.7%) Reduction in the weight of the liver was statistically significant, $p < 0:001$.

5.2.2 Clinical Parameters

5.2.2.1 Glucose

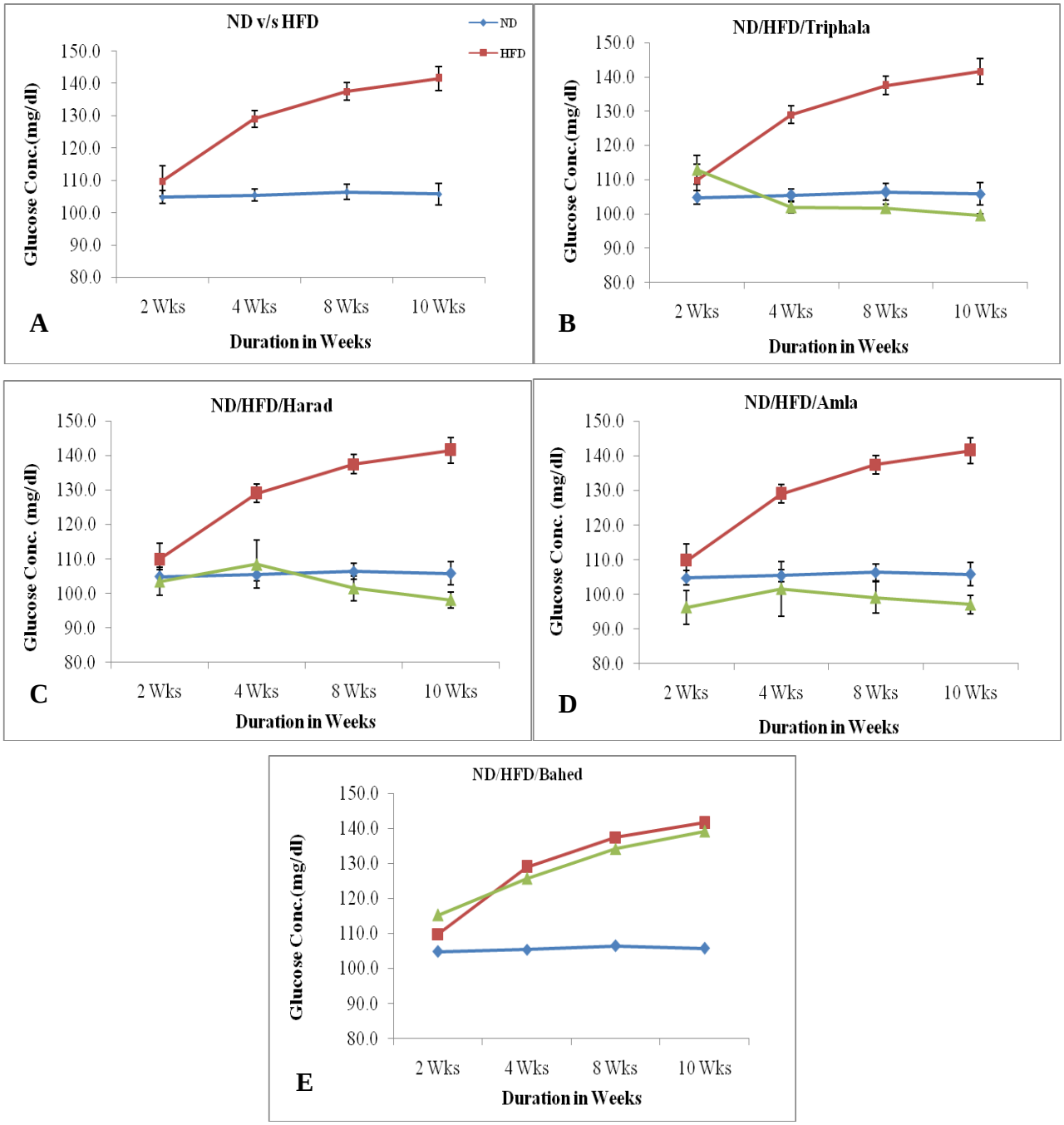


Fig: 5.10 Plasma Glucose Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean $\pm$ SEM; n=7. Means are statistically significantly different ($p < 0.05$) except for the Bahed treated group.

5.2.2.2 Cholesterol

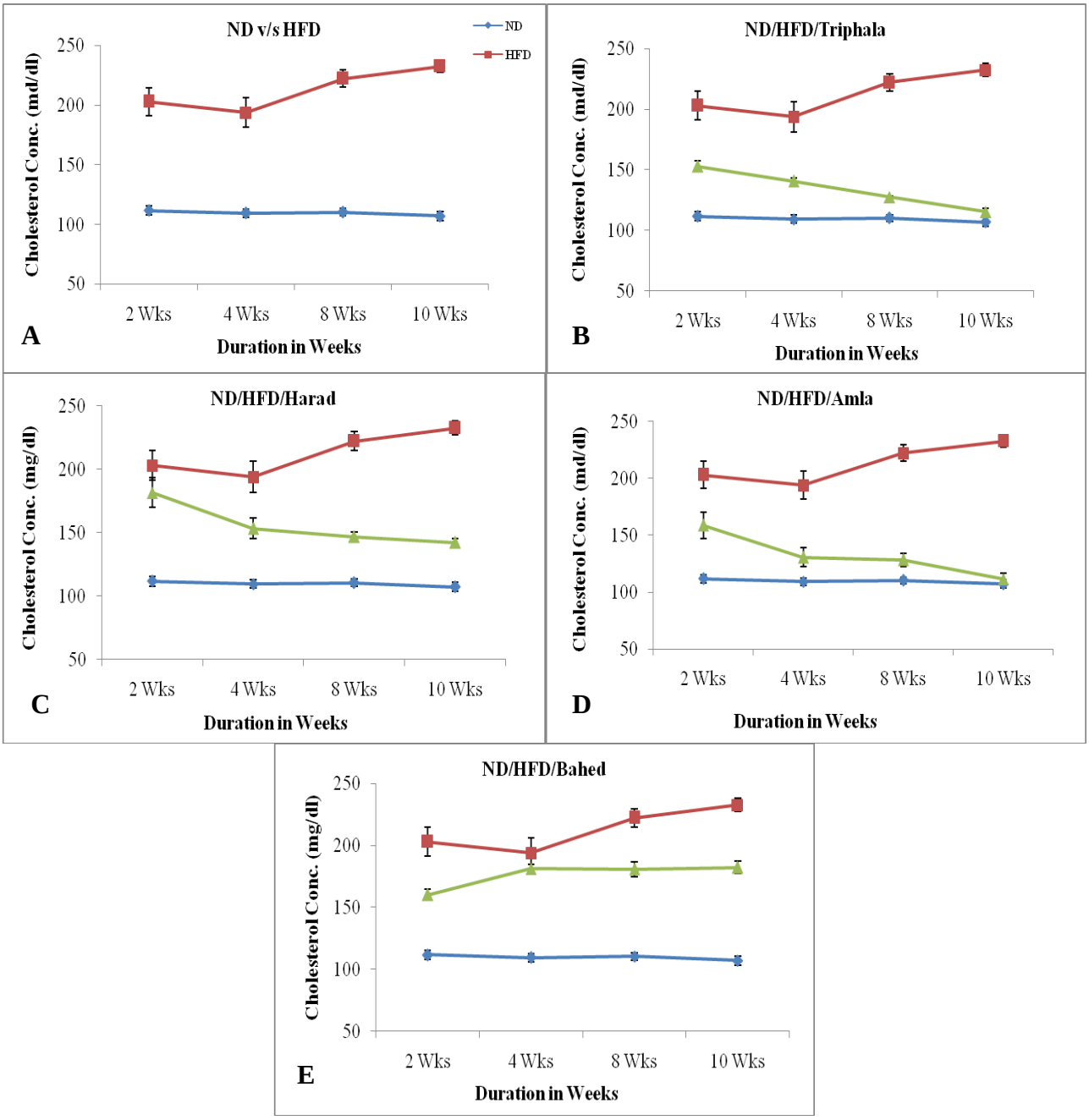


Fig: 5.11 Plasma Cholesterol Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean $\pm$ SEM; n=7. Means are statistically significantly different ($p < 0.05$).

5.2.2.3 Triglyceride

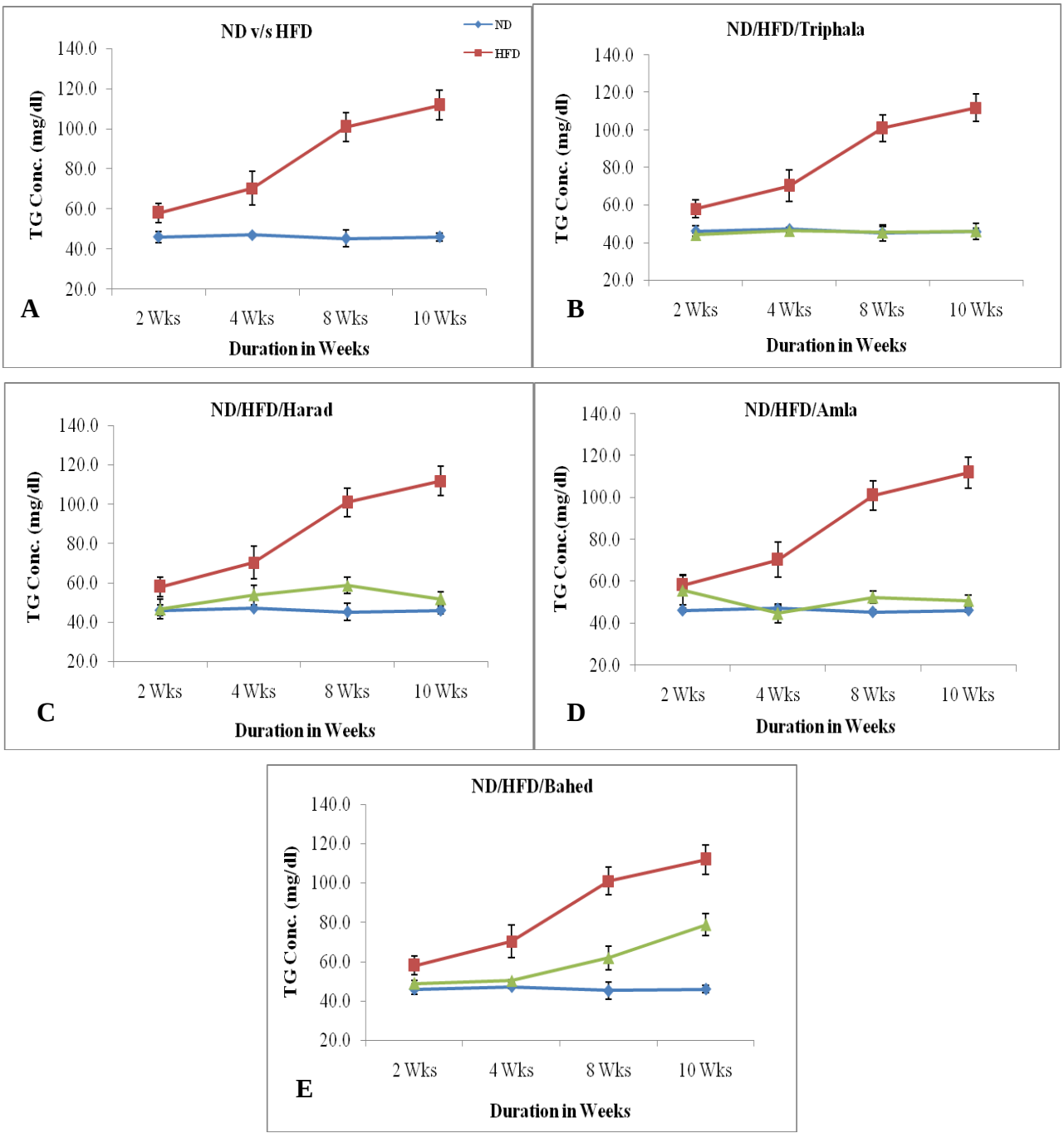


Fig: 5.12 Plasma Triglyceride Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean $\pm$ SEM; n=7. Means are statistically significantly different ($p < 0.05$).

5.2.2.4 High Density Lipoprotein (HDL)

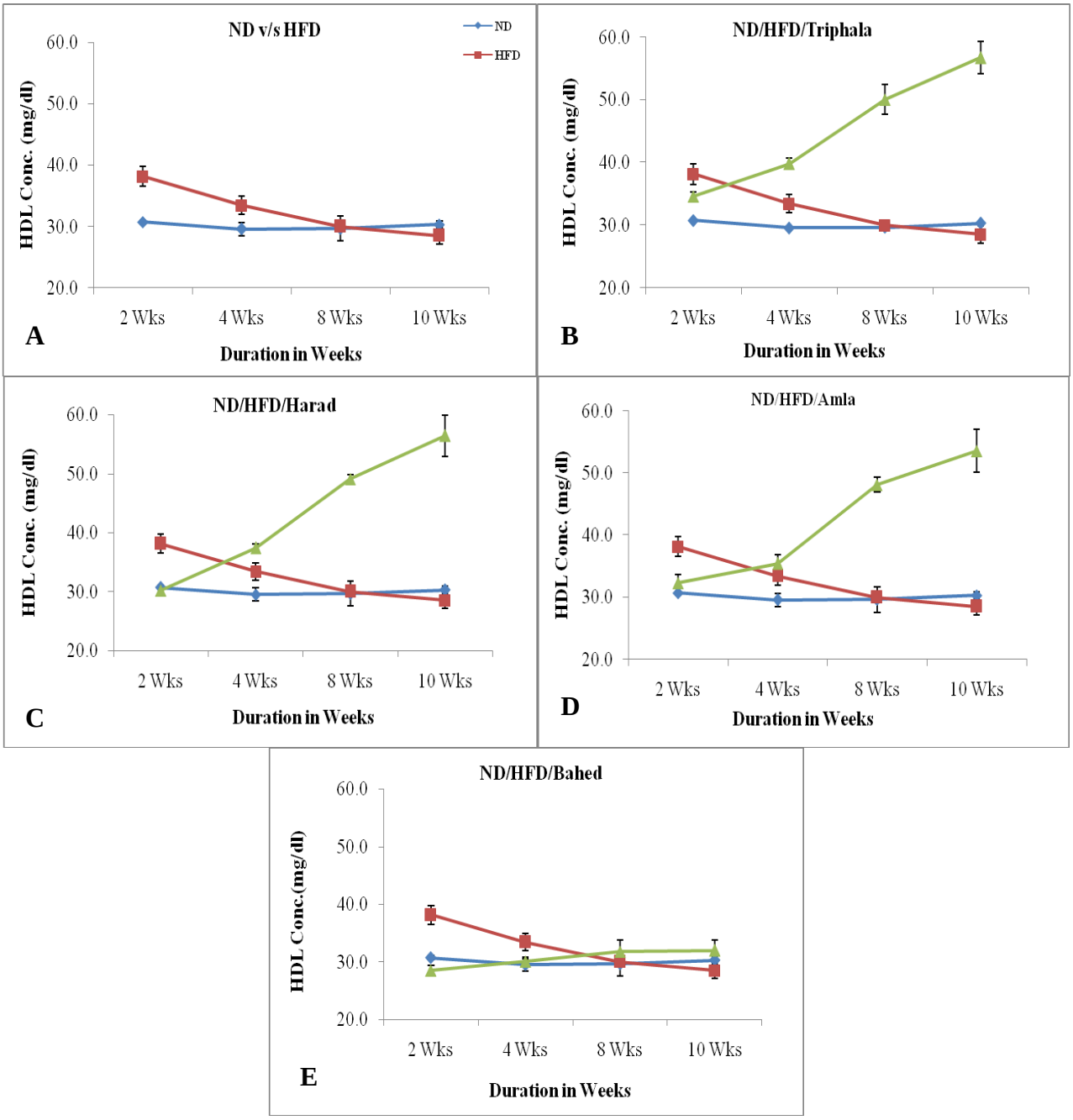


Fig: 5.13 Plasma HDL Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$) except for the Bahed treated group

5.2.2.5 Low Density Lipoprotein (LDL)

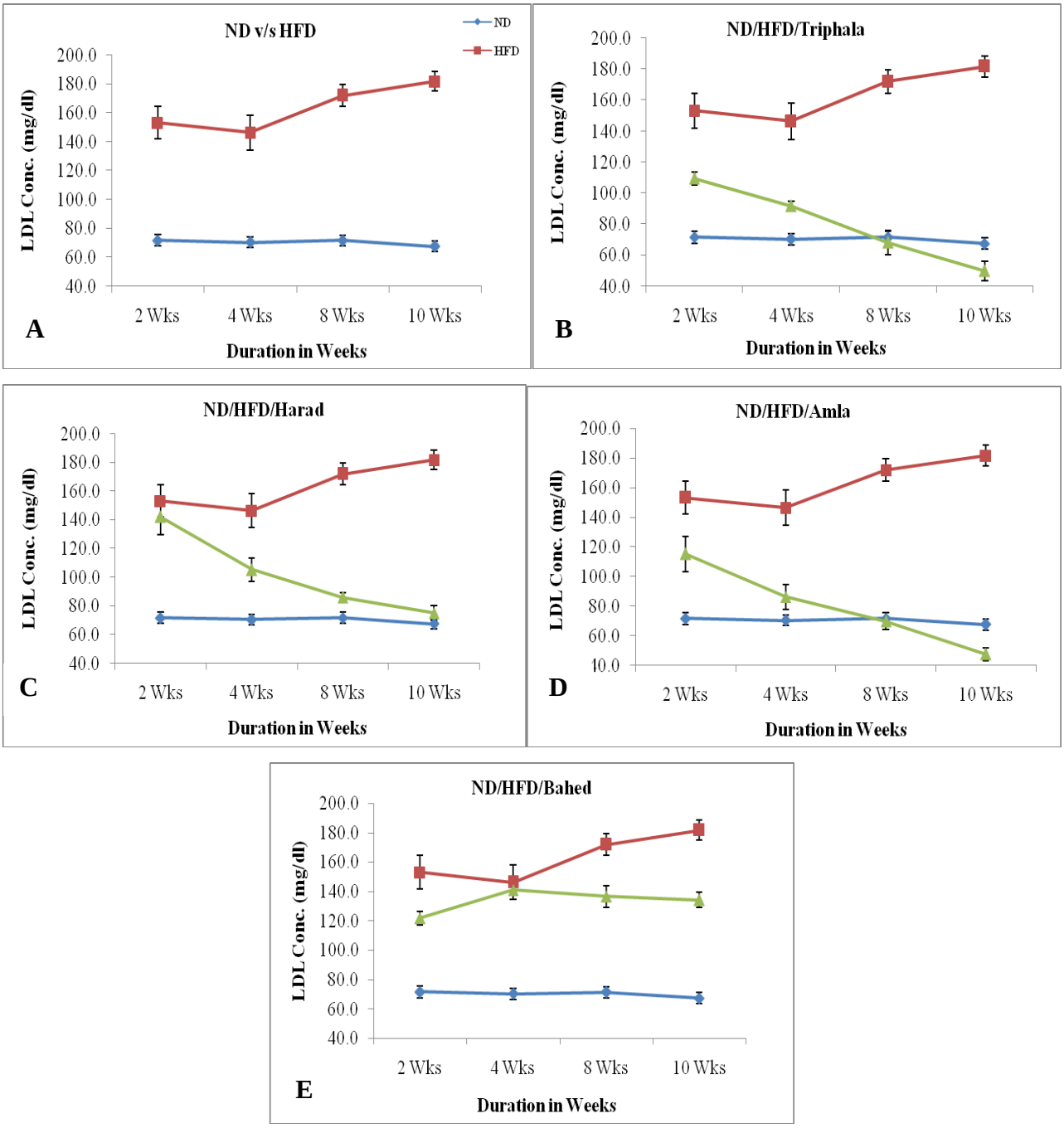


Fig: 5.14 Plasma LDL Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean $\pm$ SEM; n=7. Means are statistically significantly different ($p < 0.05$).

5.2.2.6 Very Low Density Lipoprotein (VLDL)

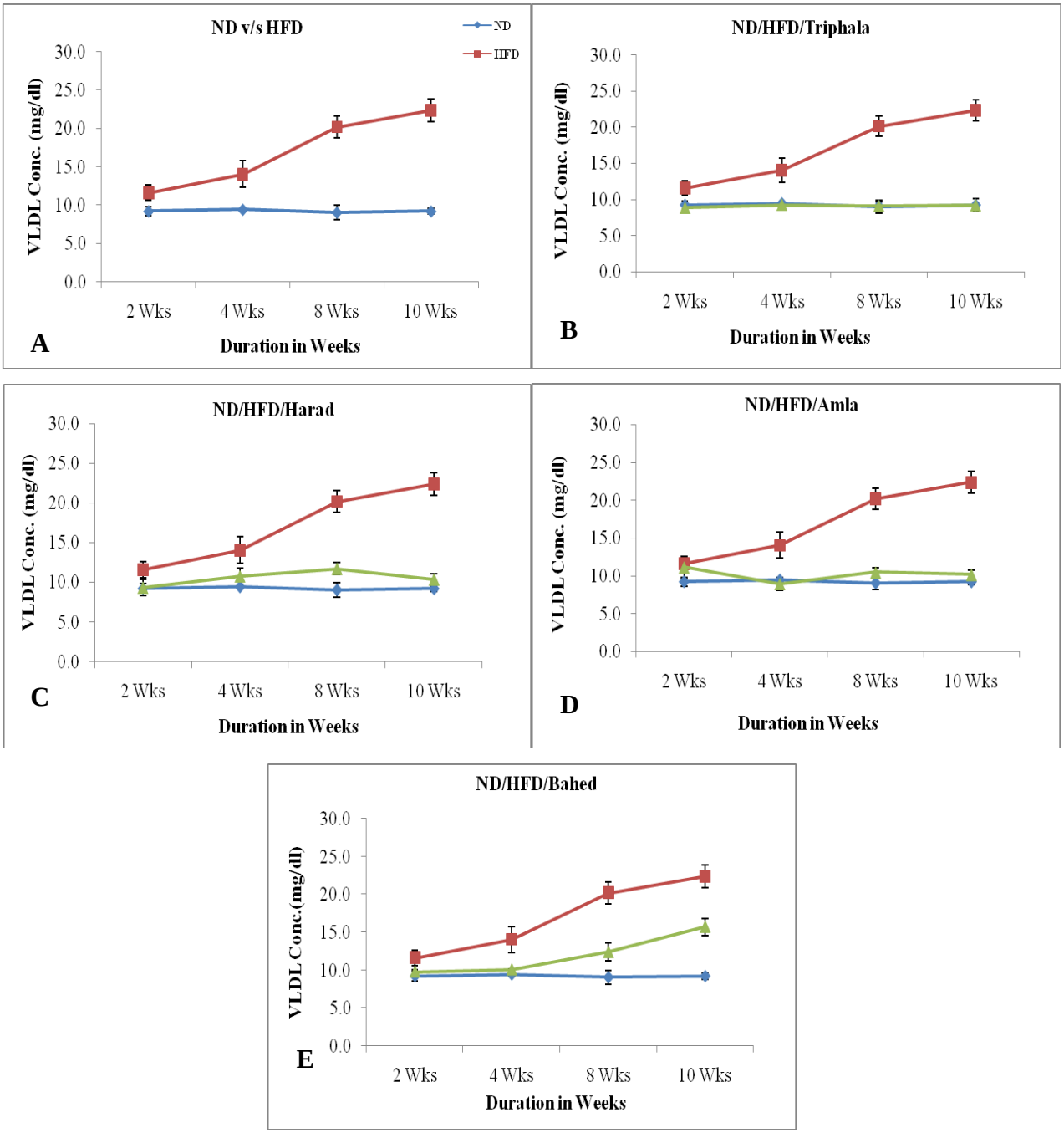


Fig: 5.15 Plasma VLDL Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

5.2.2.7 Alanine Transaminase (ALT)

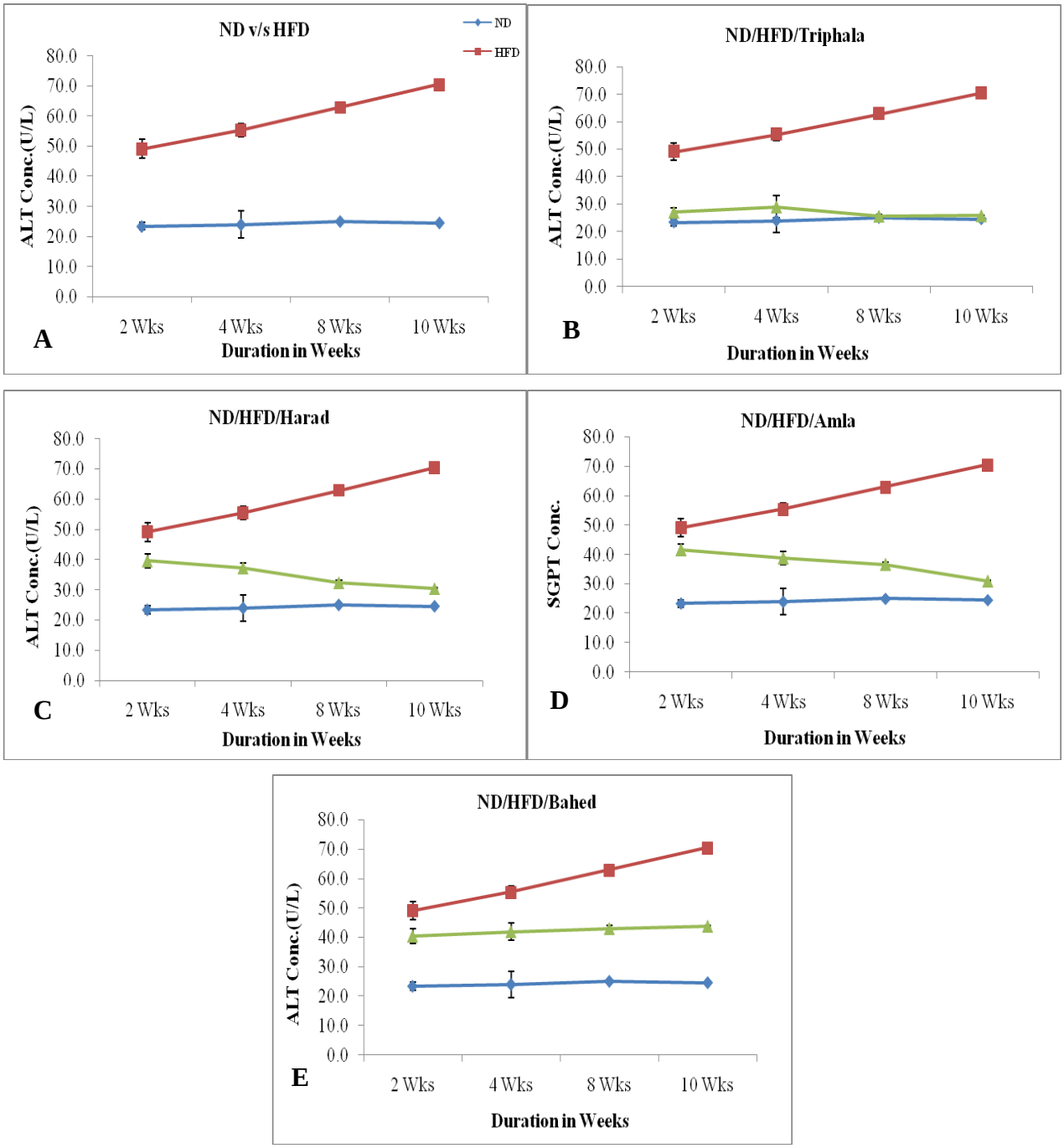


Fig: 5.16 Plasma ALT levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean $\pm$ SEM; n=7. Means are statistically significantly different ($p < 0.05$).

COMPARATIVE TABLE OF ALL CLINICAL PARAMETERS FOR ALL GROUPS

Table 5.07: Plasma Biochemistry of Mice Fed on the Experimental Diets

Group	ND	HFD	HFD+T	HFD+A	HFD+H	HFD+B
Plasma						
Glucose	105.8±3.3	141.6±3.7	99.6±0.3**	97.1±2.4**	98.1±2.7**	139.2±1.3
T.Cholesterol	107.0 ± 3.5	232.6 ± 5.4	115.5 ± 3.1**	111.3 ± 5.2**	142.2 ± 3.2**	182.1 ±5.1*
Triglyceride	46.0±1.9	111.9±7.4	46.1±4.4**	50.7±2.8**	51.9±3.7**	78.7±5.6
HDL	30.3±0.6	28.5±1.4	56.4±2.6**	53.6±3.4**	56.7±3.5**	32.0±1.8
LDL	67.5±3.7	181.7±6.8	49.9±6.3**	47.6±4.4**	75.1±4.9**	134.4±5.4**
VLDL	9.2±0.4	22.4±1.5	9.2±0.9**	10.1±0.6**	10.4±0.7**	15.7±1.1*
Liver						
Serum ALT	24.5±0.2	70.5±0.4	25.8±0.2**	31.0±0.3**	30.4±0.3**	43.5±0.4**

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**$p < 0.0001$, *$p < 0.05$).

Plasma glucose concentrations were significantly lowered by treatment with Amla, Harad and Triphala 31.4%, 30.7% and 29.6% as compared to those obtained for the HFD-fed mice, $p < 0.0001$. Treatment with Bahed however did not show any significant reduction in plasma glucose levels as compared to the HFD-fed mice, (Fig: 5.10, Table 5.07).

The HFD-induced hypercholesterolemia was significantly improved by herbal treatment with Triphala, Amla, Harad and Bahed respectively. Plasma total cholesterol concentrations were 50.3%, 52.1%, 38.8% and 21.7% lower, respectively, in mice fed with HFD + T, HFD +A, HFD + H and HFD + B than those observed for the HFD-fed mice, p<0:0001, (Fig: 5.11, Table: 5.07).

The plasma triglyceride (TG) concentrations were again 58.8%, 54.6%, 53.6% and 29.6% lower, respectively, in mice fed with HFD + T, HFD + A, HFD + H and HFD + B than those observed for the HFD-fed mice, p<0.0001, (Fig: 5.12, Table: 5.07).

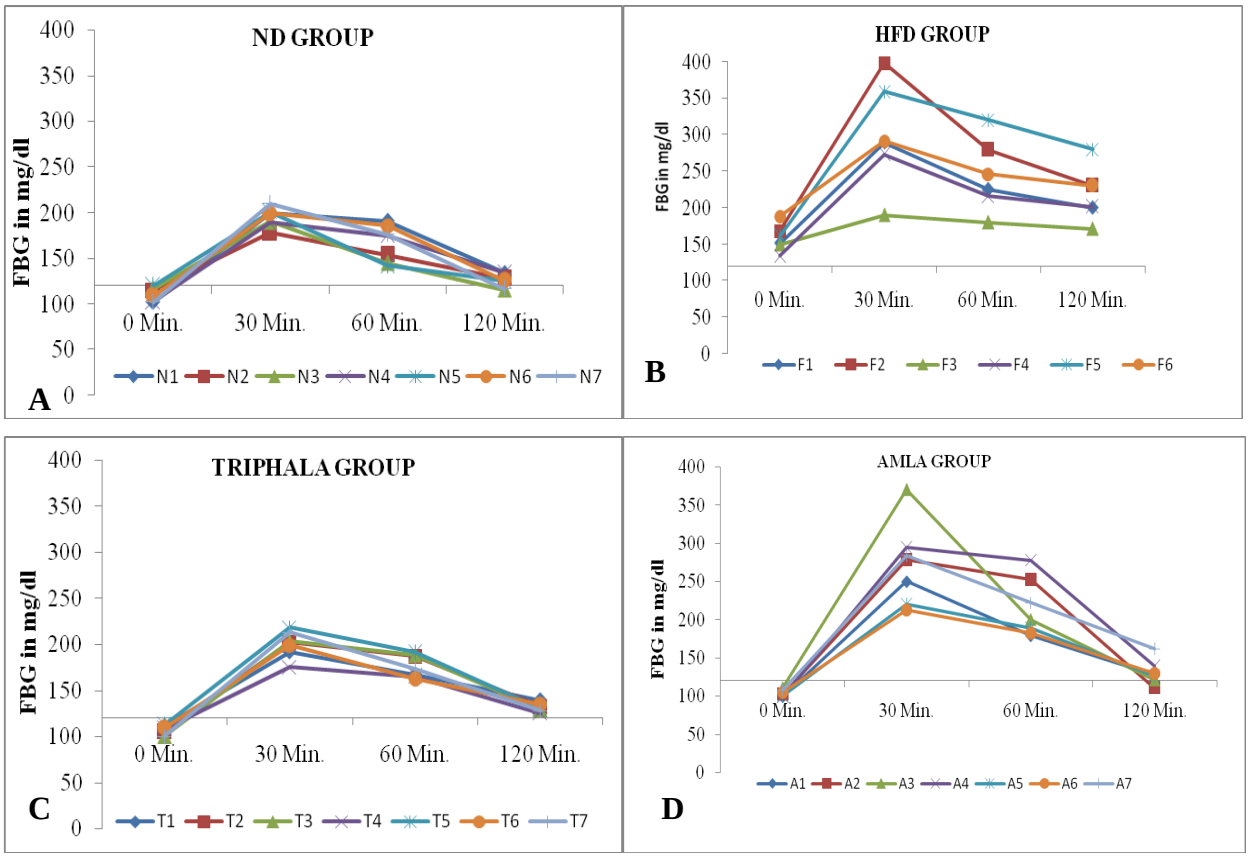
All herbal treatments increased the levels of HDL-cholesterol, which were 49.4% for HFD + T, 49.7% for HFD + H, 46.8% for HFD + A and 11% for HFD + B higher respectively, than the values observed for the HFD-fed mice, p<0.0001, (Fig: 5.13, Table: 5.07).

The same pattern was observed with LDL and VLDL levels. The LDL concentration was lowered by 72.5%, 73.8%, 58.6% and 26% in mice fed with HFD + T, HFD + A, HFD + H and HFD + B (Fig: 5.14, Table: 5.07). The treatment reduced the VLDL levels by 59%, 55%, 53.5% and 30% as compared with the HFD group (Fig: 5.15, Table: 5.07).

The plasma ALT levels were also lowered by the herbal treatments 63.4% in HFD + T group, 56% in HFD + A group, 56.8% in HFD + H group and 38.3% in HFD + B group respectively, as compared to values observed in the HFD-fed mice (Fig: 5.16, Table: 5.07).

5.2.2.8 Oral Glucose Tolerance Test (OGTT)

OGTT was done at the end of the experiment before euthanizing the animals. OGTT values (area under the curve, AUGTC) for ND and HFD group were found to be 1185.7 and 1895.7. Herbal treatment for 10 weeks in HFD + T, HFD + A, HFD + H and HFD + B resulted in significant improvement in oral glucose tolerance with the AUGTC 0–120 min being 1214, 1510.4, 1511.9, and 1582.6, respectively, versus 1895.7, in the HFD fed group of animals, Fig. 5.17.



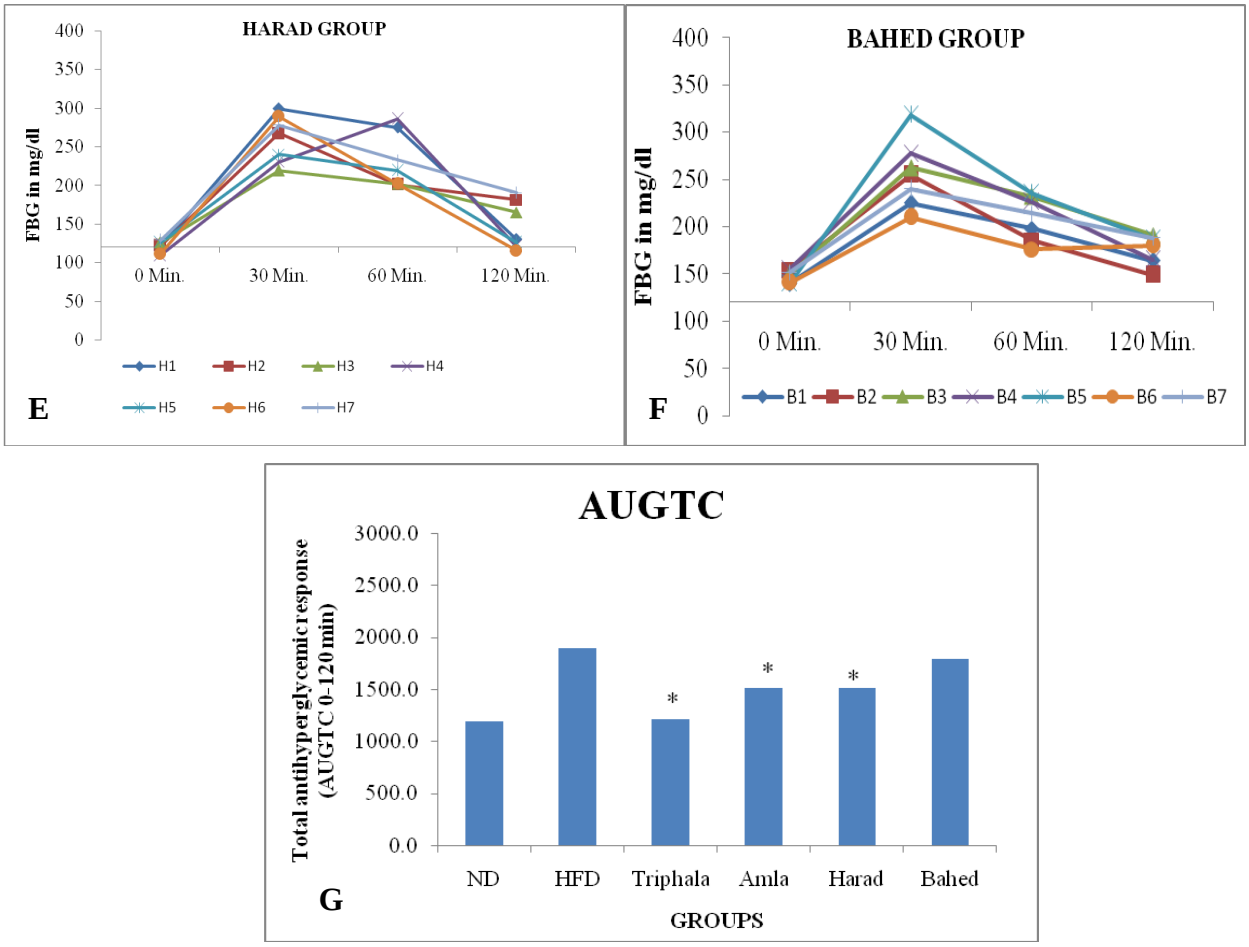


Fig 5.17: Effect of herbal treatment on oral glucose tolerance test. (A) ND Group, (B) HFD Group, (C) Triphala Group, (D) Amla Group, (E) Harad Group, (F) Bahed Group. The graph indicates the total antihyperglycemic response AUGTC 0-120 min in the herbal powder treated groups. Values are mean $\pm$ SEM; n=7 in each group. All means are statistically significantly different ($p < 0.05$).

5.2.3 Antioxidant and Oxidative Stress Parameters:

Endogenous protective mechanism against oxidative stress was investigated next. High Fat Diet altered the levels of antioxidant enzymes (Catalase and TSOD) and increased the oxidative stress (as indicated by TBARS).

5.2.3.1 Catalase

A marked increase in catalase activity was observed in Triphala and Amla group, whereas Bahed group did not show a significant change as compared with HFD group. (Fig: 5.18). Catalase concentration is measured as mMoles/mg protein/sec.

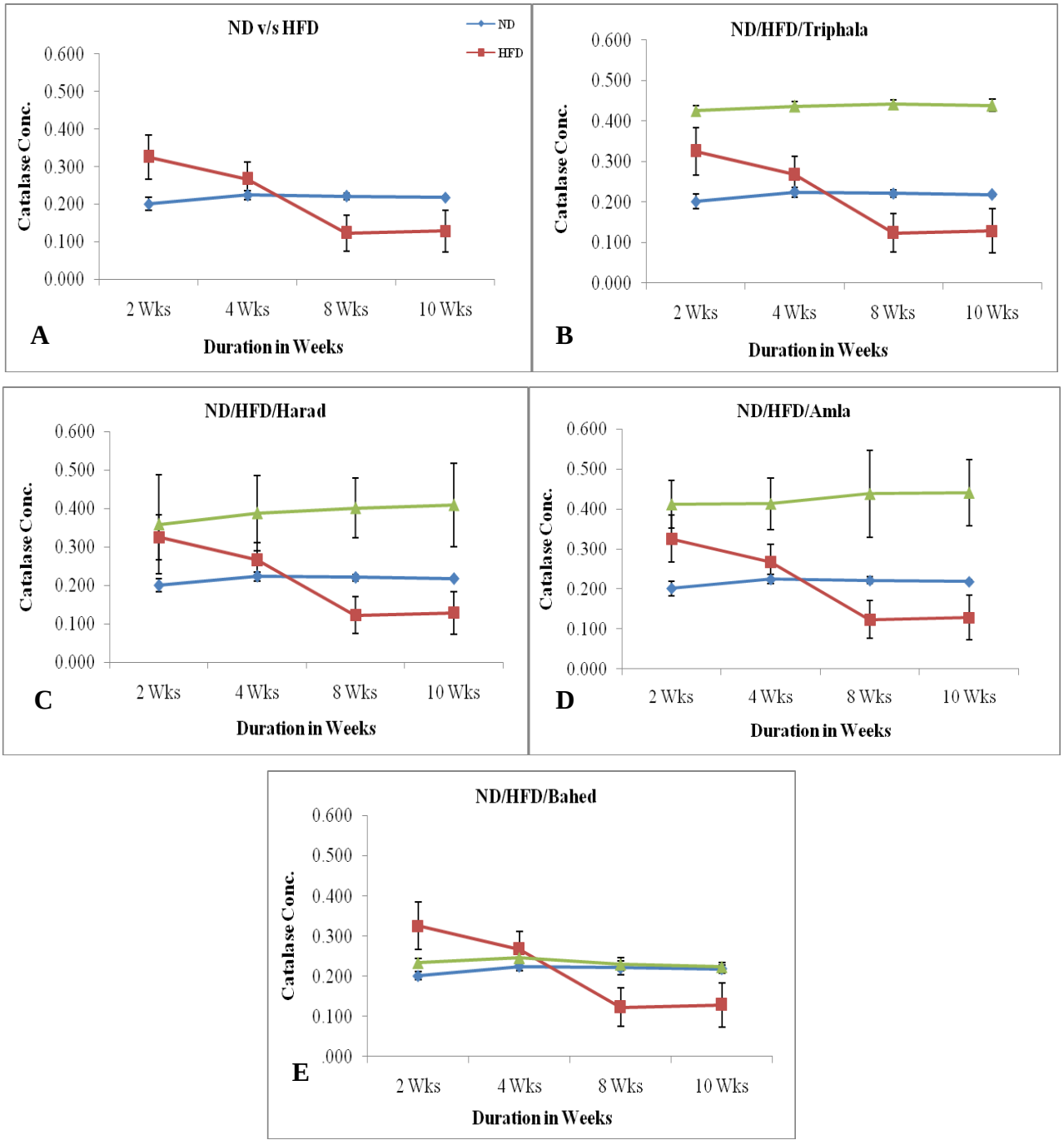


Fig 5.18: Activity of the antioxidant enzyme Catalase in mice fed on experimental diets for 10 weeks. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05).

5.2.3.2 Total Superoxide Dismutase (TSOD)

A marked increase in TSOD activity was observed in Triphala and Amla group, whereas Bahed group did not show a significant change as compared with HFD group (Fig 5.19). The TSOD conc. is measured as mU/min/mg protein.

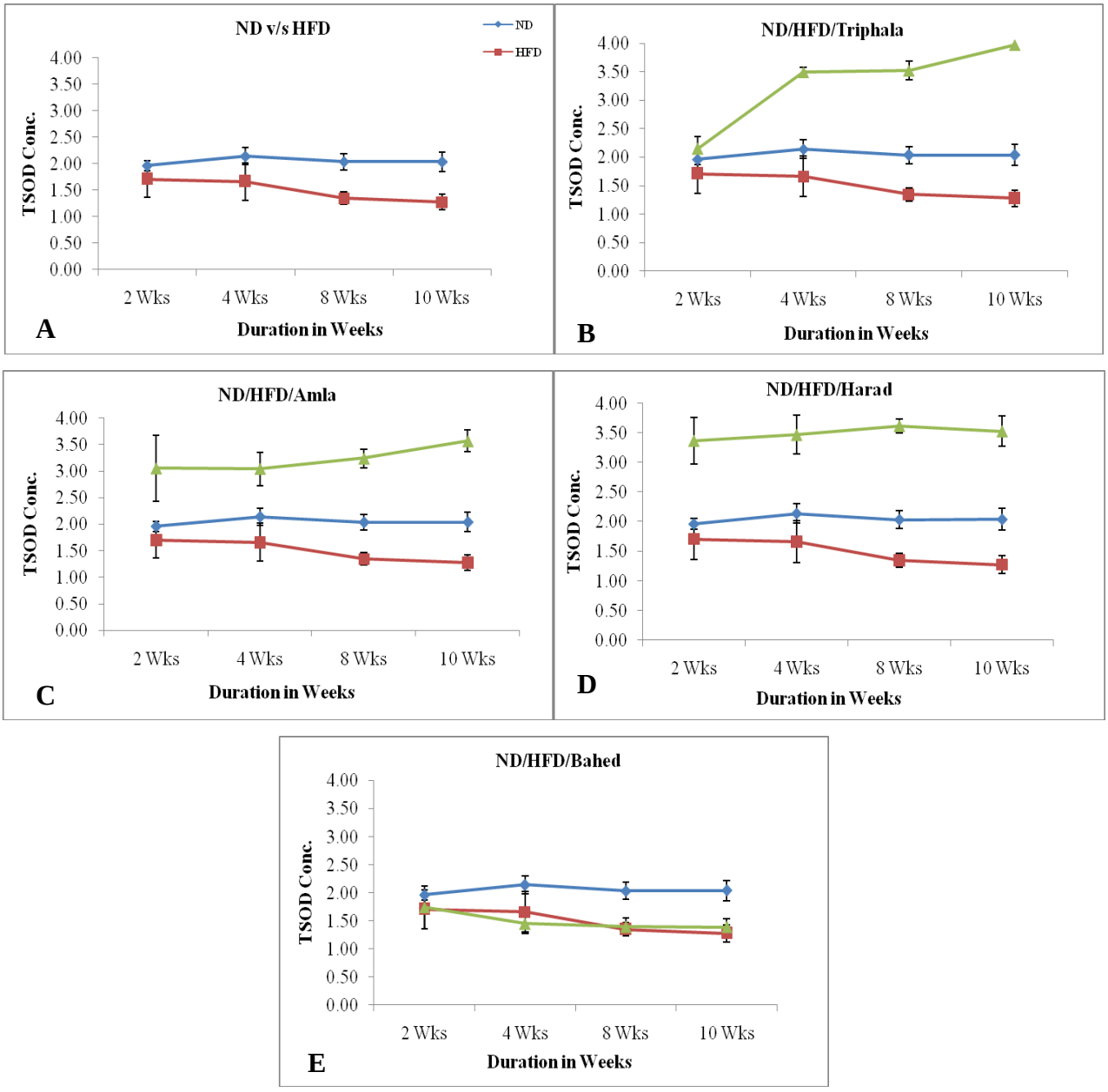


Fig 5.19: Activity of the antioxidant enzyme TSOD in mice fed on experimental diets for 10 weeks. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

5.2.3.3 Thiobarbituric Acid Reactive Species (TBARS)

A marked decrease in TBARS activity was observed in Triphala, Amla and Harad group as compared with HFD group. Bahed group was less effective in reducing the lipid peroxidation as compared to Triphala, Amla and Harad (Fig 5.20). TBARS concentration is measured as nmole/mg protein.

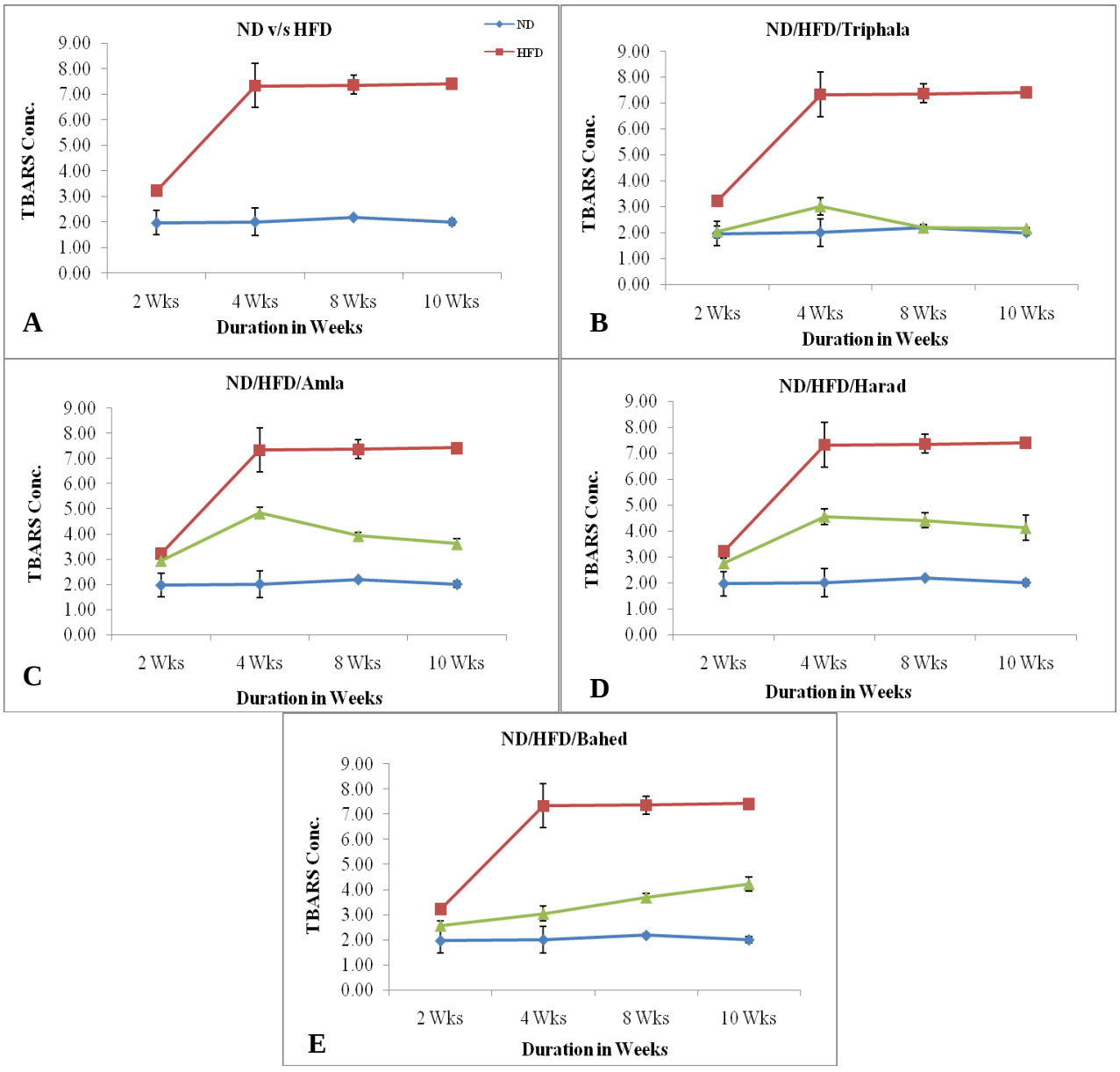


Fig 5.20: Oxidative stress activity (TBARS) in mice fed on experimental diets for 10 weeks. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

5.2.3.4 Catalase v/s HDL

The treatment improved the anti-oxidant level as well increased good cholesterol in terms of plasma HDL levels. Fig. 5.21 depicts both the parameters comparing the improvement. Triphala group showed the best prevention as compared to amla and harad groups.

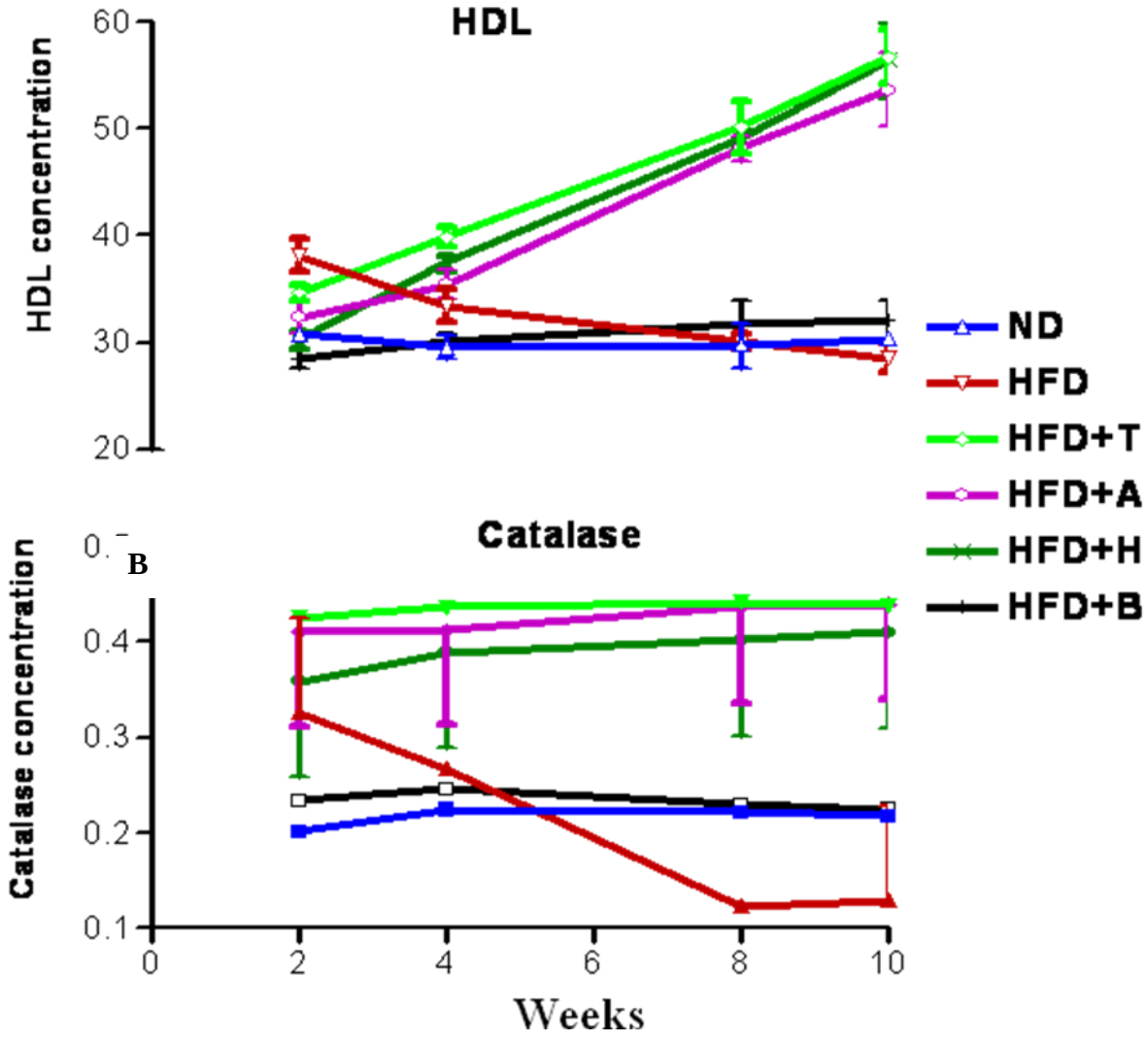


Fig 5.21: Comparative graph showing levels of anti-oxidant Catalase and HDL (good cholesterol) Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

5.2.3.5 TBARS v/s Triglycerides (TG)

The treatment proved to be effective in preventing the lipid peroxidation. Fig. 5.22 depicts TBARS levels comparing with TG concentration. Here too, treatment with triphala was most effective in reducing the lipid peroxidation as compared to amla and harad groups.

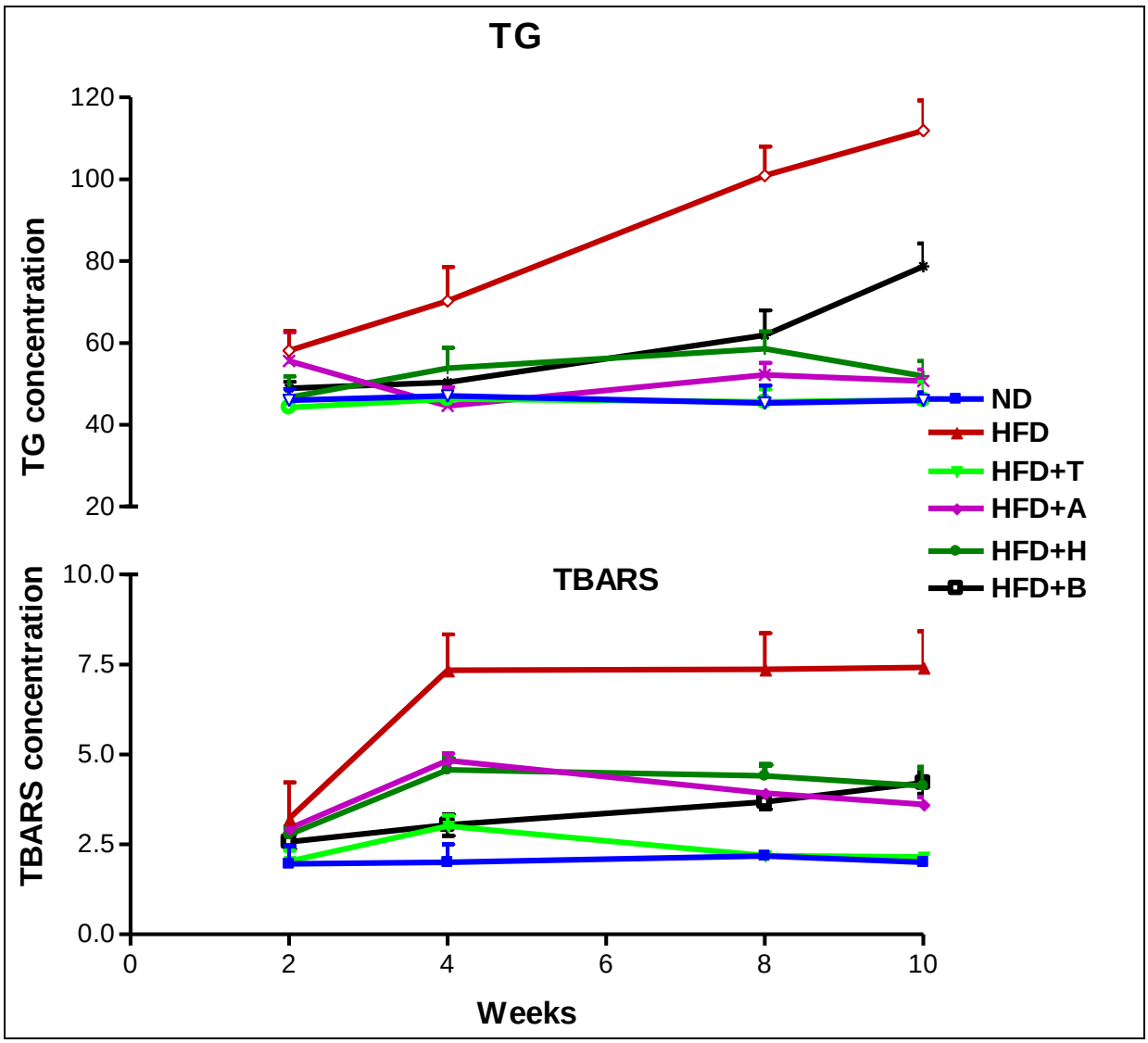


Fig 5.22: Comparative graph showing effect of Lipid peroxidation as levels of TBARS and TG.

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

5.2.3.6 TBARS v/s Low Density Lipoprotein (LDL)

The treatment proved to be effective in preventing the lipid peroxidation. Fig. 5.23 depicts TBARS levels comparing with LDL concentration. Here too, treatment with triphala was most effective in reducing the lipid peroxidation as compared to amla and harad groups.

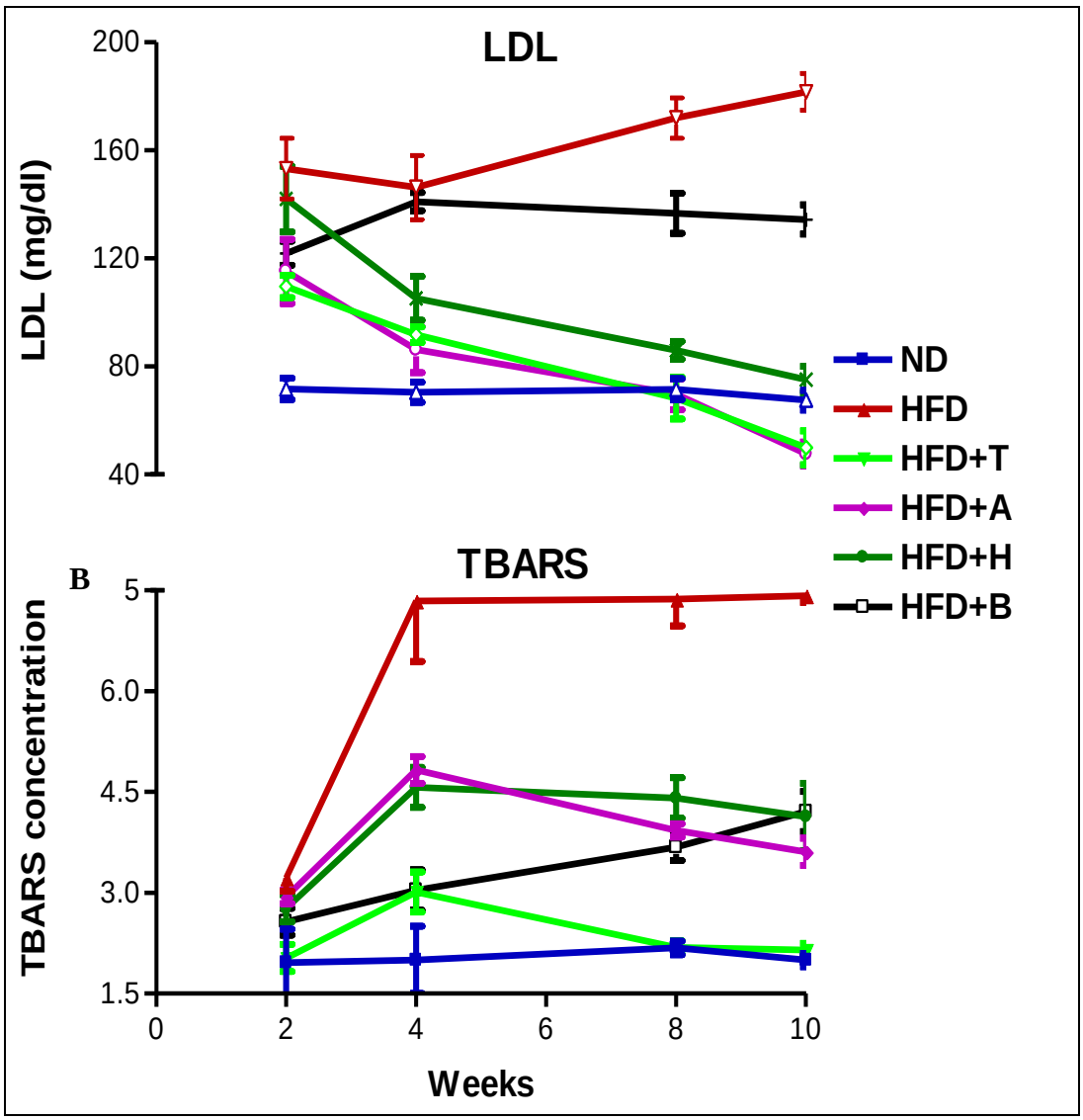


Fig 5.23: Comparative graph showing effect of Lipid peroxidation as levels of TBARS and LDL. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

COMPARATIVE TABLE OF ALL THE OXIDATIVE STRESS PARAMETERS FOR ALL THE GROUPS:

Table 5.08: Activities of the Antioxidant Enzymes and Oxidative Stress in Mice fed on Experimental Diets for 10 Weeks.

Group	ND	HFD	HFD + T	HFD + A	HFD + H	HFD + B
Catalase	0.218±0.00	0.129±0.06	0.439±0.02**	0.440±0.08**	0.410±0.11*	0.224± .01
TSOD	2.04 ± 0.2	1.28 ± 0.1	3.97 ± 0.1**	3.57 ± 0.2**	3.53 ± 0.3*	1.39 ± 0.1
TBARS	2.00 ± 0.1	7.42 ± 0.1	2.15 ± 0.1**	3.61 ± 0.2**	4.13 ± 0.5**	4.21±0.3**

Values are mean $\pm$ SEM; n=7 in each group. All means are statistically significantly different (**$p < 0.0001$, * $p < 0.05$)

Plasma catalase levels were significantly increased by treatment with Triphala, Amla, Harad and Bahed 70.6%, 70.6% , 68.5% and 42.4% as compared to those obtained for the HFD-fed mice, $p < 0.0001$, (Fig: 5.18, Table 5.08).

The plasma TSOD concentrations were again 67.7%, 64.1%, 63.7% and 8.0% higher, respectively, in mice fed with HFD + T, HFD + A, HFD + H and HFD + B than those observed for the HFD-fed mice, $p<0.0001$, (Fig: 5.19, Table: 5.08).

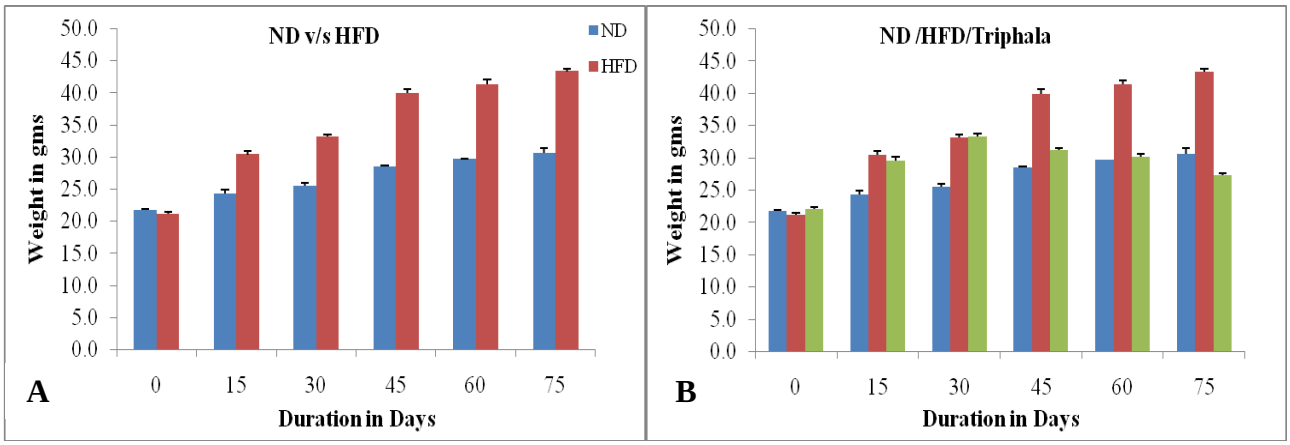
The HFD-induced lipid peroxidation was significantly improved by herbal treatment with Triphala, Amla, Harad and Bahed respectively. Plasma TBARS concentrations were 71%, 51.3%, 44.3% and 43.2% lower, respectively, in mice fed with HFD + T, HFD +A, HFD + H and HFD + B than those observed for the HFD-fed mice, p<0:0001, (Fig: 5.20, Table: 5.08).

5.3 Animal Experiment III: Triphala and Its Constituents Cure Visceral Adiposity in High Fat Diet-Induced Obesity in Mice

This experiment was designed to test the curative aspect of Triphala, Amla, Harad and Bahed powders on HFD induced obesity in mice. In this experiment, the mice were first made obese by feeding HFD for 4 weeks and then the treatment with Triphala, Amla, Harad and Bahed powders was started. To evaluate the effect of these herbal powders on visceral adiposity, anthropometric markers (BW, BMI, and Lee Index) were studied. Clinical parameters studied include estimation of glucose, OGTT, lipid profile, liver function test and oxidative stress markers. The results showed that the treatment is effective in reversing the obesogenic effects of the HFD.

5.3.1 Anthropometric Parameters

5.3.1.1 Body weight chart: Body weight for all mice was recorded every 3rd day. Fig: 5.24 shows the body weight of ND group compared with HFD group alone (Fig: 5.24A) and ND, HFD group compared with Triphala, Amla, Harad and Bahed group individually (Fig: 5.24 B, C, D, E).



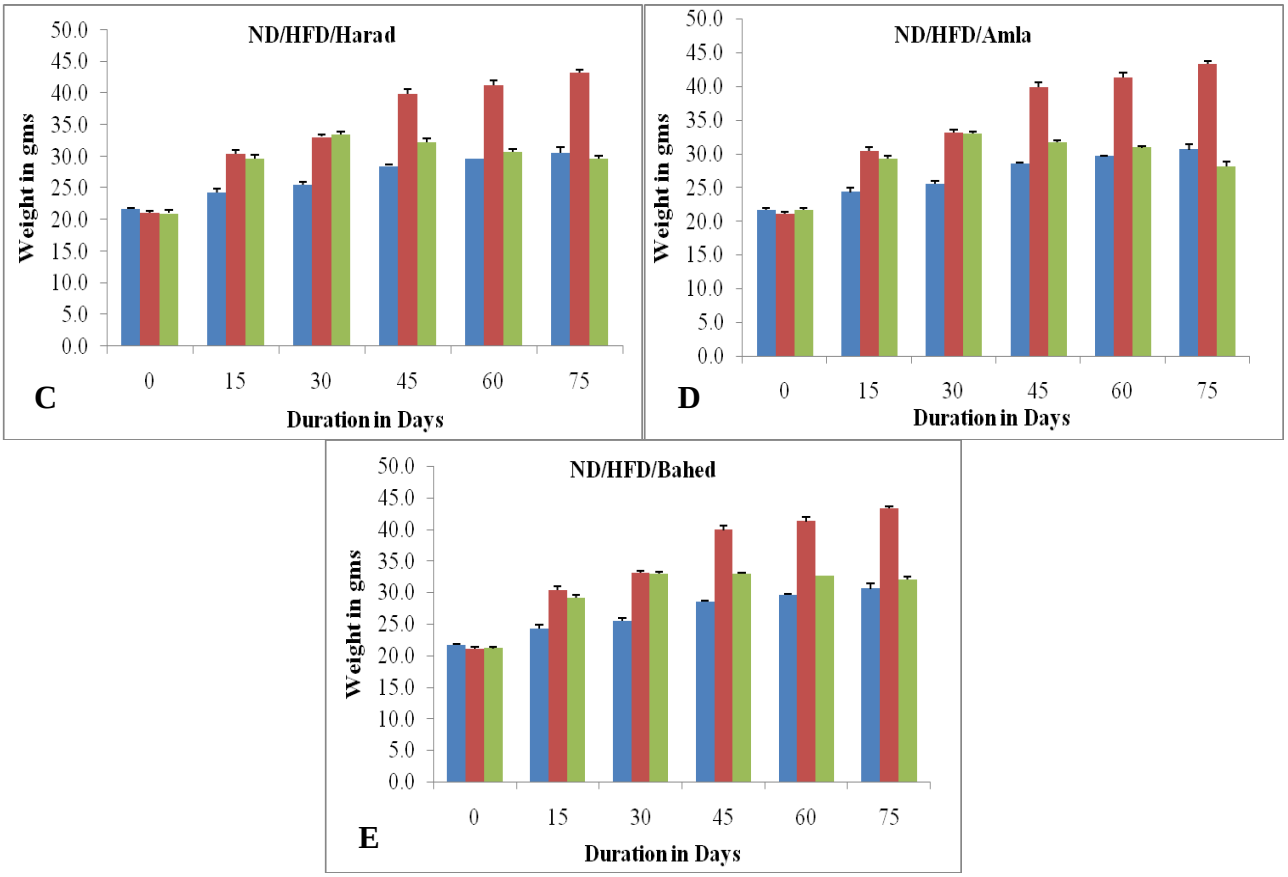


Fig: 5.24 Body Weight of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

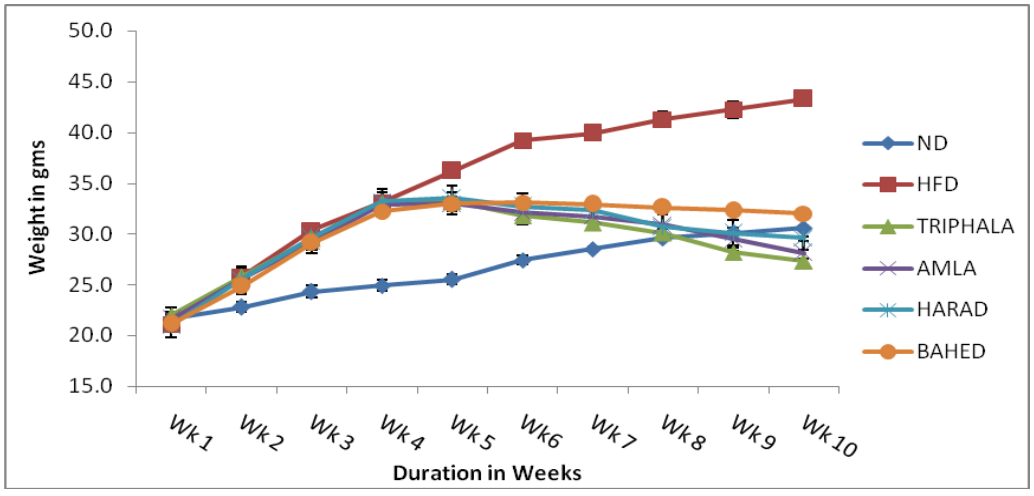


Fig: 5.25 Body Weight of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

As can be seen from Fig: 5.24 and 5.25, the body weight of animals in ND, HFD, HFD+T, HFD+A, HFD+H and HFD+B groups began to diverge from day 30 and stayed different for the remaining experimental period, ($p < 0.05$). Here the treatment was started after 4 weeks of HFD ie. after 30 days. Fig. 5.24 and 5.25 shows that the weight of mice in all the groups except the ND group showed a continous and equal increase from 0-30 days. The difference in weight began to diverge after 30 days and remain different for the remaining experimental period.

Table 5.09: Body Weight Gain in Mice Fed on the Experimental Diets for 10 Weeks

Groups	ND	HFD	HFD+T	HFD+A	HFD+H	HFD + B
0 Day	21.7±0.2	21.1±0.3	22.0±0.3	21.6±0.4	21.0±0.5	21.1±0.3
15 Day	24.3±0.6	30.4±0.6	29.5±0.6	29.2±0.4	29.6±0.6	29.5±0.6
30Day	25.5±0.5	33.1 ±0.4	33.2±0.5	33.0±0.3	33.5±0.4	33.2±0.3
45 Day	28.5±0.2	39.9±0.7	31.1±0.4	31.7±0.3	32.3±0.5	31.1±0.2
60 Day	29.6±0.1	41.3±0.7	30.1±0.4	30.9±0.3	30.7±0.4	30.1±0.1
75 day	30.6±0.8	43.3±0.4	27.3±0.3	28.1±0.7	29.6±0.5	27.3±0.5
Body weight gain g/10 wk	**8.9±0.4**	**22.2±0.6**	**5.3±0.4**	**6.5±0.4**	**8.6±0.5**	**10.9±0.3**

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (** $p < 0.0001$, * $p < 0.05$)

The Table 5.09 gives the body weight values at an interval of 0, 15th, 30th, 45th, 60th and 75th day and mean body weight for the entire experimental duration.

5.3.1.2 Feed Intake

Food intake for all groups was recorded every day. Figure 5.26 shows the food/feed intake of ND group compared with HFD group alone 5.26 A and ND, HFD group compared with Triphala, Amla, Harad and Bahed group individually (5.26 B, C, D, E).

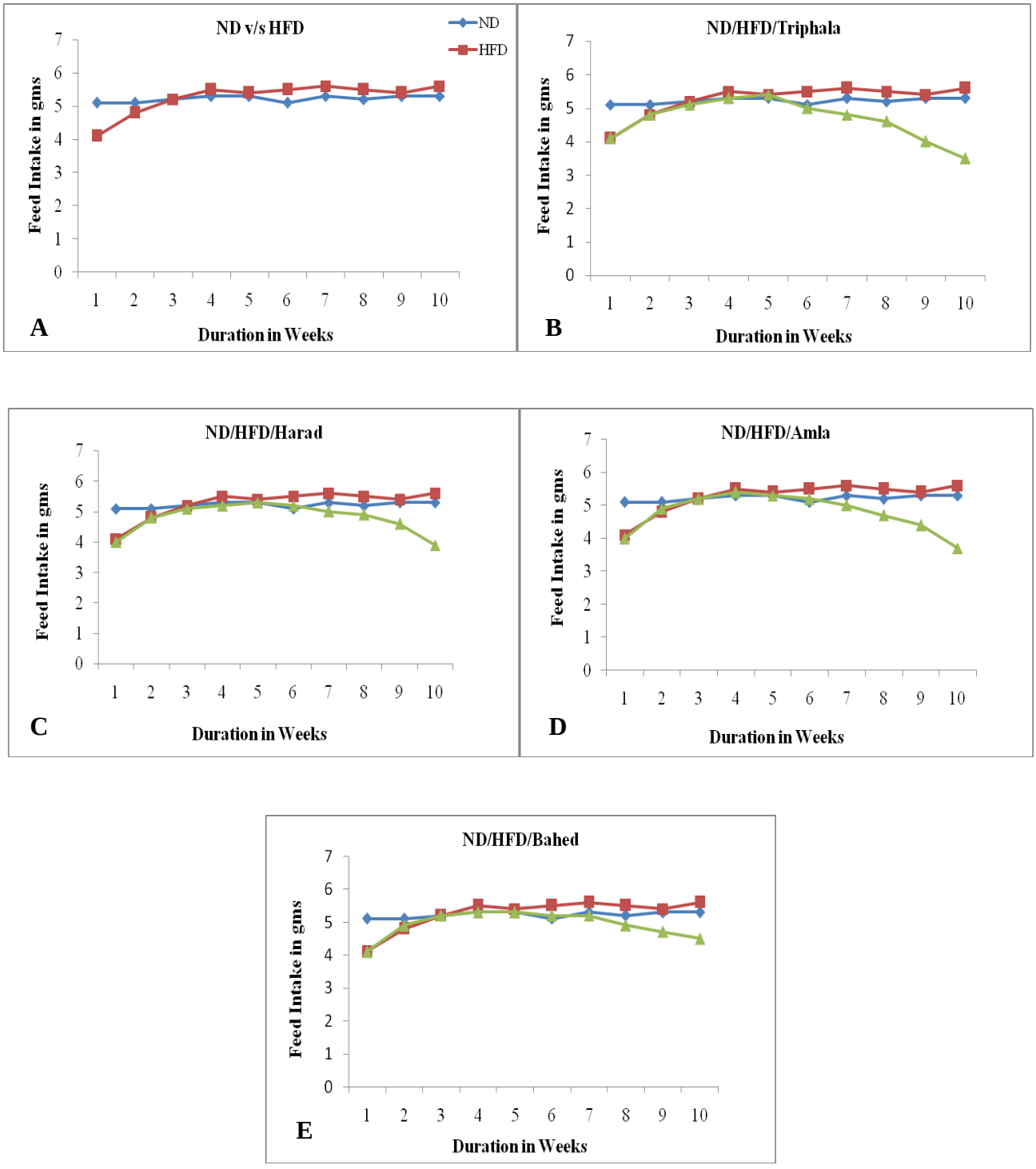


Fig: 5.26 Feed Intake of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

Table 5.10: Feed Intake in Mice Fed on the Experimental Diets for 10 Weeks

Groups / Weeks	ND	HFD	HFD+T	HFD+A	HFD+H	HFD + B
1 wk	5.1 ± 0.1	4.1 ± 0.1	4.1 ± 0.2	4.0 ± 0.1	4.0 ± 0.1	4.1 ± 0.1
2wk	5.1 ± 0.2	4.8 ± 0.2	4.8 ± 0.2	4.9 ± 0.1	4.8 ± 0.1	4.9 ± 0.1
3wk	5.2 ± 0.2	5.2 ± 0.1	5.1 ± 0.1	5.2 ± 0.1	5.1 ± 0.1	5.2 ± 0.0
4wk	5.3 ± 0.1	5.5 ± 0.1	5.3 ± 0.1	5.4 ± 0.1	5.2 ± 0.0	5.3 ± 0.1
5wk	5.3 ± 0.1	5.4 ± 0.0	5.4 ± 0.1	5.3 ± 0.0	5.3 ± 0.0	5.3 ± 0.1
6wk	5.1 ± 0.2	5.5 ± 0.1	5.0 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	5.2 ± 0.1
7wk	5.3 ± 0.1	5.6 ± 0.1	4.8 ± 0.1	5.0 ± 0.1	5.0 ± 0.1	5.2 ± 0.1
8wk	5.2 ± 0.1	5.5 ± 0.1	4.6 ± 0.1	4.7 ± 0.1	4.9 ± 0.1	4.9 ± 0.1
9wk	5.3 ± 0.1	5.4 ± 0.1	4.0 ± 0.1	4.4 ± 0.1	4.6 ± 0.1	4.7 ± 0.1
10wk	5.3 ± 0.1	5.6 ± 0.1	3.5 ± 0.1	3.7 ± 0.1	3.9 ± 0.2	4.5 ± 0.1
Food intake g/d	**5.2 ± 0.1**	**5.6 ± 0.1**	**4.7 ± 0.1**	**4.8 ± 0.1**	**4.8 ± 0.1**	**4.9 ± 0.1**

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different ($p < 0.05$)

The Table 5.10 gives the food intake values at a weekly interval of the 10 week period and mean food intake for the entire experimental duration. A similar trend was observed in feed intake as was observed in case of body weight.

5.3.1.3 Energy Intake

Energy intake for all groups was recorded every day. Figure 5.27 shows the energy intake of ND group compared with HFD group alone 5.27A and ND, HFD group compared with Triphala, Amla, Harad and Bahed groups individually (5.27 B, C, D, E).

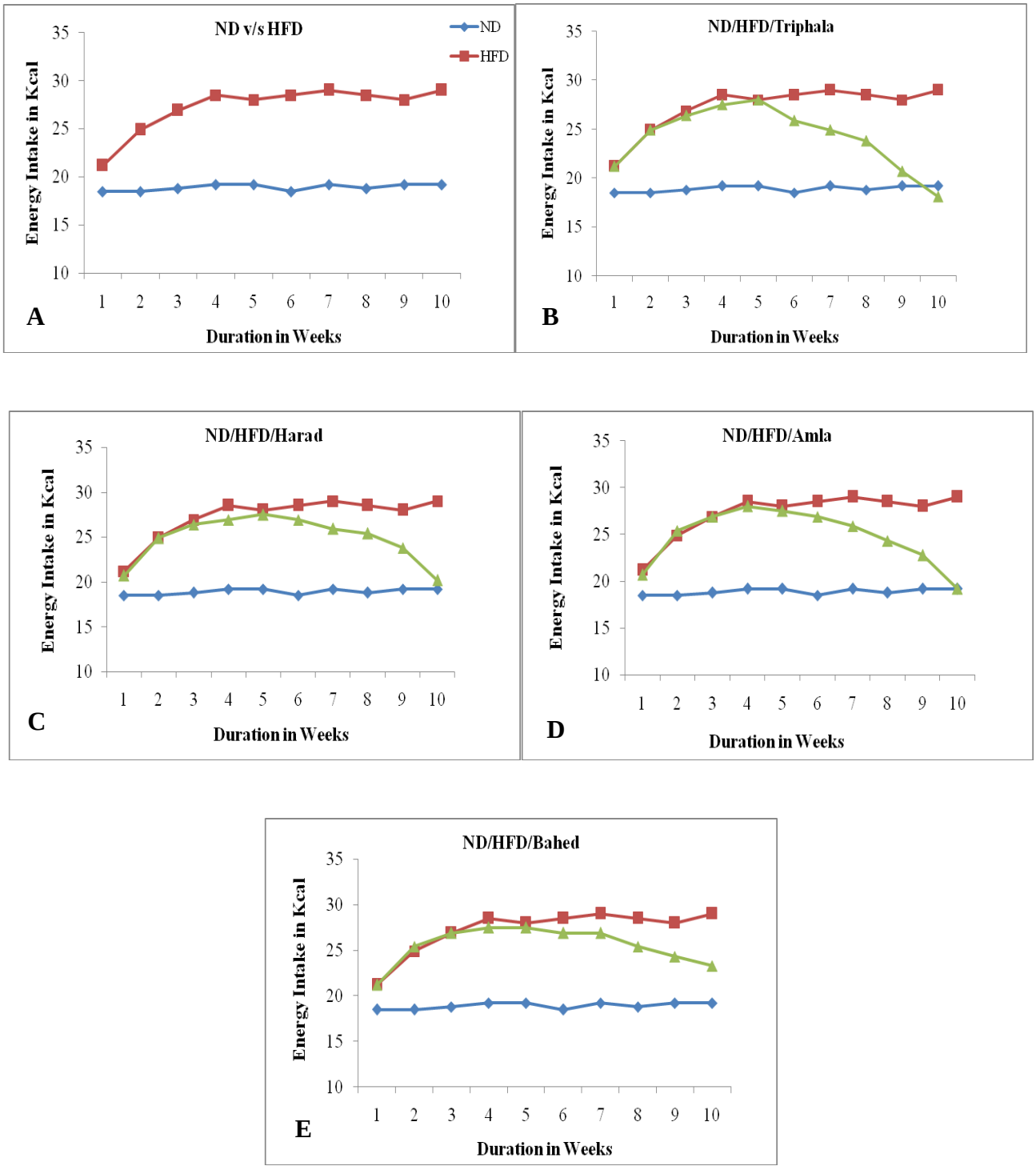


Fig: 5.27 Energy Intake of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$).

Table 5.11: Energy Intake in Mice Fed on the Experimental Diets for 10 Weeks

Groups / Weeks	ND	HFD	HFD+T	HFD+A	HFD+H	HFD + B
1 wk	18.5 ± 0.1	21.2 ± 0.1	21.2 ± 0.2	20.7 ± 0.1	20.7 ± 0.1	21.2 ± 0.1
2wk	18.5 ± 0.2	24.9 ± 0.2	24.9 ± 0.2	25.4 ± 0.1	24.9 ± 0.1	25.4 ± 0.1
3wk	18.8 ± 0.2	26.9 ± 0.1	26.4 ± 0.1	26.9 ± 0.1	26.4 ± 0.1	26.9 ± 0.0
4wk	19.2 ± 0.1	28.5 ± 0.1	27.5 ± 0.1	28.0 ± 0.1	26.9 ± 0.0	27.5 ± 0.1
5wk	19.2 ± 0.1	28.0 ± 0.0	28.0 ± 0.1	27.5 ± 0.0	27.5 ± 0.0	27.5 ± 0.1
6wk	18.5 ± 0.2	28.5 ± 0.1	25.9 ± 0.1	26.9 ± 0.1	26.9 ± 0.1	26.9 ± 0.1
7wk	19.2 ± 0.1	29.0 ± 0.1	24.9 ± 0.1	25.9 ± 0.1	25.9 ± 0.1	26.9 ± 0.1
8wk	18.8 ± 0.1	28.5 ± 0.1	23.8 ± 0.1	24.3 ± 0.1	25.4 ± 0.1	25.4 ± 0.1
9wk	19.2 ± 0.1	28.0 ± 0.1	20.7 ± 0.1	22.8 ± 0.1	23.8 ± 0.1	24.3 ± 0.1
10wk	19.2 ± 0.1	29.0 ± 0.1	12.7 ± 0.1	19.2 ± 0.1	20.2 ± 0.2	23.3 ± 0.1
Energy intake kcal/d	**18.9 ± 0.1**	**27.2 ± 0.1**	**23.6 ± 0.1**	**24.8 ± 0.1**	**24.9 ± 0.1**	**25.5 ± 0.1**

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different ($* p < 0.05$)

The Table 5.11 gives the energy intake values at an interval of one week (10 weeks) and mean energy intake for the entire experimental duration. A similar trend was observed in feed intake as observed in case of body weight.

5.3.1.4 BMI and Lee Index

Body weight for all groups was recorded on every3rd day. The mean BMI was calculated at the end of the entire experimental duration. The Fig: 5.28 shows the mean BMI and Fig: 5.29 shows the Lee Index for all the groups.

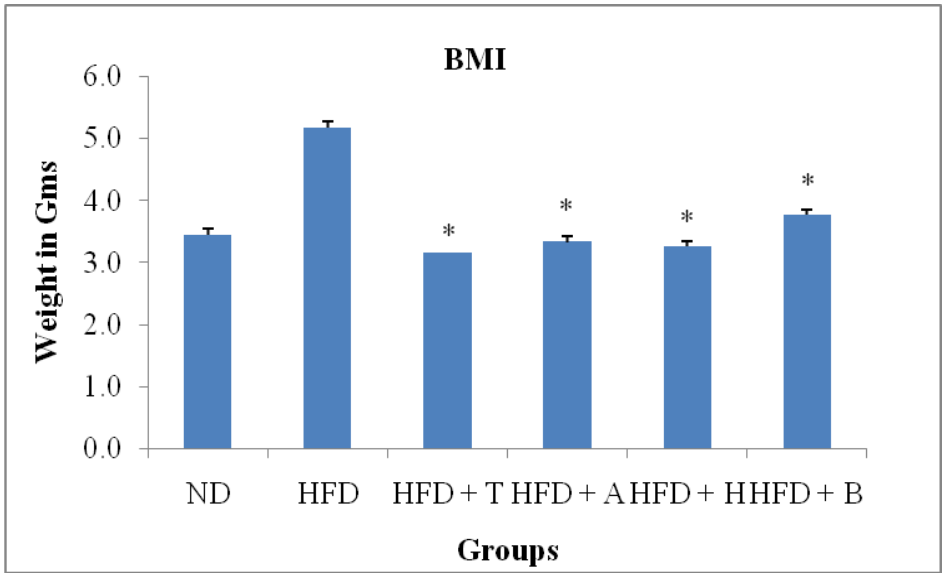


Fig: 5.28 Body Mass Index of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$)

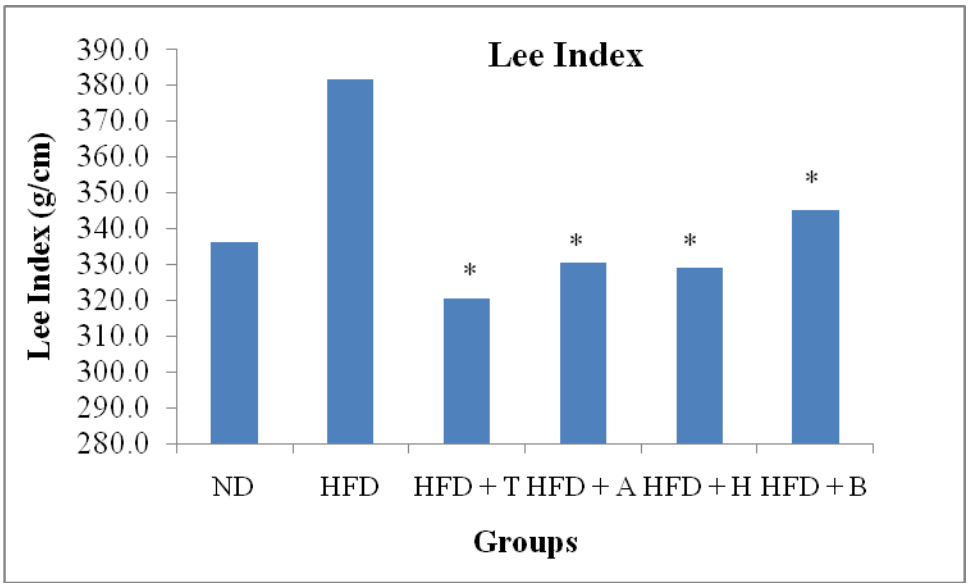


Fig: 5.29 Lee Index of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$)

5.3.1.5 Adipose Tissue (Fat pad) weight

Adipose tissue (fat pad) weight was taken at the end of the experiment after euthanisation of animals from all the groups. The fat pads were separated, washed in saline and weighed for all the mice (7 mice in each group). Figure 5.30 shows the mean fat pad weight for all the groups.

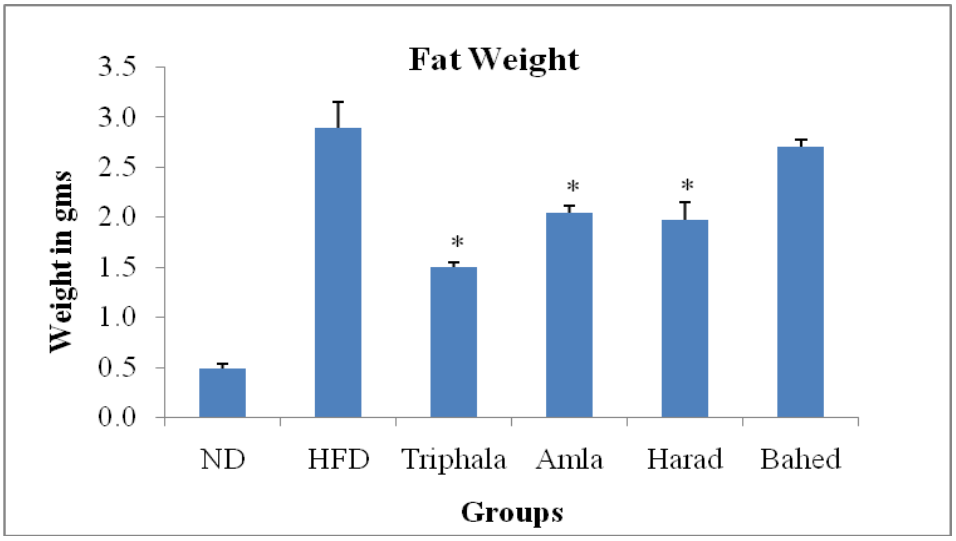


Fig: 5.30 Adipose Tissue Weight of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$)

5.3.1.6 Liver Weight

Liver weight was taken at the end of the experimental period after euthanisation of animals from all the groups. The liver was separated, washed in saline and weighed for all the mice (7 mice in each group). Figure 5.31 shows the mean liver weight for all the groups.

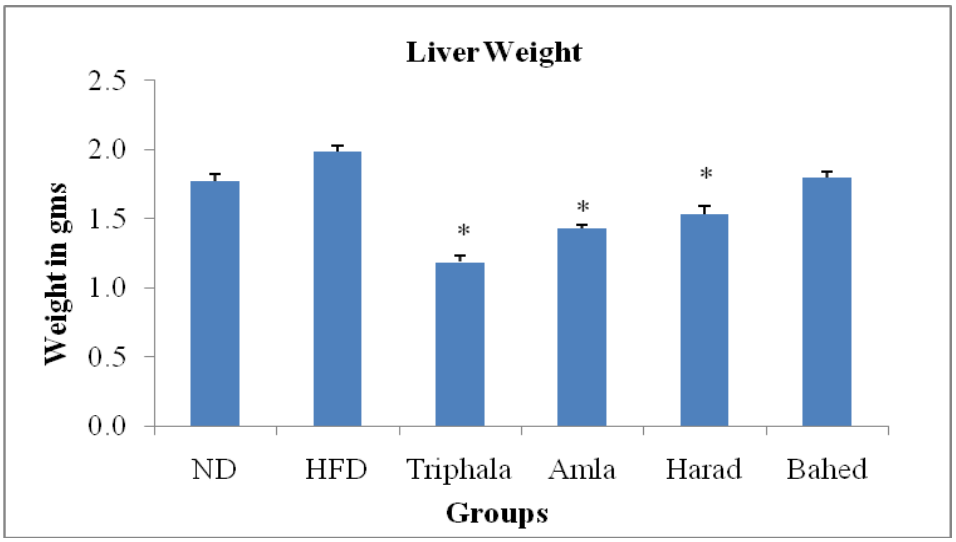


Fig: 5.31 Liver Weight of Mice Fed on the Experimental Diets for 10 weeks. Values are mean ± SEM; n=7. Means are significantly different ($p < 0.05$).

Table 5.12: Comparative Table showing the Anthropometric Parameters and Weight of certain organs in Mice Fed on the Experimental Diets for 10 Weeks

Group	ND	HFD	HFD+T	HFD+A	HFD+H	HFD + B
Initial body weight g	21.7±0.2	21.1 ± 0.3	22.0 ± 0.3	21.6 ± 0.4	21.0 ± 0.5	21.1 ± 0.3
Final body weight g	30.6 ± 0.8	43.3 ± 0.4	27.3±0.3**	28.1±0.7**	29.6 ±0.5**	32.0±0.5**
Body weight gain g/10 wk	8.9 ± 0.4	22.2± 0.5	5.3±0.4**	6.5±0.4**	8.6±0.5**	10.9±0.3**
Food intake g/d	5.2 ± 0.1	5.3 ± 0.1	4.7 ± 0.1*	4.8 ± 0.1*	4.8 ± 0.1*	4.9 ± 0.1*
Energy intake kcal/d	18.9 ± 0.1	27.2 ± 0.1	23.6 ± 0.1**	24.8 ± 0.1**	24.9 ± 0.1**	25.5 ± 0.1**
FER	1.7	4.1	0.6**	1.7**	3.8*	2.2**
Lee Index g/cm	336.3	381.7	320.3**	330.5**	329.1**	345.1**
Liver weight	1.8 ± 0.05	2.0 ± 0.04	1.2 ± 0.05**	1.4 ± 0.03**	1.5 ± 0.06**	1.8 ± 0.04
Weight of Fat pad	0.5 ± 0.04	2.9 ± 0.27	1.5 ± 0.04**	2.0 ± 0.07*	2.0 ± 0.19*	2.7 ± 0.08

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**$p < 0.0001$, * $p < 0.05$)

The BW at 10-wk after treatment and the body weight gain at the end of the experiment in the HFD group were 41.5% and 149.4% greater respectively, than the equivalent values observed in the ND group (Table 5.12). Treatment with the herbal powders significantly reduced the final body weight by 37% (Triphala), 35% (Amla), 32% (Harad) and 26.1% (Bahed) when compared with ND group and was 76%, 71%, 61%, and 51.3% respectively lower than the HFD group (Table 5.12). Food intake, energy intake and food efficiency ratio (FER) of mice in the HFD+T, HFD + A and HFD + H groups were significantly lower than the values observed for the HFD group, $p < 0.05$, as summarized in Table 5.12. The relative weight of the total adipose tissue was significantly higher (480% greater) in HFD fed mice than the value obtained for the ND mice, $p < 0.005$ and were significantly lowered 48.2%, 31.1%, 31%, 24.13% by supplementing with the herbal powders, Triphala, Amla, Harad and Bahed respectively, $p < 0.0001$. The enlargement of liver and accumulation of fat in the liver of mice fed HFD were considerably ameliorated by herbal treatment in all groups, namely HFD+T (40%), HFD + A (35%), HFD + H (25%) and HFD+B (15%). Reduction in the weight of the liver was statistically significant, $p < 0:001$. The weight of the fatty liver was significantly higher (20% greater) in HFD fed mice than the value obtained for the ND mice, $p < 0.005$. The anthropometric results in this experiment clearly showed that treatment with Triphala and its constituents proved to be effective in ameliorating the visceral adiposity.

5.3.2 Clinical Parameters (0 day in all the clinical parameters is the start of herbal treatment)

5.3.2.1 Glucose

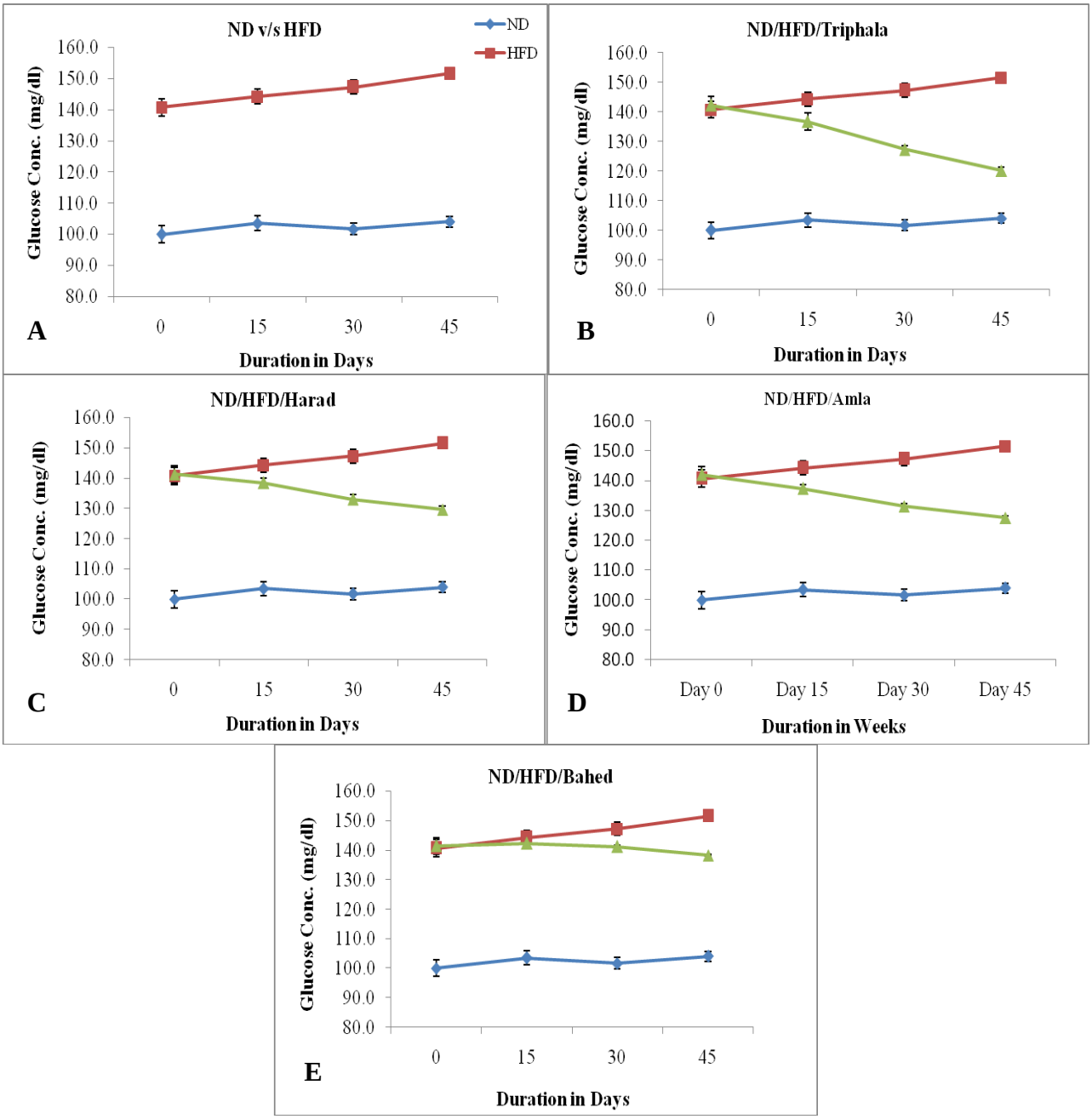


Fig: 5.32 Plasma Glucose Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$) except for the Bahed treated group

5.3.2.2 Cholesterol

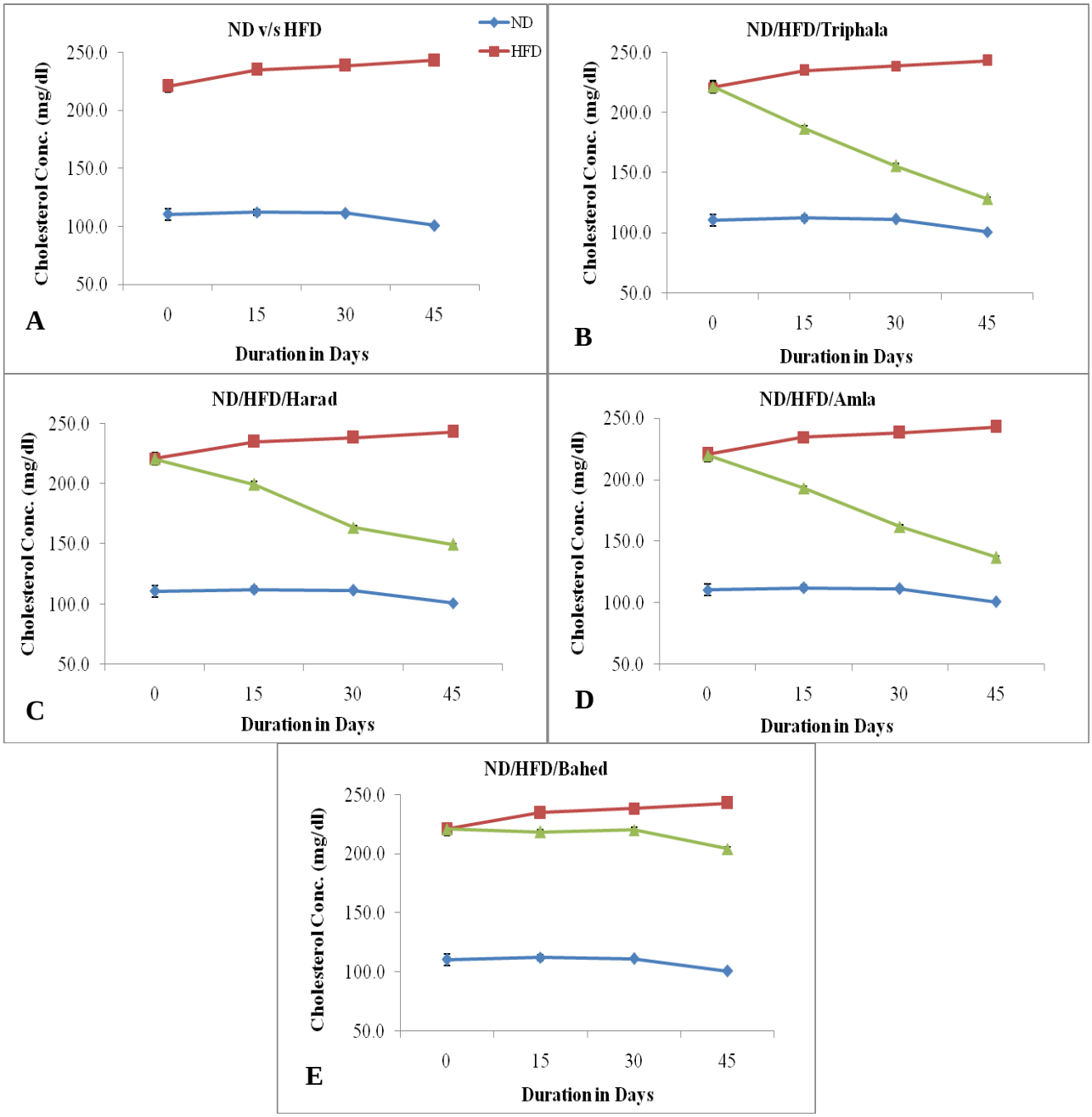


Fig: 5.33 Plasma Cholesterol Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$) except for the Bahed treated group

5.3.2.3 Triglyceride

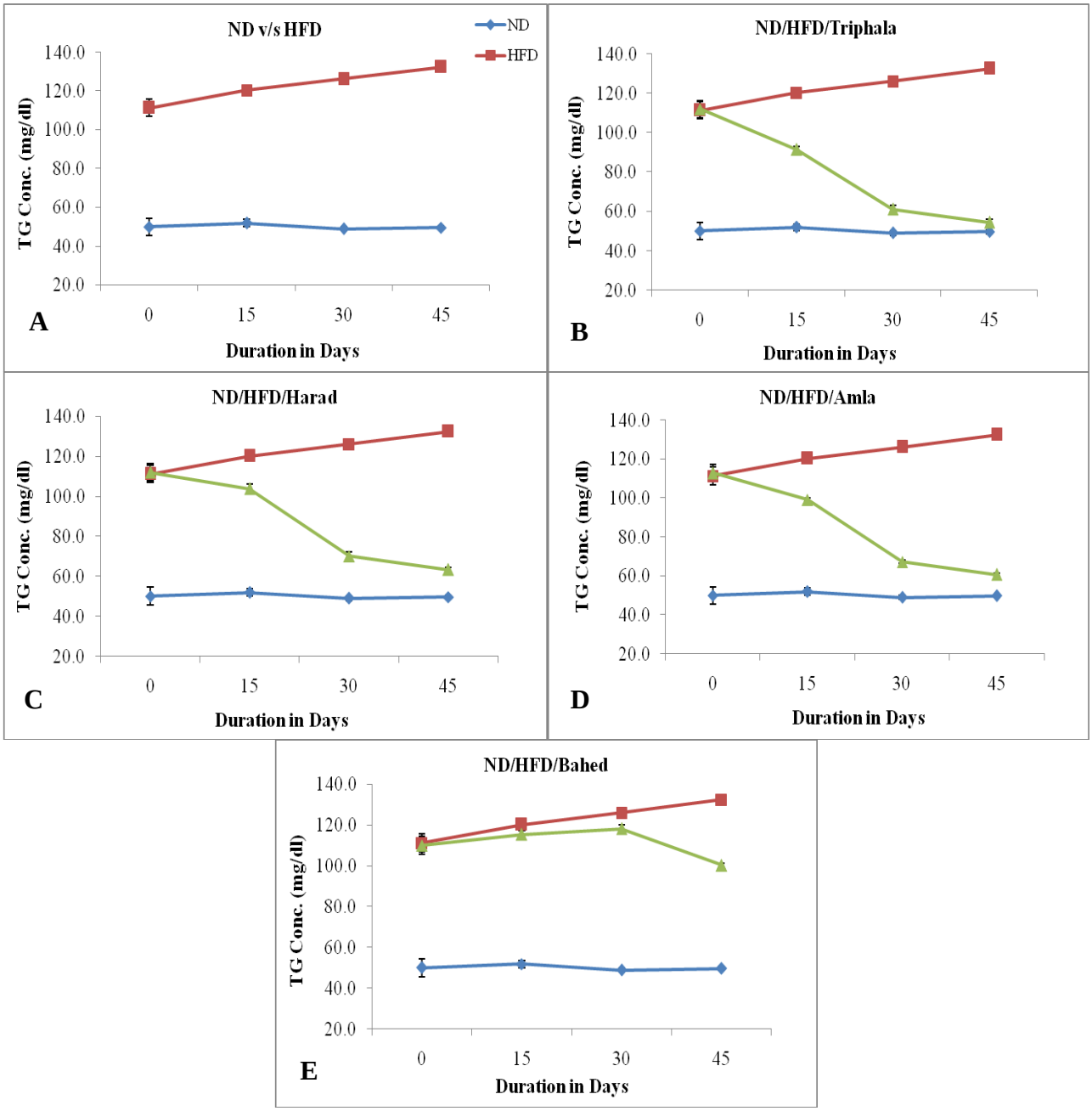


Fig: 5.34 Plasma Triglyceride Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean $\pm$ SEM; n=7. Means are statistically significantly different ($p < 0.05$) except for the Bahed treated group.

5.3.2.4 High Density Lipoprotein (HDL)

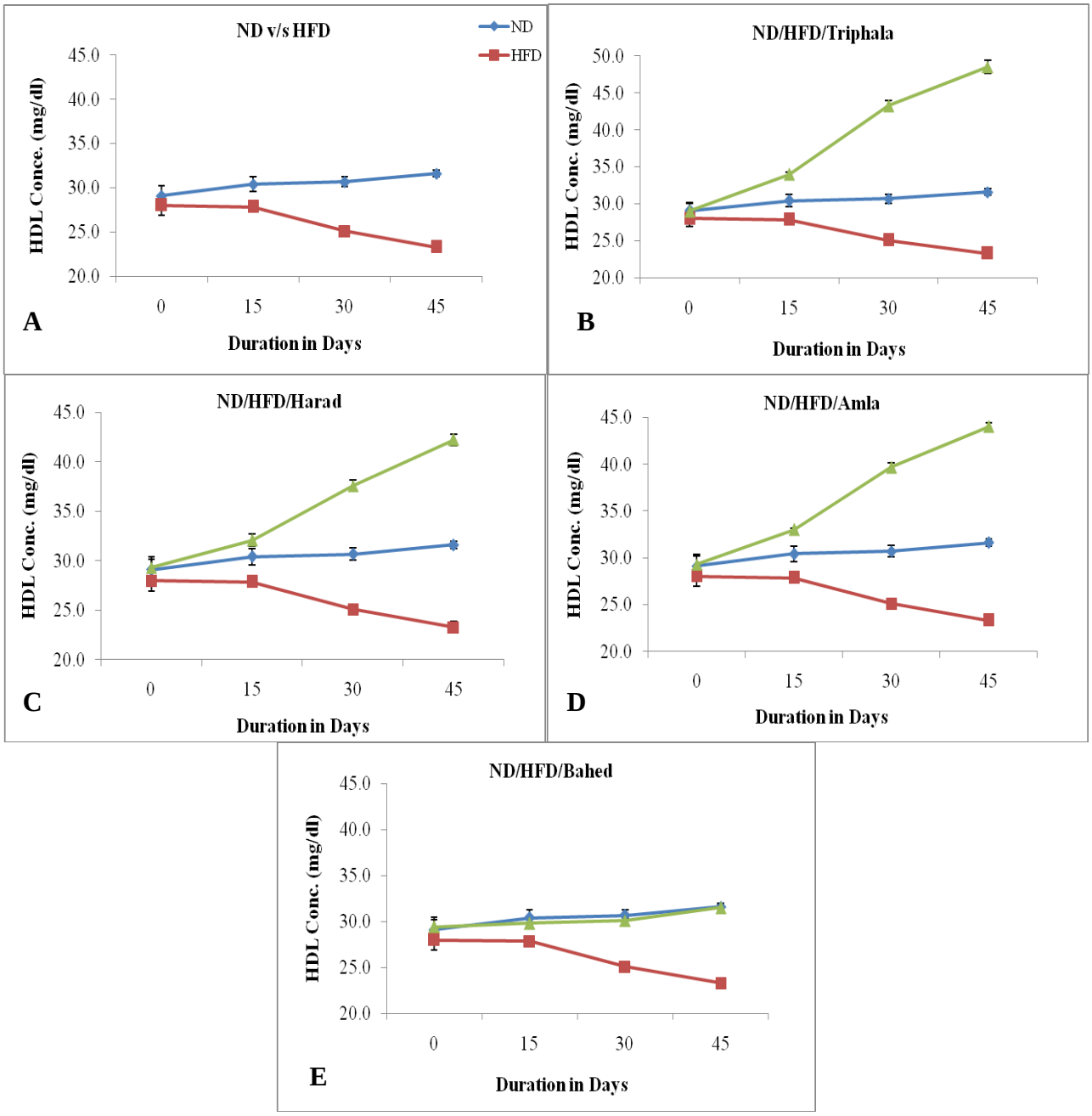


Fig: 5.35 Plasma HDL Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean $\pm$ SEM; n=7. Means are statistically significantly different ($p < 0.05$) except for the Bahed treated group.

5.3.2.5 Low Density Lipoprotein (LDL)

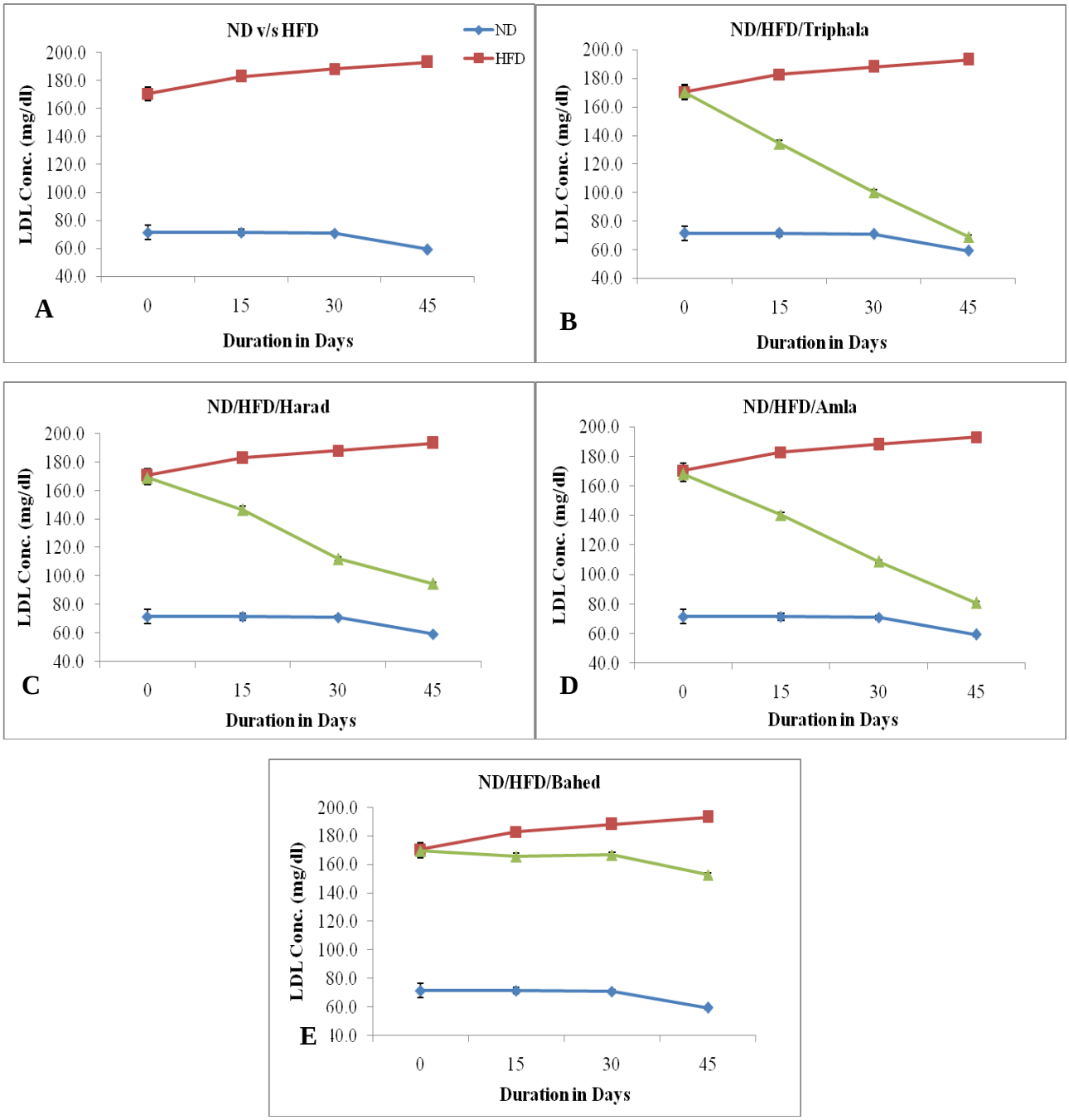


Fig: 5.36 Plasma LDL Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are significantly different ($p < 0.05$) except for the Bahed treated group.

5.3.2.6 Very Low Density Lipoprotein (VLDL)

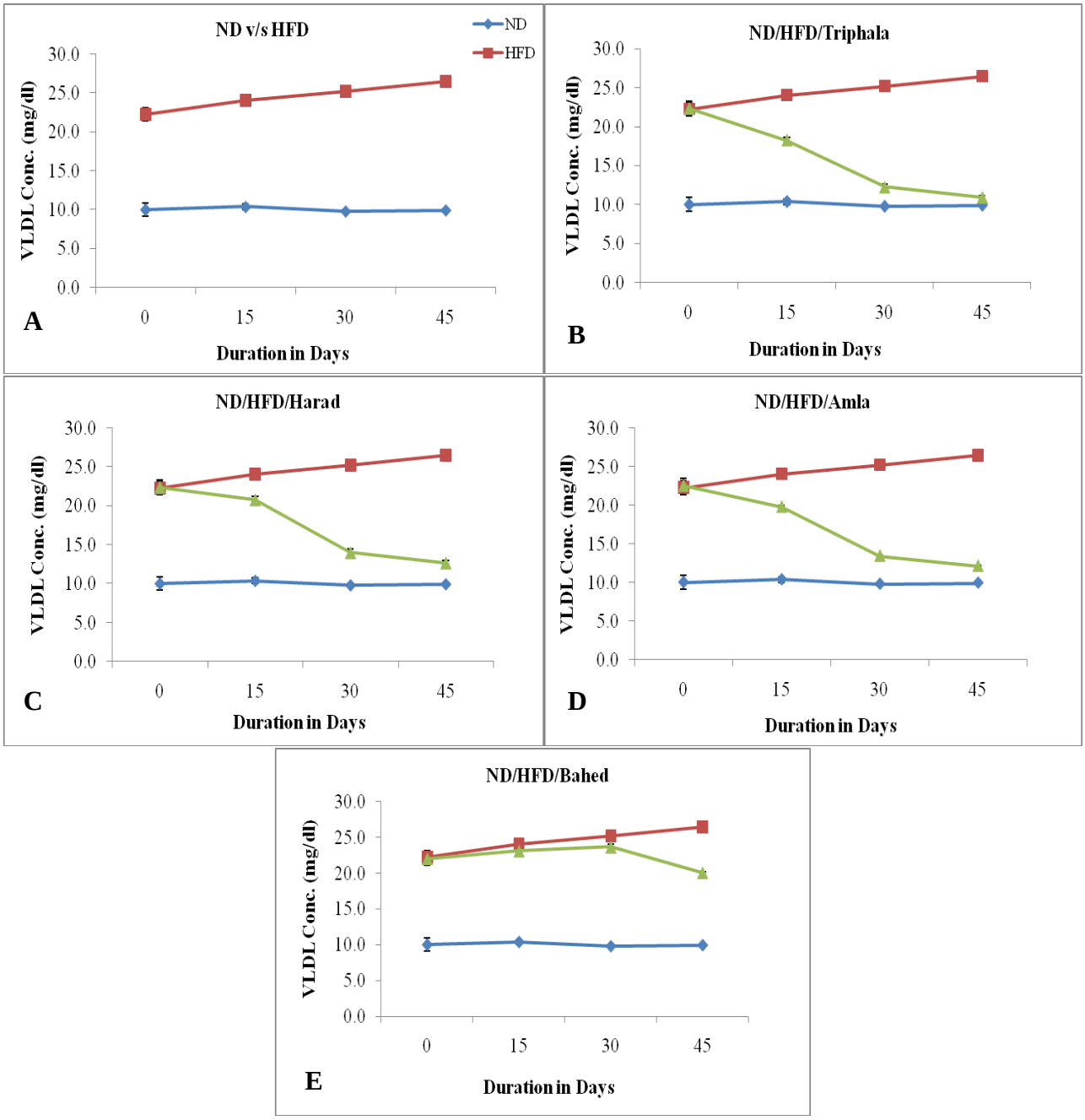


Fig: 5.37 Plasma VLDL Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are significantly different ($p < 0.05$) except for the Bahed treated group.

5.3.2.7 Alanine Transaminase (ALT)

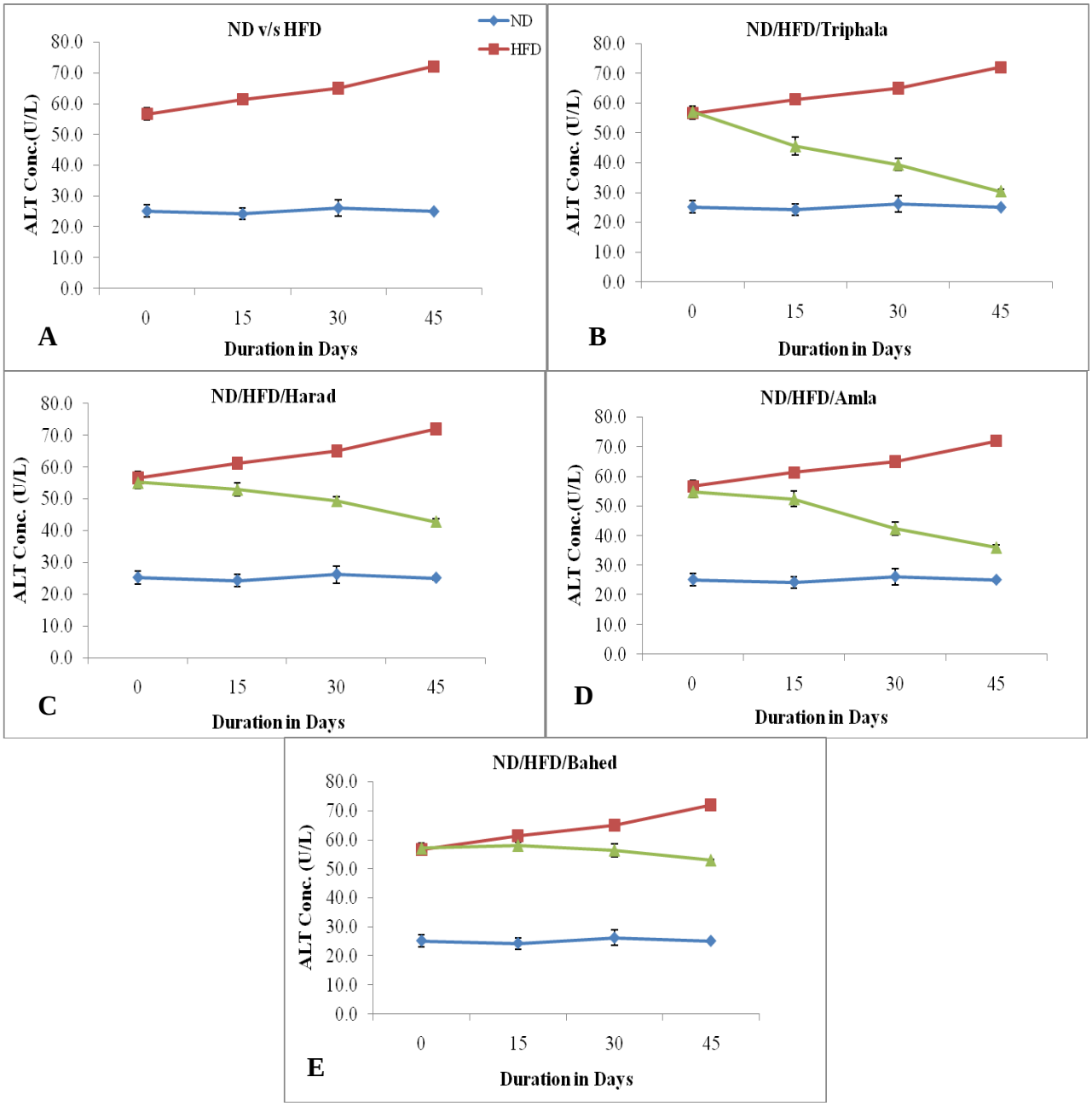


Fig: 5.38 Serum ALT Levels of Mice Fed on the Experimental Diets for 10 weeks (A) ND v/s HFD Group, (B) ND-HFD-Triphala Group, (C) ND-HFD-Harad Group, (D) ND-HFD-Amla Group, (E) ND-HFD-Bahed Group. Values are mean ± SEM; n=7. Means are statistically significantly different ($p < 0.05$) except for the Bahed treated group.

COMPARATIVE TABLE OF ALL CLINICAL PARAMETERS FOR ALL GROUPS

Table 5.13: Plasma Biochemistry of Mice Fed on the Experimental Diets

Group	ND	HFD	HFD+T	HFD+A	HFD+H	HFD+B
Plasma						
Glucose	104.0±1.7	151.5±1.2	120.2±1.1**	127.6±0.7**	129.6±1.1**	138.3±0.3**
T.Cholesterol	100.9±0.7	243.0±1.5	128.6±0.8**	137.1±1.0**	149.7±0.4**	204.4±1.3**
Triglyceride	49.7±1.0	132.4±0.7	54.4±1.3**	60.7±0.6**	63.4±1.2**	100.3±0.8**
HDL	31.6±0.4	23.3±0.6	48.5±0.9**	44.0±0.4**	42.2±0.6**	31.5±0.3**
LDL	59.4±0.9	193.2±1.4	69.2±1.4**	81.0±1.1**	94.8±0.8**	152.8±1.5**
VLDL	9.9±0.2	26.5±0.1	10.9±0.3**	12.1±0.1**	12.7±0.2**	20.1±0.2**
Liver						
Serum ALT	25.1±0.6	72.0±0.6	30.4±0.6**	36.0±0.8**	42.8±1.0**	53.0±0.2**

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**$p < 0.0001$, *$p < 0.05$)

Plasma glucose concentrations were significantly lowered by treatment with Triphala, Amla, Harad and were 20%, 15.77% and 14.45% lower respectively, as compared to those obtained for the HFD-fed mice, $p < 0.0001$. Treatment with Bahed however did not show any significant reduction in plasma glucose levels as compared to the HFD-fed mice, (Fig: 5.32, Table 5.13).

The HFD-induced hypercholesterolemia was significantly improved by herbal treatment with Triphala, Amla, Harad and Bahed respectively. Plasma total cholesterol concentrations were 47.1%, 43.58%, 38.3% and 15.88% lower, respectively, in mice fed with HFD + T, HFD +A, HFD + H and HFD + B than those observed for the HFD-fed mice, p<0:0001, (Fig: 5.33, Table: 5.13).

The plasma triglyceride (TG) concentrations were again 58.8%, 54.2%, 52.1% and 24.2% lower, respectively, in mice fed with HFD + T, HFD + A, HFD + H and HFD + B than those observed for the HFD-fed mice, p<0.0001, (Fig: 5.34, Table: 5.13).

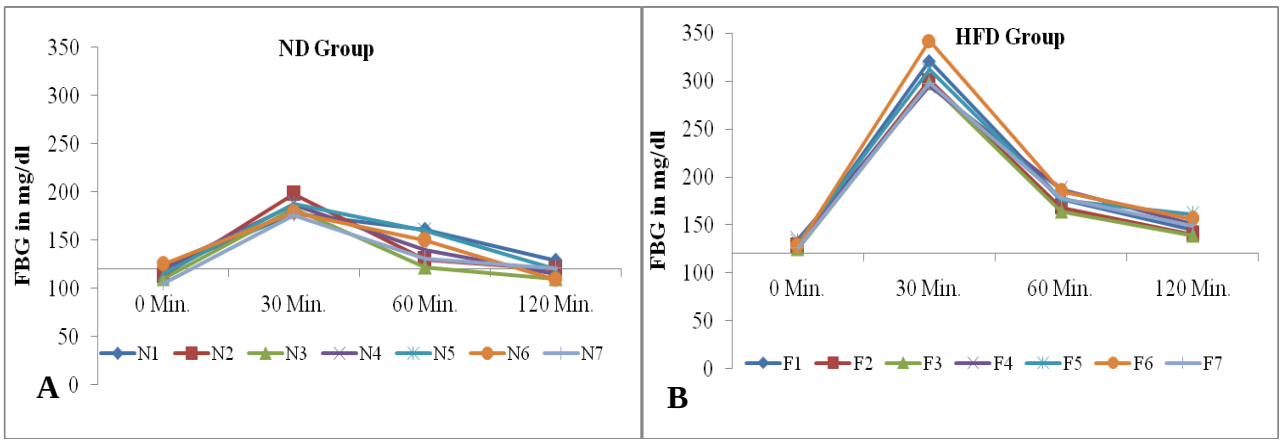
All herbal treatments increased the levels of HDL-cholesterol, which were 52% for HFD + T, 50% for HFD + H, 41% for HFD + A and 26.4% for HFD+B higher respectively, than the values observed for the HFD-fed mice, $p<0.0001$, (Fig: 5.35, Table: 5.13).

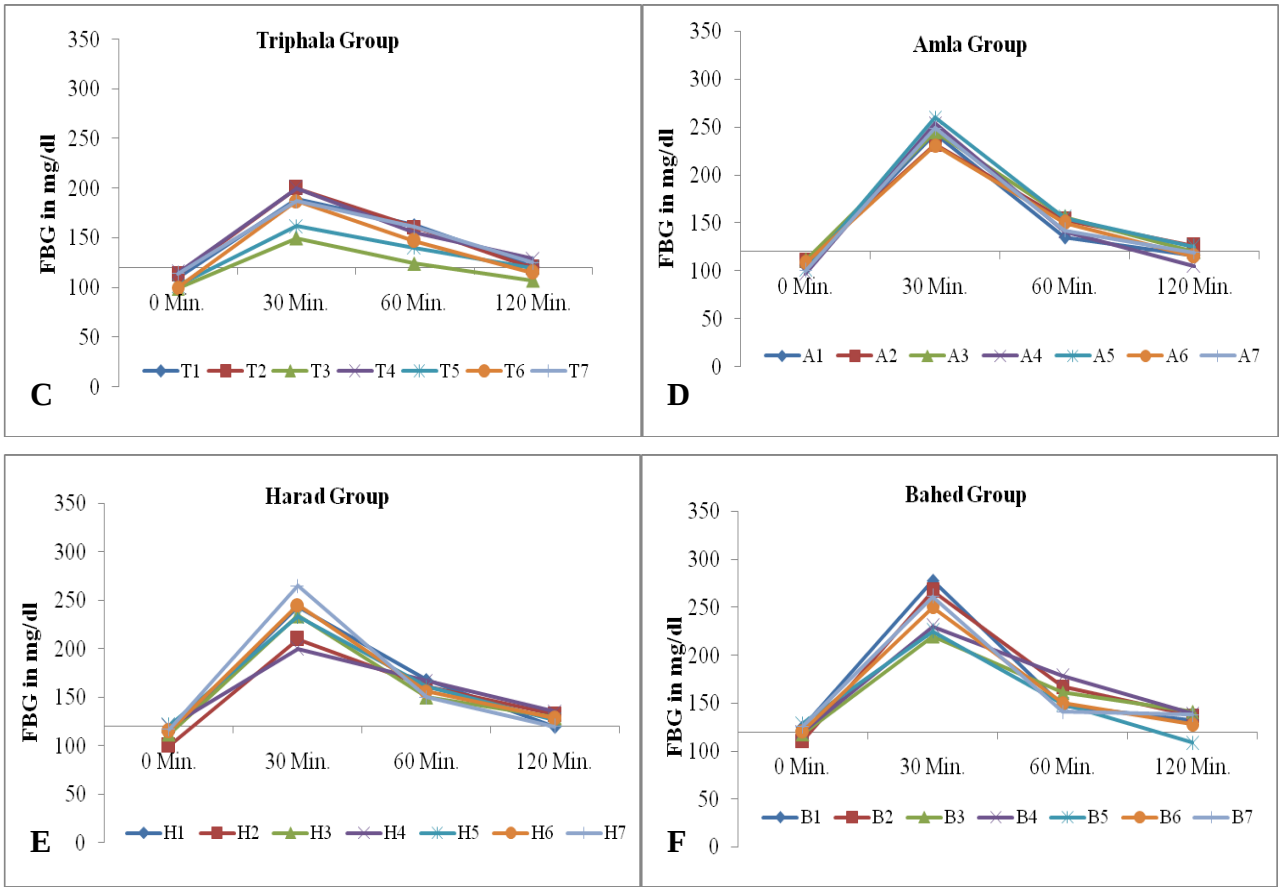
The same pattern was observed with LDL and VLDL levels. The LDL concentration was lowered by 64.1%, 58%, 51.1% and 21% in mice fed with HFD + T, HFD + A, HFD + H and HFD + B (Fig: 5.36, Table: 5.13). The treatment reduced the VLDL levels by 59%, 54%, 52% and 24% as compared with the HFD group (Fig: 5.37, Table: 5.13).

Serum ALT levels were also lowered by the herbal treatments 57.6% in HFD + T group, 50% in HFD+A group, 41% in HFD + H group and 26.4% in HFD + B group respectively, as compared to values observed in the HFD-fed mice (Fig: 5.38, Table: 5.13).

5.3.2.8 Oral Glucose Tolerance Test (OGTT)

OGTT was done at the end of the experiment before euthanisation of animals. OGTT values (area under the curve, AUGTC) for ND and HFD group were found to be 1118.6 and 1716.7. Herbal treatment for 10 weeks in HFD + T, HFD + A, HFD + H and HFD + B resulted in significant improvement in oral glucose tolerance with the AUGTC 0–120 min being 1114.2, 1359.6, 1341.4, and 1415.5, respectively, versus 1716.7, in the HFD group, Fig. 5.39.





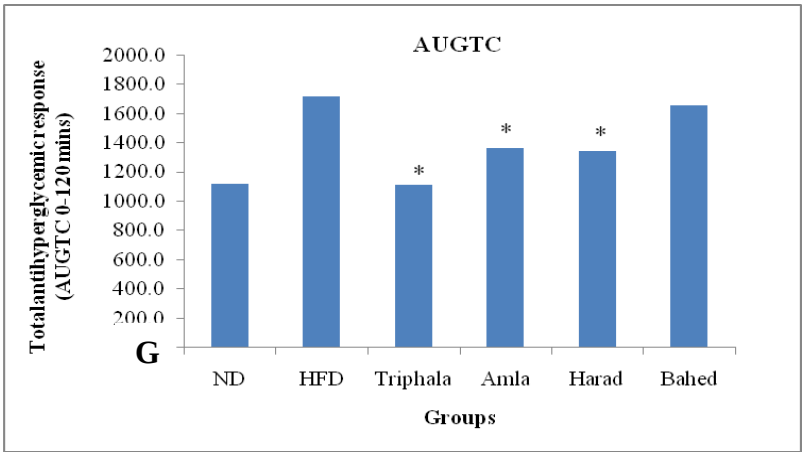


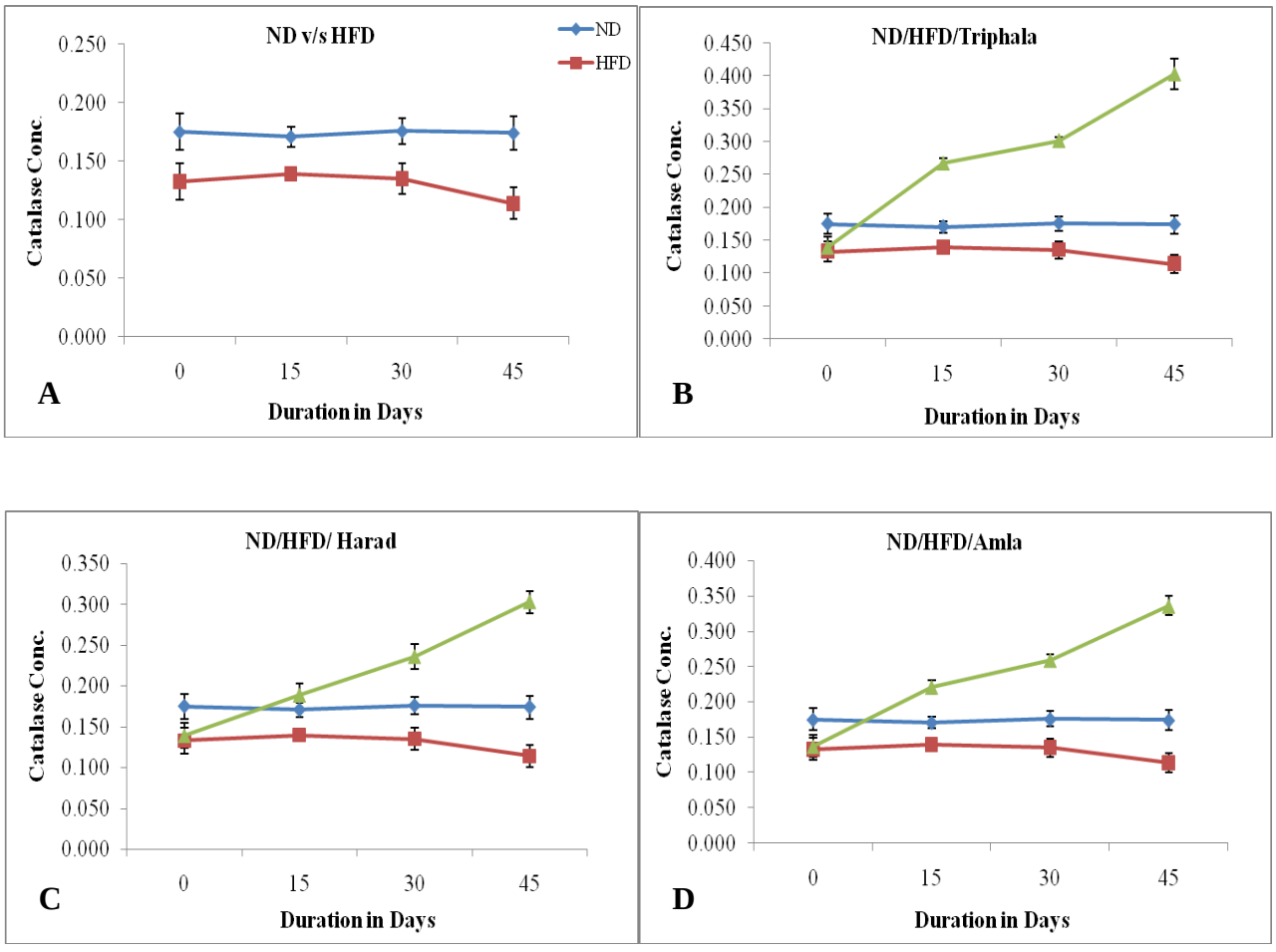
Fig 5.39: Effect of herbal treatment on oral glucose tolerance test. (A) ND Group, (B) HFD Group, (C)Triphala Group, (D) Amla Group, (E) Harad Group, (F) Bahed Group. The graph indicates the total antihyperglycemic response AUGTC 0-120 min in all the herbal powder treated groups. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different ($p < 0.05$)

5.3.3 Antioxidant and Oxidative Stress Parameters:

Endogenous protective mechanism against oxidative stress was investigated next. High Fat Diet altered the levels of antioxidant enzymes (Catalase and TSOD) and increased the oxidative stress (as measured with TBARS).

5.3.3.1 Catalase

A marked increase in catalase activity was observed in Triphala and Amla group, whereas Bahed group did not show a significant change as compared with HFD group (Fig 5.40). Catalase concentration is measured as mMoles/mg protein/sec.



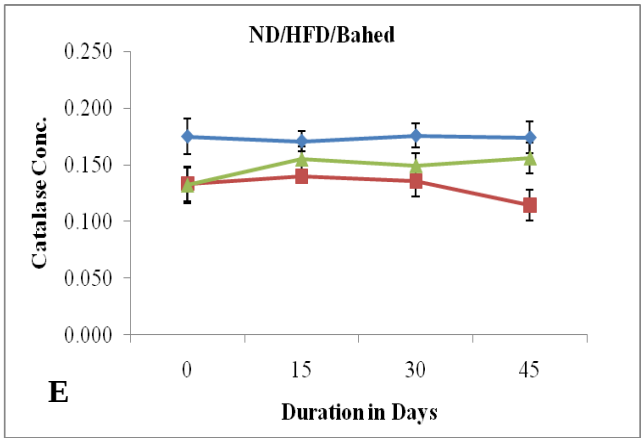
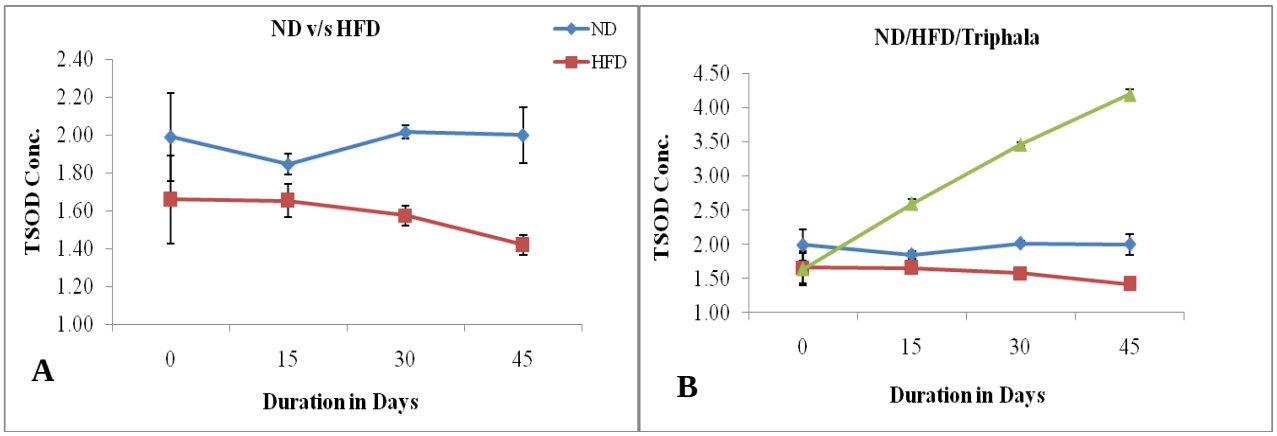


Fig 5.40: Activity of the antioxidant enzyme Catalase in mice fed on experimental diets for 10 weeks. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

5.3.3.2 Total Superoxide Dismutase (TSOD)

A marked increase in catalase activity was observed in Triphala and Amla group, whereas Bahed group did not show a significant change as compared with HFD group (Fig 5.41). The TSOD conc. is measured as mU/min/mg protein.



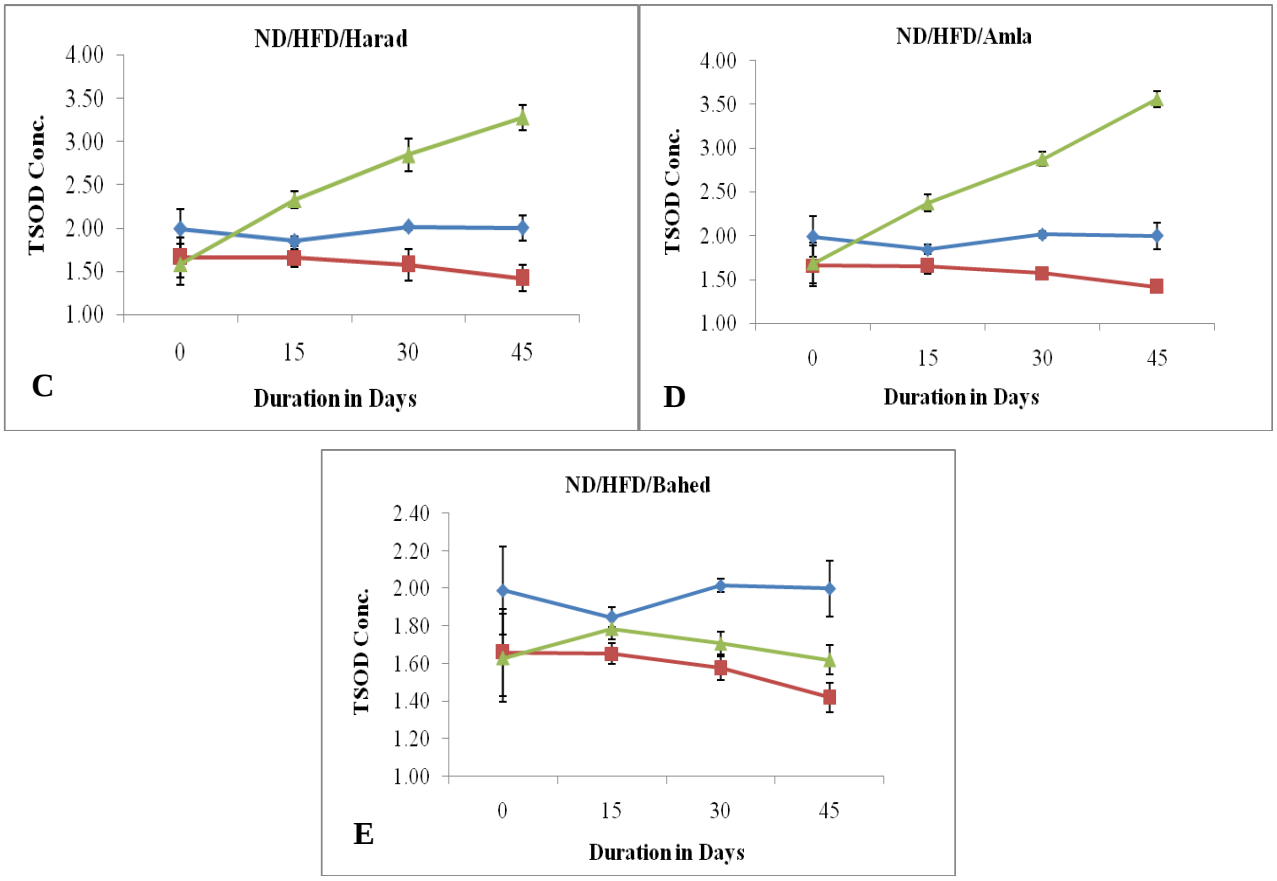
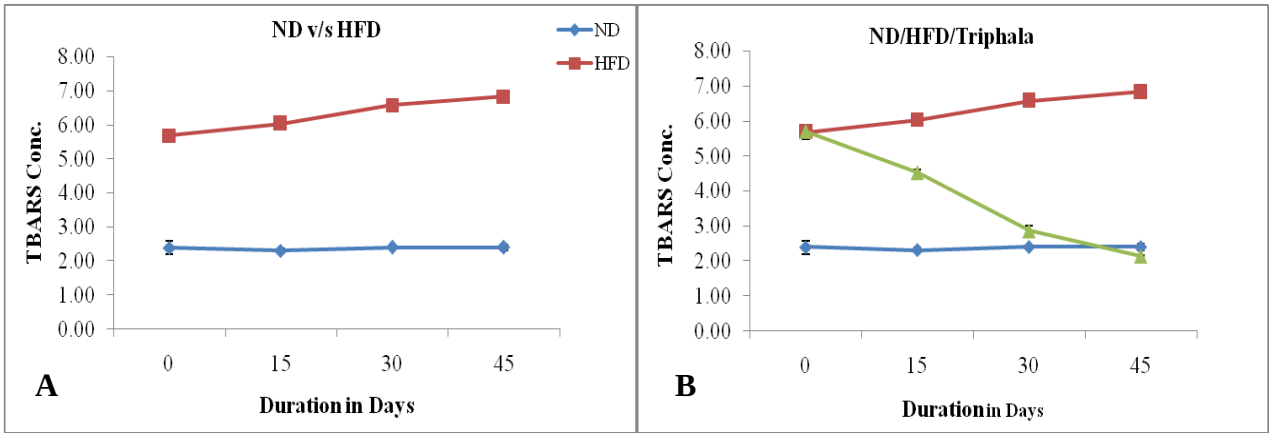


Fig 5.41: Activity of the antioxidant enzyme TSOD in mice fed on experimental diets for 10 weeks. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

5.3.3.3 Thiobarbituric Acid Reactive Species (TBARS)

A similar trend was observed for TBARS. TBARS concentration is measured as nmole/mg protein.



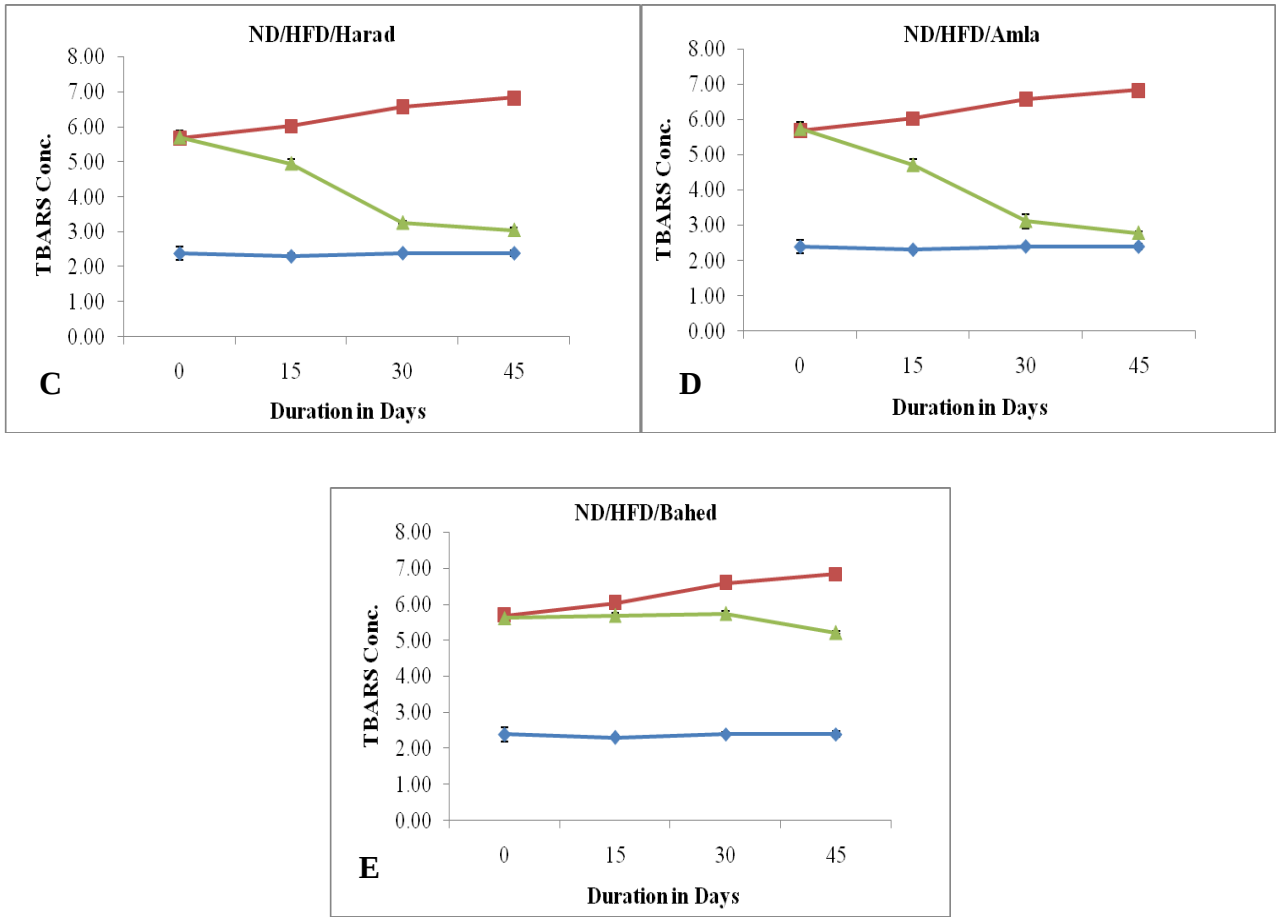


Fig 5.42: Oxidative stress activity (TBARS) in mice fed on experimental diets for 10 weeks. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

5.3.3.4 Catalase v/s HDL

The treatment improved the anti-oxidant level as well increased good cholesterol in terms of HDL. Fig. 5.43 depicts both the parameters comparing the improvement. Triphala group showed the best prevention as compared to amla and harad groups.

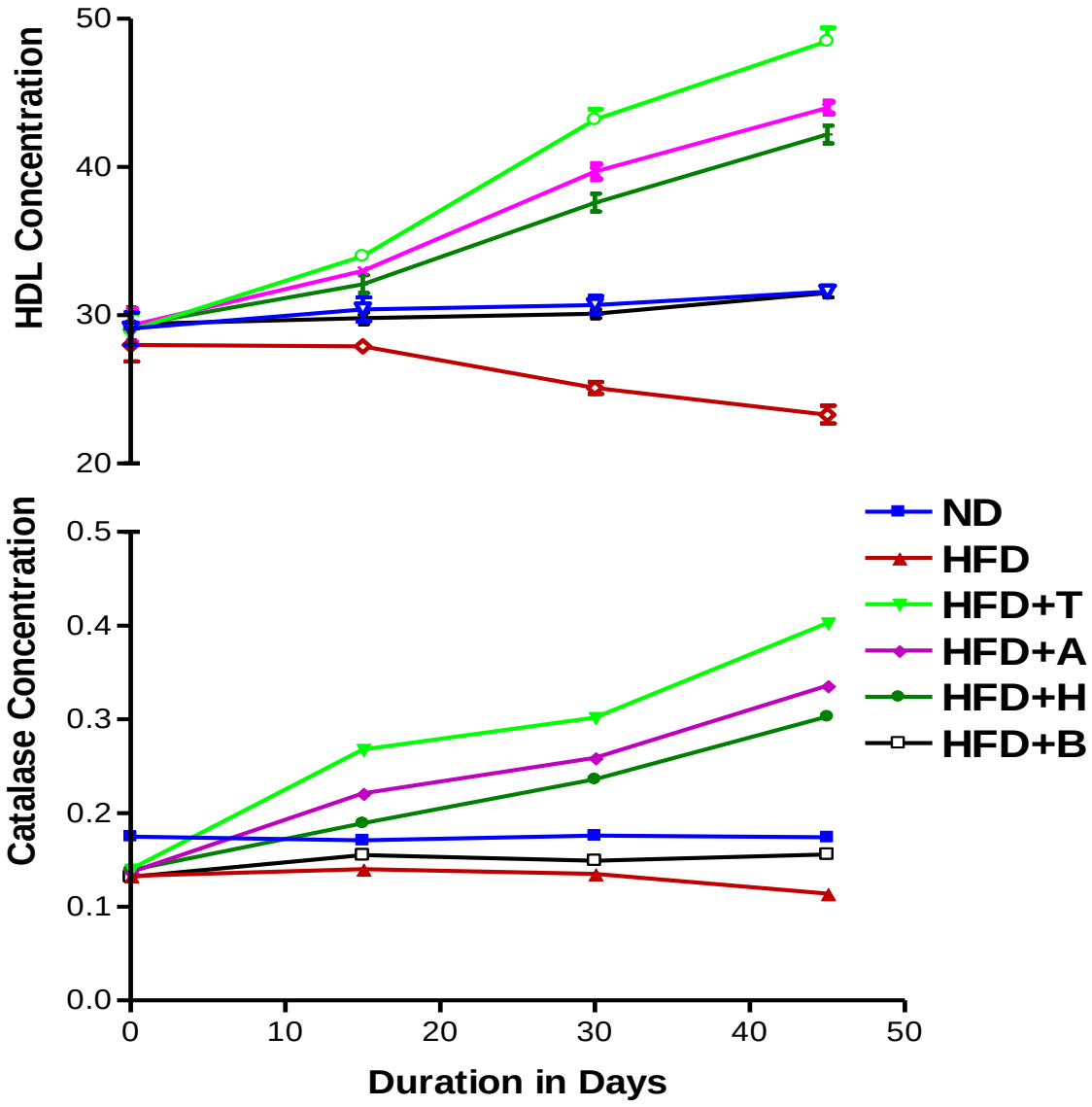


Fig 5.43: Comparative graph showing levels of anti-oxidant Catalase and HDL (good cholesterol) Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

5.3.3.5 TBARS v/s TG

The treatment proved to be effective in preventing the lipid peroxidation. Fig. 5.44 depicts TBARS levels comparing with TG concentration. Here too, treatment with triphala was most effective in reducing the lipid peroxidation as compared to amla and harad groups.

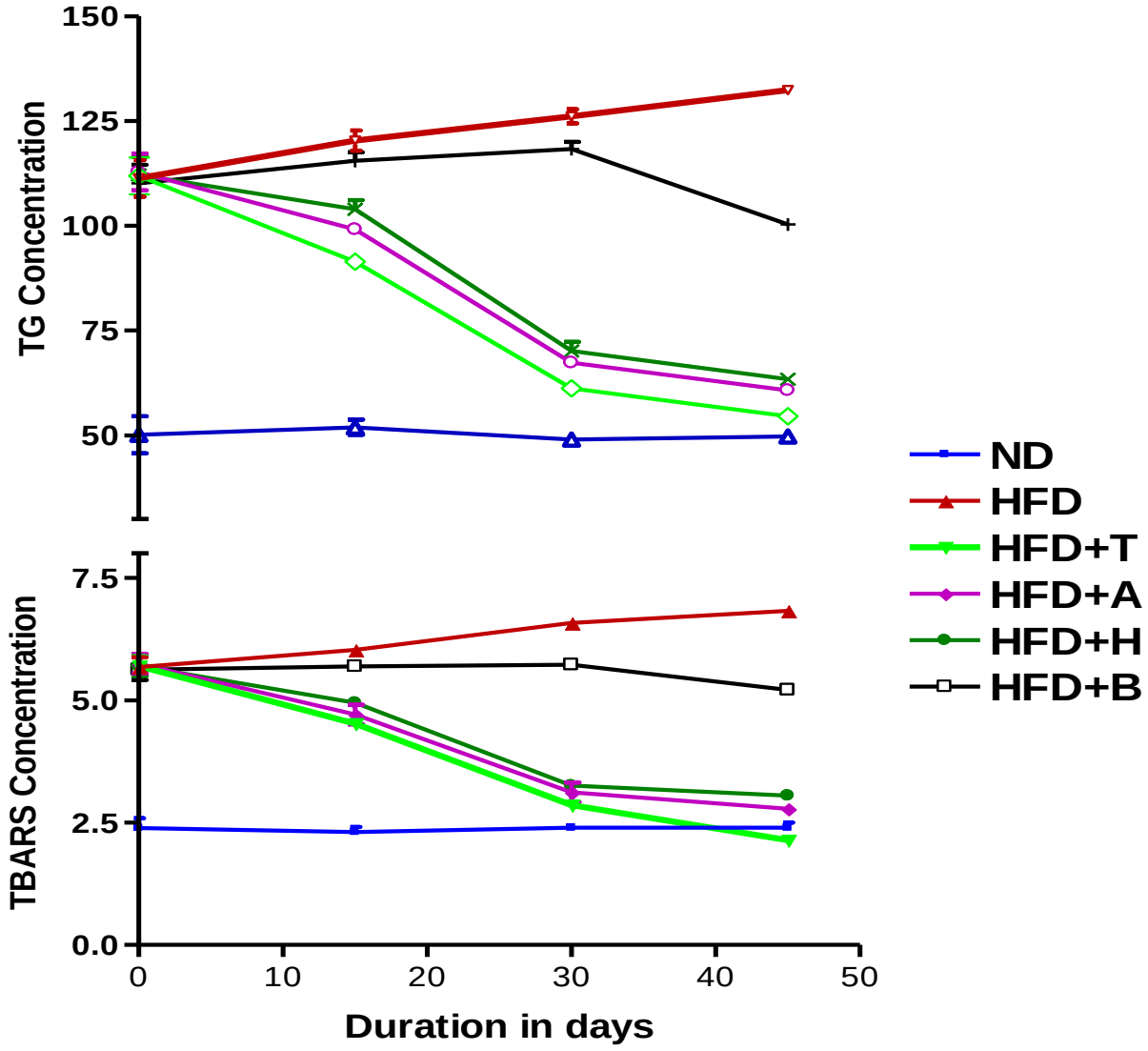


Fig 5.44: Comparative graph showing effect of Lipid peroxidation as levels of TBARS and TG. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

5.3.3.6 TBARS v/s LDL

The treatment proved to be effective in preventing the lipid peroxidation. Fig. 5.45 depicts TBARS levels comparing with LDL concentration. Here too, treatment with triphala was most effective in reducing the lipid peroxidation as compared to amla and harad groups.

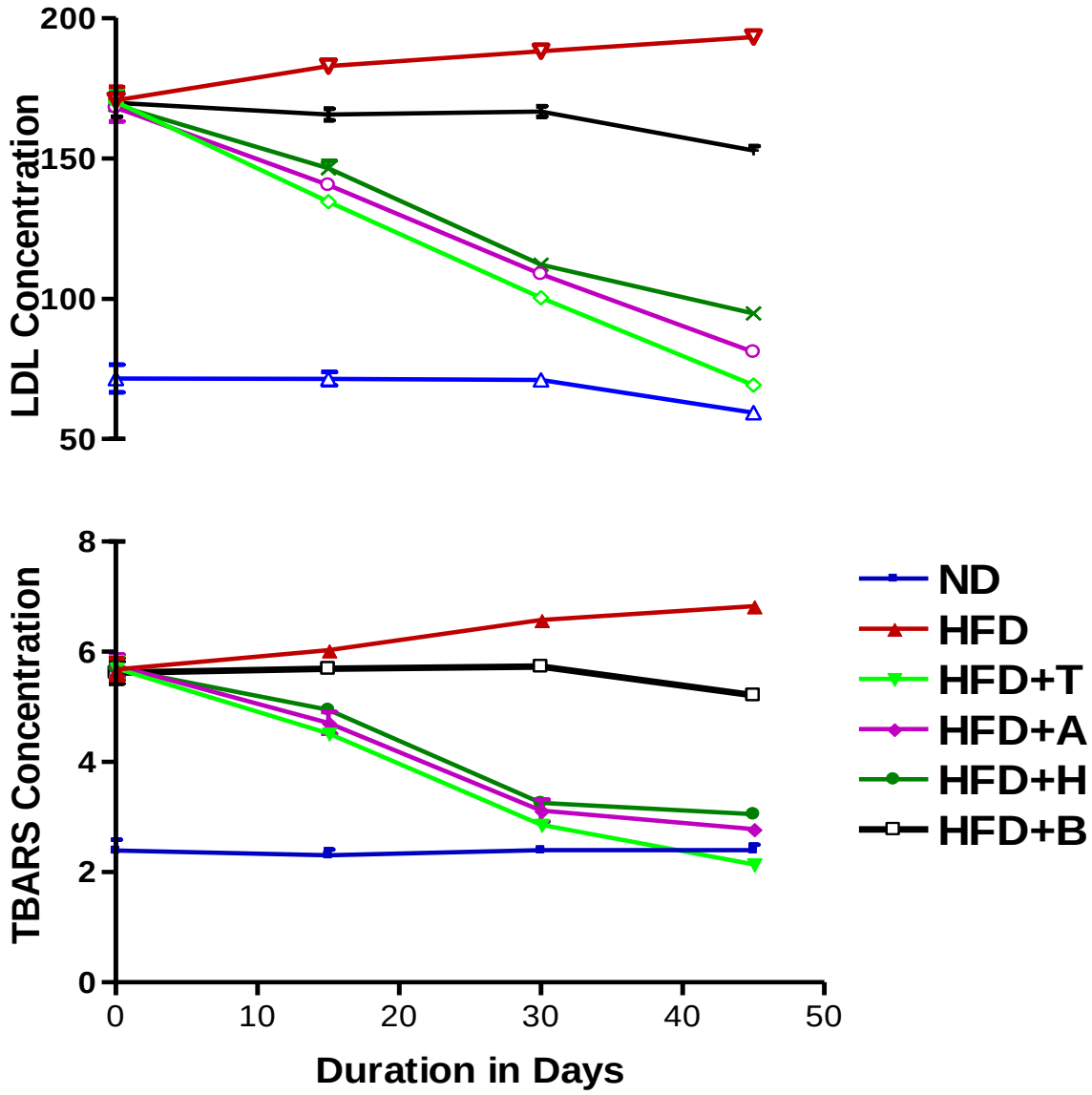


Fig 5.45: Comparative graph showing effect of Lipid peroxidation as levels of TBARS and LDL. Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**p < 0.0001, * p < 0.05)

COMPARATIVE TABLE OF ALL OXIDATIVE STRESS PARAMETERS FOR ALL GROUPS:

Table 5.14: Activities of the Antioxidant Enzymes and Oxidative Stress in Mice fed on Experimental Diets for 10 weeks.

Group	ND	HFD	HFD + T	HFD + A	HFD + H	HFD + B
Catalase	0.174±0.01	0.114±0.01	0.403±0.02**	0.336±0.01**	0.303±0.01**	0.156±0.01*
TSOD	2.00 ± 0.1	1.42 ± 0.1	4.19 ± 0.1**	3.56 ± 0.2**	3.28 ± 0.1**	1.62 ± 0.1
TBARS	2.400 ± 0.1	6.83 ± 0.1	2.14 ± 0.04**	2.78 ± 0.1**	3.05 ± 0.1**	5.21±0.04**

Values are mean ± SEM; n=7 in each group. All means are statistically significantly different (**$p < 0.0001$, * $p < 0.05$)

Plasma catalase levels were significantly increased by treatment with Triphala, Amla, Harad and Bahed 71.7%, 66% , 62.3% and 27% as compared to those obtained for the HFD-fed mice, $p < 0.0001$, (Fig: 5.40, Table 5.14).

The plasma TSOD concentrations were again 195%, 150.7% and 130% higher, respectively, in mice fed with HFD + T, HFD + A and HFD + H than those observed for the HFD-fed mice, $p<0.0001$, (Fig: 5.41, Table: 5.14).

The HFD-induced lipid peroxidation was significantly improved by herbal treatment with Triphala, Amla, Harad and Bahed respectively. Plasma TBARS concentrations were 68.6%, 59%, 55.3% and 23.7% lower, respectively, in mice fed with HFD + T, HFD +A, HFD + H and HFD + B than those observed for the HFD-fed mice, p<0:0001, (Fig: 5.42, Table: 5.14).

Chapter 6

Results: Fecal Pellet Culture & Tissue Histology

Chapter 6

Results: Fecal Pellet Culture & Tissue Histology

6.1 Microbial Contamination Estimation: Microbial contamination test revealed that the powders were sterile and the observation are summarized in Table 6.01. According to US pharmacopeia, for traditional microbiological plate count methods, an acceptable range for bacteria and yeast counts is 25–250 CFU. On the other hand, the limits suggested by WHO is less than 10^5CFU per ml or per dose. The results obtained in the present study lie within the limits suggested by both US pharmacopeia and WHO.

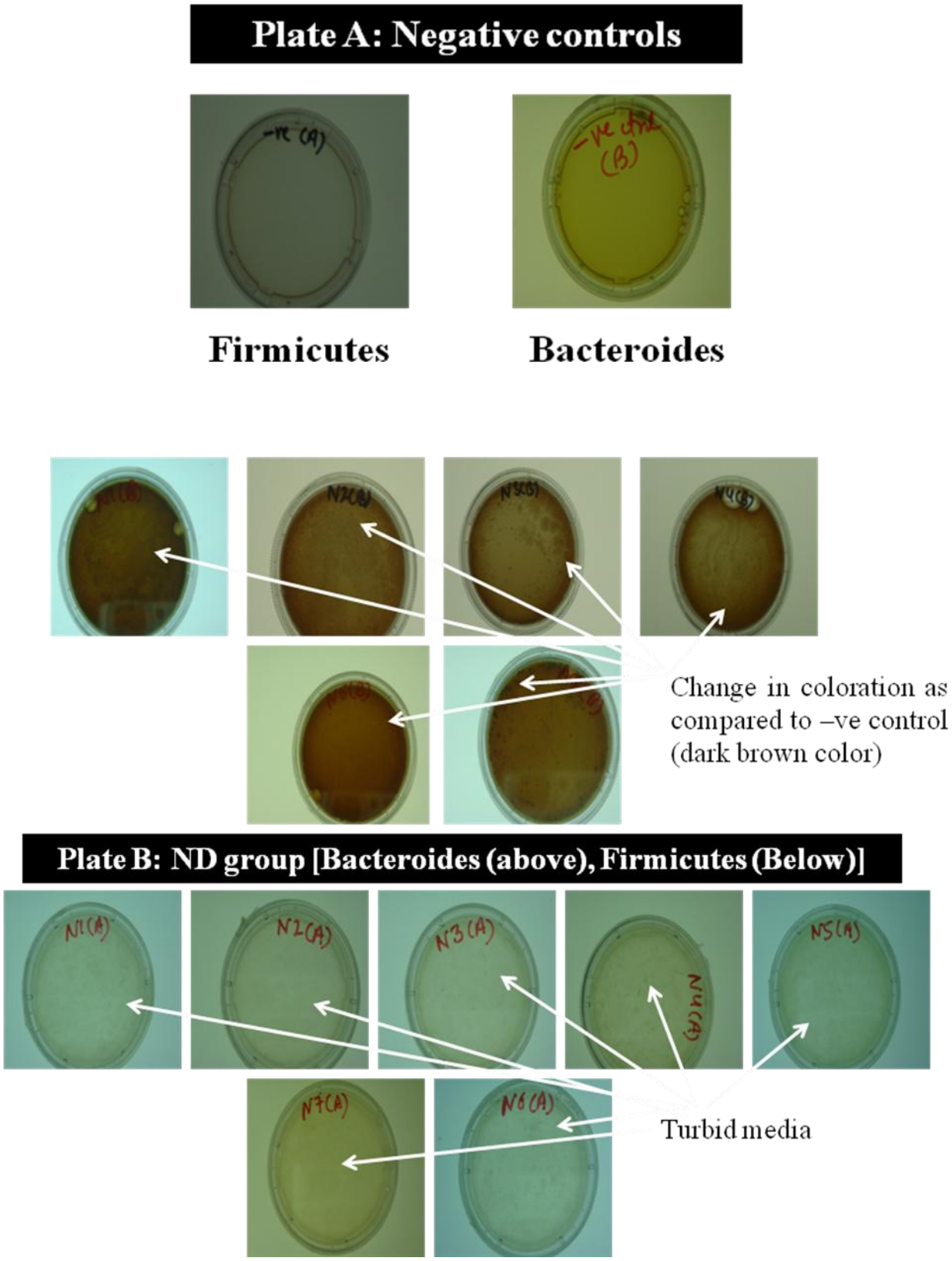
Table 6.01: Microbial Contamination in various Herbal Powders used in the present study

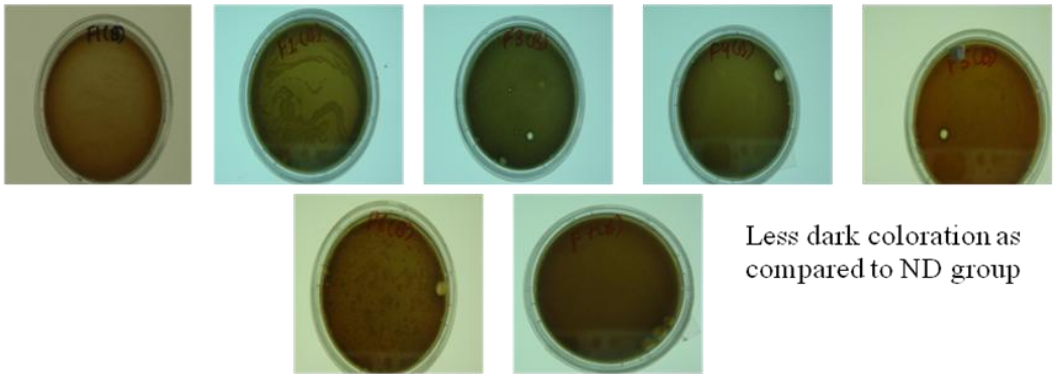
Plant Powders	TAMC (CFU)	TYMC (CFU)
Amla	100	5
Harad	60	10
Bahed	70	210

6.2 Fecal Pellet Culture: In order to estimate the presence of Bacteroides and Firmicutes in the gut, the fecal pellet from each mouse was used for culturing bacteria on Thioglycolate and Bile Esculin media.

Figure 6.01 illustrates the microbial plate culture for each group. The presence of Bacteroides is evident from the darkening of media in the Esculin media culture plate. The darkening of media is due to Esculin hydrolysis, which produces esculetin and dextrose. The esculetin reacts with the iron salt (ferric ammonium citrate) contained in the medium to produce a dark brown to black complex that appears in the medium surrounding the colonies of members of Bacteroides. To our surprise, the fecal pellets from the treated groups (Amla, Harad and Bahed) showed the presence of more Bacteroides as compared to Firmicutes. The proportion (percentage) of firmicutes was more in HFD group. The ND group on the other hand showed the presence of both Bacteroides and firmicutes.

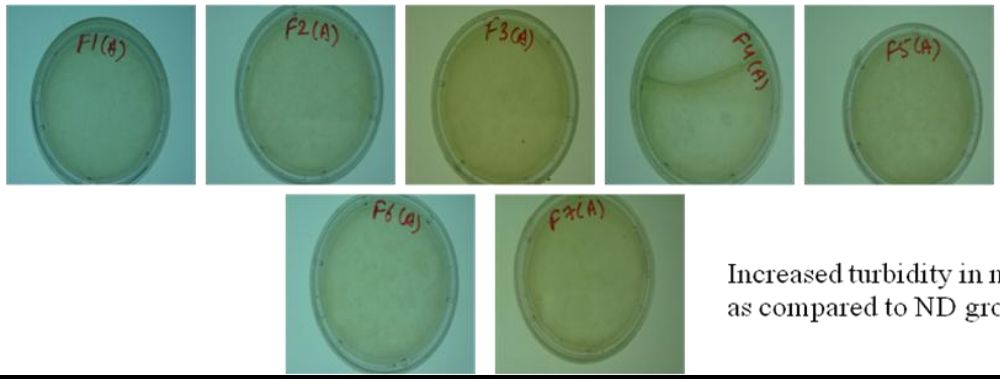
Figure 6.01: Plate A (-ve control), Plate B (ND group), Plate C (HFD group), Plate D (Triphala group), Plate E (Amla group), Plate F (Harad group) and Plate G (Bahed group).



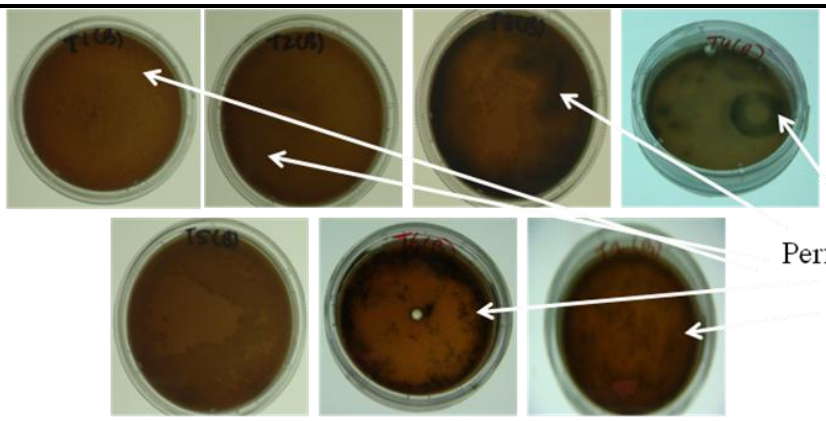


Less dark coloration as compared to ND group

Plate C: HFD group [Bacteroides (above), Firmicutes (below)]

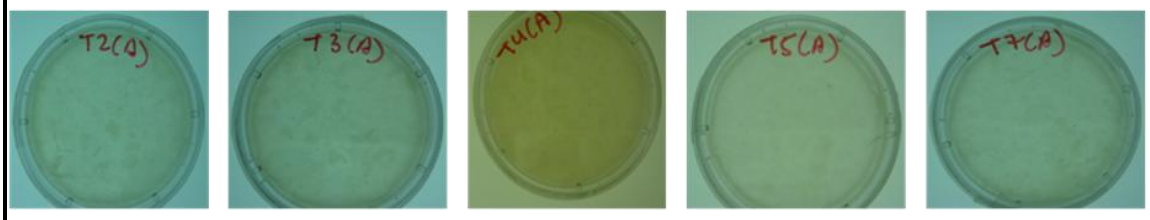


Increased turbidity in media as compared to ND group

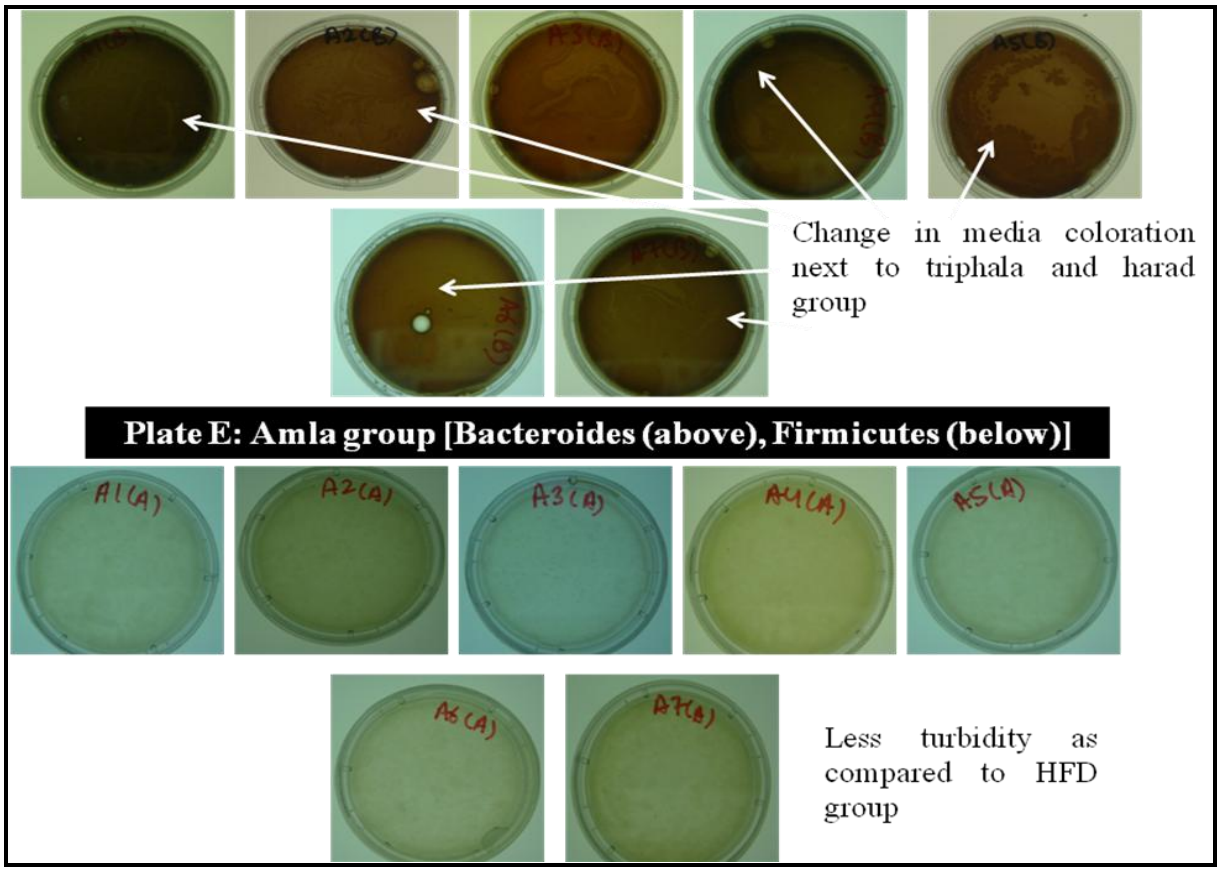


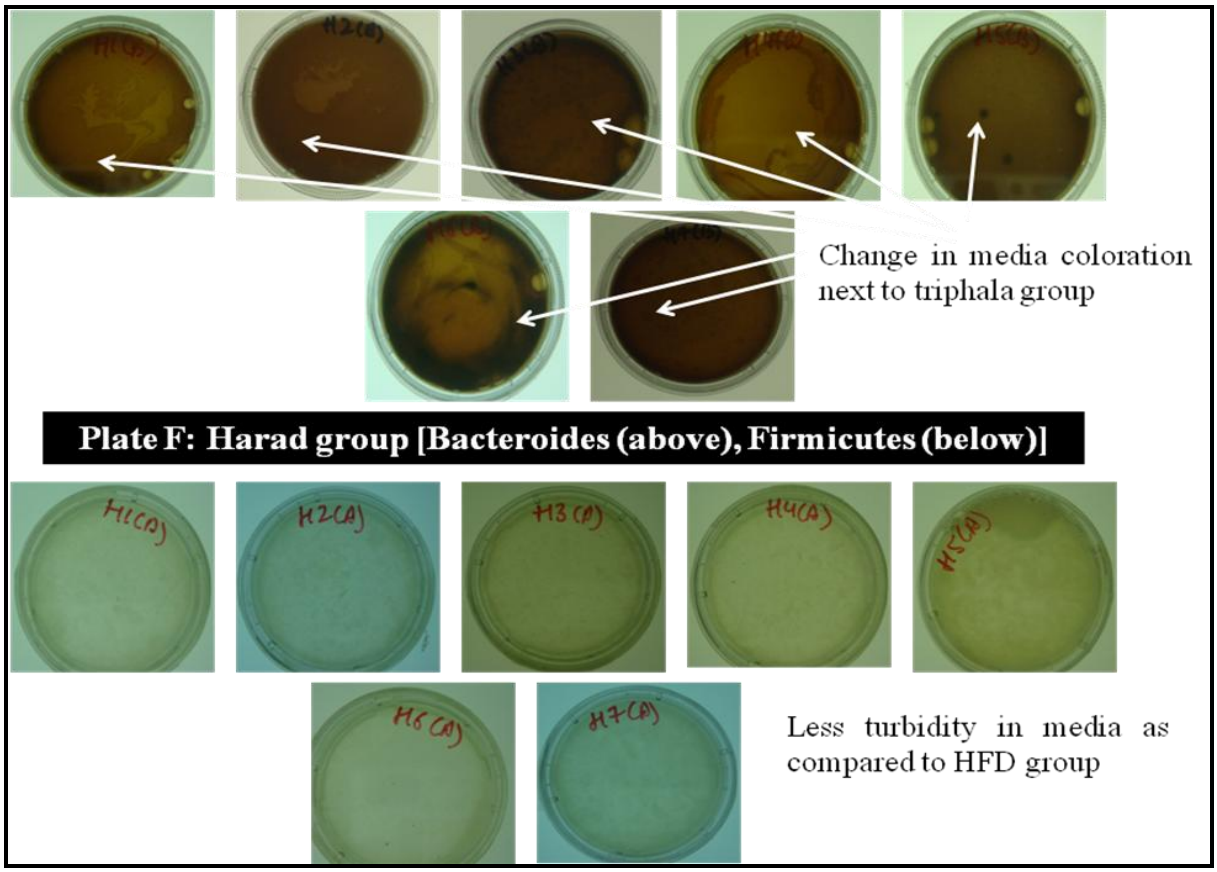
Perfect representation of Bacteroides presence

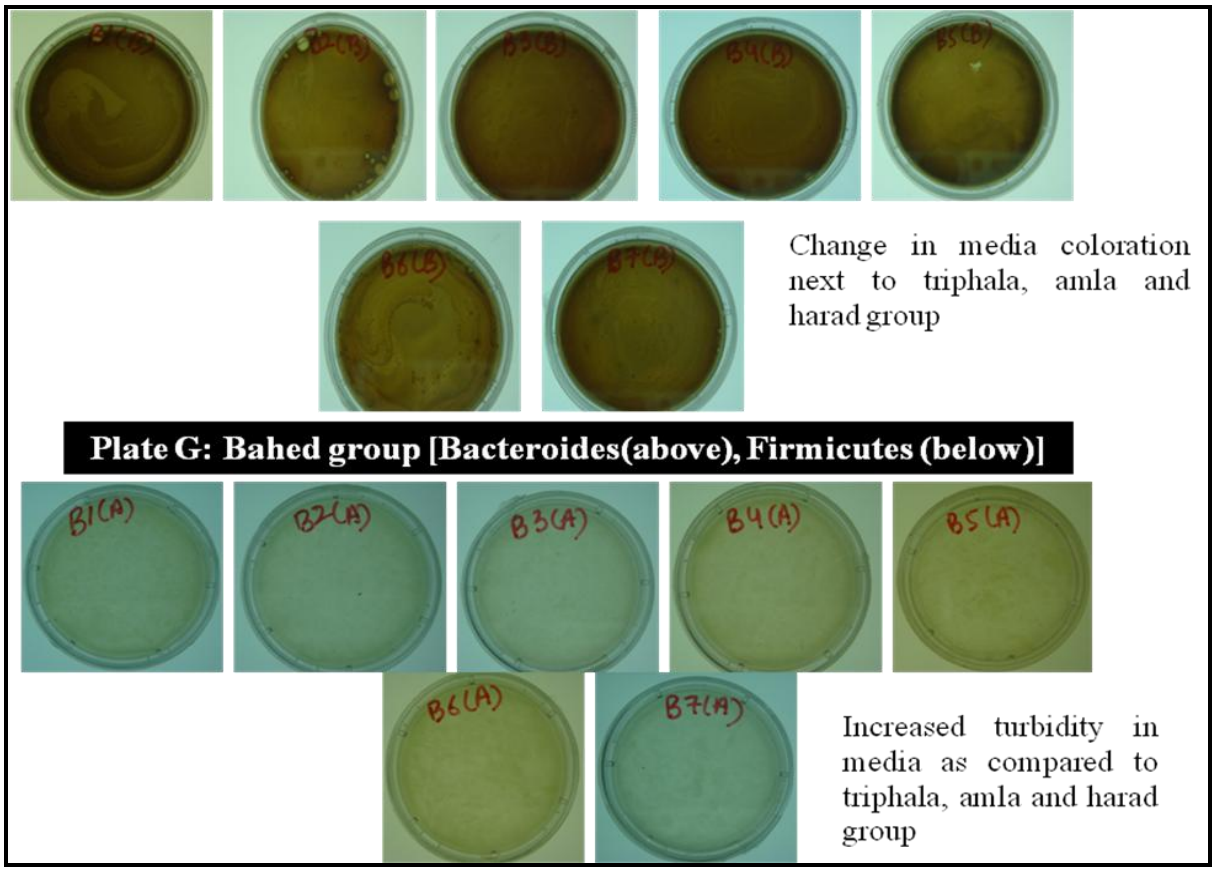
Plate D: Triphala group [Bacteroides (above), Firmicutes (below)]



Less turbidity in media as compared to ND group


Change in media coloration next to triphala and harad group
Plate E: Amla group [Bacteroides (above), Firmicutes (below)]
Less turbidity as compared to HFD group


Change in media coloration next to triphala group
Plate F: Harad group [Bacteroides (above), Firmicutes (below)]
Less turbidity in media as compared to HFD group


Change in media coloration next to triphala, amla and harad group
Plate G: Bahed group [Bacteroides(above), Firmicutes (below)]
Increased turbidity in media as compared to triphala, amla and harad group

6.3: Whole mount ND, HFD and Herbal Powder Treated Mice: The increase in weight of mice after feeding the High Fat Diet was visible through naked eyes. HFD fed mice were bulkier than the ND and the treated mice. Figure 6.02 shows the pictures of mice on normal diet, treatment group and high fat diet groups.

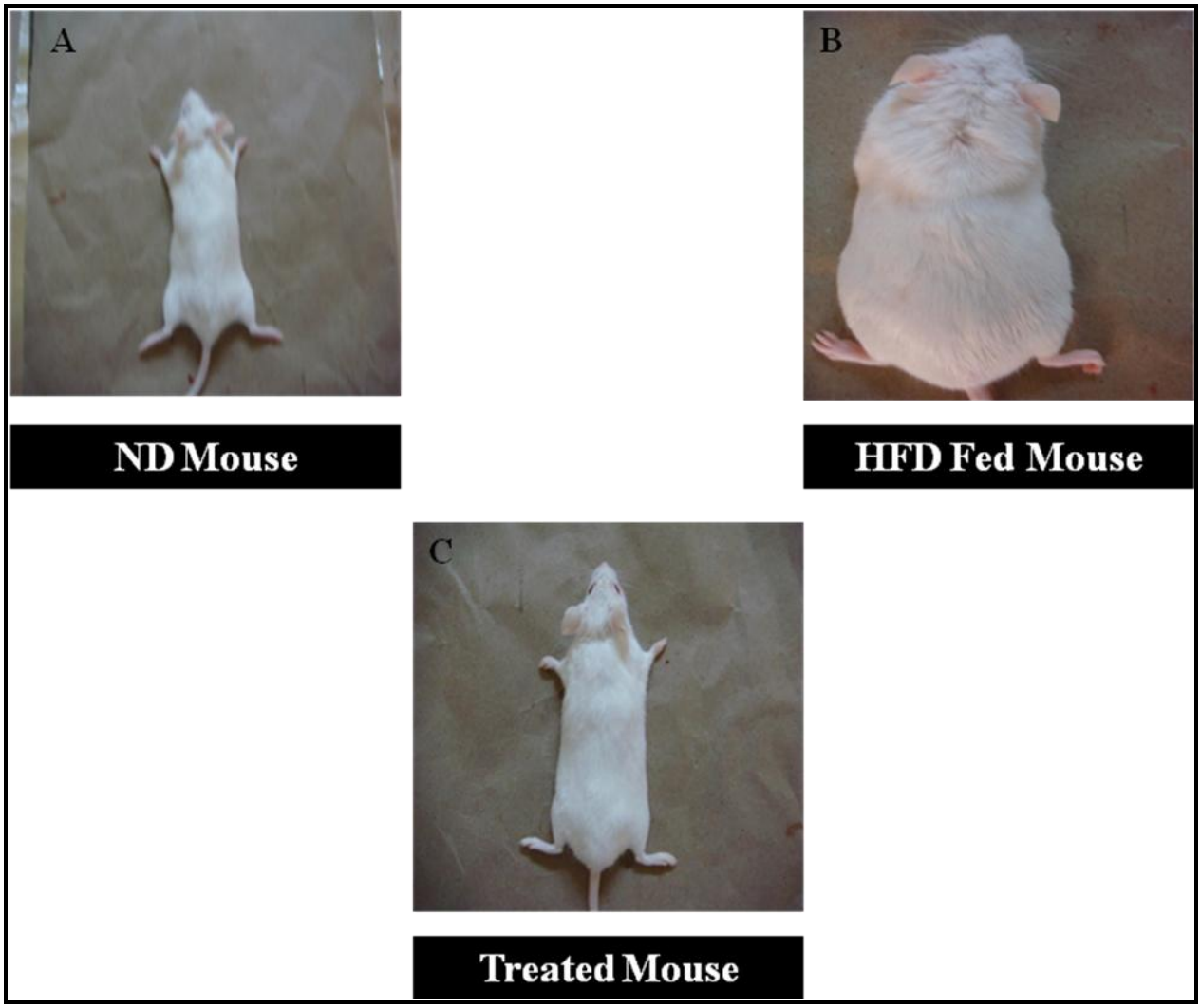


Figure 6.02: Whole mount ND mice (A), Whole mount HFD mice (B) and HFD + Herbal Powder Treated Mice (C)

6.4: Abdominal Fat Depots and Fatty Liver: Dissected figures of ND and HFD fed mice: The increase in accumulation of abdominal fat on feeding mice with high fat diet became further evident on dissecting the animals (Fig: 6.03). The increase in the fat deposit was evident even through the naked eyes. The differentiation between the fatty liver and normal liver could also be made through naked eyes (Fig: 6.03).

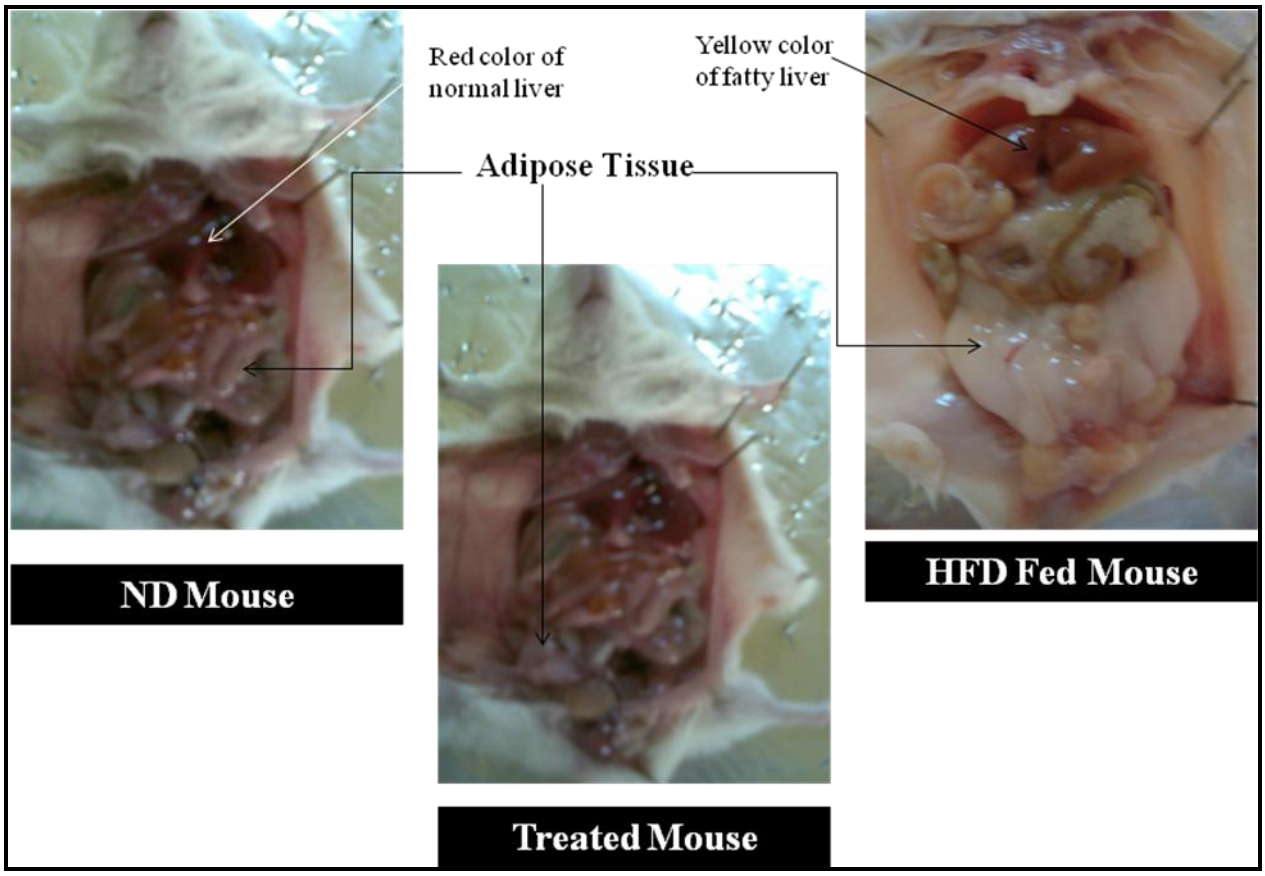


Figure 6.03: Dissected animals: ND mice, HFD + Herbal Powder Treated mice and HFD mice.

6.5 Histological Examination: The liver in all animals of the HFD group were enlarged and yellowish in color, indicative of liver steatosis. On administration of herbal powders in the HFD + T, HFD + A and HFD + H group, the liver remained reddish brown in color and appeared normal in texture and color with no abdominal or extra-hepatic fat deposition. Histological analysis revealed that the liver of the HFD group exhibited typical signs of fatty liver showing accumulation of many fat droplets in the liver acini, while animals treated with triphala, amla, and harad showed none or very small degree of lipid accumulation or other pathological changes. As Plate 6.04 shows, treatment with bahed did not seem to inhibit hepatic fat accumulation as compared to triphala, amla, and harad. Similarly, there was significant increase in size of adipose tissue cells in HFD group as compared to the ND group as well as HFD + T, HFD + A, HFD + H and HFD + B group (Plate 6.04: Exp II, Plate 6.05 :Exp III).

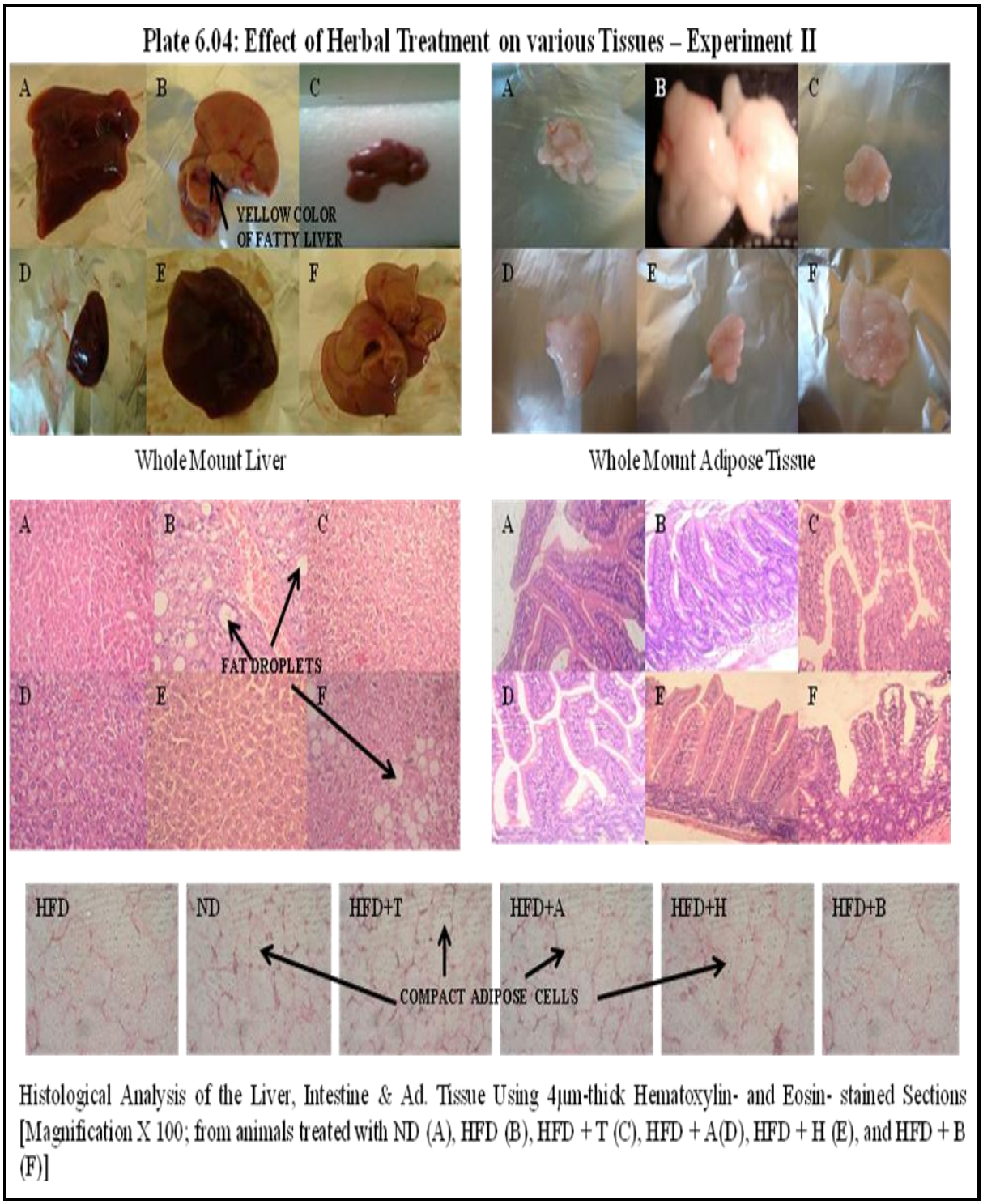


Plate 6.04: Effect of Herbal Treatment on various Tissues- Experiment II.

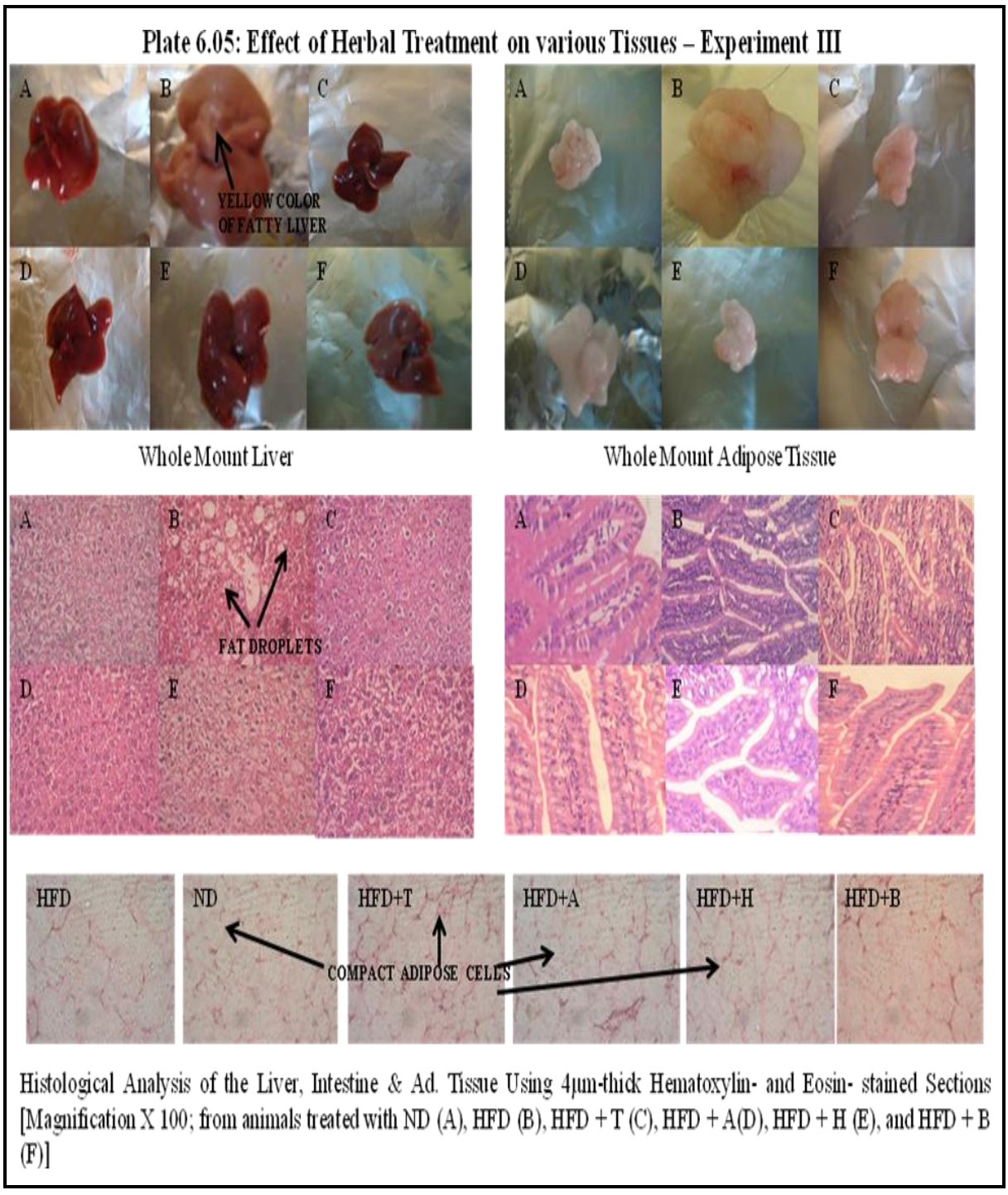


Plate 6.05: Effect of Herbal Treatment on various Tissues- Experiment III.

6.6 Gut microbiota analysis by Real Time PCR (RT-PCR): PCR based DNA amplification was used to determine the fluctuations in the population of bacteriodes and firmicutes and expressed as a ratio of Bacteroides to Firmicutes. The Figure 6.05 (A & B) shows the B/F ratio.

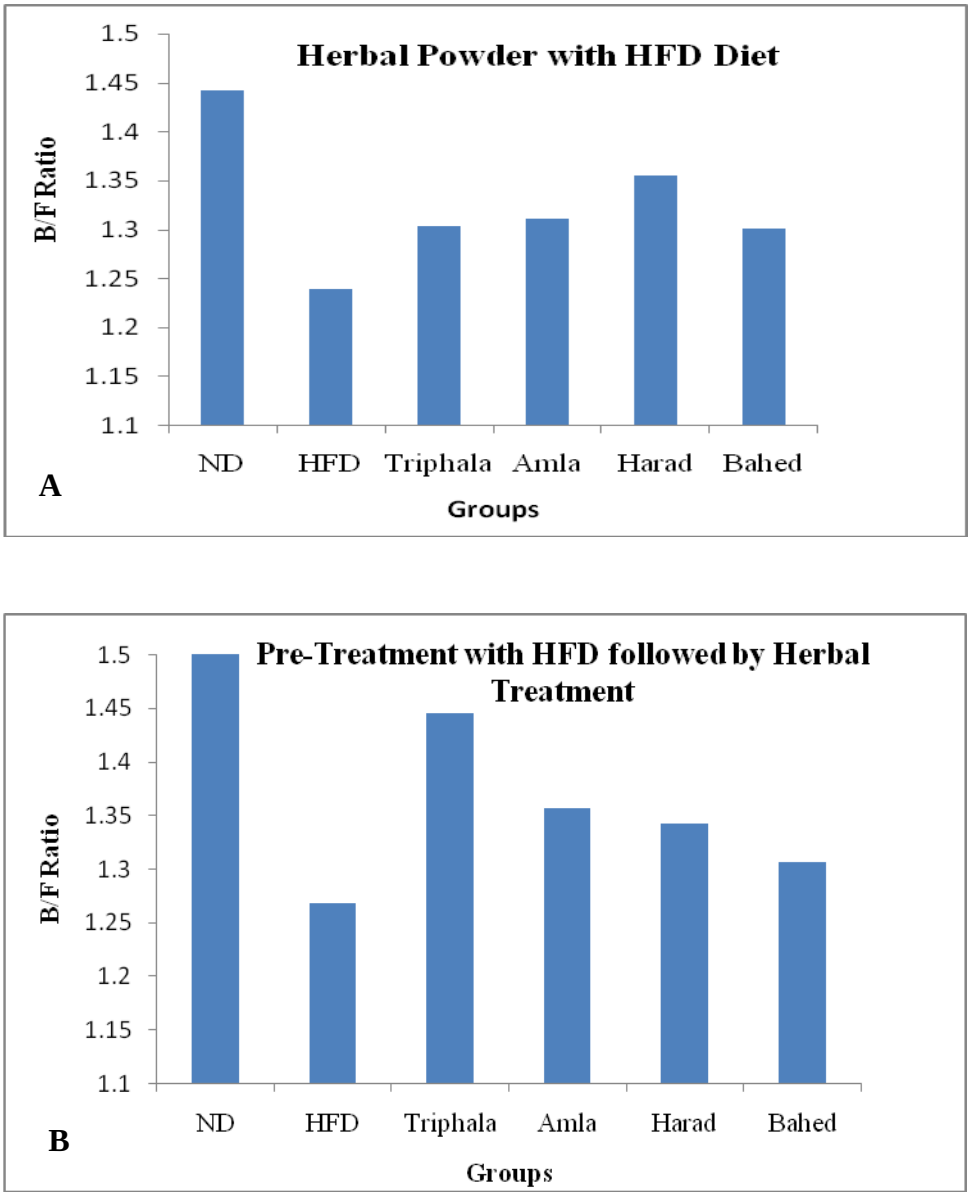


Figure 6.06: Bacteroides/Firmicutes ratio in different groups of ND, HFD and HFD + Herbal Powder treated mice

Chapter 7

Discussion

Discussion

The present study involves the screening of herbal powders for the treatment for visceral adiposity/ abdominal obesity along with the understanding the relation between obesity and gut microbiota. The present study assessed the preventive as well as the curative aspect of these herbal powders together as a formulation or separately in high-fat diet induced mice model for obesity. The relationship between anthropometric markers along with the effect on clinical and oxidative stress markers was also assessed. Interestingly these powders proved to be significantly effective in preventing as well as curing the metabolic syndrome i.e. obesity. Many anecdotal reports have documented triphala to be effective against obesity but these results have never been put under scientific investigation in recent years. Further, the effect of individual components of Triphala has never been tested for obesity.

In the present study the herbal powders viz., Triphala, Amla, Harad and Bahed were screened for the presence of microbial contamination as the relation between gut microbes and obesity was also being assessed in the present study. Whether these microbes are ingested along with these herbal powders or the ingestion of these powders manipulates the gut flora needs to be answered. The powders were procured from the GMP certified company and also screened for microbes according to the guidelines from US pharmacopeia. It was observed that these powders were free from any kind of microbial contamination. Thus it could be concluded here that may be the herbal treatment influences the gut flora once being ingested in the body and helps in combating the metabolic syndrome.

Once the powders passed the microbial contamination screening, these powders were then assessed for toxicity in swiss albino mice. In order to evaluate whether Amla, Harad and Bahed exhibited any acute toxic effects, the animals from the toxicity group were evaluated by following their body weight, several biochemical parameters (Glucose, cholesterol, triglyceride, urea, creatinine, alanine-aminotransferase, aspartate-aminotransferase and albumin) in serum using commercially available kits as per the manufacturers' instructions. The mortality rate was 0% and growth of all the experimental mice was normal as compared to the control animals. As far as clinical biochemistry was concerned, the values for all the biochemical parameters were in the normal range and comparable to those observed in the

control group. There was no difference between the treated groups and the control group. Thus it could be concluded that the plant powders were not toxic to mice.

Anti-obesity effect of Triphala, Amla, Harad and Bahed on High Fat-Diet Induced Mice model:

Anti-obesity effect of Triphala, Amla, Harad and Bahed was assessed both in terms of prevention of obesity as well as in curing the metabolic syndrome. Surprisingly, these powders proved to be effective both as preventive and curative. Although in both the experiments, combination of Amla, Harad and Bahed i.e. Triphala proved to be the most effective.

In the present study, a suppressed food intake does not appear to be the only cause of weight loss in the mice treated with herbal preparations, since in addition to food intake, the treatments also significantly lowered FER in these groups compared to the HFD mice. The initial body weights of all the groups were not significantly different (average body weight, 21 g); however, after 4 weeks, body weights began to diverge in the preventive experiment (Fig: 5.3). The body weight of ND group was significantly lower as compared to the HFD group. In the treatment groups, the body weight was significantly less as compared to the HFD group (Table 5.6). In the curative experiment, body weight began to diverge after the fifth week of the experimental duration i.e after one week of the start of the treatment (Fig: 5.25). The average weight gain and FER were significantly higher in the HFD group than in the ND and treatment groups (Table 5.12). As per ayurvedic text, Amla can dissolve accumulated fat within the body [Qureshi SA, 2009]. Kizhakkeveettil et al have reported treatment with Amla lead to reduced appetite and weight loss [Kizhakkeveettil, 2011]. Triphala and its constituents can reduce food consumption in human and rodent models of obesity, possibly by diverting carbohydrates and fatty acids that would have become fat in the liver into hepatic glycogen [Murali YK, 2007; Yokozawa T, 2007]. This metabolic change may send signal to the brain resulting in a reduced appetite. Phytochemical analysis of Amla, Harad and Bahed [Vani T, 1997] shows the presence of Polyphenols, tannins, flavinoides and glycosides; out of which tannins being the major proportion. Tannin content was reported to be 21% in *Terminalia belerica*, 30-32% in *Terminalia chebula* and 28% in *Emblica officinalis* [Vani T, 1997].

Researchers have shown the presence of other polyphenols viz. ellagic acid and gallic acid [Dharmananda S, 2007]. Recent studies have shown that presence of combination of marker compounds (i.e., gallic acid and ellagic acid) had the same antiobesity effect as the use of the whole crude extract itself thus indicating that gallic acid, ellagic acid represents the majority, if not all, of the responsible components that causes significant weight loss through unknown mechanisms [Cabrera et al, 2006; Wolfram et al, 2006; Thielecke, 2009]. Although the mechanisms of action are unknown, it is suspected that the observed anti-obesity effect is probably caused by multiple components with possible synergistic interactions at multiple sites of actions. For example, gallic acid has been reported to be an inhibitor of alpha-glucosidase, which may further reduce the breakdown of carbohydrates to simple sugars such as glucose and fructose also reported anti-obesity effect after administration of gallic acid at the dose of 100 mg/kg of body weight [Li et al, 2007; Hsu et al, 2007]. Ellagic acid, indeed, may be partially responsible in the anti-obesity effect in mice after the administration of pomegranate leaf extract containing 10.6% w/w of ellagic acid [Lei et al., 2007]. Gallic acid could play a role as an angiogenesis inhibitor and could cause reduced blood vessel formation and growth in the adipose tissues, thus leading to a reduced fat accumulation. Indeed recent report showed a close relationship between angiogenesis and obesity [Brakenhielm and Cao, 2008), a hypothesis can be formed that these powders may suppress the accumulation of adipose tissues thereby leading to weight loss. In support of this hypothesis, gallic acid has been shown to reduce the activity of α-amylase and bacterial activities, which may further decrease energy absorption by blocking the nutrient breakdown in the colon. Therefore, in addition to angiogenesis inhibition, we believe that the multiple components from Triphala, Amla, Harad and Bahed may work simultaneously in producing a significant weight loss effect via more than one specific mechanism. These possible multiple mechanisms of action, multiple action sites, and synergism of different bioactive components warrant further investigations. Regardless, it is safe to conclude that the current results verified the hypothesis.

Diet-induced obesity model is the simplest obesity-induction model, and possibly the one that most closely resembles the reality of obesity in humans. The use of high-fat diet (HFD) to induce obesity is well documented. The first description of use of HFD to induce obesity by a nutritional intervention was in 1959 [Masek and Fabry, 1959]. HFD diet significantly promotes the development of obesity [Kim et al., 2005; Saiki et al., 2007; de-Wit et al., 2008;

Kizelsztein et al., 2009]. Rats fed with HFD showed distinctive visceral adiposity, hyperglycemia, dyslipidemia, hyperinsulinemia, and hepatic steatosis, which are typically associated with human obesity [Pang et al., 2008]. Thus, in the present study, high fat diet (HFD) for 10 weeks was used to produced obesity and dyslipidemia in swiss albino mice. It is well established that fat over-consumption lead to obesity in number of animals models including mice [West and York, 1998]. Mice are frequently used as animal models for studying adverse effects of obesity [Iossa et al., 1999].

Obesity is usually taken as significant increase in body weight or energy content relative to control animals [Rothwell and Stock, 1981]. It is well documented that high fat diet produces rapid weight gain in mice as compared to standard diet fed mice [Milagroet al., 2006; Pang et al., 2008]. In our present study body weight was significantly increased in HFD fed mice as compared to ND mice. The energy intake is also an important factor in the regulation of body weight. It is documented that the average energy intake of high fat diet fed mice was higher than that of control diet fed mice [Murase et al., 2001; Milagro et al., 2006]. Our study is in agreement with the previous reports that there is increase in the energy intake (Kcal) in HFD group as compared standard diet group. The degree of obesity is most conveniently quantified by the body mass index (BMI) because of its ease of calculation and relatively accurate correlation with body fat content [Mun et al., 2001; Novelli et al., 2007]. That the BMI also is increased in HFD group as compared to normal diet group is well documented. In the present study also, the BMI was significantly increase in the HFD group.

This is the first report on the anti-obesity effect of the Triphala, Amla, Harad and Bahed on HFD induced obese mice model. Triphala and its constituents proved to be both “preventive” (prevented the weight gain when the treatment was given along with the high fat diet) as well as “curative” (caused the weight loss once the obese high fat-fed mice were given the treatment). Triphala, Amla, Harad and Bahed reduced significantly the body weight gain of the HFD induced obese mice model in the “preventive mode” experiment. In the second experiment, “curative mode”, Triphala, Amla, Harad and Bahed were again observed to reduce significantly the body weight gain of the HFD induced obese mice model. The total abdominal fat accumulation was reduced significantly both “preventively and curatively”.

Obesity may result due to increased adipose mass [Lin et al., 2000]. Studies in animals, as well as in humans, demonstrate that body fat is more closely related to the amount of fat ingested than to total caloric intake [Boozer et al., 1995; Lissner, 1995]. This is well documented that the relative weight of the total visceral fat-depots of the mice fed the HFD was significantly greater than the value for the ND mice (Pang et al., 2008), due to HFD the accumulation of visceral fat [Murase et al., 2001; Milagro et al., 2006] is also more. Therefore, the adipose tissue was weighed in the present study as an index of adiposity. Since abdominal fat accounts for the majority of weight gain in obese phenotypes, it is reasonable to state that the pronounced weight loss was in fact fat loss. An increase in liver weight is also documented in HFD fed animals [Buettner et al., 2006]. In the present study also there was an increase in the liver weight as compared to ND mice. Liver Steatosis and increased in size of adipose tissue by HFD administration in mice is also well documented [Adams, 2005; Milagro et al., 2006]. Therefore, in the present study extent of liver steatosis and size of adipose tissue was examined histologically using light microscope.

One of the most interesting results was that similar anti-obesity effects were observed by two drastically different approaches. In the first experiment, HFD fed mice were put on treatment with herbal powder along with the High Fat diet. In the second approach, HFD fed mice were allowed to become obese and then herbal treatment was started after induction of obesity. However, in both experiments the herbal powders were able to inhibit gain in body weight.

In addition to the observed anti-obesity effect, the blood glucose level was significantly improved after treatment. However, how Triphala and its constituents act as anti-obesogenic and glucose lowering effects is not clearly understood. Oxidative stress has significant effect in the etiology of obesity as well as obesity related complications in human beings [Wilson RL, 1998]. In obesity the oxidative stress co-exists with a reduction in anti-oxidant status. Oxidative stress has significant effect on the glucose transport protein (GLUT) and on insulin receptor activity [Jacqueline MS, 1997]. It is known that scavengers of oxidative stress may have an impact on reducing the increased serum glucose level in diabetes and may alleviate the other comorbid conditions associated with diabetes and reduce the secondary complications such as retinopathy and nephropathy. Treatment with Triphala significantly reduced the blood glucose level as compared to the HFD group. The constituents of triphala also exerted similar effects with Amla and Harad being more than Bahed. Similar results

were observed in case of Oral Glucose Tolerance Test. Recent studies have reported significant decrease in plasma glucose level, within 11 days in alloxan monohydrate-induced diabetes in rats [Sabu MC, 2002]. Amla, being rich in both vitamin C and polyphenols, can serve as a rich source of antioxidants, and function as a potent integral component of antioxidant protection systems to relieve oxidative stress during diabetes. Obesity and insulin resistance are interrelated. Recent studies have demonstrated the ability of gallic acid and ellagic acid to inhibit the activity of NF-κB [Liu et al, 2005) and α-amylase activity [Li et al, 2007] and both these factors are closely related to regulating glucose metabolism. Recent studies have shown decreased levels of Serum adiponectin under the conditions of obesity and insulin resistance Therefore, improvement in adiponectin levels can result in improved insulin sensitivity and glucose tolerance and can correct hyperlipidemia [Saltiel AR, 2001]. The oral administration of amla elevated the adiponectin levels in the streptozotocin-induced diabetic rats, suggesting improvement in glucose metabolism in diabetes [Rao, 2005]. These results form the scientific basis for the efficacy of triphala and its constituents in improving the glucose metabolism in HFD fed obese mice.

Obesity increases the likelihood of various diseases, particularly heart disease, type 2 diabetes, obstructive sleep apnea, certain types of cancer, osteoarthritis and asthma and is often accompanied with elevated plasma Total-C, Triglyceride and low-density lipoprotein cholesterol (LDL-C) and decreased levels of high density lipoproteincholesterol (HDL-C) [Kelley et al., 2002; Malnick and Knobler, 2006]. In general, high-fat diet significantly increases the Total-C levels in the serum and liver as compared with the normal control diet in mice [Ghasi S, 2000]. These clinical complications of obesity could be diminished when plasma lipid concentration is lowered by hypocholesterolemic agents. The effect of phytochemicals or flavonoids on serum and hepatic lipids are very relevant to cardiovascular diseases and some forms of cancer. [Stangle V, 2006]. In the present study, triphala and its constituents improved lipid profiles by lowering plasma Total-C and triglyceride concentrations, as compared with the HF group and the accumulation of hepatic droplets was also diminished. Tannin or tannin with a gallate group have been reported to bring about various physiological functions such as anti-lipidemic action in rats with hypercholesterolemia. [Choi C, 2000; Park SY, 2002; Gorinstein et al, 2000]. Many reports have suggested functional phenolic compounds, are also responsible for lipid-lowering

action. Beside antidiabetic and hypotriglyceridemic effects, the herbal treatment was also found to improve liver function as assessed by the activity of liver-specific enzyme ALT.

Oxidative stress:

Antioxidants are compounds that prevent the oxidation of essential biological macromolecules by inhibiting the propagation of the oxidizing chain reaction. Keeping in mind the adverse effects of synthetic antioxidants, researchers have channelled their interest in isolating natural antioxidants [Kuo PC, 2005] which are very effective in controlling the oxidative stress and hence prevent the initiation of disease propagation. Drugs that contain radical scavengers are well known for therapeutic activity [Yan et al, 1992; Liu and Xiao, 1994]. Certain plants exhibit efficient antioxidant properties due to their phenolic constituents [Toda et al., 1991]. Interestingly, quite a few studies on the antioxidant properties of the three plant materials, viz., T. chebula [Cheng HY, 2003; Chattopadhyay RR, 2007], T. belerica [Sabu MC, 2000; Sabu MC, 2009] and E. officinalis [Bhattacharya A, 1999; LiuXiaoli, 2008] have been done earlier. Phytochemical analysis of Triphala supports some previous observations for the presence of tannins. Tannin content was reported to be 21% in *Terminalia belerica*, 30-32% in *Terminalia chebula* and 28% in *Emblica officinalis* [Vani et al, 1997]. Tannins [Hong et al., 1995], phenols [Toda et al, 1991], lignin's and flavonoids [Faure et al, 1991] are reported to have significant antioxidant properties. The results from various free radical scavenging systems revealed that Triphala as a whole and its constituents were individually strong antioxidants. Prevention of haemolysis and mitochondrial lipid peroxidation further confirmed that it is active against the effects of free radicals on biological membranes. The antioxidant properties of Triphala can therefore be attributed to the presence of tannins. It is known that crude extracts from plants are more active pharmacologically than their isolated active principles due to the synergistic effects of the various compounds present in the extracts (Hamburger and Hostettman, 1991). All the powders suspensions (Amla, Harad, Bahed and Triphala) were equally efficient in scavenging superoxide and peroxide radicals. Free radicals cause damage in biological systems and in turn, induce cellular damage [Halliwell, 1985b] that may lead to cancer, rheumatism, liver injury, ischemic heart disease as well as ageing. Triphala can be considered a model herbal drug for combating oxidative stress.

Studies indicate that the fruit extracts contain significant amount of flavonoids and phenolic content, in the order *E.officinalis* > *T.chebula* > *T.belerica*, respectively. Both of these compounds have good antioxidant potential and their effects on human nutrition and health are considerable. The mechanism of action of flavonoids is through scavenging or chelating process [Yildirim A, 2000]. Phenolic contents are also very important plant constituents because of their scavenging ability due to their hydroxyl groups [Cook NC, 1996]. Moreover, ascorbic acid acting as a chain breaking antioxidant impairs the formation of free radicals in the process of formation of intracellular substances throughout the body, including collagen, bone matrix and tooth dentine [Beyer RE, 1994].

Oxidative stress as discussed earlier is reported to be directly or indirectly linked to both obesity and visceral adiposity. Obese patients displayed approximately 50% higher serum protein carbonyl groups concentration [Sledzinskiet al, 2008] and significantly higher MDA levels [Yesilbursaet al, 2005]. These finding were similar to the trend observed in present study. Even antioxidant enzyme levels (TSOD and Catalase) were found to be affected significantly in the present study (Table 5.8 & 5.14).Further, Crist et al, 2009 suggested that obesity and, especially abdominal adiposity is associated with elevated oxidative stress and decreased levels of total antioxidant activity and our findings are completely consistent with these observations.

It has been observed that treating mice with total extracts of medicinal plants increased the activity of all antioxidant enzymes such as SOD, CAT, GST and GSH. These enzymes are modulated in various diseases by free radical concentration, thus maintaining the balance between the rates of radical generation and scavenging. SOD and CAT are the two scavenging enzymes that remove the toxic free radicals [Wohaieb and Godin, 1987]. The first line of defense in oxidative stress is provided by SOD, an enzymatic antioxidant, which acts by quenching O_2^- (which is the first product of O2 radicals) and converting it into H_2O_2 [Pradhan *et al*, 2004] and hence diminishes the toxic effects due to this radical or other free radicals derived from secondary reactions [Arunabh et al., 1999]. Thus, they play a crucial role in maintaining the physiological levels of O2 and H2O2.Lowering of SOD occurs as more and more ROS like O_2^- are produced and subsequent inhibition of SOD activity by accumulated H_2O_2. Consequently this protective antioxidant mechanism is lowered causing continued unchecked membrane damage leading to rise in lipid and protein oxidation

products. The O2 − anion is known to inactivate CAT [Halliwell, 1984]. Catalase has been regarded as a major determinant of hepatic and cardiac antioxidant status (Wohaieb and Godin, 1987). It is known to be involved in detoxification of H2O2 concentrations (Yoshikawa et al., 1993). These enzyme activities were inactivated by ROS during obesity (Ahmed et al., 2000). In the present study, it was observed that the herbal treatment could increase the SOD and CAT activities in the high fat diet induced obese mice.

The present study supports the antioxidant potency of the fruit powders as evidenced by the increased level of these antioxidant systems in the treated mice. As shown by our present study, TBA-reactive substance levels of plasma in obese mice increased significantly, whereas the administration of Triphala and its constituent's significantly decreased the TBA-reactive substance levels. This reduction of the TBA-reactive substance level by Triphala and its constituents could be explained as an antioxidant effect of these herbal powders, which contains polyphenols, tannins, flavonoids and vitamin C. [Takako, 2007]. Amla fruits, being rich in both vitamin C and polyphenols, may be a rich source of antioxidants, and function as a potent integral component of antioxidant protection systems to relieve oxidative stress [Sabu MC, 2002]

Out of the rest of the blood parameters studied the plasma oxidative stress marker was found to be the most informative. In metabolic pathologies, such as obesity, the plasma oxidant/antioxidant profile is altered (Kłos-Rola *et al.*, 2004). The administration of herbal powders improved oxidative stress associated with obesity elevates the peroxidation of cellular lipids, which in turn are reflected by serum TBARS levels, which is a biomarker of oxidative stress [West IC, 2000; Wolff SP, 1993]. Our results also showed an elevated level of TBARS in the high fat diet induced obese mice, suggesting oxidative stress and peroxidation of lipids. The oral administration of herbal powders significantly decreased the TBARS levels, suggesting a reduction in the oxidative stress [Yamauchi T, 2003]. These results provide the scientific basis for the efficacy of triphala and its constituents in relieving oxidative stress.

Obesity and Gut microbiota:

The distal gut micro-organisms are composed of billions of bacteria and archaea [Backhed et al. 2005].Bacteroidetes and Firmicutes, which consist of more than 90% of all phylogenetic

types, are the two dominant bacterial divisions in the human and mouse gut [Eckburg et al.2005; Ley et al. 2005, 2006b]. The gut microbial communities are affected by various factors, including the food composition [Gibson 1995; Kleesen et al.2001; Duncan et al. 2007], kinship relationships [Ley et al. 2006a], environment [Pluske et al. 2007], and soon. Interestingly, recent studies reported that the microbial-community composition is affected by the body fat storage in mice [Ley et al. 2005] and humans [Ley et al.2006b]. Comparisons of the distal gut microbiota of obese and lean mice, and those of obese and lean humans revealed a statistically significant reduction in the relative abundance of Bacteroidetes and a significantly greater proportion of Firmicutes in obese animals than in lean controls [Ley et al. 2005, 2006b; Turnbaugh et al.2006].

In the present study, we quantified the proportion of Firmicutes and Bacteroidetes groups in fecal samples of control and treated mice. We found that there were lower percentages of Bacteroides division in obese mice in comparison to lean mice, and these results are consistent with previous studies in humans and mice [Ley et al. 2005, 2006b; Turnbaugh et al. 2006]. The results were consistent with recent report that increased abundance of Bacteroidetes correlated with the percentage loss of body weight in humans [Ley et al. 2006b]. However, the difference of percent Firmicutes was not significant between obese and lean mice in the present study, and these results were not coincident with Ley et al. [2005, 2006b], and may be attributed to a large number of genera and species in the Firmicutes division, or a small number of animals in this study.

Conclusions

The aim of this work was to study the anti-obesogenic effect of herbal powders viz., Triphala and its constituents separately on high fat diet induced obesity in mice model. The study also explored whether these herbal powders had any effect on gut microbiota. Information was gathered from reports on studies related to antiobesogenic effect of various herbal formulations including studies on triphala, amla, harad and bahed relating to obesity, oxidative stress, and studies on obesity and gut-microbiota to achieve this goal. To start with, a few Ayurvedic medicines used for treating obesity were selected. Based on interpretation of reported effects of Triphala, triphala and its three constituents were selected for further exploration and to study their effect in treating/ameliorating obesity. Second we explored the various diet induced obesity animal models, types of high fat diet and their availability, which required extensive data mining and literature survey.

The thesis starts with a review of all studies on obesity epidemic across the world and the various therapies used to cure obesity published before September 2012 (Chapter 2 and 3). The review in chapter 2 focuses on obesity epidemic, its prevalence (Global and Indian scenario). Various reports on future projections of obesity were studied. There are various studies which have classified obesity as a multifactorial phenotype and is characterized by various determinants. Although the epidemic of obesity really started to increase markedly in the 1980s, it is only since 1997 that WHO and many national governments have recognized the importance of obesity [Misra, 2008]. According to the WHO global estimates from 2008, more than one in ten of the world's adult population was obese [Figure 2.02]. Moreover, if the recent trends continue, by 2030 up to 57.8% of the world's adult population (3.3 billion people) could be overweight or obese.

Chapter 3 focuses on Ayurveda and ayurvedic concept of obesity. Ayurveda recognizes obesity as an imbalance in three doshas: vata, pitta and kapha. Ayurvedic approach for obesity takes into consideration the herbal medicines which not only cures the disease but also improves the entire homeostasis of the human body. When conventional medicine fails to treat chronic diseases and conditions such as obesity efficaciously and without adverse

events, many people seek unconventional therapies including herbal medicine. Although the number of randomized trials on complementary therapies has doubled every 5 years and the Cochrane library included 100 systematic reviews of unconventional interventions, none of these studies specifically mentioned herbal therapy in obesity. Triphala is one of the commonly used herbal medicines in Ayurveda. In recent years, a number of studies have found new uses for Triphala, including treatment for various forms of cancer. It is also found to have high antioxidant properties, and is useful for treatment against noise and stress induced conditions. Use of triphala as anti-obesity remedy has not yet tested clinically. This forms the basis of choosing triphala and its individual constituents for the study in this thesis. As oxidative stress is directly related to obesity, the three constituents of triphala and triphala itself is known for its anti-oxidant properties. As per Ayurvedic text, amla can dissolve accumulated fat within the body. Triphala and its constituents can reduce food consumption in humans and rodent models of obesity, possibly by diverting carbohydrates and fatty acids that would have become fat in the liver into hepatic glycogen.

Chapter 3 also focuses on the role of gut microbes in inducing obesity. Recently, it has been proposed that an alteration in the development or composition of the gut microbiota (known as dysbiosis) participates in the development of obesity.

This screening and mechanism based study of natural products for treating obesity was done on high fat diet induced mouse model (swiss albino strain). The work presented in this thesis focuses largely on the changes in anthropometric parameter, clinical parameters, oxidative stress markers and anti-oxidant markers. Clinical parameters were analyzed to study the effect of this treatment on the energy and fat metabolism. Our preliminary investigation on amelioration of visceral adiposity has been discussed in chapter 5. High fat diet significantly increased the body weight of HFD mice as compared to ND mice. Interestingly, the data from both preventive as well as curative aspect has shown similar trends. The herbal treatment showed significant reduction in body weight along with reduction in Food efficiency ratio (FER) and energy intake, and there was a marked decrease in BMI and Lee index of animals. The treatment reduced liver steatosis and adipose tissue accumulation. The present study followed hyperglycemia, hypercholesteramia and hyperlipidemia in obese mice. The treatment significantly improved the clinical parameters as compared to the HFD group. HDL concentration was significantly improved by the given herbal treatment.

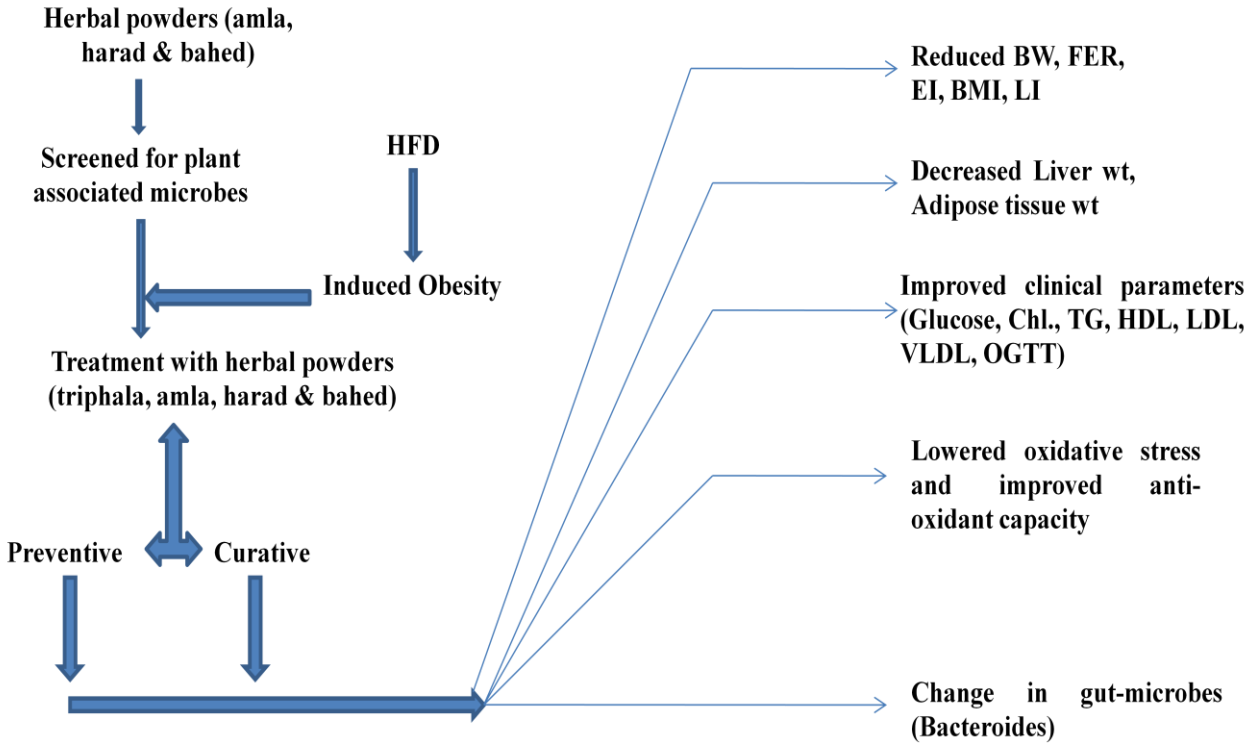
The latter half of chapter 5 investigated the plasma oxidant/antioxidant profile. The plasma oxidant/antioxidant profile was also found to be altered in HFD fed groups with significantly elevated levels of lipid peroxidation products (TBARS) and depressed antioxidant defense (TSOD and Catalase).

Treatment with triphala or its constituents was effective in reducing the body weight gain that high-fat diet induces and in improving related conditions such as fatty liver, hypercholesterolemia, dyslipidemia and oxidative stress in DIO-mice rendered obese by HFD. Amla, harad, and bahed were also effective in reducing the HFD-induced weight gain but to a lesser as compared to triphala powder. The research team herein reports for the first time the in vivo antiobesogenic effects of triphala and its constituents.

Chapter 6 of the thesis focuses on microbial culture of Bacteroides and Firmicutes from the fecal pellet from individual mice. The culture results clearly show the dominance of Bacteroides over the Firmicutes in Triphala, Amla and Harad groups with more in Triphala group as compared to others. The darkening of the media shows the presence of Bacteroides. More the darkening, the more is the abundance of Bacteroides in that particular sample/plate. The treatment has surly increased the Bacteroides population as compared to the depletion of bacteroides in HFD group. The effect was more pronounced in the triphala group. These results were further supported by the PCR analysis for presence of bacteroides and firmicutes of gut microbes. **Thus through this study, change in the ratio of Bacteroides and Firmicutes comes out to be one of the mechanism which plays a role in ameliorating the visceral fat deposition. This mechanism needs further exploration.**

Histological examination of the liver in all animals of the HFD group revealed enlarged livers, yellowish in color, indicating liver steatosis. On administration of herbal powders, the liver remained reddish brown in color and appeared normal in texture and color with no abdominal or extra hepatic fat deposition. Histological analysis revealed that the liver of the HFD group exhibited typical signs of fatty liver showing accumulation of many fat droplets in the liver acini, while animals treated with triphala, amla, and harad showed none or a very small degree of lipid accumulation or other pathological changes. The histological section shows the increase in size of adipose tissue which decreased when animals were treated with herbal powders.

The figure below summarizes the salient features of this thesis. Taken together, the results support the hypothesis that herbal treatments ameliorate visceral adiposity from a high-fat fed mice with diet-induced obesity.



The schematic presentation summarizing the work done in the thesis

Specific Contributions

The specific contributions of the present study are as follows:

- High fat diet produced an increase in food intake (kcal), body weight, body mass index (BMI), Lee index, weight of the adipose tissue, liver weight, and plasma lipid (total cholesterol, LDL, VLDL, HDL and triglycerides) and glucose levels to produce experimental obesity.
- Histologically studies showed liver steatosis and increase in the size of adipose tissue in HFD group as compared to standard diet control.
- Triphala significantly attenuated the high fat diet induced increase in body weight, FER, liver weight, hypercholesteramia, hyperlipidemia, blood glucose levels and oxidative stress.
- Triphala treatment also decreased the liver steatosis and adipose tissue size. Triphala also significantly increased the anti-oxidant capacity as compared to the HFD group.
- Individual constituents of triphala (amla, harad and bahed) also significantly attenuated the high fat diet induced increase in body weight, feed consumption, liver weight, hyperlipidemia, FER, liver weight, hypercholesteramia, hyperlipidemia, blood glucose levels and oxidative stress.
- Treatment with triphala and its individual constituents both proved to be effective as preventive and curative for obesity/metabolic syndrome.
- Treatment with triphala and its constituents also improved the Bacteroides/Firmicutes ratio as compared to that observed in HFD fed group thus forming one of the underlying mechanism in ameliorating the visceral fat deposition.
- **Collectively**, we present findings which suggest that triphala and its constituents can counter the effects of an environment (ie, high dietary intake of fats) and have the potential for use as antiobesity agents with desirable lipid-profile modulating properties and improving gut microbiota.

Future scope of the work

Future studies should continue to investigate the anti-obesity effect on other high fat diet-induced obesity animal models other than the one used in this thesis. Our findings on amelioration of visceral fat depots due to triphala and its constituents needs to be replicated with a larger sample size. As the effect of the herbal powders on reducing visceral adiposity has been proven, the underlying molecular pathways need to be investigated further. One of the well known mechanisms is mediated by Leptin gene. Leptin is an adipokine involved in body weight and food intake regulation whose promoter region presents CpG islands that could be subject to dynamic methylation. This methylation process could be affected by environmental (e.g. diet) or endogenous (e.g., adipocyte differentiation, inflammation, hypoxia) factors, and could influence adipocyte leptin gene expression. A step forward from this thesis could be investigating the role of Triphala and its constituents on leptin gene promoter methylation. It is well known that leptin methylation pattern can be influenced by diet-induced obesity, and suggest that epigenetic mechanisms could be involved in obesity by regulating the expression of important epiobesigenic genes. Another well known mechanism is mediated through Neuropeptide Y (NPY). NPY stimulates mouse and human fat growth, whereas pharmacological inhibition or fat-targeted knockdown of NPY2R is anti-angiogenic and anti-adipogenic, while reducing abdominal obesity and metabolic abnormalities. Thus, herbal powder may be involved in NPY2R activity within fat tissue and offer new ways to remodel fat and treat obesity and metabolic syndrome. Similarly the associations between herbal powders and gut microbiota demands further investigation to elucidate the biological mechanism of action and its role in disease progression.

Limitation of Study

To conclusively substantiate the role of herbal powders in treating obesity, it is necessary to replicate the findings of this study, in other obesity models. It may be possible that the effect observed due to these herbal powders may vary in terms of treatment duration in other high fat diet induced animal models. Also different types of high fat diets should be used to induce obesity. More molecular pathways should be analyzed in order to have better and clear understanding of the underlying mechanism for the observed effects of herbal powders. In animal studies a group of six or seven is statistically sufficient. As we have studied the effect gut microbes, also the effect on bacterial lipopolysaccharide [LPS], commonly called as metabolic endotoxemia, should be studied further.

References:

- Abdollahi M, Afshar-Imani B. A review on obesity and weight loss measures. *Middle East Pharmacy*. 2003; 11: 6-10.
- Abelson P, Kennedy D. The obesity epidemic. *Science* 2004; 304:1413.
- Agrawal P. Emerging Obesity in Northern Indian States: A Serious threat for Health. *IUSSP Conference, Bankik*. 2002; June: 10-12.
- Alberti K. G, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. *Lancet*. 2005; 366: 1059-62.
- Amirkhizi F, Siassi F, Minaie S, Djalali M, Rahimi A, Chamari M. Is obesity associated with increased plasma lipid peroxidación and oxidative stress in women? ARYA. *Atheroscler. J*. 2007; 2, 189–192.
- Amrithpal S. Medicinal Plants of the World, Published by Mohan Primlani for Oxford and IBH Co. Pvt, New Delhi.2006.
- Amrithpal SS. Herbalism phytochemistry and Ethanopharmacology, Science Publishers. 2011; 357-361.
- Anand KK, Singh E, Saxena AK, Chandan BK, Gupta VN. Hepatoprotective studies of a fraction from the fruits of *Terminalia belerica* Roxb. on experimental liver injury in rodents. *Phytother. Res*. 1994; 8: 287-292.
- Anila L, Vijayalakshmi NR. Antioxidant action of flavonoids from *Mangifera indica* and *Emblica officinalis* in hypercholesterolemic rats. *Food Chem.* 2003; 83, 569–574.
- Anila L, Vijayalakshmi NR. Flavonoids from *Emblica officinalis* and *Mangifera indica*-effectiveness for dyslipidemia. *J. Ethnopharmacol*. 2002; 79, 81–87.
- Anonymous. The Wealth of India. Raw Materials. CSIR, New Delhi, India. 1976; 10:168-170.
- Anonymous. The Wealth of India. Raw Materials, CSIR New Delhi, India. 1952; 3: 168.
- Anwarul HG, Arif-Ullah K, Tuba A, Saad A. Anti Spasmodic and Bronchodialatory Properties of *Terminalia belerica* Fruit, *Journal of Ethanopharmacology*. 2008; 116:528-538.
- Arif-Ullah K, Anwar HG. Pharmacodynamic Evaluation of *Terminalia belerica* for its Anti Hypertensive Effect. *Journal of Food and Drug Analysis* .2008; 16:6-14.

- Arif-Ullah K, Anwarl HG. Anti-Secretory & Analgesic Activities of *Terminalia belerica. African Journal of Biotechnology*. 2010; 9:2717-2719.
- Ascaso JF, Romero P, Real JT, et al. Abdominal obesity, insulin resistance, and metabolic syndrome in a southern European population. *Eur J Intern Med*. 2003; 14:101–106.
- Aslokar L, Kakkar KK, Chakre OJ. Supplement to Glossary of Indian Medicinal Plants with Active Principles. Directorate CSIR, New Delhi, India. 1992; 291-293.
- Asmawi MZ, Kankaanranta H, Moilanen E, Vapaatalo H. Anti-inflammatory activities of *Emblica officinalis* Gaertn. Leaf extracts. *J. Pharm. Pharmacol*. 1993; 45, 581–584.
- Aurasorn S, Pattana S, Kornkanok I. Effects of *Terminalia belerica* Roxb. Methanolic extract on mouse immune response in vitro, Maejo. *International Journal of Science and Technology*. 2008; 02(2):400-407.
- Badrul A. Antioxidant, Antimicrobial and Toxicity studies of the Different Fractions of Fruits of *Terminalia belerica* Roxb. *Global Journal of Pharmacology*. 2011; 5(1):07-17.
- Bandyopadhyay SK, Pakrashi SC, Pakrashi A. The role of antioxidant activity of *Phyllanthus emblica* fruits on prevention from indomethacin induced gastric ulcer. *J. Ethnopharmacol*. 2000; 70, 171–176.
- Barsh GS, Farooqi IS & O'Rahilly S. Genetics of body weight regulation. *Nature*. 2000; 404, 644–651.
- Basciano et al. Fructose, insulin resistance, and metabolic dyslipidemia *Nutrition & Metabolism.* 2005; 2:5.
- Bell C, Walley A, Froguel P. The genetics of human obesity. *Nat Rev Gene.* 2005;6(3):221-34
- Bhattacharya A, Chatterjee A, Ghosal S, Bhattacharya SK. Antioxidant activity of active tannoid principles of *Emblica officinalis* (amla). *Indian journal of experimental biology*. 1999; 37 (7): 676–680.
- Bimlesh K, Kalyani D, Prashant T, Manoj S, Diwakar G. Evalution of Anti-Diarrhoeal Effect of Aqueous And Ethanolic Extracts of Fruits Pulp of *Terminalia belerica* In Rats. *International Journal of Drug Development and Research*. 2010; 2(4):769-779.

- Block G, Dietrich M, Norkus EP, Morrow JD, Hudes M, Caan B, Packer L. Factors associated with oxidative stress in human populations. *Am. J. Epidemiol*. 2002; 156, 274–285.
- Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. *Diabetes*. 1997; 46(1):3-10.
- Börjeson M. The etiology of obesity in children. A study of 101 twin pairs. *Acta Paediatr Scand*. 1976; 65(3):279-87.
- Bouchard A, Tremblay J, Deapres A, Nadeau P, et al. The response to long-term overfeeding in identical twins. *New England Journal of Medicine*. 1990; 322, 1477–1482.
- Bouchard C, Despres JP, Tremblay A. Genetics of obesity and human energy metabolism. *Proc Nutr Soc* (in press).
- Bouchard C, Tremblay A, Despres JP, et al. The response to long-term overfeeding in identical twins. *N Engl J Med*. l980; 322:1477-82.
- Bouchard C. Genetic aspects of human obesities. *Proceedings of the Italian Society for the Study of Obesity*, Catania meeting. Basel, Switzerland.
- Bouchard C. Genetics of body fat, energy expenditure, and adipose tissue metabolism. *Recent advances in obesity research*.1987; 16-25.
- Bouchard C. Is obesity inherited? *Rev Prat*. l990; 20:1773-6.
- Brakenhielm E, Cao Y. Angiogenesis in Adipose Tissue. *Methods Mol. Biol*. 200; 456: 65-81.
- Buettner R, Scholmerich J, Bolheimer LC. High-fat diets: Modeling the metabolic disorders of human obesity in rodents. *Obesity*. 2007; 15:798-808.
- Butler AA, Cone RD. Knockout models resulting in the development of obesity. *Trends Genet.* 2001; 17(10):S50-4.
- Cabrera C, Artacho R, Gimenez R. Beneficial Effects of Green Tea – A Review. *J. Am. Coll. Nutr*. 2006; 25(2): 79-99.
- Capel I, Dorrell H. Abnormal antioxidant defense in some tissues of congenitally obese mice. *Biochemistry*. 1984; 219, 41–49.
- Caterson I, Gill T. Obesity: epidemiology and possible prevention. *Best Practice & Research Clinical Endocrinology & Metabolism*. 2003; 16:595 – 610.

- Chan JCN, Cheung JCK, Stehouwer CDA et al. The central roles of obesity-associated dyslipidemia, endothelial activation and cytokines in the metabolic syndrome – an analysis by structural equation modeling. *Int J Obes Relat Metab Disord*. 2002; 26: 994–1008.
- Cheng HY, Lin TC, Yu KH, Yang CM, Lin CC. Antioxidant and free radical scavenging activities of *Terminalia chebula*. *Biological and pharmaceutical bulletin*. 2003; 26, 1331–1335.
- Chicco et al. Muscle lipid metabolism and insulin secretion are altered in insulin-resistant rats fed a high sucrose diet. *Journal of Nutrition*. 2003; 133: 127–133.
- Chopra RN, Nayar SL, Chopra IC. Glossary of Indian Medicinal Plants, CSIR, New Delhi, India. 1956; 106:241-242.
- Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *Br Med J*. 2000; 320: 1240–3.
- Daly ME, Vale C, Walker M, Alberti KG, Mathers JC. Dietary carbohydrates and insulin sensitivity: a review of the evidence and clinical implications. *Am J Clin Nutr*. 1997; 66:1072-1085.
- De Wit NJ, Bosch-Vermeulen H, de Groot PJ, et al. The role of the small intestine in the development of dietary fat-induced obesity and insulin resistance in C57BL/6J mice. *BMC Med Genomics*. 2008; 1:14.
- Deng Y, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. *Ann. N. Y. Acad. Sci*. 2010; 1212, E1–E19.
- Dreon DM, Frey-Hewitt B, Ellsworth N, Williams PT, Terry RB, Wood PD. Dietary fat: carbohydrate ratio and obesity in middle-aged men. *Am J Clin Nutr*. 1988; 47:995-1000.
- Dulloo AG, Jacquet J, Solinas G, Montani JP, Schutz Y. Body composition phenotypes in pathways to obesity and the metabolic syndrome. *Int. J. Obes*. 2010; 34 (2), S4–S17.
- Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley GE. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol*. 2007; 73:1073–1078.

- Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, Flint HJ: Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes (Lond).* 2008; 32:1720-1724.
- Duvnjak M, Lerotic I, Barsic N, Tomasic V, Jukic L, Velagic V. Pathogenesis and management issues for non-alcoholic fatty liver disease. *World. J. Gastroenterol*. 2007; 13, 4539–4550.
- Eckburg PB, Bik EM, Bernstein CN, Purdom E, et al. Diversity of the human intestinal microbial flora. *Science*. 2005; 308:1635-1638.
- Elizabeth KM. Anti microbial Activity of *Terminalia belerica*. *Indian Journal of Clinical Biochemistry*. 2005; 20(2):150-153.
- El-Mekkawy S, Meselhy MR, Kusumoto IT, Kadota S, Hattori M, Namba T. Inhibitory effects of Egyptian folk medicines on human immune-deficiency virus (HIV) reverse transcriptase. *Chem. Pharm. Bull*. 1995; 43, 641–648.
- Esposito K, Ciotola M, Giugliano D. Oxidative stress in the Metabolic Syndrome. *J. Endocrinol. Invest*. 2006; 29, 791–795.
- Fall CHD. Non-industrialized countries and affluence. In type 2 diabetes: the thrifty phenotype. *British Medical Bulletin*. 2001; 60, 33–50.
- Farooqi I, O'Rahilly S. New advances in the genetics of early onset obesity. *Int J Obes (Lond)*. 2005; 29(10):1149-52.
- Faure M, Lissi E, Torres R, Videla LA. Antioxidant activities of lignans and flavanoids. *Phytochemistry*. 1991; 29: 3773-3775.
- Fonseca-Alaniz MH, Takada J, Alonso-Vale MI, Lima FB. Adipose tissue as an endocrine organ: From theory to practice. *J. Pediatr*. 2007; 83 (5), S192–S203.
- Friedman J. Modern science versus the stigma of obesity. *Nat Med*. 2004; 10 (6):563-9.
- Furukawa S. Increased oxidative stress in obesity and its impact on metabolic syndrome. *Journal of Clinical Investigation*. 2004; 114: 1752-1761.
- Gaiva MH, Couto RC, Oyama LM, Couto GE. Polyunsaturated fatty acid-rich diets: effect on adipose tissue metabolism in rats. *Br. J. Nutr*. 2001; 86(3):371-7.
- Gajda et al. Ticlopidine attenuates progression of atherosclerosis in apolipoprotein E and low density lipoprotein receptor double knockout mice. *European Journal of Pharmacology*. 2007; 556 (1-3): 129-135.

- George V, Tremblay A, Despres JP, et al. Further evidence for the presence of small and large eaters among women. *Am J Gin Nutr*. 1991; 53:425-9.
- George V, Tremblay A, Despres JP, Leblanc C, Perusse L, Bouchard C. Evidence for the existence of small eaters and large eaters of similar fat free mass and activity level. *Int J Obes*. l989; l3:43-53.
- Ghodke Y, Joshi K, Patwardhan B. Traditional medicine to modern pharmacogenomics: Ayurveda Prakriti type and CYP2C19 gene polymorphism associated with the metabolic variability. *Evid Based Complement Alternat Med.* 2009.
- Gibson GR, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr.* 1995; 125: 1401-12.
- Gopalani A, Sarmandal BA. Obesity. Kottakkal Ayurveda Series 67. Kottakkal: *Arya Vaidya Sala*. 2007; 84.
- Gopinath N, Chadha SL, Jain P, Shekhawat S, Tandon R. An epidemiological study of obesity in adults in the urban population of Delhi. *J Assoc Physicians India*. 1994; 42: 212–215.
- Guarner F, Malagelada R. Gut flora in health and disease. *The Lancet*. 2003, 361: 512-519.
- Guarner F: Inulin and oligofructose: impact on intestinal diseases and disorders. *Br J Nutr*. 2005; 93(1):S61-S65.
- Guo X, Xia X, Tang R, Zhou J, Zhao H, Wang K. Development of a real-time PCR method for Firmicutes and Bacteroidetes in faeces and its application to quantify intestinal population of obese and lean pigs. Letters in Applied Microbiology .2008; 47(5): 367–373.
- Gupta AK, Ahmad AJ. Childhood obesity and hypertension. *Indian Pediatr*. 1990; 27: 333–337.
- Gupta KA, Upadhyaya YN. Vagbhata's Ashtangahridayam Vidyotini commentary. Varanasi, India: *Chaukhambha Prakashana*. 2007.
- Gupta KA. Vagbhata's Ashtanga Samgraha. Bombay, India: *Nirnayasagar Press*. 1951.
- Gupta R, Gupta VP, Sarna M, Prakash H, Rastogi S, Gupta KD. Serial epidemiological surveys in an urban Indian population demonstrate increasing

coronary risk factors among the lower socioeconomic strata. *J Assoc Physicians India*. 2003; 51: 470–477.

- Habib-ur-Rehman, Yasin KA, Choudhary MA, et al. Studies on the chemical constituents of Phyllanthus emblica. *Nat. Prod. Res*. 2007; 21 (9): 775–81.
- Halliwell B, Gutteridge JMC. Free radicals ageing and disease. *Free radicals in Biology and Medicine*. Clarendon, Oxford. 1985a; 279-315.
- Hamburger M, Hostettmann K. Bioactivity in plants. The link between phytochemistry and medicine. *Phytochemistry*. 1991; 30: 3864-3874.
- Hardeman W, Griffn S, Johnston M, Kinmonth AL, Wareham NJ. Interventions to prevent weight gain: a systematic review of psychological models and behaviour change methods. Int J Obes Relat Metab Disord. 2000; 24: 131-143.
- Hardeman W, Griffn S, Johnston M, Kinmonth AL.Wareham of psychological models and behaviour change methods. *Int J Obes Relat Metab Disord*. 2000; 24: 131-143 NJ.
- Hartwich J, Goralska J, Siedlecka D, Gruca A, Trzos M, Dembinska-Kiec, A. Effect of supplementation with vitamin E and C on plasma hsCPR level and cobalt-albumin binding score as markers of plasma oxidative stress in obesity. Genes Nutr. 2007; 2, 151-154.
- Hasani-Ranjbar S, Larijani B, Abdollahi M. A systematic review of Iranian medicinal plants useful in diabetes mellitus. *Arch Med Sci*. 2008; 4: 285-292.
- Hasani-Ranjbar S, Nayebi N, Larijani B, Abdollahi M. A systemic review of the efficacy and safety of herbal medicines used in the treatment of obesity. *World J Gastroenterol*. 2009; 15(25): 3073-3085.
- Haslam D, James P. Obesity. *Lancet*. 2005; 366: 1197–1209.
- Hatakka K, Saxelin M: Probiotics in intestinal and non-intestinal infectious diseases — clinical evidence. *Curr Pharm Des.* 2008; 14:1351-1367.
- Hebebrand J, Friedel S, Schäuble N, Geller F, Hinney A. Perspectives: molecular genetic research in human obesity. *Obes Rev*. 2003; 4(3):139-46.
- Heilbronn LK, Campbell LV. Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. *Curr Pharm Des*. 2008; 14: 1225-30.
- Higdon J, Frei B. Obesity and oxidative stress: A direct link to CVD? *Arterioscler. Tromb. Vasc. Biol*. 2003; 23, 365–367.

- Hill J, Peters J. Environmental contributions to the obesity epidemic. *Science*. 1998; 280 (5368):1371-4.
- Hong CY, Wang CP, Huang SS, Hsu Fe. The inhibitory effect of tannins on lipid. peroxidation of heart mitochondria. *J. Pharm. Pharmacal*. 1995; 47: 138-142.
- Hsiao WW, Fraser-Liggett CM: Human Microbiome Project — paving the way to a better understanding of ourselves and our microbes. *Drug Discov Today*. 2009; 14:331-333.
- Hsu C, Yen G. Effect of Gallic Acid on High Fat Diet-induced Dyslipidaemia, Hepatosteatosis and Oxidative Stress in Rats. *Br. J. Nutr*. 2007; 98: 727-735.
- IIPS and Macro International. National Family Health Survey (NFHS-3), 2005–06: India. *International Institute for Population Sciences: Mumbai*. 2007.
- Ikemoto, K. Geffard, M. Maeda,T. Electron-microscopic study of the dopaminergic structures in the medial subdivision of the monkey nucleus accumbens . *Exp. Brain. Res.* 1996; 111 (1): 41-50.
- Indian Herbal Pharmacopoeia Revised New Edition. Published by *Indian Drug Manufacturer's Association, Mumbai*. 2002; 429-438.
- James WPT, Ferro-Luzzi A, Waterlow JC. Definition of chronic energy deficiency in adults. Report of a Working Party of the International Dietary Energy Consultative Group. *Eur J Clin Nut*. 1988; 42: 969–81.
- James WPT. The epidemiology of obesity: the size of the problem. *Journal of Internal Medicine*. 2008; 1365-2796.
- Jequier E, Schutz Y. New evidence for a thermogenic defect in human obesity. *Regulation of energy expenditure*. 1985; 1-7.
- Jia W, Li H, Zhao L, Nicholson JK: Gut microbiota: a potential new territory for drug targeting. *Nat Rev Drug Discov*. 2008; 7:123-129.
- Jones, Walker. Bacterial DNA Isolation using CTAB method. *Maniatis*. 1963; 1:662.
- Jose JK, Kuttan G, Kuttan R. Antitumor activity of *Emblica officinalis*. *J. Ethnopharmacol*. 2001; 75, 65–69.
- Jose JK, Kuttan R. Antioxidant activity of *E. officinalis. J. Clin. Biochem. Nutr*. 1995; 19, 63–70.

- Juang LJ, Sheu SJ, Lin TC. Determination of hydrolyzable tannins in the fruit of *Terminalia chebula* Retz by high-performance liquid chromatography and capillary electrophoresis. *Journal of Separation Science*. 2004; 27, 718–724.
- Keaney JF, Larson MG, Vasan RS, Wilson PWF, Lipinska I, et al. Obesity and systemic oxidative stress: Clinical correlates of oxidative stress in the Framingham study. *Arterioscler. Tromb. Vasc. Biol*. 2003; 23, 434–439.
- Kelly T, Yang W, Chen CS, Reynolds K, He1 J. Global burden of obesity in 2005 and projections to 2030. *International Journal of Obesity*. 2008; 32, 1431–1437.
- Keun-Young KIM, Hye Nam LEE, Yun Jung KIM, Taesun PARK. Garcinia cambogia Extract Ameliorates Visceral Adiposity in C57BL/6J Mice Fed on a High-Fat Diet. *Biosci. Biotechnol. Biochem*. 2008; 72 (7), 1772–1780.
- Khan N, Naz L, Yasmeen G. Obesity: An independent risk factor systemic oxidative stress. *J. Pharm. Sci*. 2006; 19, 62–69.
- Kim JH, Hahm DH, Yang DC et al. Effect of crude saponin of Korean red ginseng on high-fat diet-induced obesity in the rat. *J Pharmacol Sci*. 2005; 97(1): 124-31.
- Kirtikar KR, Basu BD. Indian Medicinal Plants, Allahabad, India. 1991; 2:1017-2220.
- Kizhakkeveettil A, Jayagopal PS, Rose KK. Hypercholesterolemia and Ayurvedic Medicine: A Case Report. *Topics in Integrative Health Care*. 2011; 2(2).
- Kleesen B, Hartmann L, Blaut M. Oligofructose and long chain inulin: influence on the gut microbial ecology of rats associated with a human fecal flora. *Br J Nutr*. 2001; 86: 375–382.
- Knowler W, Pettitt D, Saad M, Bennett P. Diabetes mellitus in the Pima Indians: incidence, risk factors and pathogenesis. *Diabetes Metab Rev*. 1990; 6(1):1-27.
- Korner J, Woods S, Woodworth K. Regulation of energy homeostasis and health consequences in obesity. *Am J Med*. 2009; 122(4-1):S12-8.
- Kuzawa C. The developmental origins of adult health: intergenerational inertia in adaptation and disease. Evolutionary Medicine and Health: New Perspective. *Oxford University Press: USA*. 2007; 325–349.
- Lara-Villoslada F, Olivares M, Sierra S, Rodriguez JM, Boza J, Xaus J: Beneficial effects of probiotic bacteria isolated from breast milk. *Br J Nutr*. 2007; 98(1):S96-100.

- Latha PCR, Daisy P. Influence of Terminalia belerica Roxb. Fruits Extract on Biochemical Parameters in Streptozotocin Diabetic Rats. *International Journal of Pharmacology*. 2010; 06:89-96.
- Lee HS, Jung SH, Yun BS, Lee KW. Isolation of chebulic acid from *Terminalia chebula* Retz. And its antioxidant effect in isolated rat hepatocytes. *Archives of Toxicology*. 2006; 81 (3): 211–218.
- Lee HS, Koo YC, Suh HJ, Kim KY, Lee KW. Preventive effects of chebulic acid isolated from *Terminalia chebula* on advanced glycation endproduct-induced endothelial cell dysfunction. *Journal of Ethnopharmacology*. 2010; 131 (3): 567–574.
- Lee SH, Ry SY, Choi SU, Lee CO, No ZS, Kim SK. et al. Hydrolysable tannins and related compound having cytotoxic activity from the fruits of *Terminalia chebula*. *Archives of Pharmacal Research*. 1995; 18, 118–120.
- Lei F, Zhang XN, Wang W, Xing DM, Xie WD, Su H, Du IJ. Evidence of Antiobesity Effects of the Pomegranate Leaf Extract in High-fat Diet Induced Obese Mice. *Int. J. Obesity*. 2007; 31:1023-1029.
- Lemmens RHMJ, Bunyapraphatsara N. Plant resources of South-East 2003; Asia No. 12 (3): Medicinal and poisonous plants (p. 3). Leiden: Backhuys Publishers.
- Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI: Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA*. 2005; 102:11070-11075.
- Ley RE, Turnbaugh PJ, Klein S, Gordon JI: Microbial ecology: human gut microbes associated with obesity. *Nature*. 2006; 444:1022-1023.
- Li HB, Jiang Y, Wong CC, Cheng KW, Chen F. Evaluation of Two Methods for the Extraction of Antioxidants from Medicinal Plants. *Anal. Bioanal. Chem*. 2007; 388: 483-488.
- Lin S, Thomas TC, Storlien LH, Huang XF. Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. *Int J Obes Relat Metab Disord*. 2000; 24: 639-646.
- Liu J, Xiao PG. Recent advances in the study of antioxidative effects of Chinese medicinal plants. *Phytother. Res*. 1994; 8: 445-451.
- Liu JP, Yang M, Du XM. Herbal medicines for viral myocarditis. *Cochrane Database Syst Rev*. 2004; CD003711.

- Liu JP, Zhang M, Wang WY, Grimsgaard S. Chinese herbal medicines for type 2 diabetes mellitus. *Cochrane Database Syst Rev*. 2004; CD003642.
- Lyon H, Hirschhorn J. Genetics of common forms of obesity: a brief overview. *Am J ClinNutr*. 2005; 82(1):215S-17S.
- Macia L, Viltart O, Verwaerde C, Delacre M, Delanoye A, Grangette C. Genes involved in Obesity: Adipocytes, Brain and Microflora. *Genes and Nutrition*. 2006, 1: 189-212.
- Madani A, Jain SK. Anti-Salmonella Activity of *Terminalia belerica* In vitro and in vivo Studies, Indian Journal of Experimental Biology .2008; 46:817-821.
- Mahan LK, Escott-Stump S. Krause's food, nutrition, and diet therapy. *12th ed. Philadelphia: WB Saunders*. 2008.
- Malekzadeh F, Ehsanifar H, Shahamat M, Levin M, Colwell RR. Antibacterial activity of black myrobalan (*Terminalia chebula* Retz) against Helicobacter pylori. *International Journal of Antimicrobial Agents*. 2001; 18, 85–88.
- Manyam BV. Diabetes mellitus, Ayurveda, and yoga. *J Altern Complement Med*. 2004; 10:223–225.
- Margareto J, Larrarte E, Marti A, Martinez JA. Up-regulation of a thermogenesis-related gene (ucp1) and down-regulation of ppargamma and ap2 genes in adipose tissue: Possible features of the antiobesity effects of a beta3-adrenergic agonist. *Biochem Pharmacol*. 2001; 61: 1471-1478.
- Matarese G, Mantzoros C, La CA. Leptin and adipocytokines: bridging the gap between immunity and atherosclerosis. *Curr Pharm Des*. 2007; 13: 3676-80.
- McIntyre A. Herbal treatment of children: Western and Ayurvedic perspectives. *Elsevier Health Sciences*. 2005; 278–978.
- Misra A, Khurana L. Obesity and the Metabolic Syndrome in Developing Countries. *J Clin Endocrinol Metab*. 2008; 93 (11):S9-S30.
- Misra A. Corrigendum to High prevalence of diabetes, obesity and dyslipidemia in urban slum population in northern India. *International Journal of Obesity Related Metabolic Disorders*. 2001; 25: 1281.
- Mohan V, Shanthirani S, Deepa R, Premalatha G, Sastry NG, Saroja R. Intra-urban differences in the prevalence of the metabolic syndrome in southern India – the Chennai Urban Population Study (CUPS No. 4). *Diabet Med*. 2001; 18: 280–287.

- Monga S. Obesity among School Children (7–9 Years Old) in India: Prevalence and Related Factors. *American Public Health Association*: Washington, DC. 2004.
- Monteiro R, Azevedo I. Chronic inflammation in obesity and the metabolic syndrome. Mediators. *Inflamm*. 2010; 289-645.
- Morrow J. Is a oxidative stress a connection between obesity and atherosclerosis. *Arterioscler. Tromb. Vasc. Biol*. 2003; 23, 368–370.
- Murali YK, Anand P, Tandon V, Singh R, Chandra R, Murthy PS. Long-term effects of Terminalia chebula Retz. on hyperglycemia and associated hyperlipidemia, tissue glycogen content and in vitro release of insulin in streptozotocin induced diabetic rats. Exp Clin Endocrinol Diabetes. 2007; 115 (10):641-646.
- Nadkarni KM. Indian Meteria Medica, Published by Ramdas Bhatkal for Popular Prakashan Pvt.Ltd.Mumbai. 2002; 01:202-1205
- Nakamura T, Tokunaga K, Shimomura I, et al. Contribution of visceral fat accumulation to the development of coronary artery disease in non-obese men. *Atherosclerosis*. 1994; 107(2):239-246.
- Nierenstein M, Potter J. The distribution of myrobalanitannin. *The Biochemical journal*. 1945; 39 (5): 390–392.
- NNMB. Diet and nutritional status of population and prevalence of hypertension amongst adults in rural areas. *National Nutrition Monitoring Bureau Technical Report No: 24.* 2007.
- O'Rahilly S, Farooqi I. Human obesity: a heritable neurobehavioral disorder that is highly sensitive to environmental conditions. *Diabetes*. 2008; 57(11):2905-10.
- Ozata M, Mergen M, Oktenli C, Aydin A, Sanisoglu SY, et al. Increased oxidative stress and hypozincemia in male obesity. *Clin. Biochem*. 2002; 35, 627–631.
- Pagliassotti MJ, Wei Y, Bizeau ME. Glucose-6-phosphatase activity is not suppressed but the mRNA level is increased by a sucrose-enriched meal in rats. *J Nutr*. 2000; 133:32-37.
- Patel C, Ghanim H, Ravishankar S, Sia CL, Viswanathan P, Mohantym, P, Dandona P. Prolonged reactive oxygen species generation and Nuclear Factor- kB activation after a high-fat, high-carbohydrate meal in the obese. *J. Clin. Endocrinol. Metab*. 2007; 92, 4476–4479.

- Patrice D Cani, Nathalie M Delzenne. The Role of the Gut Microbiota in Energy Metabolism and Metabolic Disease. *Current Pharmaceutical Design.* 2009; 15 : 1546-1558.
- Patwardhan B, Bodeker G. Ayurvedic genomics: Establishing a genetic basis for mind-body typologies. *J Altern Complement Med*. 2008; 14:571–576.
- Patwardhan B, Joshi K, Chopra A. Classification of human population based on HLA gene polymorphism and the concept of Prakriti in Ayurveda. *J Altern Complement Med*. 2005; 11:349–353.
- Pihl E, Zilmer K, Kullisaar T, Kairane C, Magi A, Zilmer M. Atherogenic inflammatory and oxidative stress markers in relation to overweight values in male former athletes. *Int. J. Obesity*. 2006; 30, 141–146.
- Pluske JR, Durmic Z, Payne HG, Mansfield J, Mullan BP, Hampson DJ, Vercoe PE. Microbial diversity in the large intestine of pigs born and reared in different environments. *Livest Sci*. 2007; 108:113–116.
- Popkin BM, Doak CM. The obesity epidemic is a worldwide phenomenon. *Nutr Rev*. 1998; 56: 106–114.
- Pradhan AK, Shukla AK, Reddy MVR, Garg N. Assessment of oxidative stress and antioxidant status in age related cataract in a rural population. *IJCB*. 2004;19(1):83-87
- Prasher B, Negi S, Aggarwal S, et al. Whole genome expression and biochemical correlates of extreme constitutional types defined in Ayurveda. *J Transl Med*. 2008; 6:48.
- Prentice A. Obesity and its potential mechanistic basis. In type 2 diabetes: the thrifty phenotype. *British Medical Bulletin*. 2001; 60, 51–67.
- Prentice A. The emerging epidemic of obesity in developing countries. *Int J Epidemiol*. 2006; 35: 92–99.
- Rajarama Rao MR, Siddiqui HH. Pharmacological studies on *Emblica officinalis* Gaertn. *Indian Exp.Biol.* 1964; 2:29-31.
- Ramachandran A, Snehalatha C, Kapur A, Vijay V, Mohan V, et al. Diabetes Epidemiology Study Group in India (DESI) High prevalence of diabetes and impaired glucose tolerance in India: National Urban Diabetes Survey. *Diabetologia*. 2001; 44: 1094–1101.

- Ramachandran A, Snehalatha C, Latha E, Vijay V, Viswanathan M. Rising prevalence of NIDDM in an urban population in India. *Diabetologia*. 1997; 40: 232–237.
- Ramachandran A, Snehalatha C, Satyavani K, Sivasankari S, Vijay V. Cosegregation of obesity with familial aggregation of type 2 diabetes mellitus. *Diabetes Obes Metab*. 2000; 2: 149–154.
- Rao TP, Sakaguchi N, Juneja LR, Wada E, Yokozawa T. Amla (Emblica officinalis Gaertn.) Extracts Reduce Oxidative Stress in Streptozotocin-Induced Diabetic Rats. *J Med Food*. 2005; 8 (3): 362–368.
- Ravussin E, Lillioja, Knowler WC, et al. Reduced rate of energy expenditure as a risk factor for body weight gain. *N Engl J Med*. l988; 3 18:467-72.
- Reddy VR, et al. Cardiotonic activity of the fruit of *Terminalia chebula. Fitoterapia LXI*. 1990; 517-525.
- Romieu I, Willett WC, Stampfer M, et al. Energy intake and other determinants of relative weight. *Am J Gin Nutr*. l988; 47:406-12.
- Roos C, Lidfeldt J, Agardh C, Nyberg P, Nerbrand C, Samsioe G, et al. Insulin resistance and self-rated symptoms of depression in Swedish women with risk factors for diabetes: the Women's Health in the Lund Area study. *Metabolism*. 2007; 56(6):825-9.
- Roth J, Qiang X, Marbán S, Redelt H, Lowell B. The obesity pandemic: where have we been and where are we going? *Obes Res*. 2004; 12(2):88-101.
- Round JL, Mazmanian SK: The gutmicrobiota shapes intestinal immune responses during health and disease. Nat Rev Immunol 2009, 9:313-323. Neish AS: Microbes in gastrointestinal health and disease. *Gastroenterology*. 2009; 136:65-80.
- Roy AK, Dhir H, Talukdar G. *Phyllanthus emblica* fruit extract and ascorbic acid modify hepatotoxic and renotoxic effects of metals in mice. *Int. J. Pharmacog*. 1991, 29: 117-126.
- Sabu MC, Kuttan R. Anti-diabetic activity of medicinal plants and its relationship with their antioxidant property. *J. Ethnopharmacol*. 2002; 81: 155–160.
- Saiki R, Okazaki M, Iwai S, Kumai T, Kobayashi S, Oguchi K. Effects of pioglitazone on increases in visceral fat accumulation and oxidative stress in

spontaneously hypertensive hyperlipidemic rats fed a high-fat diet and sucrose solution. *J Pharmacol Sci*. 2007; 105: 157–167.

- Saleem A, Husheem M, Härkönen P, Pihlaja K. Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* retz. Fruit. *Journal of Ethnopharmacology*. 2002; 81 (3): 327–336.
- Sánchez F, Garcí R, Alarcón F, Cruz M. Adipocinas, tejido adiposo y su relación con células del sistema inmune. *Gac. Méd. Méx*. 2005; 141, 505–512.
- Sangeetha S, Anjana J, Monika B. Protective effect of *Terminalia belerica* Roxb, and gallic acid agaist carbontetra chloride induced damage in albino rats. *Journal of Ethanopharmacology*. 2006; 109:214-218.
- Schousboe K, Willemsen G, Kyvik K, Mortensen J, Boomsma D, Cornes B, et al. Sex differences in heritability of BMI: a comparative study of results from twin studies in eight countries. *Twin Res*. 2003; 6(5):409-21.
- Sharma H, Chandola HM, Singh G, Basisht G. Utilization of Ayurveda in health care: An approach for prevention, health promotion, and treatment of disease. Part 1—Ayurveda, the Science of Life. *J Altern Complement Med.* 2007; 13: 1011–1019.
- Sharma H, Chandola HM. Ayurvedic Concept of Obesity, Metabolic Syndrome and Diabetes Mellitus. *J Alt. Comp. Med*. 2011; 17 (6): 549–552.
- Sharma H, Chandola HM. Prameha in Ayurveda: Correlation with Obesity, Metabolic Syndrome and Diabetes Mellitus. Part 1–Etiology, Classification, and Pathogenesis. *J Alt. Comp. Med*. 2011; 17(6):491–496.
- Sharma H, Clark C. Contemporary Ayurveda. London: Churchill Livingstone, 1998.
- Sharma HM. Contemporary Ayurveda. In: Micozzi MS, ed. Fundamentals of Complementary and Alternative Medicine, 4th ed. St. Louis: Saunders Elsevier. 2011; 495–508.
- Shastri A. Sushruta Samhita, Ayurveda-Tattva-Samdipika commentary, 14th ed. Varanasi, India: Chaukhambha Publications. 2003.
- Shastri KN, Chaturvedi GN, Agnivesha, Charaka Samhita, Vidyotini commentary. Varanasi, India: Chaukhamba Bharati Academy. 2004.
- Shukla VD, Tripathi RD. Agnivesha, Charaka Samhita, Vaidyamanorama Hindi commentary. Delhi, India: Chaukhamba Sanskrit Pratishthana. 2002.

- Sikaris K. The clinical biochemistry of obesity. *Clin. Biochem. Rev*. 2004; 25, 165–181.
- Silveira PP, Portella AK, Goldani MZ, Barbieri MA. Developmental origins of health and disease (DOHaD). *J Pediatr (Rio J).* 2007; 83: 494–504.
- Sims EAH. Destiny rides again as twins overeat. *New England Journal of Medicine*. 1990; 322, 1522–1523.
- Sinclair KD, Lea RG, Rees WD, Young LE. The developmental origins of health and disease: current theories and epigenetic mechanisms. *Soc Reprod Fertil Suppl*. 2007; 64: 425–443.
- Smith J., Al-Amri M., Dorairaj P., Snideman A. The adipocytes life cycle hypothesis. *Clinical Science*. 2006; 110: 1-19.
- Srikumar R, Jeya Parthasarathy N, Sheela Devi R. Immunomodulatory activity of triphala on neutrophil functions. *Biological & Pharmaceutical Bulletin.* 2005; 28(8):1398-40.3
- Srinivasan K, Viswanad B, Asrat L, Kaul CL, Ramarao P. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: A model for type 2 diabetes and pharmacological screening. *Pharmacol. Res.* 2005; 52: 313–320.
- Stefanadis C. The implication of obesity on total antioxidant capacity apparently healthy men and women: The ATTICA study. *Nutr. Metab. Cardiovasc. Dis*. 2007; 17, 590–597. 27.
- Steppan CM, Bailey ST, Bhat S, et al. The hormone resistin links obesity to diabetes. *Nature*. 2001; 409:307–312.
- Stunkard A, Foch T, Hrubec Z. A twin study of human obesity. *JAMA.* 1986; 256(1):51-4.
- Sturm R. The effects of obesity, smoking, and drinking on medical problems and costs. *Health Aff (Millwood).* 2002; 21(2):245-53.
- Tarwadi K, Agte V. "Antioxidant and micronutrient potential of common fruits available in the Indian subcontinent". *Int J Food Sci Nutr*. 2007; 58 (5): 341–9.
- The Ayurvedic Pharmacopoiea of India,1st edition, Published by The controller of Publications, Civil Lines, New Delhi .2001;part-1,01: 252.
- The Ayurvedic Pharmacopoiea of India,1st edition, Published by The controller of Publications, Civil Lines, New Delhi .2001;part-1,01: 252.

- The Ayurvedic Pharmacopoiea of India, 1st edition, Published by The controller. 2001; part-1, 01: 252.
- Thielecke F, Boschmann M. The Potential Role of Green Tea Catechins in the Prevention of Metabolic Syndrome – A Review. *Phytochem*. 2009; 70:11-24.
- Thresher JS. et al. Comparison of the effects of sucrose and fructose on insulin action and glucose tolerance. American Journal of Physiology. *Regulatory, Integrative and Comparative Physiology*. 2000; 279(4): 1334-40.
- Toda S, Kumura M, Ohinishi M. Effect of phenolcarboxylic acids on superoxide anion and lipid peroxidation induced by superoxide anion. *Planta Med*. 1991; 57:8-10.
- Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009; 457:480-484.
- Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI: The Human Microbiome Project. *Nature*. 2007; 449:804-810.
- Vaidyaratnam PS. Varier's, Indian Medicinal Plants, Published by Orient Longman Private Ltd. Chennai.2004; 05:258-262.
- Van HM, Compton DS, France CF, et al: Diet-induced obese mice develop peripheral, but not central, resistance to leptin. *J Clin Invest*. 1999; 9: 385-390.
- Vani T, Rajani M, Sarkar S, Shishoo CJ. Antioxidant properties of the ayurvedic formulation triphala and its constituents International. *Journal of Pharmacognosy*. 1997; 35(5):313-317.
- Vincent H, Vincent K, Bourguignon C, Braith R. Obesity and post exercise oxidative stress in older women. *Med. Sci. Sports Exer*. 2005; 37, 213–219.
- Walley A, Asher J, Froguel P. The genetic contribution to non-syndromic human obesity. *Nat Rev Genet*. 2009; 10(7):431-42.
- Wang Y, Beydoun M, Liang L, Caballero B, Kumanyika S. Will all Americans become overweight or obese? Estimating the progression and cost of the US obesity epidemic. *Obesity (Silver Spring)*. 2008; 16 (10):2323-30.
- Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. *Int J Pediatr Obes*. 2006; 1: 11–25.
- Wang Y, Mi J, Shan XY, Wang QJ, Ge KY. Is China facing an obesity epidemic and the consequences? The trends in obesity and chronic disease in China. *Int J Obes*. 2007; 31: 177–188.

- WHO. Obesity: Preventing and Managing the Global Epidemic. Report of a WHO Consultation Geneva. *World Health Organization*: Geneva. 2000.
- Wolfram S, Wang Y, Thielecke F. Anti-obesity Effects of Green Tea: From Bedside to Bench. *Mol. Nutr. Food Res*. 2006; 50:176-187.
- World Health Organization (WHO). Physical Status: the Use and Interpretation of Anthropometry. *Tech. Rep. Series, Geneva: WHO*. 1995; 854.
- World Health Organization. Obesity: preventing and managing the global epidemic. Report of a WHO consultation on obesity. Geneva: *World Health Organization*. 1997.
- Yajnik C S. Obesity epidemic in India: intrauterine origins? Proceedings of the Nutrition Society. *The Nutrition Society*. 2007; 63: 387-396.
- Yajnik CS. Obesity epidemic in India: intrauterine origins? *Proceedings of the Nutrition Society*. 2004; 387–396.
- Yan J, Gyogan Z, Long C, Ma Xingynan. Influence of ginsenosides Rb 1, Rb 2 and Rb 3 on electric and contractile activities of normal and damaged cultured myocytes. *Acta Pharmacolog. Sin*. 1992; 13: 403-406.
- Yokozawa T, Kim HY, Kim HJ, Okubo T, Chu DC, Juneja LR. Amla (Emblica officinalis Gaertn.) prevents dyslipidemia and oxidative stress in the ageing process. *Br J Nutr*. 2007; 97(6):1187-1195.
- Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, et al. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci U S A*. 2009; 106:2365-2370.
- Zhang YJ, Abe T, Tanaka T, Yang CR, Kouno I. Phyllanemblinins A-F, new ellagitannins from *Phyllanthus emblica. Journal of natural products*. 2001; 64 (12): 1527–1532.
- Zimmet P, Dowse G, Finch C, Serjeantson S, King H. The epidemiology and natural history of NIDDM--lessons from the South Pacific. *Diabetes Metab Rev*. 1990; 6(2):91-124.
- Zurlo F, Lillioja S. Esposito-Del Priente A, et al. A low ratio of fat to carbohydrate oxidation as a predictor of weight gain: study of 24-hour RQ in Pima Indians. *Am J Physiol*. 1990; 22:650-7.

List of publications

- **S Gurjar**, A Pal, S Kapur. Triphala and Its Constituents Ameliorate Visceral Adiposity From a High Fat Diet in Mice With Diet-induced Obesity. *Alternative Therapies*, Sept-Oct 2012; 18(5): 38-45.

- S Kapur, A Pal, **S Gurjar**, V.V.S Pavan, S Sharad. Isolation and characterization of secondary metabolites "Saponins" from explants of Indian medicinal plant Chlorophytum borivillianum. *IJPFR*, July-Sep 2011; 1(2): 14-22.

- **Shaifali Gurjar** and Suman Kapur. Insight into the roles of gut microbes. *Pharmacologyonline*. 2010; 3: 548-559.

List of presentations

- **Shaifali Gurjar** and Suman Kapur. Obesity and Gut Microbiota, presented at National conference on Emerging trends in Life sciences organized by Biological Sciences and Pharmacy Group (sponsored by UGC) of Birla Institute of Technology and science, Pilani from 6-7th March'2009.

- Kuldeep Gupta, **Shaifali Gurjar**, K. Pushkala and Suman Kapur, Reduction of fat storage in mice fed on high-fat diet after treatment with Mangifera indica seed Kernel ethanolic extract, , presented at IPS-2010 held at Hyderabad from 14th to 16th December, 2010.

- Suman Kapur, Pareek, R. P., Aggarwal, R.P, Khinvasara, R.K, Manav Kapoor, **Shaifali Gurjar**, Urvashi Dubey and Shashwat Sharad, APOE and Ob Gene: Correlation of Genotypes with Various Clinical and Anthropometric Parameters in Type 2 Diabetes Mellitus, presented at 5th Winter Symposium held at CMC, Vellore on 9th-11th January 2007.

- Suman Kapur, R P Pareek, R P Aggarwal, R. K. Khinvasara, **Shaifali Gurjar**, Manav Kapoor, APOE Gene: Correlation of Genotypes with Various Clinical and Anthropometric Parameters seen in Type 2 Diabetes, , presented at the 34th Annual Conference of Research Society for the Study of Diabetes in India at Delhi between 3rd-5th November 2006.

Brief Biography of the Candidate

Shaifali Gurjar is a PhD student in Biological Sciences at Birla Institute of Technology and Sciences, Pilani. She received her M.Sc. degree in Biotechnology from Banasthali Vidyapeeth Rajasthan. Her M.Sc. thesis involved the In vitro studies on *Withania somnifera* (L.) dunal along with effect of hormones and sugars on shoot regeneration and withanoloid production under in-vivo and in- vitro conditions. After joining BITS, Pilani in 2006 she was awarded BITS - JRF fellowship and worked on several projects investigating the isolation and characterization of secondary metabolites "Saponins" from explants of Indian medicinal plant *Chlorophytum borivillianum*. In her thesis she aimed at screening and knowing mechanism based study for use of natural products for treating obesity. Her work has been published in different peer reviewed scientific journals and timely presented in national and international meetings such as NCETL and IPS.

She has been pursuing the field of plant tissue culture and exploring the medicinal and therapeutic properties of medicinal plants such as Chlorophytum borivillianum, Emblica officinalis, Terminalia bellerica and Terminalia chebula. She has actively worked on UGC funded project "Medical and Therapeutic Characterization of Induced Somaclonal Variation in a Medicinal Plant *Chlorophytum borivillianum*. She was also the part of project "callus regeneration Establishment and multiplication of shoot cultures of *C. borivillianum"*. She has good experience of working with animal models (Swiss albino mice). She has worked on antidiabetic activity using streptozotocin induced diabetic rodent model.

She has good command over statistics tools and data handling. Currently, as BITS-SRF she is involved in developing clonal variants of Indian Medicinal plants and screening natural products for anti-diabetic, anti-inflammatory and anti-obesity activities in specific animal models for these diseases.

Brief biography of Dr. Suman Kapur

Dr. Suman Kapur joined BITS, Pilani on 17th July 2004 as Professor in the Center for Biotechnology, Biological Sciences Group. She has worked in the capacity of Unit Chief, Community Welfare and International Relations since 1st January 2007. From 16th April 2010 she took over charge as Dean, Research & Consultancy at the Hyderabad Campus of BITS and since June 2012 she has been serving as the University-wide Dean for International Programmes and Collaboration Division. Dr. Kapur is a popular teacher at BITS, Pilani and has been instrumental in introducing several new courses, namely MPH G513, MPH G522, MPH G692, MPH G539, MPH G521, MPH G681, BIO G515 and developed the curriculum for a new degree program "Master of Public Health", incorporating learning through field visits and interdisciplinary teaching. Several students, trained by her have gone to make excellent careers for themselves as CEO's of start up companies and faculty at some of the best institutions in USA.

Dr. Kapur with her team of a dozen research scholars has been instrumental in building a state of the art Human Genomics laboratory from funds (Rs 362.19 lacs) received as Principal and/or Co-Investigator of now more than eighteen grants awarded since her joining BITS in 2004. As a mentor she has been able to motivate younger faculty to submit and execute independent grants in the form Women scientist (DST), Research Associate and senior research fellows (ICMR & CSIR). She has published more than 80 research articles in International and national journals.

Dr. Kapur's research interests lie in identifying biomarkers for unraveling the genetic basis of human diseases such as psychiatric disorders like depression, schizophrenia, addiction and Alzheimer's disease and metabolic disorders such as diabetes (T2DM), obesity, cataract and metabolic syndrome. Early and specific diagnosis is the backbone of effective treatment and reduction of both disease associated morbidity and mortality. Modern day integration of electronics and biological possibilities on an integrated chip can be successfully used to develop POC devices, especially suited for low-cost settings and our group has already developed two such devices which will be launched in 2013

and 2014. Attempts for early, non-invasive diagnosis of urinary bladder and prostate cancer are underway

In modern times several research approaches suggest that liability to complex inherited illnesses like obesity, diabetes, mental disorders and neurodegenerative diseases is influenced by several genes. Study of the involved genes will shed light on genetic architecture of these illnesses. More over the genetic profile of different populations for the complex disorders will serve as platform to diagnose the at risk individuals at an early age and help design strategies for early and timely intervention of the disease. The group is specifically studying several genes, viz., *APOE*, *CAPN*, *PPARγ*, *ALDH2*, *ADH1B*, *ADH1C*, *OPRM1*, *OB*, *TPH*, *CRYGA*, *CRYGB*, *D2*, *D5*, *ADCY4*, *ADCY3*, *CCKAR*, *CCKBR*, *CFTR*, *CF508*, *SPNK*-1, PS-1, *CYP2E1*, *CTSB*, *HSP70*, *TNFα*, *PRSS*-1 and several micro-satellite markers on chromosome segments 2, 6 and 10. Chronic diseases have a long latency period and genetic markers can be effectively used for identifying individuals at an increased risk for developing these diseases and advocating appropriate lifestyle measures to delay the onset and progression of such diseases. Ours is the first group to show that in the Indian population a mutation in the mu Opiate receptor is linked to risk for addiction to opiates and alcohol and a mutation in the Ob (leptin) gene is linked to hypertension in depressed subjects.

Revival of research on Traditional Medicine/Herbal Remedies with a locally-relevant evidence-based, disease-oriented approach is particularly relevant for India. Her group is also involved in developing clonal variants of Indian Medicinal plants and screening natural products for anti-diabetic, anti-inflammatory and anti-obesity activities in specific animal models for these diseases. Several industry sponsored projects are also underway for bio-conversion, -remediation & effluent treatment using consortia of microbial populations.