FENUGREEK GALACTOMANNAN: MEDICINAL APPLICATIONS AND DESIGNER FOOD PRODUCT DEVELOPMENT

THESIS

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By

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CERTIFICATE

This is to certify that the thesis entitled **"Fenugreek Galactomannan: Medicinal Applications and Designer Food Product Development"** and submitted by **Roshan Issarani, ID. No. 2000 PHXF 008,** for the award of Ph.D. Degree of the Institute embodies original work done by him under my supervision.

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LIST OF ABBREVIATIONS

FGM	Fenugreek galactomannan
GG	Guar gum
LBG	Locust bean gum
DGG	Depolymerized guar gum
DFGM	Depolymerized fenugreek galactomannan
LD	Lethal dose
SCFAs	Short chain fatty acids
NSPS	Non-starch polysaccharides
G: M ratio	Galactose: mannose ratio
LDL	Low density lipids
HDL	High density lipids
TG	Triglycerides
IDDM	Insulin dependent diabetes mellitus
NIDDM	Non- insulin dependent diabetes mellitus
IHD	Ischemic heart disease
C_{max}	Maximum blood glucose concentration
T_{max}	Time to reach maximum blood conc.
F.O.S.	Fructo-oligosaccharides
PP	Post parandial
OD	Optical density
rpm	Revolutions per minute
cps	Centipoises
GRAS	Generally regarded as safe
RTD	Ready to drink
PKU	Phenylketoneurea
EMP	Emden Meyerhof pathway

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CHAPTER 1
INTRODUCTION

1: INTRODUCTION

The use of plants and animals as source of medicine and food is as old as humanity. One such noted food plant being used since ages and having immense medicinal and industrial potential is fenugreek.

Polymer Science is a branch of Science that has developed since 1940 due to the pioneering work of Nobel laureates Dr. H. Mark, Dr. P. J. Flory and Dr. Staudinger (1). Polymers are high molecular weight natural, semi - synthetic, or synthetic compounds which find extensive applications in our day-to-day life. Applications of our concerned interest include both as Pharmaceutical adjuvant and as active ingredients possessing medical uses. The present project shall explore the medicinal applications.

Gums, polymers of saccharides, or simply polysaccharides, have the property to disperse in water to form a slimy / mucilaginous colloidal dispersion which may sometimes gel (2). Considering the Chemistry of a specific saccharide, the hexose, it is seen that the hexoses can exist in enatiomeric forms due to the presence of asymmetric carbons (3). However, only three predominantly occur in nature. These include glucose, galactose and mannose. The importance of glucose is well known, while that of galactose and mannose lies in their selection by the nature in the form galactomannans. The galactomannans are unique, compared to the more common polysaccharides (cellulose, starch, and glucomannans) by differing in spatial disposition of their OH groups. Mannose and galactose each have a pair of OH groups on the same side (cis position) of the pyran ring (4). This cis disposition, offers unique hydrogen bonding property by which

they reinforce each other. This structural feature of galactomannans has not been touched upon by any of the workers in their studies with galactomannans.

Galactomannans are found as reserve food material in the endosperms of leguminous plant seeds. These are referred to as galactomannans, because they consist of β-(1-4)- mannose backbone having single α - (1-6) - galactose side chains. The various seed galactomannans differ from each other in galactose - mannose (G: M) ratios, molecular weight, fine structure regarding the distribution of galactose side groups on the main chain (5).

The use of plants and animals as source of medicine and food is as old as humanity (6). One such noted food plant being used since ages and having immense medicinal and industrial potential is fenugreek.

Fenugreek (***Trigonella foenum-graceum*** family: Fabaceae, subfamily: Papillionateae) is an annual legume (7 - 9). There are numerous (about 30 based on the literature surveyed) medicinal applications of Fenugreek. It can be used internally in powder form or as an extract or applied externally in the form of a poultice.

India is a world exporter of *Methi* and major supply comes from Rajasthan (10). Particularly, Jodhpur, on the world map is known for its guar gum exports.

The literature surveyed on fenugreek does report studies of its medicinal applications (11 - 12). The mechanism and the specific constituent, responsible for most of its activities, are not known or simply the whole seed powder is reported to have the particular activity in question in most of the cases. Therefore, medical science has yet to discover the correlation between the constituents of this little seed responsible for its numerous therapeutic actions.

Work on other fenugreek constituents like its alkaloids, saponins, glycosides, oil has been carried out; but the work on fenugreek galactomannan is lacking. The work on galactomannans from guar (13) has been extensively carried out and that on galactomannans from other leguminous seeds to some extent has been carried out (14 - 18).

Prior to the introduction, cultivation and commercialization of guar gum which has a G: M ratio of 1: 2, locust bean gum was industrially the most widely used galactomannan which has a G: M ratio of 1: 4. Fenugreek offers a Galactomannan that has a G: M ratio of 1: 1 (19).

The medicinal activities of guar galactomannan are believed to be a result of its high viscosity. This notion needs reconsideration. Because according to our hypothesis the mechanism of action of galactomannans also has to do with interaction of glucose, cholesterol and bile salts with *cis*-OH groups. Based on this hypothesis, the depolymerized galactomannans should be effective as well in reducing the absorption of glucose and cholesterol. Generally, the modification i.e. depolymerization is achieved by methods using chemicals, enzymes, heat etc (20, 21). Here, in Jodhpur, there is a gamma radiation plant at Defense Laboratory. This facility can be used for the purpose of depolymerization of the fenugreek galactomannan. Achieving depolymerization of galactomannans by gamma irradiation shall be a novel approach (22).

Atherosclerosis, a disease of modern day civilization, results due to deposition of cholesterol crystals in arteries, impeding the flow of blood (23). Different mechanisms appear to underlie the varying levels of blood cholesterol in normal population. Use of soluble dietary fibre, like galactomannans, is the only safe and natural way of lowering blood cholesterol and reducing the possibility of developing atherosclerotic plaque.

It is desired that an ideal hypolcholesterolimic agent should lower not only the low-density lipid levels but should also maintain the high-density lipid levels constant. However, the studies performed with agents that lower blood cholesterol, focus on the total cholesterol reduction.

Fenugreek is reported to lower serum triglyceride levels (24 - 27). This work has been carried out using whole seed powder and no mechanism has been suggested. Steroidal saponins from Fenugreek have been found to reduce total blood cholesterol levels (28, 29).

Diabetes is a condition of glycosuria accompanied with hyperglycemia, primarily due to lack of insulin. If left untreated it is damaging to the tissues (30). The damage of tissues is now thought, to be a result of the binding of glucose with the body proteins, just as in the formation of glycosylated hemoglobin (31). The approach in the management of diabetes is, therefore, to keep the high blood glucose concentration within physiological limits. This can best be achieved by incorporating a soluble dietary fibre in the diet of a diabetic patient.

Fenugreek is reported to have hypoglycemic activity (32 - 35). Here again the whole seed powder has been tested for the activity in question.

Current evidence indicates that consumption of food naturally rich in non-starch polysaccharides (NSPs) is associated with human longetivity and healthy life. Studies related to physico-chemical properties of NSPs in relation to their physiological effects can help in development of Pharmaceutical preparations of diet rich in food fibre, for treatment of diabetes, hyperlipidemia, hypertension etc. However, NSPs will have little therapeutic value if they are unpalatable and therefore not consumed by patients on a regular basis. Much work is needed to improve the sensory properties of polysaccharide gums if they are to be incorporated into suitable dosage forms at pharmacological doses.

In this study, attempt shall be made to fill in the above-mentioned gaps. The specific objectives of this study therefore include the following:

- To extract and specify the fraction of the seed, namely galactomannan, responsible for the hypoglycemic and antihyperlipidemic effects.
- To study the effect of fenugreek galactomannan on HDL / LDL ratio.
- To depolymerize fenugreek galactomannan by gamma radiation and study its hypoglycemic and antihyperlipidemic activity.
- To study the role of G: M ratio on the medicinal effects of galactomannans having different G: M ratios.
- To incorporate fenugreek galactomannan in a suitable dosage form.

A. J. Whistler, a noted authority on galactomannans, had predicted a very bright industrial potential for guar gum, which has already come true (36). Similarly, he has predicted that, chitosan, hemicellulose and fenugreek have a potential for industrial commercialization. The same successful story, like guar gum, is yet to become a reality for fenugreek.

Industrially, seed volume of fenugreek is a strong incentive to make dual use of this seed by removing spice component and allowing the endosperm to become a source of a potentially useful gum. Also, the seed size, larger than *Cassia tora* and comparable to guar gum, is well suited to the dehusking and grinding machines presently used in industry for guar gum production. Hence, fenugreek shall be easily accepted by the industry.

There is going to be a major impact on Pharmaceutical products, due to acceptance of WTO proposal. So far, the Indian industry has not geared up to carry out research on basic molecules as new drugs, which is highly cost oriented and time - bound work. In order to meet the initial challenges, everyone looks at medicinal herbs for potential drug development projects (37, 38).

CHAPTER 2
LITERATURE REVIEW

2.1: A REVIEW ON GALACTOMANNANS

The Galactomannans are unique, compared to the more common polysaccharides (cellulose, starch, and glucomannans) by differing in spatial disposition of their OH groups on the pyran ring which offers unique hydrogen bonding property by which they reinforce each other.

Definition

Polysaccharide based hydrocolloids having the property to disperse in water to form a slimy / mucilaginous colloidal dispersion which may sometimes gel, are referred to as 'gums' (39).

The gums in literature are generally classified according to the nature of their origin (40); such a classification of gums is given below in Table-1.

Classification

Table 1: Classification of gums based on origin

Origin	Gum
Plant exudates	Gum acacia
Plant seeds	Guar gum, Cassia gum, Starch, Tamarind, Psyllium, Fenugreek
Plant extract	Pectin
Animal extract	Chitin
Marine	Agar
Microbial	Xanthan
Semi – synthetic	Cellulose & starch derivatives

The leguminous plant seeds contain polysaccharides as reserve food material in their endosperms. One specific class of these chemical substances, referred to as 'galactomannan' has found several industrial and medicinal applications during the past years (41).

These are referred to as galactomannans, because they consist of β-(1-4)- mannose backbone having single α - (1-6) - galactose side chains. The various seed galactomannans differ from each other in galactose - mannose ratios, molecular weight, and fine structure regarding distribution of galactose side groups on the main chain (42).

Structure (43 - 46)

With the help of well-established methodologies for structure elucidation of polysaccharides, Rafique, Smith, and Whistler et al determined the structure of guaran. Complete acid hydrolysis of polysaccharide yielded a mannose - galactose ratio of 2:1. Typical structure of a galactomannan is shown in Figure – 1 on the following page.

Earlier it was believed that D - galactose units are joined to every alternate D - mannose, but the present notion is that there are 'smooth' and 'feathery' regions on the main chain. Smooth refers to no galactose units on that particular region and feathery refers to presence of galactose units continuously on every next mannose unit. Another notion is that the galactose units may link together to form short galactose chains (referred to as ramification), which then join to the main mannose backbone. This postulation explains the rather unusual high viscosity behavior of galactomannans (47).

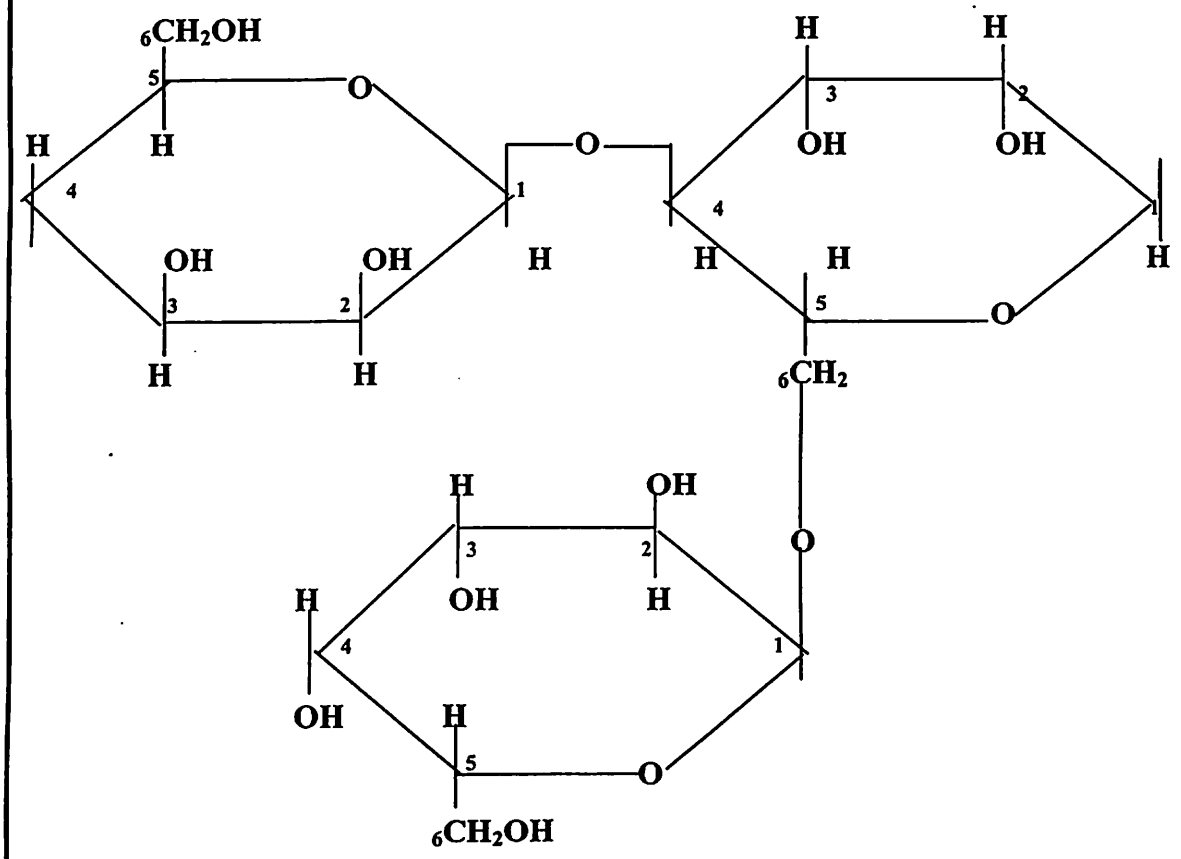


Figure 1: Linking of galactose and mannose units to form galactomannan polymer

Properties

i. Galactomannans rapidly hydrate when dispersed in cold or hot water to form hydrophilic colloidal sols of very high viscosity. Viscosity of some of the gums is given in Table – 2 below.

Table - 2: Viscosity of some gums

Gum	% w/v for 800 cps
Tragacanth USP	2.75
Sodium alginate	1.15
Guar gum	0.2
Methyl cellulose (1500 cps)	1.7
Methyl cellulose (4000 cps)	1.35
Sodium CMC (medium viscosity)	1.9
Sodium CMC (high viscosity)	0.7
Carbopol 934	0.17

ii. Galactomannans are insoluble in organic solvents.

iii. Hydrogen bonding activity; thus used as flocculent in extremely small concentration.

iv. Film formation which is flexible and resistant to organic solvents e.g. triacetate and butyrate films (48).

v. Synergistic increase of viscosity with other gums (49). This behaviour of galactomannans is depicted in Table- 3.

Table - 3: Depicting synergistic increase in viscosity

Gums	gm % in dispersion	Viscosity (cps)		
		Neutral	Acidic	Alkaline
C. fistula HP Guar	1 1	3050	2950	2250
C. fistula CM Guar	1 1	7400	5600	3600
C. fistula Carob	1 1	1800	1650	1700
C. fistula Tara	1 1	10600	9800	8100
C. fistula Xanthan	1 1	15000	26000	45000

vi. Their viscosity behavior is dependent on various factors like - concentration of gum, temperature of dispersion, time, pH, presence of electrolytes, and rate of shear (see Fig. 2). Very dilute solutions of galactomannans (0.1 %) show Newtonian flow behavior but at higher concentrations they become pseudo plastic and thixotrophic (50 - 52).

vii. Non- ionic character, thus stable in presence of electrolytes. The gums, in fact, disperse / hydrate rapidly in Sodium and Calcium chloride solutions. Sodium benzoate a preservative (food grade) accelerates hydration and viscosity (39).

viii. Stable over practically usable range of pH from 4.5 to 10.5, viscosity being maximum between pH 7 - 9.

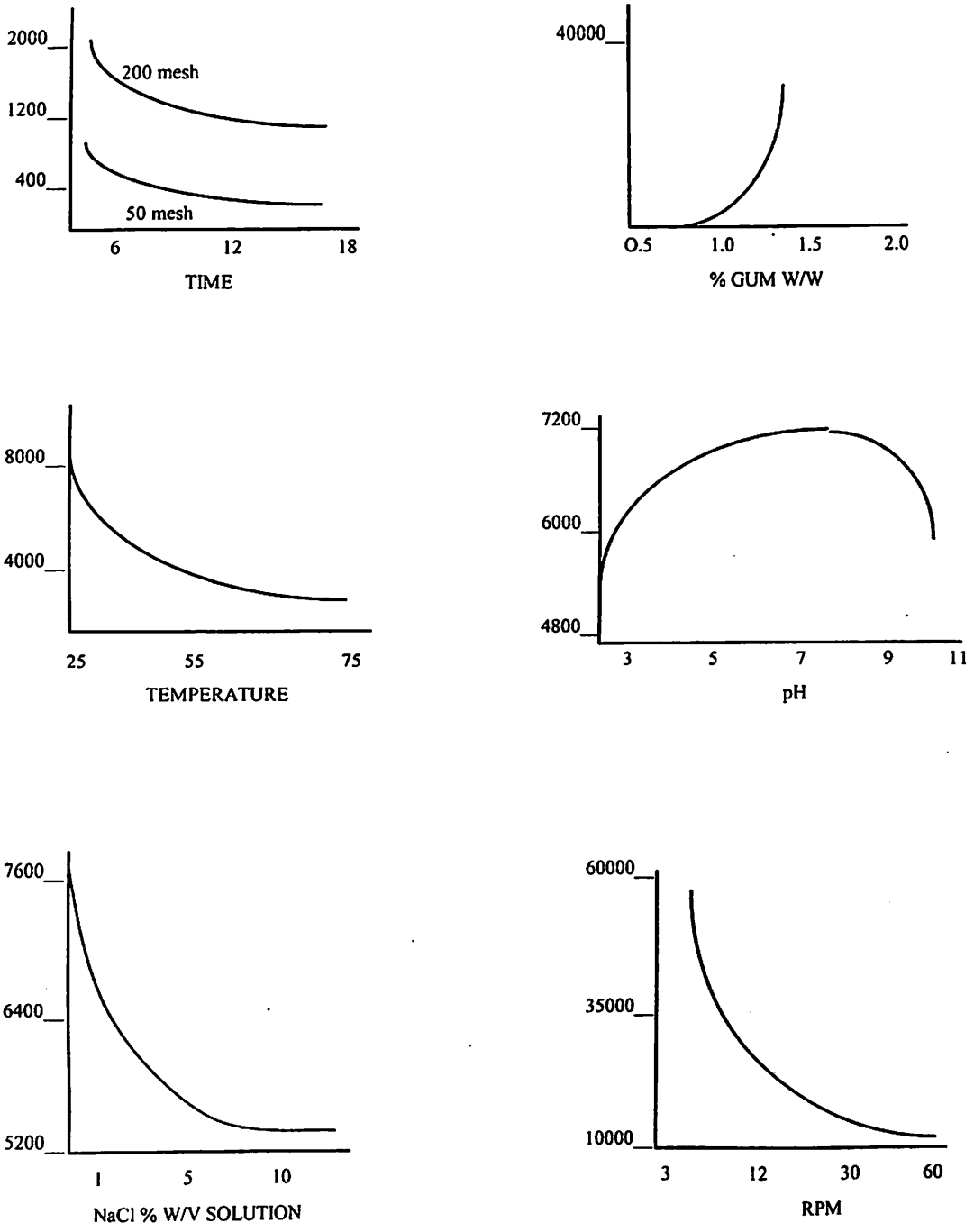


Figure-2: Factors affecting the viscosity behaviour of a galactomannans

(In all the graphs viscosity in centipoise is plotted on the y - axis)

Depolymerization

The unusually high viscosity of galactomannan gums is in a way a limitation for their use in both food and non - food industries. Hence, there is a need to modify them by depolymerization to offset this limitation and suit the requirements.

The limitations of unmodified gum include:

- The quantity of high viscosity gum employed as food additive (0.1% of bulk) is too insufficient to make it effective dietary fibre (12 - 28 gm / day).
- In dairy products, it provides a thickening that is very unnatural.
- High viscosity gum does not work well in pharmaceutical applications like coating of granular and spray dried products.
- An ideal viscosity builder should have a fast rate of hydration and should maintain a consistent viscosity under variable shear rate and temperature. This is not possible with a very high viscosity gum product.
- It is the low molecular weight galactomannan that finds application in preparation of adsorbents for chromatography (53).

The various ways and means of modifying the native gum are enumerated below.

i) Enzymatic method: These methods employ the enzymes - galactomannanase, mannosidase or galactosidase. A Japanese company - Danippon Pharmaceuticals, has used a process based on this method to produce depolymerized guar gum.

ii) Acid catalyzed hydrolysis: In this method, powdered sample is impregnated with a volatile acid like methanolic HCl and the mixture is heated for a period of time (54, 55). Adding NaOH can arrest the

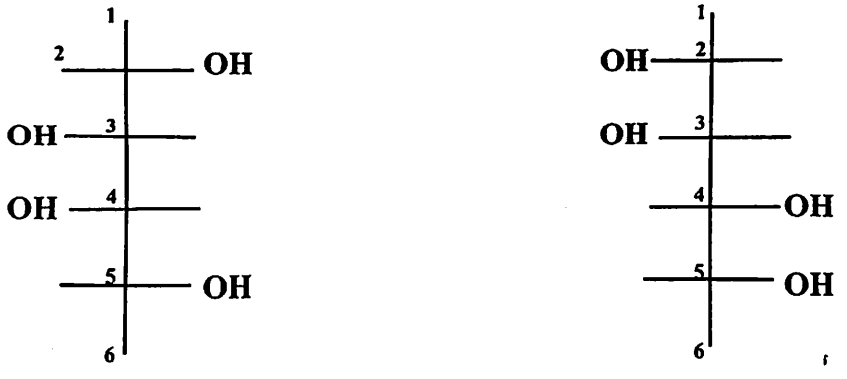
depolymerization reaction. The process variables include: time, temperature and concentration of acid (56).

iii) Thermal methods: The gum sample is heated to 100 - 150 ° C for 3 - 30 hours.

iv) High energy radiation: (method indigenous to our laboratory). The sample is exposed to γ - radiation, the variables being, time period and intensity of radiation.

Chemistry

***i) cis - OH disposition*:** The galactomannans are unique, compared to the more common polysaccharides (cellulose, starch, and glucomannans) by differing in spatial disposition of their OH groups. Mannose and galactose each have a pair of OH groups on the same side (*cis* position) of the pyran ring, (see Fig. – 3) (57). This *cis* disposition, offers unique hydrogen bonding property by which they reinforce each other.



D - Galactose **D - Mannose**

Figure - 3: *cis* - OH disposition of galactose and mannose

ii) Specific Chemical interactions: The interactions of galactomannans are characteristic of and due to the abundant presence of OH groups. These include:

- ***Esterification***: Reacting with acetic anhydride in pyridine, a triacetate is obtained which can be cast into strong, flexible films. Likewise, tributyrate and nitrate esters can be obtained.

- ***Etherification***: Alkoxylation with ethylene or propylene oxides yield the corresponding ethers. Other examples include carboxyalkyl and cyanoalkyl ethers.

- ***Oxidation***: This is achieved by the use of oxidizing agent like periodic acids, peroxides, hypochlorites. The oxidation results in lowering of mannose and galactose ratio. Also, molecular weight and viscosity are reduced. It imparts anionic character and alkali tolerance.

- ***Complexing reactions***: These lead to cross - linking of molecules resulting in three-dimensional network, which manifests itself in gel formation (58).

The galactomannans (having an abundance of adjacent OH groups in *cis* position) enter into complex formation with a number of ions like Ca^{2+}, Cu^{2+}, Al^{3+}, Ti^{2+}, and Sb^{3+}. However, the most noted interaction takes place with the borate ion, shown in Figure - 4.

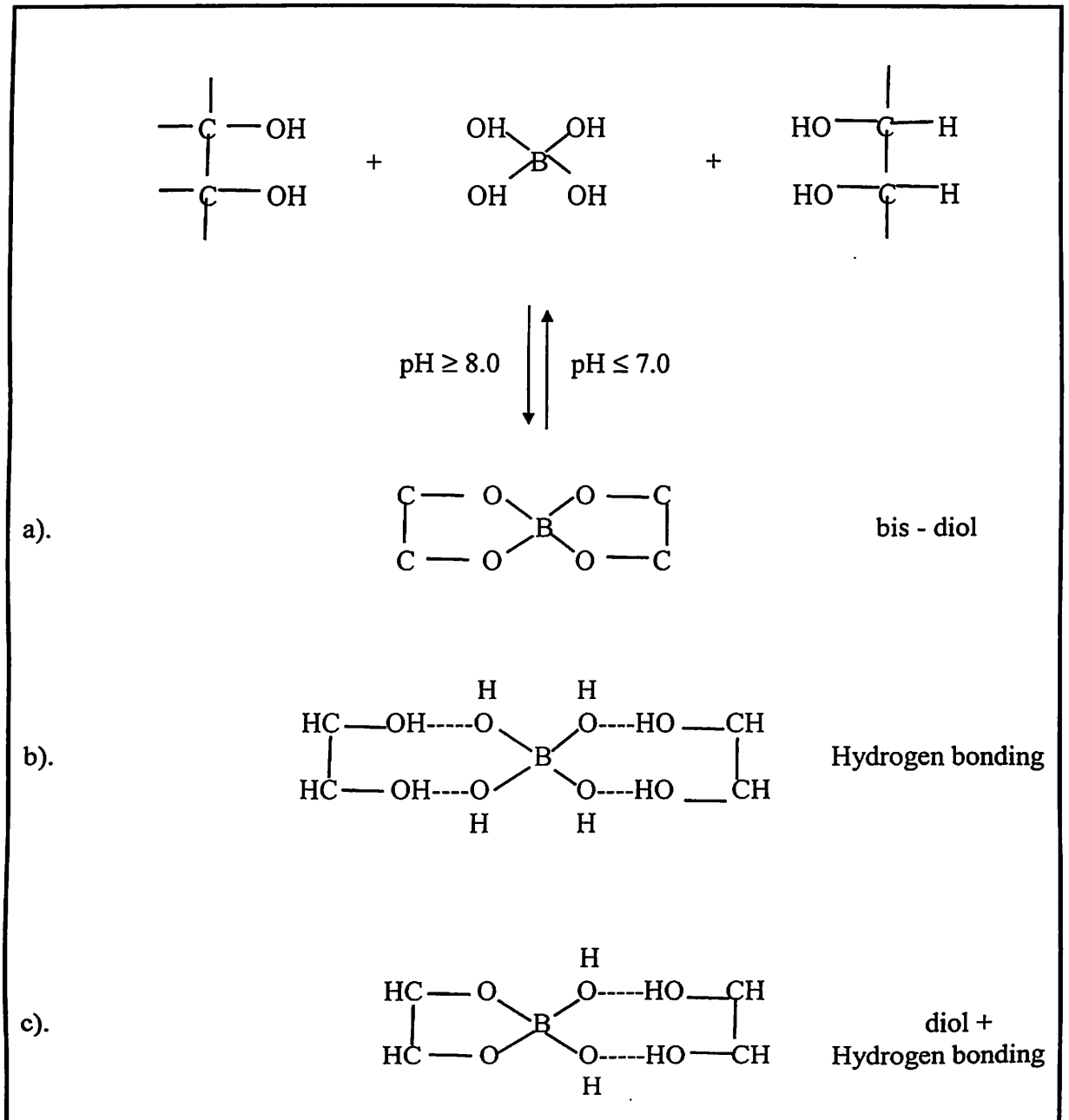


Figure - 4: Depicting two point interaction of galactomannans

Derivatization

The native form of galactomannans is derivatized chemically and the derivatives so obtained find useful applications in Pharmaceutical and other industries. The various derivatives are given below in Table – 4 (59 - 66).

Table - 4: Derivatization of galactomannans

Type of derivative	Change in properties
Oxidation a) Periodate b) Peroxide and hypochlorite	Lowering of mannose: galactose ratio and reduced mol. wt. and viscosity; increased anionic nature and alkali tolerance; increased thermo plasticity
Esterification a) Nitrate esters b) Triacetate, tributyrate	Increased pliability of films
Etherification, using a) Chloroacetic acid b) Epoxides CH_2--CH_2--CH_2X \ / O X = Cl, amino, sulphonic, quaternary ammonium, thiol, alkyl, alkoxy, etc. c) Cyanoethylation CH_2=CH-CN	Anionic polymer, water dispersible or cross - linked water insoluble. Cationic polymer water-soluble or cross - linked water insoluble. Polymer containing specific functional groups; increased compatibility with organic solvents.
Grafting of vinyl monomer on polymer backbone using free radicals or high-energy radiation. CH2=CH-X X = C_6H_5, $COOCH_3$, CN, $O.COCH_3$	Change in hydrophilic - hydrophobic balance.
Binding of fibre reactive dye	Chromophoric reagent for lectin detection.

Potential uses

i) Pharmaceutical applications

In Pharmaceutical industry, these polysaccharides are used as ***disintegrants*** in dry form and as ***binder*** in solution form for manufacturing compressed tablets (67, 68). These are used in liquid dietic preparations as a low calorie ***thickener*** to improve their mouth feel, body and pour characteristics. In appreciable concentration these are employed as ***dispersing / suspending*** agents and in low concentration act as ***flocculating*** agents (69). They possess excellent ***emulsifying*** properties. ***Microencapsulation*** of drugs and controlled released of drugs can be achieved using these agents (70 - 72).

ii) Medicinal applications

Trowell observed in societies of rural Africa, that those who consumed diets containing more dietary fibres rarely suffered from diabetes mellitus. Since then a number of studies regarding the role of dietary fibres in diabetes mellitus have appeared in literature. Guar gum is found to be the most effective.

The galactomannans are not metabolized in the human body due to lack of the required enzymes, hence used as ***dietary fibres***. The medicinal applications are due to the slowing down of sugar and lipid absorption in the intestines. Hence, recommended as ***anti- diabetic*** and ***hypocholesteremic*** agents. Another use is as ***bulk laxative***. This is due to strong water retention and to act as lubricant for intestinal contents. The minor and less reported uses include treatment of ***peptic ulcer*** and ***sore throat*** (73 - 83).

iii) Chromatography

During the past few years, there has been an increasing demand for optically active compounds in various fields. A wide difference in the biological activity of the two enantiomers is particularly important for pharmaceutical and agrochemicals. Several examples are known where the desired activity is solely due to one isomer (L - DOPA) while the other isomer produces harmful effects (thalidomide). Thus it has become important for the scientific community to develop methods to obtain optically pure compounds.

Resolution of racemates is the oldest approach to obtain compounds that are optically enriched. Amongst the various methods, chromatography is the only effective and quickest method, which has been developed for resolution. It is now known that in most of the chromatographic enantioseparations, operating interactions belong to one of the following few classes:

- Hydrogen bonding
- Metal complexation
- Charge - transfer complexation and
- Host - guest relationship.

Galactomannans have chiral discriminating properties, which do not occur with starch or cellulose. This has been attributed to the sterically selective dual bonding (a two point contact), due to presence of a pair of cis - OH groups on mannose. The chiral selectivity is further enhanced by complexing borate ion with the guaran. Hence, borate - gelled - guaran can be put to ***enantioseparation*** of racemate mixtures (84 - 87).

iv) Other industrial applications

- Food Industry: as binder, thickener, suspending agent in ice creams, dairy products, bakery products, sauces, beverages.
- Paper Industry: It produces an improved sheet with greater strength.
- Petroleum industry:
- Textiles Industry: As print paste thickener.
- Mining Industry: Flocculates clay and silica, depresses froth.
- Explosives Industry:
- Cosmetic Industry: used to manufacture toothpastes, shaving creams (imparts slip for easy extrusion), creams, lotions (as protective colloid), shampoos, etc.
- Ceramic Industry: binder for enamels, porcelain.

v) Other minor uses

Apart from the above-mentioned major uses, there are numerous and very interesting minor uses of galactomannans especially of guar gum (88). Namely, these include: civil disorder and traffic control management (by making slippery roads), firefighting, inducing galactosidase production in *Penicillium ochrochloron* cultures (89), electric cables, photography, lectin research, water and effluent treatment, etc. to name just a few.

Summary

Gums and mucilages are polysaccharide hydrocolloids obtained from vegetable sources. These substances are extensively used in numerous industries as adhesives, emulsifiers, stabilizers, lubricants, binders or suspending agents.

Acacia and Tragacanth, the traditionally used ones, are slowly getting replaced by newer ones. Finding an alternative for the popular gums has been a source of constant inspiration for many Pharmaceutical scientists in India (90).

India's contribution in this regard seems to be fairly large due to the vast natural resources at our disposal. During the last four decades a number of gums and mucilages from Indian flora have been studied for their potential uses as Pharmaceutical adjuvants.

Among the various sources of gums, the galactomannans specifically, are surely here to stay (91). This is because of their unique characteristic feature of cis - OH disposition, unlike starch, cellulose, and glucomannans.

Among the galactomannans, Locust Bean gum had been the only commercial source of galactomannans, till the discovery of guar gum in 1945. The next best new sources of galactomannans that have been commercialized in India during the past decade are gums from *Cassia tora* and *Cassia dencha.*

The next best two sources of galactomannan gums that have been identified are fenugreek seed gum and tamarind seed gum.

Presently, *C. tora* is used as pet food and its Pharmaceutical, medicinal, chromatographic applications need to be established.

It is therefore of utmost importance for us Indian scientists to take up active research work in identifying, characterizing, and standardizing the gums from various sources and have their uses established and patented.

2.2: A TO Z OF FENUGREEK

> Medical science has yet to discover the correlation between the constituents of this little seed responsible for the numerous therapeutic actions it shows.

Introduction

The use of plants and animals as source of medicine and food is as old as humanity. The therapeutic hints from remote mists of time hold key of the treasures of medical knowledge. From the large array of hints and claims, the investigator of today has to promulgate the best and beneficial. One such noted food plant being used since ages and having immense medicinal and industrial potential is fenugreek (92).

Fenugreek (*Trigonella foenum-graceum* family: Fabaceae, subfamily: Papillionateae) is an annual legume with white flowers (triangular in shape hence the name) and long slender pods with a pronounced beak. Also, commonly, known as: bird's foot and Greek hayseed. The most commonly used part is the seed. Its most common habitat is the countries on the eastern shores of the Mediterranean. Fenugreek is cultivated in India, Africa, Egypt, Morocco, and occasionally in England. It is a native of Southern Europe and Asia.

The name comes from *foenum-graecum*, meaning Greek hay, the plant being used to scent inferior hay. The name of the genus, *Trigonella*, consisting of about 70 species, is derived from the old Greek name, denoting 'three-angled,' from the form of its corolla. The seeds of Fenugreek have been used medicinally all through the ages and were held in high repute among the Egyptians, Greeks and Romans for medicinal and culinary purposes.

The plant is grown for forage and ornamental purposes. The small seeds, having a strong and characteristic smell, are used in India for curries. If the seeds are placed in water, the inner seed coat swells up into thick mucilage and bursts the testa. There are numerous (about 30 based on the literature surveyed) medicinal applications of fenugreek. It can be used internally or applied externally. Most importantly the seeds yield diosgenin, a precursor of steroids. Fenugreek seeds were formerly official in our Pharmacopoeia and official in Australia, Germany, Portugal, and Switzerland.

General characters and commerce

Fenugreek is an erect annual herb, growing about 2 feet high, similar in habit to Lucerne. Fenugreek seeds of commerce are small, hard, angular, somewhat compressed, with a light brown or brownish yellow colour externally, and yellow internally. The seeds are brownish, about 1/8 inch long, oblong, rhomboidal, with a deep furrow dividing them into two unequal lobes. They are contained, ten to twenty together, in long, narrow, sickle-like pods. They have a somewhat oily and farinaceous taste peculiar, to lovage or celery, accompanied by a slight bitterness, and feeble melilot flavour, and a strong peculiar odour, which is suggestive of melilot or of coumarin. They give out the whole of their odour and taste to alcohol and when boiled with water form thick slimy mucilage (93 - 97).

The seeds are chiefly exported to Europe from Egypt and India. In Egypt, fenugreek is known as *Helbeh* and in India under the Sanskrit name of *Methi*. India produced 32000 tones of fenugreek in 1992, and the major supply came from Rajasthan (97, 98). The main markets for Indian Fenugreek seeds are Japan, Singapore, besides Sri Lanka, Saudi Arabia, and Nepal. The principal constituents of Fenugreek seeds are given in Table - 5.

Table - 5: Constituents of fenugreek seeds

S.No.	Constituent	g %
1	**Carbohydrates**	
	Mucilage	44.1
	(Galactomannan)	28
2	**Fat**	5.8
3	**Fatty (fixed) oil**	6 – 8
	(bitter component)	
4	**Essential oil**	0.02
5	**Flavonoid Glycosides and aglycones of**	6 – 8
	Quercitin & Lutteolin	0.02
6	**Proteins**	26.2
	Albumin	22
	Globulin	-
	Prolamine	-
	nucleoalbumin betain	-
	Trimethylamine,	-
	Neurin	-
7	**Vitamins**	
	A, D, C, B complex	-
	Nicotinic Acid	1.1*

S.No.	Constituent	g %
8	**Alkaloids**	
	Trigonelline,	0.38
	Choline	-
9	**Furostanol saponoin** (99)	
	Trigonesides Ia, Ib,IIa, IIb, IIIa, IIIb.	
	Glycoside D,	
	Trigofoenoside A.	
10	**Steroidal Saponins**	
	Diosgenin	0 .1
	Gitogenin	-
	Tiogenin, Yamogenin	
11	**Minerals**	
	Calcium	160*
	Zinc	10*
	Phosphorus	370*
	Iron	14*
	Sodium	19*
	Potassium	530*
12	**Tannic acid**	-
13	**Yellow colouring matter**	-

*mg/100gm

Moisture: 13.7 %	**Fibre**: 7.2 %	**Ash**: 3.0 %.

Medicinal Uses

Fenugreek has been used since ancient times both as a food and medicine by the people living on the shores of Mediterranean and across Asia. Known through out the world, the first recorded medical uses of Fenugreek were made by the ancient Egyptians.

Fenugreek has excellent medicinal virtues. Its regular use helps keep the body clean and healthy. The leaves of Fenugreek are aromatic, cooling and a mild laxative. The seeds have soothing effect on the skin. They increase the secretion and discharge of urine and promote lactation in nursing mothers. They are the best cleanser within the body, highly mucous solvent and soothing agents. Fenugreek has the ability to soften and dissolve hardened masses of accumulated mucous. It helps to expel toxic wastes through lymphatic systems. It expels mucous and phlegm from the bronchial tubes.

It has antiseptic properties and kills infection in the lungs. Fenugreek contains lecithin which dissolves cholesterol and contains lipotropic (fat dissolving) substances, which dissolves deposits of fat, prevents fatty accumulates and water retention. The constituents in the seeds contain a saponin closely related to those in Yucca. Fenugreek is also considered an aphrodisiac.

During the early stages of any of the respiratory tract infections such as bronchitis, influenza, sinusitis, catarrh and suspected pneumonia, fenugreek tea helps to perspire, dispel toxicity and shorten gestation period of fever. One can take up to four cups of fenugreek tea. The quantity may be reduced as the condition improves. To improve flavour few drops of lemon juice can be used. During the treatment no other food or nourishment must be taken as fasting aids the body to correct respiratory problems in few days.

Tea made from fenugreek seeds is equal in value to quinine in reducing fevers. Fenugreek used with lemon juice and honey also helps reduce fevers. Seeds when moistened with water become slightly mucilaginous and the tea made from this has the power to dissolve sticky substance like mucous.

Fenugreek tea has a soothing effect on the inflamed stomach and intestines. It cleans the stomach, bowels and kidneys. It helps healing peptic ulcers by providing coating of mucilaginous matter. Fenugreek leaves (preferably fresh) are beneficial in the treatment of indigestion, flatulence and a sluggish liver. Fenugreek with other spices is found to enhance pancreatic lipase, amylase, and chymotrypsin activity. This accounts for the digestive stimulant action of the spices, including fenugreek (100).

The leaves of fenugreek help in blood formation. The cooked leaves prevent anemia and run down condition in girls usually associated with the onset of puberty and a sudden spurt in growth. The seeds also help in recovering from anemia, being rich in iron. For this purpose the bread in Egypt is fortified with 4 % fenugreek flour (101). The seeds help restore the senses of taste and smell. The sense of taste becomes dull due to improper functioning of salivary glands which often becomes clogged with mucous and accumulated juices causing swelling. Similarly the sense of smell is obstructed due to prolonged accumulation of mucous and other impurities in the nose where the olfactory nerves (the special sensory nerve of smell) are based.

The tea is beneficial for the bad breath and body odour. Unpleasant odours emanate from the body due to accumulations of hardened mucous and other toxins in the nasal and oral passages, the gastrointestinal tract, the urinary tract, the blood and vagina. Fenugreek tea taken regularly helps

remove these accumulates from the spots where mouthwash and soap can never penetrate.

Fenugreek seeds can also be taken for diabetes (102 - 06) and for reducing blood lipid (cholesterol) levels (107-08). According to a study fenugreek given in a daily dose 2.5 g twice daily for three months to healthy individuals did not affect the blood lipids and blood glucose levels. However, administered in the same daily dose for the same duration to coronary artery disease patients and non - insulin dependent diabetic patients, fenugreek reduced significantly the blood lipid and blood sugar levels. Therefore, the normal dose is 2 tsp. of powdered seeds taken daily in broth or milk. Two teaspoons can also be swallowed as whole daily or they can be soaked in a cup of water at night and the water taken in the morning.

Fenugreek seeds are also used for removing dandruff. Two table spoons should be soaked overnight in water. In the morning softened seeds can be ground to a fine paste and applied on scalp and left on for half an hour. The hair is then thoroughly washed with soap-nut (*ritha* nut) solution or *shikakai*. A paste of fenugreek leaves applied over the scalp regularly before washing the scalp also cures dandruff.

An infusion of the leaves is used as a gargle for recurrent mouth ulcers. A gargle made from the seeds is best for ordinary sore throat. The solution used to gargle should be stronger than tea. Two tablespoons of Fenugreek seeds are put in a litre of water and allowed to simmer for half an hour over a low flame. It is then cooled to room temperature and strained. Then the whole liquid is used as gargle.

Fenugreek tea is used as a douche in curing leucorrhoea. The solution is prepared in the same way as the throat gargle.

Poultice made with Fenugreek leaves can be used for external and internal swellings. It can also be used for burns due to its cooling properties.

The seeds are employed in the preparation of emollient cataplasms, ointments and plasters.

They give strong mucilage, which is emollient and a decoction of 1-ounce seeds to 1-pint water is used internally in inflamed conditions of the stomach and intestines. Externally it is used as a poultice for abscesses, boils, carbuncles, etc. It can be employed as a substitute for cod-liver oil in scrofula, rickets, anemia, and debility following infectious diseases. For neurasthenia, gout and diabetes it can be combined with insulin. It possesses the advantage of being cheap and readily taken by children, if its bitter taste is disguised by taking 1 or 2 teaspoonful of the powder daily in jam etc.

Fenugreek seeds made in gruel, given to nursing mothers increase the flow of milk.

Fenugreek seed contains many proteins, fats, carbohydrates, and saponins, which are nutritionally beneficial during convalescence from tuberculosis, pneumonia, and other debilitating conditions.

Research has validated other properties of fenugreek seed including uterine and intestinal stimulant, oxytocic, and spermicidal action.

In some parts of the world, fenugreek is used as an aphrodisiac, but this property has not been verified.

Paste of fresh fenugreek leaves applied over the scalp regularly before bath helps hair grow, preserves natural colour and keeps hair silky. The same paste can be applied on face every night before going to bed and then washed with warm water in the morning. This will prevent one from getting pimples, black heads, and dryness of face and early appearance of wrinkles. It improves complexion and make one look younger.

The ground seeds are used also to give maple flavouring to confectionery. The powder is also employed as a spice in curry.

The cattle like the flavour of fenugreek in their forage. At the present day, the ground seeds are utilized to an enormous extent in the manufactures of condition powders for horses and cattle. Fenugreek is the principal ingredient in most of the quack nostrums, which find so much favour among grooms and housekeepers. It has a powerful odour of coumarin and is largely used for flavouring cattle foods and to make damaged hay palatable.

Steaming is considered the best method of cooking leaves (109). In this, the vitamins are retained and the vegetables become palatable. The drained leaves can be compared to pulses for their protein content.

In Indian homes, fenugreek seeds are generally used as a condiment and the fresh plant as an esculent for flavouring. They form an ingredient of curry powder. Its leaves can be used in salads.

Internally: 2-6 g, in 2-3 doses. Externally: 50 g powdered drug boiled with 250 ml water and applied as a moist warm poultice.

The aqueous extracts of the seeds show antibiotic activity against *Micrococcus pyogens* var *aureus*.

Fenugreek mucilage has been tried as a sizing material for paper. The dried mucilage has remarkable swelling properties, and may find applications as an adjuvant in Pharmaceutical preparation, as a tablet disintegrant.

Drug interactions

May potentate antidiabetic (hypoglycemic) drugs. Hypoglycemic drugs, MAOI drugs, anticoagulants. Since fenugreek seeds have high fibre content the absorption of concomitant administration of drugs may be affected. When taken simultaneously, bulk-forming laxatives may inhibit the absorption of other drugs (e.g. aspirin, cardiac glycosides, antibiotics,

anticoagulants, etc.) and dietary nutrients (e.g. calcium, iron, zinc, sodium, potassium, etc.).

Due to the diuretic action of this herb the following drug interactions are possible: increased risk of toxicity with anti-inflammatory analgesics; if hypokalemia occurs possible antagonism with antiarrhythmics and potentiation of muscle relaxants; antagonizes antidiabetic (hypoglycemic) drugs; may potentiate and/or interfere with antihypertensives; may potentiate lithium therapy; when taken with corticosteroids there is a risk for hypokalemia; may potentiate other diuretics and increase the risk of hypokalemia.

Due to the antihypertensive (hypotensive) action of this herb the following interactions are possible: when taken with anesthetics an increased hypotensive effect; potentiation of antihypertensives; when taken with diuretics difficulty with diuresis and hypertension may result; antagonism of sympathomimetics

Constituent and activity

Maple syrup urine disease is an autosomal recessive inherited disorder of branched - chain amino acid metabolism. The disease was originally named after the characteristic sweet aroma, reminiscent of maple syrup. The same well - known flavour impact compound present in fenugreek was found to be present in the urine of patients with maple syrup disease. This compound has been identified as 4, 5, - dimethyl, 1 - 3 - hydroxy - 2 [5H] - furanone, (sotolone) (110).

Reutter has noted the presence of trimethylamine, neurin and betain; like the alkaloids in cod-liver oil, these substances stimulate the appetite by their action on the nervous system, or produce a diuretic or ureo-poietic effect.

Fenugreek with other spices is found to enhance pancreatic lipase, amylase, and chymotrypsin activity. This accounts for the digestive stimulant action of the spices, including fenugreek (100).

Fenugreek has bitter principles and contains saponins, which have dissolving, and loosening properties. This may explain why this herb has been used to help dissolve and expel thick or hardened mucous.

The unsaponifiable matter (3.9 - 4.0 %) of the fatty oil contains a lactation-stimulating factor (98). Numerous studies these days have been focused towards this application.

Fenugreek is a rich source of iron, hence indicated in anemia. In Egypt, the breads are fortified with 4 % Fenugreek flour for reducing the high prevalence of iron deficiency in wheat eating regions (101).

The seed is believed to contain a lipotropic (fat dissolving) agent. This has been identified as the steroidal saponins (furostanol type) that reduce the plasma cholesterol levels in rats (111).

However, medical science has yet to discover the correlation between the constituents of this little seed responsible for the numerous therapeutic actions it shows. Namely, its antidiabetic effect, whether it is systemic or not and its use as an antirheumatic agent.

Safety of fenugreek

Fenugreek does not produce any significant acute and cumulative toxicity. This is well evident from the fact that it has been used since ages in our diets as food, spice and in curry powder (112).

In an acute toxicity study (113), debitterized fenugreek powder administered intragastrically to mice and rats of both the sexes failed to induce any signs of toxicity or mortality up to a maximum dosage of 2 and 5 g / kg body weight, respectively. Further, no significant alterations in organ

weights or their histology were discernible at internal autopsy. In the 90-day sub chronic study, fenugreek fed to weaning rats of both sexes had no effect either on daily food intake or growth. Further, the biochemical measurements in serum and liver of such rats have revealed no appreciable changes in various parameters.

Summary

The galactomannans are here to stay because of the uniqueness of their chemical structure, of their two monomeric units, i. e. mannose and galactose. Both these hexoses have a pair of cis - hydroxyl groups (2, 3 and 3, 4 positions respectively) on their pyranose ring structures. Due to this, galactomannans have much stronger hydrogen bonding properties, than glucose, starch, and cellulose derivatives.

Looking at the chemical structure of glucose, it is readily noted that there can be 16 different possible enantiomers. But, it is observed that only three enantiomers namely, glucose, mannose and galactose, occur widely in nature. This is suggestive of molecular evolution, analogous to the biological evolution and survival of the fittest in life forms. This proves the significance and uniqueness of the galactomannans in seeds.

A. J. Whistler (114), a noted authority on galactomannans, had predicted a very bright industrial potential for guar gum, which has already come true. Similarly, he has predicted that chitosan, hemicellulose and fenugreek have a potential for industrial commercialization. Accordingly, we all are well aware of the successful investigation and establishment of chitosan as a polymeric material for drug delivery system and as medicinal agent during the last decade. The same successful story is yet to become a reality for fenugreek

Industrially, seed volume is a strong incentive to make dual use of the seed by removing spice component and allowing the endosperm to become a source of a rather unusual, potentially useful gum. Also, the seed size (larger than *Cassia tora* and comparable to guar gum) is well suited to the dehusking and grinding machines presently used in industry for guar gum production (115).

Herbs, as drugs, are known to mankind since ages and in recent times they have become increasingly popular as alternative source of medicine for different ailments. Looking into various facts of toxicity, teratogenicity and carcinogenicity of synthetic drugs and lack of modern drugs for chronic and immunological diseases, in the future usage of natural herbs to combat these problems looks very bright (116).

However, as on today the scientific validity of herbal powders and extracts, as therapeutic aids remain doubtful due to lack of proper quality control, absence of rationality of combination in polyherbal formulae and non - reproducible pharmacological and clinical efficacies.

It is believed that the medicinal herbs are used as drugs since ages and they are bound to have clinical efficacy and not much emphasis is given for providing data on the animal pharmacology or other biological tests of the herbal formulations.

Due to acceptance of WTO proposals a major impact is going to be on Pharmaceutical products. So far Indian industry has not geared up to carry out research on basic molecules as newer drugs, which is highly cost oriented and time - bound work. In order to meet initial challenges everyone looks at medicinal herbs for potential drug development projects.

Scientific literature survey provides clues for the phytochemical isolation, pharmacological efficacies, toxicological effects and clinical efficacies of individual medicinal plants as well as polyherbal combinations.

However, data on pharmacokinetics and bioavailability studies of herbal drugs is totally lacking and we do not have enough base to argue upon the bioequivalence of herbs grown in different countries.

Attempts are going on by scientific community to fill in the gaps and make rationale and scientific formulations of herbal medicines with reliable clinical efficacies. The people all over the world are looking for herbal remedies as viable answers for various chronic and other diseases like diabetes, rheumatoid arthritis, cancer, immunological diseases etc. The market for herbal products is very wide open and is going be a major part of global business. India can take lion share in this business as it has a well-established traditional system and enormous natural wealth, provided a systematic and scientific approach is adopted.

2.3: LIPIDS AND MANAGEMENT OF ATHEROSCELEROSIS

A relatively low ratio of LDL to HDL is desirable for lowering the risk for development of coronary artery disease.

Introduction

The lipids are organic substances insoluble in water but soluble in organic solvents. They are esters of fatty acids or are substances capable of forming such esters and are utilizable by the living organisms. They form important dietary constituents on account of their high calorific value and the fat-soluble vitamins. In the body, they are present in the cytoplasm as well as in the cell wall and also in specialized areas, as the fat depots. Nervous tissues are particularly rich in lipids where they serve the important function of insulating the nerve impulse. The subcutaneous fat serves the role of insulating against atmospheric heat and cold and also helps in rounding off the contours of the body (117).

The lipids are classified as follows:

i) ***Simple lipids:*** ***e.g. triglycerides.***

ii) Compound lipids: ***e.g. phospholipids.***

iii) Derived lipids: ***e.g. fatty acids, cholesterol***

Lipid metabolism (118)

Exogenous lipids

The first step in the digestion of dietary fats is to break the fat globules into small sizes so that water - soluble digestive enzymes can act on the globule surfaces. This process of emulsification is achieved by bile salts. The lipases being water-soluble can now attack the fat globules. The

end products of this enzymatic cleavage are free fatty acids and 2 - monoglycerides. Now, once again the bile salts play an important role in solubilizing the fatty acids by the phenomena of micellar solubilization. The bile salt micelles also act as transport medium to carry the monoglycerides and the free fatty acids to the intestinal epithelial cells. Likewise, the cholesterol esters and phospholipids are hydrolyzed and the bile salts play the similar "ferrying - action".

After entering the epithelial cells, the fatty acids and monoglycerides are taken up by the smooth endoplasmic reticulum and recombine to form new triglycerides. These triglycerides aggregate with cholesterol, phospholipids and small amounts of beta lipoprotein, which coat the surface of each globule. This is then excreted out of the cells and passes into the lymph. These globules are referred to as chylomicrons. The chylomicrons are then transported up the thoracic duct and emptied into the venous blood at the juncture of jugular and subclavein vein.

As the chylomicrons pass through the adipose tissue and liver, the enzyme lipoprotein lipase hydrolyzes the triglycerides into fatty acids and glycerol. Over 95 % of all the plasma lipids are in the form of lipoproteins, containing triglycerides, phospholipids, and cholesterol. The total concentration of lipoproteins in the plasma averages 700 mg /100 ml. This average plasma concentration of lipoproteins is broken down to the following individual constituents, as depicted in Table – 6, on the following page.

Table - 6: Average plasma concentration of lipoproteins.

S. No.	Constituent	Plasma Conc. (mg/dl)
1	Cholesterol	180
2	Phospholipid	160
3	Triglyceride	160
4	Protein	200

Endogenous lipids

Endogenously synthesized cholesterol and triglycerides are lipids that circulate in the blood. They being fats are insoluble in water. Therefore, to circulate through the blood, which is mainly water, they must be carried by protein packages called apoproteins. The combination of apoprotein and Lipid is known as lipoproteins.

In blood, there are three major classes plasma of lipoproteins. Plasma lipoproteins contain polar lipids (phospholipids) and non - polar lipids (triacylglycerols), as well as cholesterol and its esters. The triacylglycerols and cholesterol are hidden inside an outer coat of water - soluble, hydrophilic segments of the polypeptide chains and the hydrophilic polar heads of phosphoglycerides molecules. This arrangement provides for the transport of the lipids absorbed from the intestine to the fat depots and tissues via the blood.

Thus the dietary lipids are carried by chylomicrons, and the endogenously synthesized triacylglycerols are transported from the liver to adipose tissue by very - low - density lipoproteins (VLDL). After delivering its content of triacylglycerols, VLDL is converted into intermediate - density lipoprotein (IDL) and then into low - density lipoprotein (LDL).

IDL and LDL carry cholesterol esters, primarily cholesterol lineolate. Liver and peripheral tissue cells take up LDL by receptor - mediated endocytosis. The LDL receptor, a genetically controlled protein spanning the plasma membrane of these cells, binds LDL and mediates its entry into the cell (119).

Lipoproteins

The lipoproteins are classified on the basis of their density, which in turn depends on their lipid content. The plasma also contains chylomicrons, particularly after fat - rich diets, which are pure triacylglycerols coated with a very thin layer of protein, to be transported to fat depots (120). Their composition in blood is given below in Table – 7 below.

Table 7: Composition of blood plasma lipoproteins (121)

Type	Density g / ml	Protein %	Triacyl glycerol %	Phospho lipid %	Choleste rol %	Diameter in nm	mg / 100 ml
Chylo microns	0.92 - 0.96	1.7	96	0.8	1.7	300 – 5000	100 – 250
Very low density lipid (VLDL)	0.95 - 1.00	10	60	18	15	300 – 750	130 – 200
Low density lipid (LDL)	1.00 - 1.06	25	10	22	45	200 – 250	210 – 400
High density lipid (HDL)	1.06 - 1.21	50	3	30	18	100 – 150	50 - 130

Predisposing factors to atherosclerosis

We need cholesterol for a number of body functions to manufacture adrenal and sex hormones, to produce bile acids used in digestion, to build cell walls, and to form protective sheath around nerves. Because cholesterol is so important, the body makes its own supply from the liver.

But, cholesterol is a pre-dominant substance in atherosclerotic plaque, which may develop in arteries and impede the flow of blood. Different mechanisms appear to underlie the varying levels of blood cholesterol in normal population.

Low-density lipoproteins (the so called 'bad cholesterol') provide cholesterol for necessary body functions but in excessive amounts, it promotes cholesterol accumulation in the artery walls. High-density cholesterol (the so called 'good cholesterol') tends to help remove excess cholesterol from blood. Therefore, a relatively **low ratio of LDL to HDL is desirable** for lowering the risk for development of coronary artery disease.

Why do high levels of cholesterol in the blood lead to heart disease? A common explanation is the injury theory. The disease begins when the thin protective layer of cells, the endothelium, that lines the arteries become damaged where thick deposits of cholesterol form on the inner surface of blood vessels. This damage can result from high blood pressure, excessive smoking, alcohol, sedentary lifestyle, and high cholesterol levels (122).

Carbohydrates should account for at least 55% of the total caloric intake with the remainder coming from protein. The glycemic index measures how much a given carbohydrate containing food raises the sugar level in the blood. High glycemic index foods have been associated with lower HDL cholesterol levels, higher triglyceride levels and more chances of heart disease.

Some people will always be able to eat anything and stay out of danger; others however, remain cholesterol high due to genetic factors. One reason is the absence of the LDL receptor, which leads to a markedly elevated plasma level of LDL cholesterol, deposition of cholesterol on blood vessel walls, and increased probability of heart attacks in childhood.

Fat in the diet also affects the cholesterol level. There are four different types of fats in our diet: Saturated fats, monounsaturated fats, polyunsaturated fats and a fourth type which goes by several names including trans fats, hydrogenated fats and partially hydrogenated fats. Trans fats occur rarely in nature and are produced artificially from naturally occurring unsaturated fats in order to convert oils to a solid or semisolid state.

While saturated and unsaturated fatty acids have nearly equal calorific value, consumption of a diet rich in saturated animal fats (myristic and palmitic), but poor in polyunsaturated fats tends to reduce the concentration of HDL and increase the concentration of LDL.

Oxidized LDL now appears to be an important risk factor; since LDL needs to be oxidized before it can be deposited in the atherosclerotic plaques.

One of the leading causes of atherosclerosis is stress. Due to stress, the level of fat and cholesterol in the blood stream increases, which could melt the membranes of the cells, lining the arteries, causing damage.

Management of Atherosclerosis

Lowering the cholesterol level has been scientifically proven to prevent heart disease and prolongs life. We can bring menacing levels down through dietary reforms, exercise and other modifications in life style.

Dietary fibre helps to expel the bile salts and cholesterol from the intestine into the feaces; therefore, the liver converts more cholesterol into bile salt thus reducing the total amount of cholesterol considerably. This is the only way of lowering cholesterol.

Relaxation and stress reduction calm the nervous system, lower blood pressure, reduce blood levels of stress hormones (adrenaline), cholesterol, and reduces platelet stickiness.

Physical exercise has a significant effect in the development and progression of atherosclerosis. It raises the level of HDL, which is cardio protective and lowers LDL, which contributes to building of plaque in the arteries. It lowers the level of triglycerides, the fraction of blood fat that is converted into LDL by the liver. Eventually the blood reaches a point where it begins to dissolve the fat in plaques in the arteries.

In general, it is prudent to eat a diet high in fruits and vegetables. These types of diets are definitely associated with lower cholesterol levels and a reduced incidence of heart disease. In addition to containing fibre, they also contain antioxidant substances including polyphenols; vitamins A, C, and E; as well as phytoestrogens, isoflavones which lower cholesterol and act as antioxidants.

Fruits and vegetables also contain sterols (sitosterol) and stanols (sitostanol), which block the absorption of dietary cholesterol from the intestine to help lower the cholesterol level. Oats lower LDL (the 'bad cholesterol') blood cholesterol levels. HDL (the 'good cholesterol') cholesterol is not affected. A number of studies have actually found

increased HDL cholesterol levels with oat consumption. That's one smart grain! Though most of its cholesterol-lowering power comes from soluble fibre, other components of oats - such as the protein and natural antioxidants - also contribute to its phenomenal effectiveness in reducing blood cholesterol.

Lipid lowering drugs

The drug therapy is usually used in conjunction with diet, and is instituted only after maximal efforts to control serum lipids by dietary measures alone prove unsatisfactory. The currently used antihyperlipidemic agents are briefly discussed below (123-24).

i) Cholestramine is an anion - exchange resin that binds bile acids in the intestine. The bound bile acids are lost in the feaces and the depletion of the bile acid pool stimulates conversion of cholesterol to bile acid. The result is that the LDL levels fall by 20 - 25 %. Colestipol is similar to cholestramine.

ii) Nicotinic acid and derivatives lower plasma triglyceride and cholesterol concentrations. These act as antilipolytic agents in the adipose tissue, reducing the availability of free fatty acids for hepatic triglyceride synthesis. Acipimox and Nicofuranose are the two better-tolerated nicotinic acid derivatives.

iii) Fibric acid derivatives inhibit lipid synthesis, causing plasma cholesterol to decline by 10 - 15 % and at the same time raise the HDL - cholesterol levels. The group includes: Bezafibrate, Fenofibrate, Gemfibrozil, and Clofibrate.

iv) Statin, Simvastatin, inhibits the rate-limiting enzyme in endogenous cholesterol synthesis, Hydroxy - methyl - glutaryl co - enzyme A (HMG CoA) reductase. This results in increased synthesis of LDL - receptors in the liver and clearing of LDL from the circulation. Likewise, ***Mevinolin*** inhibits the enzyme HMG - CoA, thereby reducing the rate of cholesterol synthesis and can reduce the LDL levels by 25 - 45 %.

v) Probucol lowers the plasma cholesterol levels. It reduces the cholesterol in both LDL and HDL fractions. This is achieved by increased excretion of bile acids in the feaces and inhibition of early stages of cholesterol biosynthesis.

vi) Dextrothyroxine Sodium stimulates the liver to increase catabolism and excretion of cholesterol via the biliary route into the feaces. This results in lowering of plasma cholesterol and LDL levels.

Fish (salmon) oil contains a special type of unsaturated fat called ***omega-3 fatty acids***. Omega 3 fatty acids lower triglyceride levels and have a blood thinning effect that can help protect against heart disease.

Summary

It is ironic that an essential constituent of body tissues can also be deadly. But the fact is that for each 1 mg / dl decrease in LDL cholesterol in plasma, there is approximately a 2 % decrease in mortality from atherosclerotic heart disease.

In the author's opinion, the very cause of the hyperlipidemias is associated with an improper diet containing either a high percentage of saturated fatty acids or foods with high glycemic index. The treatment lies in controlling the diet. For the purpose, nature has provided us with galactomannans. These substances can effectively bind with cholesterol, fatty acids, and bile acids and thereby reduce the absorption of the "lethal cholesterol".

The importance of Galactomannans lies in their selection by the nature i.e. the hexoses have six asymmetric carbons therefore there must be $2^4 = 16$ enantiomers; however, only three predominantly occur in nature. These include glucose, galactose ands mannose. The importance of glucose is well known and that of galactomannans becomes evident here.

2.4: DIABETES MELLITUS AND ITS MANAGEMENT

The addition of gel forming soluble unabsorbable fibres to the diet of diabetics reduces carbohydrate absorption.

Introduction

Blood sugar is α - β D - glucose. The fasting blood glucose level, in case of a normal individual, remains steady upto70 - 110 mg/dl during the 24 hours. Following food it increases up to 140 to 150 mg/dl within one hour and then after two hours, due to the action of insulin it drops to the normal level. Afterwards it does not fall below 70 mg/dl due to the antagonizing action of other hormones such as thyroxin, epinephrine, glucagon, etc. When the blood glucose sugar level rises, the kidney exerts a regulatory effect by filtering out the glucose in the urine. This is termed as glycosuria and occurs when blood sugar exceeds 170 - 180 mg/dl, the renal threshold of glucose (125).

Diabetes is a disease that occurs when the body doesn't make enough of a hormone called insulin, or if the body doesn't use insulin the right way. It is a condition of glycosuria accompanied with hyperglycemia, primarily due to lack of insulin, caused by degeneration or hypo activity of the beta cells of the islets of Langerhans. If left untreated, it may result in blindness, heart attacks, strokes, kidney failure and amputations. Only half of the people who have diabetes are diagnosed, because in the early stages of diabetes, there are few symptoms, or the manifested symptoms may be the same as in other health conditions.

Early symptoms of diabetes may include the following:

- Extreme thirst
- Frequent urination
- Unexplained weight loss
- Blurry vision that changes from day to day
- Unusual tiredness or drowsiness
- Tingling or numbness in the hands or feet
- Frequent or recurring skin, gum or bladder infections

An individual is at a risk for having diabetes if one is:

- Older than 45 years of age
- Overweight
- Doesn't exercise regularly
- Having a parent, brother or sister who has diabetes
- Had a baby that weighed more than 9 pounds or had gestational diabetes while pregnant
- Black, Hispanic, Native American, Asian or a Pacific Islander

Causes of diabetes mellitus

i) ***Due to insulin lack:*** This may give rise to juvenile diabetes. Here the pathology of pancreas Beta cells is common and insulin content of the pancreas is low. This is also referred to as the Type I or insulin dependent diabetes mellitus (IDDM).

ii) ***Type II or non - insulin dependent diabetes mellitus (NIDDM):*** which develops late in life, hence also called maturity onset diabetes, often occurs in obese people who retain the capacity to secrete insulin but who are resistant to its action.

iii) Hyperpetuitarism: gigantism, due to hyper secretion of growth hormone.

iv) Hyperthyroidism: Grave's disease.

v) Hyper functioning of adrenal cortex: Cushing's syndrome.

vi) Genetic / hereditary.

Metabolism in diabetes mellitus (126)

i) Carbohydrate metabolism: Due to absence of insulin, glycogen formation is depressed and glycogen content in the liver is lowered. There is an impairment of glucose oxidation and tissues utilize fats. The rate of gluconeogensis increases, wherein glucogenic amino acids are converted to glucose by the liver and hyperglycemia, is enhanced. Glucose tolerance is lowered, the effect of which is illustrated in Figure – 5 below.

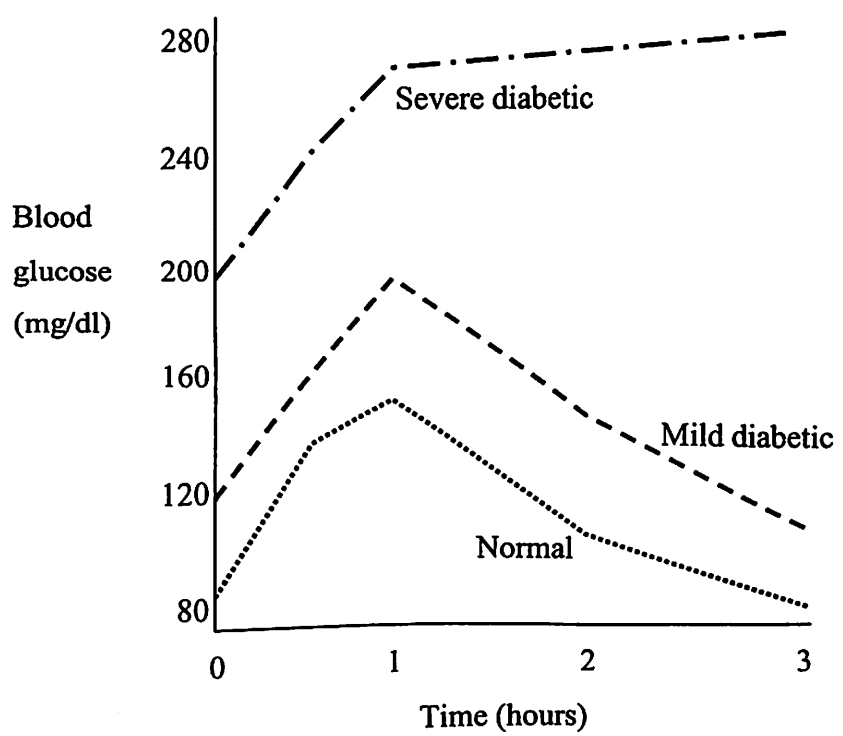


Figure 5: Glucose tolerance curves of diabetics and normal subjects.

ii) Fat metabolism: The depot fats are mobilized and the liver gets loaded with fats. In the liver fatty acids are converted into acetyl CoA. These two carbon units condense to form four-carbon aceto - acetic acid or ketone bodies. Increase in blood ketone bodies leads to metabolic acidosis, which causes deep and rapid breathing; and finally diabetic coma and death. The glycerol liberated from fats is converted into sugar and blood cholesterol rises.

iii) Protein metabolism: Gluconeogensis, conversion of non - carbohydrates to glucose, occurs at a faster rate. If enough protein is not given in the diet, the tissue proteins will be mobilized and converted into sugar, leading to marked weight loss.

iv) Urine changes: Presence of sugar in urine (glycosuria) increases the osmotic pressure, retards water absorption hence polyuria and consequently increased thirst (polydipsia).

v) Infections: There is reduced resistance to infections due to altered immunological response.

Insulin receptors

Insulin is bound to receptors on the surface of the target cells (liver, muscle, fat) and the insulin / receptor complex enters the cell. Receptors vary in number inversely with the insulin concentration, to which they are exposed, i.e. with high insulin concentration, the number of receptors declines (down regulation) and responsiveness to insulin declines (insulin resistance). With a low insulin concentration the number of receptors increases (up regulation) and responsiveness to insulin increases. Thus

obese Type II (NIDDM) patients having hyper secretion due to overeating may recover insulin responsiveness as a result of dieting so that the insulin secretion diminishes, cellular receptors increases and insulin sensitivity is restored (127).

Antidiabetic drugs

Patients having diabetes can be treated with one of the following:

- **Diet** alone
- **Diet** plus oral hypoglycemic agents
- **Diet** plus insulin (with or without oral hypoglycemic agent).

Type I (IDDM) patients require exogenous ***human insulin***. Type II (NIDDM) can be managed with oral antidiabetic agents. These are of two kinds, sulphonylureas and biguanides. The ***sulphonylureas*** activate receptors on the β - islet cells of the pancreas to release more stored insulin in response to glucose. Thus they are ineffective in insulin deficient patients. Examples include: tolbutamide, chlorproamide, glibenclamide.

The mechanism of ***biguanides*** is not well understood but their effect seems to result from reduced production of blood glucose in the liver due to gluconeogensis. Also they enhance the peripheral effect of insulin i.e. increase glucose uptake in peripheral tissues. The example includes: metformin.

Dietary fibres: The addition of gel forming soluble unabsorbable fibres to the diet of diabetics reduces carbohydrate absorption.

α - glucosidase inhibitor : Acarbose inhibits this enzyme in the gut, reducing the breakdown and absorption of carbohydrate, the agents are popularly known as 'starch blockers'.

Summary

The approach in management of diabetes is to keep the high blood glucose concentration within physiological limits. This can best be achieved by incorporating a soluble dietary fibre in the daily, routine diet of a diabetic patient. This measure alone can control the high glucose levels in some cases of diabetics. The damage of tissues, as seen in diabetes, is now thought, to be a result of the binding of glucose with the body proteins, just as in the formation of glycosylated hemoglobin. Therefore, the presence of a fibre like galactomannan in diet can possibly reduce the absorption of sugar.

If a patient has to be managed with insulin or oral hypoglycemic agents, then **the addition of galactomannans to the diet of such diabetics can possibly reduce the required dose of expensive humulin.** Likewise, **the dose of oral hypoglycemic agents can be reduced,** the use of which is hazardous in the elderly and in patients with heart disease.

In author's opinion, there is a need to recognize the potential of galactomannans as dietary food fibre and advocate its daily use in the diet of diabetics. For the purpose, there is a need to formulate a suitable dosage form.

2.5: RHEOLOGY

Gums, polymers of saccharides, or simply polysaccharides, have the property to disperse in water to form a slimy / mucilaginous colloidal dispersion which may sometimes gel.

Introduction

The term rheology, from the Greek *rheo* (to flow) and logos (science), was suggested by Bingham and Crawford and is defined as the science of the deformation and flow of matter and describes mainly the material properties of fluid and semi-solid materials (128). Rheology is used to describe the properties of a wide variety of materials such as oils, foods, inks, **polymers**, clays, concrete, etc. The common factor is that these materials exhibit some sort of flow and, therefore, cannot be treated as solids.

One of the properties often dealt with in rheology is viscosity, which measures how thick a fluid is or simply its resistance to flow. Rheology plays an important role in almost all the industries including the Pharmaceutical industry where rheological findings are of fundamental importance for the development, manufacture and processing of Pharmaceutical products.

Rheology concerns the flow and deformation of substances and in particular their behavior in the transient area between solids and fluids. Rheology attempts to define a relationship between the stress acting on a sample substance and the resulting kinematic values of deformation or flow.

Types of fluids

The fluids may be of two types i.e. Newtonian or non - Newtonian. Newton was the first to recognize that higher the viscosity of a fluid, the greater the force required per unit area (shearing stress) to produce a certain velocity gradient (rate of shear). The consistency curves for different types of fluids are shown in Figure - 6 below.

The flow of fluids is described by an empirical power law referred to as the Ostwald de Waele equation and is give below: (129)

$$F^N = nG$$

If N = 1, flow is Newtonian, or plastic (with a yield value) e. g. simple liquids in case of former or flocculated suspensions in case of latter if N < 1 , flow is dilatant e.g. concentrated deflocculated suspensions and if N > 1 , flow is pseudoplastic e.g. polymer dispersions.

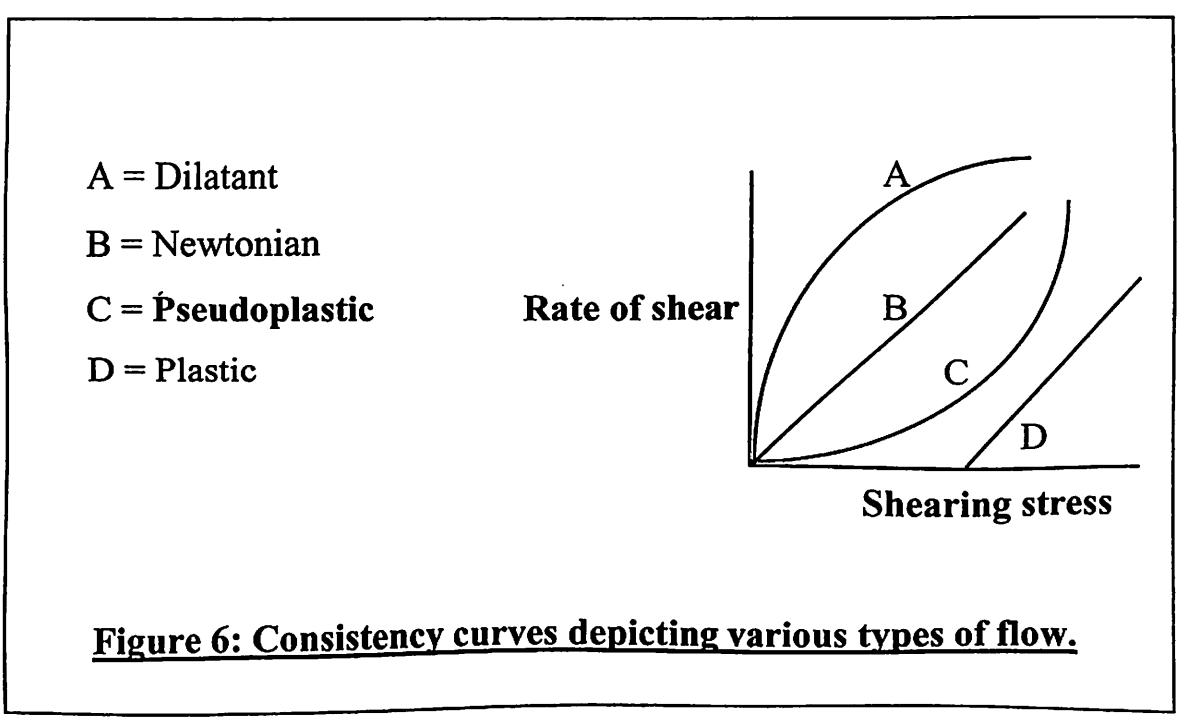


Figure 6: Consistency curves depicting various types of flow.

As a general rule, polymers in solution exhibit pseudoplastic flow. The consistency curve - C, shown above in Figure - 6, for a pseudoplastic material begins at the origin and concaves towards the shear rate axis (reduced consistency) with increasing shear stress. Hence, these systems are also referred to as shear thinning systems.

Polymers are high molecular weight natural, semi - synthetic or synthetic compounds which find extensive applications in our day-to-day life. Gums, polymers of saccharides, or simply polysaccharides, have the property to disperse in water to form a slimy / mucilaginous colloidal dispersion which may sometimes gel.

Pseudoplasticity: mechanistic explanation

The curved rheogram for pseudoplastic materials results from a shearing action on the long - chain molecules of materials such as linear polymers. As the shearing stress is increased, the normally disarranged molecules begin to align their long axes in the direction of flow. This orientation reduces the internal resistance of the material and allows greater rate of shear at each successive shearing stress. In addition some of the solvent associated with the molecules may be released, resulting in an effective lowering of the concentration and the size of the dispersed molecules. This is schematically depicted in Figure - 7 (130).

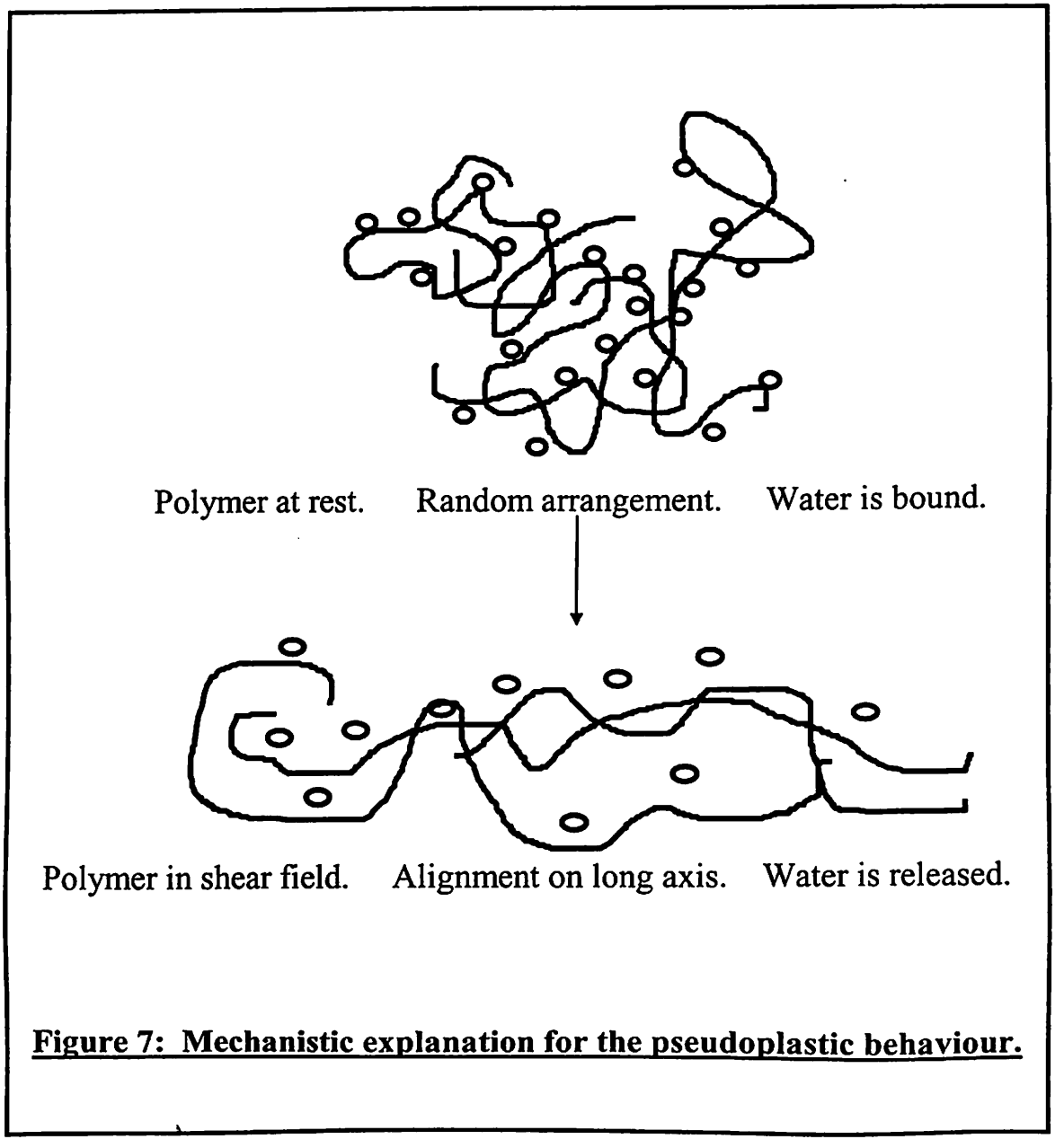


Figure 7: Mechanistic explanation for the pseudoplastic behaviour.

Factors affecting viscosity

The factors which influence viscosity can be discussed under two headings as follows (131):

Intrinsic factors

i) Molecular weight: The heavier the molecule, the greater shall be the viscosity.

ii) Molecular size / Shape: Large and irregularly shaped molecules tend to be more viscous compared to small and symmetric molecules. Spherical shaped molecules slide past one another and thus have low viscosity.

iii) Intermolecular forces: The stronger the intermolecular forces, the higher shall be the tendency of molecules to stick together thereby increasing the viscosity.

Extrinsic factors

i) Temperature: As the temperature increases, cohesive forces reduce leading to reduced viscosity.

ii) Pressure: Higher the pressure higher the cohesive forces of interaction resulting in increased viscosity.

iii) Added substance: In general, addition of nonelectrolytes increases viscosity while that of electrolytes reduces viscosity.

Determination of viscosity

In case of Newtonian systems, the rate of shear is directly proportional to the shearing stress. Therefore, single point viscometer i.e. the equipment that works at a single rate of shear, is sufficient. For the evaluation of non - Newtonian fluids, multipoint viscometers are required,

because the apparent viscosity is to be determined at several rates of shear to get the entire consistency curve.

The single point viscometers are used for determining the viscosity of Newtonian fluids. These include:

i) Capillary viscometers exemplified by: Ostwald viscometer

ii) Falling sphere viscometers.

The multipoint rotational viscometers are used to determine the viscosity of non- Newtonian fluids. These include:

i) Cup and bob type and

ii) Cone and plate type.

Principle

Since, a dispersion of galactomannans will exhibit pseudoplastic flow; it shall be relevant to consider the working principle of rotational viscometers.

Cup and bob type

The sample is placed in the cup and the co - axial bob is placed in the cup up to an appropriate height. The sample is accommodated between the gap of cup and bob. Now, either the cup or the bob is made to rotate and the torque resulting from the viscous drag is measured by a spring or sensor in the drive of the bob.

These type of viscometers are exemplified by:

a) Couette type : revolving Cup e. g. Mac Michael

b) Searle type: revolving Bob e. g. Stormer, Hercules, Brookefield.

Cone and plate type

The sample is placed at the centre of the plate, which is then raised into position under the cone. The cone is driven by a variable - speed motor and the sample is sheared in the narrow gap between the stationary plate and rotating cone. The rate of shear in rpm is increased and decreased by a selector dial and the viscous traction or torque produced on the cone is read on the indicator scale. A plot of rpm (rate of shear) versus scale reading (shearing stress) may thus be plotted.

Rheology of galactomannans

According to the literature surveyed the rheological study of the following galactomannans has been carried out:

Guar gum (132), Xanthan gum, Locust Bean gum (133 - 35), *Cassia spectabilis* (136), *Crotalaria intermeridia*(137), *Cassia fistula*(138), *Cassia nodosa*(139), *Cassia javanica* (140)

The viscosity of some galactomannans is presented in Table – 8 below, for ready comparison of their relative viscosities.

Table 8: Viscosity of some galactomannans at 1 % w/v concentration.

S. No.	Galactomannan	Viscosity cps	G : M ratio*
1	*Crotolaria intermedia*	7200	1 : 1
2	*Cyamopis tetragonolobus*	6800	1 : 2
3	*Cassia marilandica*	app. 2500	-
4	*Cassia fistula*	app. 100	1 : 4

*Galactose : Mannose ratio

Approximate hydration rate of fine mesh guar powder as measured by Brookefield's viscometer at 20 rpm at 25°C can be determined by the following empirical equation:

$$\eta = 452 + 656 \ln T$$

Where η is the apparent viscosity and T is hydration time.

Empirical relationship has also been derived between viscosity-concentration for course and fine mesh guar powders. These are:

$$\eta = 3104 \times [C]^{3.11}$$ for fine mesh powder (sieve # 250)

$$\eta = 2274 \times [C]^{3.27}$$ for course mesh powder (sieve # 100)

where C is the concentration and η the apparent viscosity at 25°C.

These equations show that the viscosity increase is 8-10 folds when the concentration is doubled.

An empirical relation between the molecular weight and intrinsic viscosity has also been worked out as given below:

$$[\eta] = 3.8 \times 10^{-4} \times M^{0.723}$$

where M is the molecular weight of polymer and $[\eta]$ the intrinsic viscosity.

In aqueous solution the viscosity of galactomannans is reduced in presence of salts and hydrophilic liquids/solids due to their competition for binding with water.

Summary

The conclusion drawn from the above literature is that work on galactomannans from fenugreek is lacking in general and on its rheological properties in specific. The viscosity of fenugreek galactomannans needs to be compared with other galactomannans. The factors affecting its viscosity namely temperature, pH, concentration, rpm, and the effect of added substances like polymers, electrolytes and nonelectrolytes need to be studied.

Further, the relationship between viscosity, galactose: mannose ratio, molecular weight and the medicinal use of the galactomannans needs to be studied. Fenugreek has a galactose: mannose ratio of 1:1, therefore it can be expected to possess a higher viscosity as compared to galactomannans from other sources. The medicinal value of depolymerized, reduced viscosity fenugreek galactomannan also needs to be studied.

2.6: DIETARY FIBRES: NON - STARCH POLYSACCHARIDES

A factor for the cause of diseases like constipation, obesity, hemorrhoids, cardiovascular diseases, hypertension, diabetes and colo - rectal cancer can be co - related with a diet low in fibre content.

Introduction

The six basic constituents of food are: the fats, carbohydrates, proteins, vitamins, minerals, and water. Among the carbohydrates, food contains mono - and di - saccharides, which are metabolized in the human system via the glucose pathway and serve as a source of energy.

There are polysaccharides that are not metabolized in the human being but are either excreted unchanged, or are partially fermented by colonic flora to short chain fatty acids (SCFAs) like acetate, propionate or butyrate. Unlike starch, (metabolizable polysaccharide), these nonmetabolizable, non - starch polysaccharides (NSPs) are collectively known as food or dietary fibre (141).

Definition and classification

The food fibres are broadly classified as

i) Insoluble food fibre e.g.: cellulose

ii) Soluble food fibres e.g.: guar, tamarind, psyllium, Gum Arabic, xanthan gum, carraginnan, pectin, etc.

Association of Official Analytical Chemists (AOAC) has defined soluble food fibres as the alcohol precipitated fraction of water extractable portion of food, which has been suitably subjected to degrade the proteins.

Thus a large number of cell wall, reserve and structural polysaccharides constitute food fibres.

Health benefits of food fibres

A factor for the cause of diseases like constipation, obesity, hemorrhoids, cardiovascular diseases, hypertension, diabetes and colo - rectal cancer can be co - related with a diet low in fibre content. Individuals predisposed to such diseases would be anticipated to benefit from a diet rich in NSPs. Soluble food fibres perform certain important physiological functions which are beneficial to sustain good health (142). Some of these are considered below.

***i) Source of energy*:** Carbohydrates yield energy in two forms, as monosaccharides and as SCFAs in variable proportions which is in the range of 6 - 9 KJ / gm. i.e. about half of that produced by glucose, which is 16 - 17 KJ / gm. (143). The availability of energy from NSPs is related to the extent of their degradation by microorganisms in the large intestine.

ii) Obesity management: NSPs are satiating and decrease food consumption (144 – 45). Thus they can be employed in the management of obesity. Studies concerned with the effect of NSPs on food intake need to be long term, which at present are lacking. An important attribute of foods containing NSPs is their potential to prevent the constipation that may occur when less food is eaten. Additionally, individuals attempting to slim aim to starve. In such cases, the NSPs can maintain the supply of energy to the colon during slimming.

iii) Energy supply to the colon: The end products of fermentation of NSPs in the colon are the SCFAs: acetic, propionic, butyric. While acetic and propionic acids are absorbed and transported for metabolism by tissues, butyric acid serves as the fuel for the mucosal cells lining the colon (146). The absence of butyrate seems to result in the colonic mucosa becoming inflamed. A number of studies lend support to a view that butyric acid is essential to maintain a healthy colon (147 - 48).

iv) Diarrhea: The incidence of diarrhea in hospital patients fed with diets devoid of NSPs may often be as high as 30 %. Here also, the supply of energy as butyrate might be expected to maintain the colonic tissue and so the absorptive capacity of the colon.

***v) Constipation*:** This is quite a common complaint affecting the masses. It often is a problem in individuals attempting to slim on low - residue diets. The laxative effect of NSPs is well known and insoluble NSP is more effective than soluble NSP.

***vi) Bacterial translocation, sepsis and gut strength*:** The prevention of sepsis and bacterial endotoxemia might be prevented by SCFAs and difficult - to - digest carbohydrates (148). Such substrates stimulate gut growth and so may maintain the gut barrier to infection. The effect of NSPs in preventing bacterial translocation from the gut lumen to the circulation is variable and is dependent on the extent of fermentation and proportion of SCFAs produced. SCFAs can possibly improve the tone of the bowel, which generally decreases as the age advances (149). However, the physico - chemical properties of the NSPs that best maintain the gut barrier have yet to be defined.

vii) Colorectal cancer: Butyrate production has been suggested as the protective factor against colon cancer. Butyric acid influences gene expression has a differentiating effect on human cancer cell lines and increases the doubling time for tumors (150, 151).

viii) Control of glucose metabolism: NSPs influence the entry of glucose into the circulation by a number of mechanisms. First, by slowing the digestion of nutrients, due possibly to the impaired mixing of digesta with enzymes, and diffusion of the hydrolyzed products. Secondly, by controlling the insulin dependent glucose metabolism post parandially, (152 - 53) possibly by improving the insulin sensitivity. Insulin sensitivity is usually decreased in obesity, Type II diabetes, and CVS disease. Additionally, it might be due simply to the lower intake of food in response to feeding NSPs.

ix) Blood pressure: NSPs in general and guar gum in particular has been reported to decrease blood pressure and urinary sodium and potassium excretion (154 - 55). However, the blood pressure is under control only so long as a high fibre dietary treatment is continued.

x) Lipid metabolism: NSPs have been suggested to prevent IHD. Studies with NSPs suggest decrease in plasma cholesterol concentration may be accompanied by selective increases in HDL - cholesterol or decrease in LDL - cholesterol (156). In this respect, soluble NSPs are thought to be most effective. However, the metabolic response to difficult to digest carbohydrates is dependent on the nature of carbohydrates and its interaction with gut flora (157 - 59).

Summary

Foods rich in difficult to digest carbohydrates, or non - starch polysaccharides, referred to as dietary fibres, are associated with lower incidence of morbidity and mortality. Therefore, NSPs have potential therapeutic value and the relative potencies of the NSPs have yet to be established (160).

There is a new insight to the fact that NSPs in the gut are fermented, producing short chain fatty acids like acetic, propionic, and butyric acids. More studies to assess the therapeutic value of butyrate are needed, but the observations to date are consistent with the view that butyrate may be essential to maintain a healthy colon while acetate and propionate are absorbed and utilized by the tissues.

It is also possible that one type of NSP may be effective whereas other is not. Not all soluble NSPs have an effect on plasma cholesterol levels. The efficacy of NSPs may depend on the viscosity, fermentability and saccharide composition. Therefore, it is not possible to raise claims for NSPs in general. **A health claim for a particular NSP can be raised only and only if it has been investigated.**

It has been observed that polysaccharides having linear galactose or mannose backbone are most effective among the various NSPs. This may possibly be due to their unique structural feature of having a pair of cis - hydroxyl group on their pyranose ring.

However, doses of viscous polysaccharides needed to influence blood glucose are quite high that cannot be easily achieved in the diet. Therefore, ways have to be devised to incorporate therapeutic doses of gums into dosage forms or foods, which should be palatable and accepted by the patient as well.

Since, fenugreek is an indigenous food product, there is an urgent need to investigate the potential medicinal applications of its galactomannan and formulate a suitable dosage form or a designer food product.

2.7: FERMENTATION OF COMPLEX CARBOHYDRATES

The action of bacterial fermentation produces a range of compounds that are bioactive and may have significant effects on the human host.

Introduction

The most widely accepted action of complex carbohydrates on the human colon is to increase stool output but not all complex carbohydrates are effective stool bulkers. Some of which promise to be effective, by having a large water holding capacity, are often ineffective because they are fermented by the bacteria in the colon and so loose their fecal bulking effect. The action of bacterial fermentation produces a range of compounds that are bioactive and may have significant effects on the human host (161).

When considering the action of carbohydrates on the human colon it is important to consider the following factors:

i. The digestibility of carbohydrate in the small intestine.

ii. The fermentability of carbohydrate in the colon.

iii.The water holding capacity of fermented residue.

iv.The rate and site of fermentation

v. The profile of fermentation products.

Carbohydrate digestibility

Carbohydrates escape digestion in the small intestine because of malabsorption or the resistance of chemical bonds or tertiary structure to pancreatic enzymes. Such carbohydrates include those contained within the definition of dietary fibres.

Carbohydrate fermentability

The complex carbohydrates that enter the colon can be divided broadly into the following three groups:

i) Those that are easily fermented and have little effect on stool output but increase colonic SCFA production e.g. guar, pectin, and gum Arabic.

ii) Those that are very slowly fermented and have the greatest effect on stool output. e.g. ispaghula, cellulose, gellan.

iii) Those that are partially fermented and have effects on both colonic SCFA and stool output e.g. wheat bran, gum Tragacanth, Karya gum, and Xanthan gum.

The flora of human colon is a large complex community of about 10^{11} organisms per gram content, made up of 400 - 500 bacterial species (162). The organisms are predominantly anaerobic. Complex carbohydrates that enter the colon can be divided into those that are water soluble e.g. pectin and guar and those that are water insoluble e.g. cellulose and wheat bran.

Water holding capacity

It was thought initially that the water holding capacity of a complex carbohydrate was the major determinant of its action on stool output, but it is now recognized that it is the water holding capacity after subjection to bacterial fermentation that has the major influence on stool bulking activity (163). Water-soluble carbohydrates, such as guar gum, are more likely to be fermented than insoluble carbohydrates, such as wheat bran (164). Fermentation results in the loss of water holding capacity and thus reduces the effect on stool output.

Rate and site of fermentation

The rate and site of fermentation of soluble fibres is also critical to their effects on output. Those fibres that are fermented at a more distal site in the colon (Group iii fibres as classified above) appear to influence the stool water content the most perhaps due to the greater osmotic pressure exerted by the produced SCFAs. Fibres that are easily fermented at a proximal site in the colon (group i fibres as classified above) produce SCFAs that are very rapidly absorbed, exerting a negligible osmotic pressure for stool water content. While fibres that are very slowly fermented (Group ii fibres as classified above), have the greatest effect on stool output probably due to the intact physical structure and water holding capacity

The profile of fermentation products

Carbohydrates are fermented by the Emden - Meyerhof Pathway (EMP) to short chain fatty acids (SCFAs), gases, CO_2, H_2, CH_4, H_2S, and produce a low pH.

The major SCFAs produced in the adult human colon are: acetate approximately 60%, propionate less than 20 %, and butyrate less than 20 %. These SCFAs have a variety of biological actions as shown in Table - 9. Many actions are shared by all SCFAs, but each SCFA has its own role to play.

pH: Rapid fermentation of carbohydrates may result in a decrease of colonic pH (171). A low pH will precipitate or encourage sequestration of certain bioactive molecules such as bile acids, thereby reducing their reabsorption and increasing excretion. A low pH will also increase the availability of calcium ions, which may inhibit carcinogenesis (172).

Gas Production: The most obvious action of gas production is distention. There may be adaptation to complex carbohydrates and distention and flatulence appear to decrease with time (173). CO_2 is the major gas produced with smaller amounts of hydrogen, methane, and hydrogen sulfide. Since hydrogen is detectable in the breath, it can be used as an index of carbohydrate fermentation (174) and to determine the mouth to ceacum transit time (175).

Table - 9: Possible effects of short chain fatty acids

S. No	Effect	SCFA	Ref.
1.	Energy source for body	Acetate	165
2.	Anti - nepotistic effect	Butyrate	166
3.	Energy source fort gut mucosal cells	Butyrate	167
4.	Modulation of liver metabolism of glucose and lipid	Propionate	168-9
5.	Stimulation of cell proliferation throughout gut	Butyrate	-
6.	Modulation of colonic motility	Butyrate	170
7.	Promotion of water and electrolyte absorption	All SCFAs	-
8.	Modulation of stool water	All SCFAs	-

Summary

Carbohydrates that enter the human colon are subjected to fermentation by the bacterial flora. The main products of fermentation are short chain fatty acids (SCFA) and gases. These products have a wide range of physiological activities.

The major SCFAs produced are acetate, propionate, and butyrate, which are useful as a source of energy for body, to regulate metabolism of glucose and lipid, and to maintain healthy colon respectively.

As seen from the above account, the consequences of feeding carbohydrates that are not absorbed in the small intestine are important and diverse. Each carbohydrate has its own physiological effect and it is very difficult to predict the action of a particular carbohydrate unless it is studied.

The potency of a particular complex carbohydrate in comparison to other complex carbohydrates has to be assessed. Also, the effect of incorporating these complex carbohydrates in combination with diet of an individual need to be assessed.

2.8: SHORT CHAIN FATTY ACIDS: FERMENTATION PRODUCTS OF COMPLEX CARBOHYDRATES

It is suggested that SCFAs could contribute to the lipidemic and glycemic effects of dietary fibres in man.

Introduction

The increase in the consumption of dietary fibres by man is associated with an improvement in glucid and lipid metabolisms. Various mechanisms have been put forward to explain these effects. The dietary fibres alter the digestive secretions and digestion and absorption of nutrients, they also modify the morpho - histological structure of the gut and transit time in the g.i.t. The alterations in the digestibility of glucido - lipidic substrates at small intestine level are thought to induce an alteration in the absorption rate of nutrients and in their concentrations at portal level. They also induce the secretion of hormones. This is bound to affect the nutrient cellular bioavailability, the regulation of enzyme activities and altogether alter the metabolism of carbohydrates and lipids (176).

Recently, a number of research workers have put forward the hypothesis of involvement of short chain fatty acids (SCFAs), the by - products of colonic dietary fibre fermentation, in fibre - induced metabolic changes (177).

Nature and origin of SCFAs

The SCFAs or volatile fatty acids are saturated fatty acids made of 1 to 6 carbon atoms produced by the fermentation of colonic micro flora (178). Each molecule of SCFA presents only one carboxylic organic acid

functional group: -COOH. The SCFAs are weak organic acids with a pKa of 4 - 5. Acetate, propionate, butyrate having 2, 3, 4, carbon atoms, respectively, constitute the main SCFAs. These substances are found in their native state in our every day diet and environment. Besides the exogenous source of fatty acids from the food, most of the SCFAs are produced endogenously by the host flora from a number of fermentable substances. The major fermentable food substrates are the non-starch polysaccharides, (NSPs) which are, defined as 'dietary fibre' e.g. cellulose, pectin guar gum, xanthan gum etc. Starch and its hydrolyzed by - products not digestible in the small intestine, referred to as resistant starches, proteins and glycoproteins (mucins) constitute substrates for bacterial fermentation. In addition to these, the low calorie glucidic ingredients: fructo - oligosaccharides (F.O.S.) and polydextrose constitute substrates for bacterial fermentation.

The necessary daily amount of fermentable substrates to sustain the colonic bacterial population has been estimated at 70 gm.

Production of SCFAs

The predominant bacterial species in the human colon are the anaerobes from the genera *Bacteroides, Eubacterium, Bifidobacterium, Peptostreptococcus,* and *Fusobacterium.* These bacteria degrade the polysaccharides into constitutive monomers (hexose or pentose). Hexoses are then degraded by anaerobic glycolysis and rejoined by pentoses (xylose) at glyceraldehyde triphosphate level. Acetate is made from acetyl - CoA which itself is made from pyruvate. Butyrate is also made from acetyl - CoA, whereas propionate is obtained by the decarboxylation of succinate. (See Fig. - 8).

The molar ratios between acetate, propionate and butyrate are 60:15:25 respectively. In fact, the physicochemical nature of fibres and fermentable substrates affect the amount and profile of the SCFAs produced (179 - 80). The production profile of SCFAs from various polysaccharides is tabulate in Table - 10.

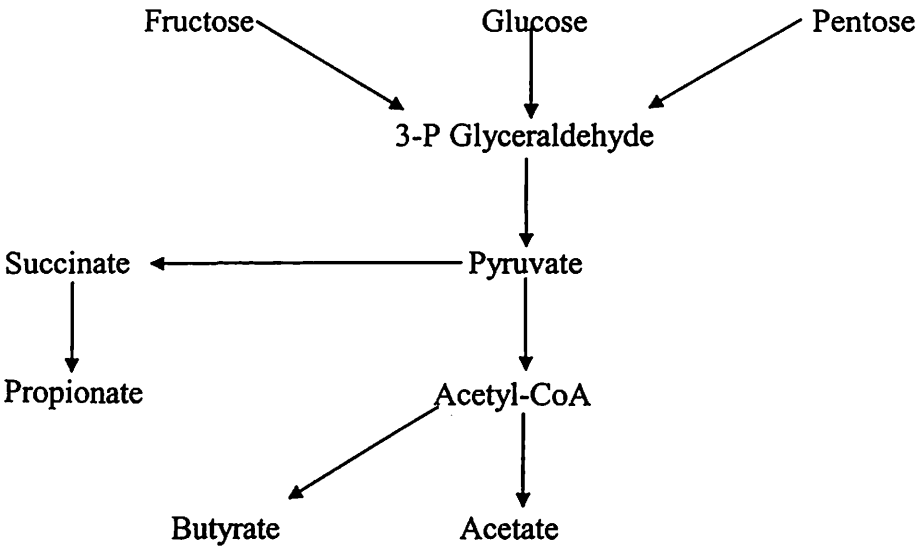


Figure - 8: Production of short chain fatty acids by colonic bacteria.

Table 10: Percentage of the short chain fatty acids produced from degradation of various substrates.

S. no	Substrate	SCFA mmol/gm	% of each SCFA produced				% substrate fermented
			Acetate	Propionate	Butyrate	Valerate	
1.	Glucose	7.4	62	16	22	-	100
2.	F.O.S.	8.4	74	11	15	-	100
3.	Corn starch	7.8	61.4	14.9	22.1	1.6	93
4.	Resistant starch	4.0	52.5	22.5	25	-	30
5.	Pectin	6.4	90	7	3	-	93
6..	Gum Arabic	7.3	68.5	20.5	8.2	2.7	91.8
7.	Guar gum	6.9	59.4	27.5	8.7	4.3	-
8.	Beet fibre	1.2	93	6.6	0.7	-	37.6
9.	Soy fibre	1.9	71	21	8	-	65.2
10	Wheat bran	0.6	63	30	7	-	23.6
11	Oat fibre	0.4	88	11	1	-	5

The most fermentable substrates are fructo - oligosaccharides (F.O.S.), pectins and gums. These produce 6.4 to 8.4 mmol. SCFAs for each gram of incubated fibre.

Insoluble fibres like beet, soy, wheat bran, and pea or oat fibres are scarcely degraded and produce 0.4 to 1.9 mmol SCFAs for each gram of incubated fibre. Acetate is always the predominant fatty acid produced. It is the by-product of starches and FOS. Guar gum and Gum Arabic produce large amounts of propionate. The other fatty acids such as valerate are produced in small amounts.

Digestive absorption of SCFAs

There is no competition between these three SCFAs, namely acetate, propionate and butyrate, with regards to their absorption. It has been proved that in man the SCFAs are absorbed from the colon (181 - 82). The mechanism of their absorption has not been completely elucidated but the works of Cummings and Ruppin seem to indicate that absorption of SCFAs takes place both in ionic and in non-ionic forms. The fatty acid colonic absorption is a crucial factor of the organism hydro electrolytic homeostasis. The transfer of SCFAs is always associated with the appearance of bicarbonate ions and a decline of CO_2 pressure. The bicarbonate ions seem to stabilize intraluminal pH, ensuring optimal conditions for the microbial activity and thus stimulating the production of fatty acids. The SCFAs, along with their own absorption, stimulate the absorption of sodium and water. Hence, a number of authors even recommend the adjunction of acetate to orally taken rehydration salts, in view of its capacity to reabsorb water and sodium (183).

Summary

SCFAs are the major end products of dietary fibre degradation by anaerobic bacteria in the large bowel. The principal SCFAs are acetate, propionate and butyrate. They are rapidly absorbed, stimulate salt and water absorption and provide energy for man. The mean energy values of SCFAs are estimated to be 2.68 Kcal. for acetate and 4.73 Kcal. for butyrate. However, it is suggested that SCFAs could contribute to the lipidemic and glycemic effects of dietary fibres in man (184 - 94).

All the SCFAs have the potential to act as an energy source for the body but since acetic acid is produced in greater amounts and is the only SCFA to circulate around the body; this is the major energy source from bacterial fermentation. As far as propionate is concerned, the *in vitro* and *in vivo* studies confirm the tendency of propionate to deplete glycemia in fasting subjects and to enhance their sensitivity to insulin. Butyrate is mostly utilized as an energetic substrate by the colonocytes. It provides 60 - 70 % of the colon energy needs and constitutes a factor of cellular differentiation for the human colorectal cells. It inhibits the proliferation of malignant cells, and stimulates the proliferation of normal colonic cells.

As far as the part played by SCFAs in lipid metabolism is concerned, further investigations in man are still required to be carried out.

2.9: ANIMAL MODELS FOR SCREENING ANTI - HYPERLIPIDEMIC AGENTS.

Any attempt to measure the different lipids in the blood to evaluate agents, interfering with their levels must take into account of the fact that lipid patterns in most laboratory animals differ from that in human beings.

Introduction

The present interest in drugs affecting lipid levels in plasma is based on the widely held opinion that these levels influence the initiation and progress of atherosclerosis and ischemic heart disease. Lipids are present in large amounts in atherosclerotic lesions, and it is possible to induce arterial lesions in experimental animal by administration of lipids.

Most of the data available from human and animal studies are about the relation between atherosclerosis and blood cholesterol levels. All the procedures for producing experimental atherosclerosis include cholesterol feeding. Atherogenic diets induce an increase in all lipid fractions, i.e. an increase in cholesterol, phospholipid and triglyceride levels.

The commonly employed test methods involving the use of animals for evaluating the cholesterol - lowering agents are briefly considered below.

Models for testing antihyperlipidemic agents (195)

i) Normal and hypercholesterolaemic animals

The use of rabbits on cholesterol - rich diet is useful for testing drugs able to interfere with cholesterol absorption, degradation or excretion or with general lipoproteins production and secretion into serum. This test is of

no value for investigating drugs affecting cholesterol synthesis, because hypercholesterolemia is exogenous in nature and itself reduces synthesis to low levels.

The hypercholesterolemia should be as high as possible for the production of arterial lesions, but to test potential cholesterol depressants, the cholesterol levels need not to be raised to the highest possible levels. Because such a high cholesterol concentration may interfere with lipoprotein production or even with cholesterol absorption and the conditions of lipid transport may be so different from the physiological situation as not to give valid information of the kind required.

In order to produce hypercholesterolemia, cholesterol feeding needs to be continued for a period of two to three weeks and to induce atherosclerotic lesions and calcification feeding has to be continued for up to three months. Having induced hypercholesterolemia, the ability of drug under study to reduce the increased levels can be co - related to its cholesterol depressant action.

It has been observed that feeding rabbits with saturated fat alone can induce a hypercholesterolemia more closely related to human conditions (196).

ii) Triton induced hyperlipidemia

The administration of the surface-active agent, Triton WR 1339, a polymer of polyoxyethylenephenol, induces hypercholesterolemia in many species. The physicochemical properties of lipoproteins modified by Triton involve an increase in the low - density lipids. It also interferes with the uptake of plasma lipids by the tissues. As a result of this impaired uptake, endogenous cholesterol biosynthesis is rapidly increased in the liver.

The test may therefore be divided into two phases. In the first phase the drug to be tested is given immediately after Triton injection, and the reduced levels of blood lipids for periods up to 8 hr. are measured as criteria for activity. In the second phase, the drug is give after 22 hr. after the Triton injection and the slope of the blood cholesterol curve is then observed. On the second part of the curve appear drugs interfering with cholesterol breakdown or excretion, in contrast with the first phase, in which drugs blocking endogenous synthesis are active.

This test has the advantage of being simple and rapid, but it is doubtful if the nature of hypercholesterolemia induced can be compared with that which occurs under physiological conditions.

iii) Estrogen - treated fowls

The administration of estrogen hormones to hens induces a rise in blood lipid and atherosclerotic lesions. This hyperlipaemia is endogenous in nature. The mechanism of action is not clear. It has, however, been observed that the changes in plasma triglyceride levels are even greater than the change in cholesterol levels (197).

iv) Hypercholesterolemia in unweaned rats

Unweaned rats show much higher cholesterol levels in blood than do adult rats. Their hypercholesterolemia may be greatly increased by the addition of propylthiouracil to their diets.

v) Castration of rats

Castration of rats involving a minor surgical operation is also found to induce hyperlipidemia. The probable mechanism is that the gonad which

is one of the major organs utilizing cholesterol, is removed resulting in an increase in the blood cholesterol levels.

Quantitative determination of cholesterol (198 - 99)

The various methods by which cholesterol can be determined quantitatively are described below.

i) Colourimetric - Watson method.

Cholesterol is determined by a simple colourimetric method commonly known as Watson method. The principle of the test based on the fact that cholesterol reacts with acetic anhydride in the presence of glacial acetic acid, 2, 5 - dimethyl benzene sulphonic acid and concentrated sulfuric acid to form a green coloured complex. The intensity of the colour measured at 575 nm is proportional to the cholesterol concentration. The reaction is very sensitive to temperature. Grossly haemolysed and highly icteric sera are not suitable for the test.

In order to determine high-density lipids (HDL), low-density lipids (LDL) and very low-density lipids (VLDL) are first precipitated by adding phosphotungstic acid and magnesium chloride to serum sample. Centrifugation leaves only the HDL in supernatant, which is determined by the usual method.

Another colourimetric reagent for the quantitative estimation of serum cholesterol using ferric chloride has also been used by workers (200).

ii) Enzymatic method - application on autoanalyzer

Cholesterol esters are hydrolyzed by cholesterol ester hydrolase to free cholesterol and fatty acids. The free cholesterol produced and pre - existing one are oxidized by cholesterol oxidase to cholestenone - 4 en - 3 -

one and hydrogen peroxide. Peroxidase acts on hydrogen peroxide and liberated oxygen reacts with the chromogen (4 - amino phenazone) to form a red coloured compound, which is determined at 510 nm.

iii) Agarose electrophoresis

The lipoproteins are colloidal in nature. At pH 8.6 when subjected to an electrical current, all the proteins behave like anions and move towards the anode. The rate of migration depends upon their different molecular weights and sizes. By using a densitometer, quantitative determinations of various lipoproteins can be made (201).

Quantitatively, the various lipid fractions are related by the following equation:

Total cholesterol = HDL + LDL + VLDL
Total glycerides = 5 X VLDL

iv) Radio - isotope labeled cholesterol

Alternatively, cholesterol can radioactively be labeled and simultaneously administered with the test drug (202). Then determining the total radioactivity of lymph, biosynthetic intermediates (before and after mevalonic acid stage), different lipid fractions, or of cholic acid respectively gives an idea of drugs affecting cholesterol absorption, biosynthesis(203), catabolism (204 - 05) or excretion (206).

Summary

Atherogenic diets induce an increase in all lipid fractions, not only the cholesterol plasma levels. Any attempt to measure the different lipids in the blood to evaluate agents, interfering with their levels must take into account of the fact that lipid patterns in most laboratory animals differ from that in human beings. Animals have lower total lipid levels and a much higher percentage of high-density lipoproteins. Another difference is that individual lipid fractions show considerable species differences: dietary cholesterol has no evident effect on cholesterolaemia in rat, dog or man, but a marked and rapid effect in the rabbit. A difference also exists in the bile acids and their conjugates, which are in some species mostly taurine conjugates and in others glycine conjugates.

Each lipoprotein contains a fairly constant series of lipid classes (cholesterol, phospholipids, and triglycerides). A change in one lipoprotein class implies a change of all the lipid components. It is therefore not sound to attack the hyperlipidemic diseases by changing blood cholesterol only, especially since several drugs able to reduce blood cholesterol levels at the same time increase the triglycerides in blood and liver.

In conclusion it is appropriate to mention that cholesterol levels in blood are regulated by several mechanisms including endogenous biosynthesis, absorption, transport, excretion and catabolism. All these mechanisms are under feed back hormonal or nervous control and drugs may be used to interfere with specific steps in each metabolic process.

2.10: ANIMAL MODELS FOR SCREENING HYPOGLYCEMIC AGENTS

It is tempting to determine the activity of a hypoglycemic drug in the experimentally induced diabetic animal.

Introduction

Methods of testing for hypoglycemic action can be said to have begun when investigators aimed at isolating purer and purer preparations of insulin from crude extract of pancreas. In the present state of our knowledge two main groups of substances are recognized as having hypoglycemic action: they are arylsulphonamides or sulphonylureas and derivatives of guanidines.

Choice of species

Laboratory animals of various species like mouse, rat, guinea pig, rabbit, dog, cat, hamster, chicken, duck, goose, toad, snake and rhesus monkey have been used for studies of hypoglycemic substances (207 - 210).

In general, the trials have been carried out on white rats (Wistar, Sprague - Dawley) of the same age and sex, of almost identical weight, fed on the same diet for several months and born in the same breeding colony. The rat, being omnivorous, resembles man nutritionally. Blood from a rat can be taken either by puncture of the tail vein or by cutting the end of the tail. Before any test the rats should be fasted for 18 hours to ensure stable blood sugar regulation.

Certain experimenters have used rabbit, which is herbivorous,, although its blood sugar regulation is less stable than that of the rat. In general the samples of blood are taken by puncture of marginal ear vein. This can be done frequently. At the beginning of the test the animals should

have been fasted for 24 hours. In the rabbit, digestive absorption lasts longer than in the rat.

The dog is excellent for this type of investigation. Its blood sugar regulation is more stable and predictable than that of either the rat or the rabbit. Samples of blood can be taken easily from the dog's jugular vein without anesthesia. This can be frequently repeated for several days without upsetting the animal. Food should be withdrawn 18 hours before beginning the experiment.

The guinea pig has also been used. The samples of blood can be taken from the jugular vein. However, because of the difficulties of this method, cardiac puncture is often used.

The mouse has only seldom been used.

The rhesus monkey is the animal whose metabolism most nearly approximates to that of man, and its sensitivity to drugs is also close to that of man. Experiments on the monkey are important for investigating the possibility of extrapolating to man the results of tests already done on other animals.

It is to be kept in mind that different species of animals are not equally sensitive to the same hypoglycemic agents.

Route of administration

Any usual route of administration can be used. For hypoglycemic substances two routes seem to be the most important.

i) Alimentary tract

The product is introduced by gastric intubation in an amount proportional to the weight of the animal. There is a choice between using a

solution whose pH should be as near 7.0 as possible or a suspension in a solution of 5 % gum acacia or a gel of 2 % methylcellulose.

ii) Intra - venous route

This makes it possible to determine more rapidly the activity of a given quantity of substance than does the alimentary route, which is a general rule.

Action on diabetic animals

It is tempting to determine the activity of a hypoglycemic drug in the experimentally induced diabetic animal. The various methods of producing experimental diabetes are considered below.

i) Diabetes due to total excision of pancreas

Total excision of the pancreas can successfully be carried out on the dog or the cat. Some have been successful with the rat as well operating under the microscope. Diabetes after total removal of the pancreas is severe and leads to the animal's death at its maximal intensity.

ii) Diabetes due to partial excision of pancreas

This form of diabetes is produced in the dog by removal of nine - tenths of the pancreas.

iii) Alloxan induced diabetes

This form of diabetes can be induced in various species of animals by the intravenous injection of alloxan (211 - 13). The dose varies with species, age and route of administration. In the dog, the dose is about 50mg/kg body weight and is given intravenously. In the rabbit it is 150mg/kg i.v.

The diabetes that appears in the hours after injection of alloxan varies in intensity, depending on the number of β cells destroyed in the islets of Langerhans by alloxan.

iv) Streptozotocin induced diabetes

Streptozotocin in a dose of 65 mg / kg administered intraperitoneally in 0.1 M citrate buffer of pH = 4.5 induces diabetes in the animal within two days (214 - 15). These days, research workers prefer the streptozotocin-induced diabetes in animals due to its lower rate of mortality compared with the alloxan-induced diabetes in animals. The only disadvantage of Streptozotocin is its high cost.

v) Dithizone-induced diabetes

Various chelators like, dithizone, 8-(p-toluene-sulfonylamino)-quinoline (8-TSQ) and 8-(p-benzene-sulfonylamino)-quinoline (8-BSQ) in a single i.v. dose of 40-100mg/kg body weight to cats, rabbits, golden hamsters and mice induce permanent diabetes after 24-72 hours .

vi) Hypophysial diabetes

This form diabetes appears in the dog and the cat using injections of growth hormones. The doses to be injected vary according to the animals used and should be progressively increased.

vi) Metahypophysial diabetes

This permanent form of diabetes persists, after normal animals, dogs or cats, have had a course of large doses of growth hormones for several weeks and has characteristics closely resembling alloxan diabetes.

vii) Adrenocorticoid induced diabetes

This form of diabetes appears in certain species during administration of gluccocorticoids. Diabetes is corrected after steroid injections are stopped.

ix) Diabetes due to insulin antibodies

Intravenous injection of 0.25-1.0 ml guinea pig anti-insulin serum to rats induces a dose-dependent increase of blood glucose reaching values up to 300mg%

x) Virus induced diabetes

The D-variant of encephalomyocarditis virus (EMC-D) selectively infects and destroys pancreatic beta cells in susceptible mouse strains similar to human insulin dependent-diabetes (216).

xi) Genetically diabetic animals

Certain animal species, notably rodents, such as the Chinese hamster, the obese mice, the obese hyperglycemic Zucker rat, various sub strains of KK-mice, BB (Bio Breeding) rat etc are described to exhibit spontaneous diabetes mellitus on hereditary basis (217). It is possible to study the action of certain hypoglycemic drugs in these species in conditions that can be said to resemble, to a certain extent, those observed in diabetic man.

Apart from studying the blood glucose lowering activity of antidiabetic drugs on experimentally induced diabetic animal models as described above, the hypoglycemic activity can also be studied using the following methods:

xii) Studies in glucose loaded rabbit or rats

The rabbit has been used since many years for standardization of insulin. Therefore, it has been chosen as primary screening model of blood glucose lowering compounds as well as for establishing time-response curves and relative activities.

Rabbits of either sex are treated either once (0.5 hours after test compound) or twice (0.5 and 2.5 hours after test compound) orally with 2gm glucose/kg body weight in 50% solution. Oral blood glucose lowering substances are applied by gavage in 0.4% starch suspension. Blood glucose is determined over a time period.

In case of rats glucose is administered in dose of 1gm/kg body weight either orally 5 minutes after oral administration or subcutaneously 5minutes after intraperitoneal administration of the test compound.

xiii) Perfusion of isolated rat pancreas

The in vitro perfusion of rat pancreas offers the advantage to study the influence of antidiabetic drugs not only on insulin secretion but also on glucagon and somatostatin without interference of secondary effects resulting from changes in hepatic, pituitary or adrenal functions.

xiv) Receptor binding of sulphonylureas

Sulphonylureas block ATP-dependent K^+ channels in the beta cell plasma membrane. Binding to the receptor and depolarization of he

membrane initiates a chain of events leading to the release of insulin. Binding studies of sulphonylureas and other drugs can be performed on isolated pancreatic islets, isolated insulinoma cells, isolated intact membranes or solubilized membranes.

Quantitative blood sugar determination

The various test methods for determining blood glucose are considered below (218 - 19).

i) Orthotoludine method

The test principle involves a reaction of glucose with orthotoludine in hot acidic medium to form a green coloured complex. The intensity of final colour produced is measured by using a photometer at 620 - 660 nm. The measure intensity is directly proportional to the concentration of glucose in the specimen. The method has good efficacy and precision. The procedure is linear up to 300 mg / dl of glucose.

ii) Glucose - oxidase method: The aldehyde group of glucose is oxidized by the enzyme glucose oxidase to give gluconic acid and hydrogen peroxide. The hydrogen peroxide is broken down to water and oxygen by peroxidase. The liberated oxygen reacts with 4 - aminophenazone in the presence of phenol to form a pink coloured compound, intensity of which is determined at 530 nm.

iii) Determination of glucose using autoanalyzer

The above reagent is mixed with plasma or serum sample and kept for 15 minutes at a temperature of 37° C. The intensities of the colour produced are measured at 505 nm. The method is linear up to 350 mg/dl.

iv) UV - kinetic method

In this test method, the reagent consists of ATP, hexokinase, NADP, and Glucose - 6 - phosphate dehydrogenase (G_6 - PD) in ethanol amine buffer (pH = 7.5). When plasma or serum is mixed with the reagent, following reactions take place:

Glucose (in sample) + ATP $\Leftrightarrow$ HK $\Leftrightarrow$ G - 6 P + ADP

G-6P + NADP $\Leftrightarrow$ G - 6 PD $\Leftrightarrow$ Gluconate - 6 P + $NADPH_2$.

Increase in O. D. is measured after a fixed interval of minute.

v) Glucose tolerance test

Glucose tolerance means ability of the body to utilize glucose in blood circulation. Blood sugar in the case of a normal individual remains fairly constant throughout the day at about 1 mg / ml. Following food, there is a temporary rise in blood sugar, the extent of which depends on the type of food taken. If there is diminished glucose tolerance, this increase, however, does not return to normal fasting levels, which should be reached within 2 - 3 hours normally. In order to study this effect, patient is given 75gm (1.75 gm/kg body weight) glucose dissolved in water. Five blood samples are collected: one prior to the administration of glucose, (fasting blood sample), and four more samples are collected after glucose has been drunk at half hourly intervals. From the type of curve obtained, it can be inferred whether the patient has (or has not) diminished glucose tolerance.

vi) Determination of glycosylated hemoglobin (220 - 21)

The red cells of normal human adults and children contain three hemoglobin species: HbA1 or HbA -abc, HbA2, and HbF comprising 90%, 2.5% and 0.5% respectively. Studies on the role of HbA1 indicate that, it reflects the average blood sugar concentration for an extended time period. HbA1 levels may better reflect carbohydrate imbalance than fasting glucose tolerance test, without resorting to glucose loading.

To determine, glycosylated hemoglobin, interfering substances are removed by washing the red blood cells 4 - 6 times with normal saline. Haemolysate is prepared by using carbon tetra chloride. Hexoses bound to hemoglobin are hydrolyzed by heating the haemolysate at 100°C in the presence of oxalic acid. Resultant chromogen formed is measured at 443 nm.

Glycosylated hemoglobin can also be determined by ion exchange method using commercially available kits.

2.11: DESIGNER FOOD PRODUCTS

> As the role of food in the etiology and alleviation of various clinical conditions continues to expand, the distinction between food and medicine is becoming less and less defined.

Introduction

The concept of food as medicine is certainly not new. Hippocrates is quoted in his famous aphorism, "Food is thy friend and enemy". Galen, also felt that good medicine could only be found in the diet. Thousands of years ago, civilizations around the world learned by trial and error that certain foods contain ingredients that not only support recovery from illness, but also help maintain optimal health. Throughout Asia, virtually all aspects of traditional or folk remedies involved ingestion or avoidance of certain foods, and until the early 1900's, dietary regimens remained an essential component in the management of diseases (222).

The term nutraceuticals was first used in 1989 by Dr. Stephen DeFelice, of the Foundation for Innovation in Medicine, to describe a rapidly developing area of biomedical research. Nutraceuticals are also known as functional foods or designer foods. A nutraceutical is any substance that is a food or a part of a food and promoted as healthful or nutritious diet and provides medical or health benefits including the prevention and treatment of diseases. Such products may range from isolated nutrients, dietary supplements such as folic acid and chicken soup to genetically engineered designer foods, herbal products, and processed foods such as cereals, soups and beverages. This definition also includes a genetically engineered designer vegetable food, rich in antioxidant ingredients, and a stimulant functional food or Pharmafood (223).

Nutrients such as vitamins and minerals have been used to fortify foods and drinks for many years, while functional foods are bioactive and work in still undiscovered and unidentifiable ways (224).

There are two categories of functional foods (225).

i) Phytochemicals

These are plant foods that naturally contain biologically active, non-nutrient compounds and provide health benefits.

ii) Designer foods

Food products specifically formulated to have higher amounts of nutrients or phytochemicals than would naturally occur in that food.

Phytochemicals are plant chemicals that differ from nutrients in some important ways. Essential nutrients that include protein, fats, minerals, and vitamins are essential for life. Without them, people develop acute deficiency diseases that can eventually cause death. Phytochemicals are not necessary for life but they help to promote optimal health by lowering risk for chronic diseases, such as cancer and heart disease. They are found only in plant foods. Fruits, vegetables are among the best sources of these compounds. Phytochemicals are believed to have many health benefits.

While nutraceuticals is catchall term, the term functional foods is commonly used for food products specifically formulated and scientifically proven to provide health benefits beyond basic nutrition. One classic example of a functional food is orange juice fortified with calcium (226).

The concept of foods having medicinal functions, however, fell out of favor over the last century, due to the overwhelming advances of surgery and synthetic drugs which overshadowed the more subtle effects of diet and

food in the management of diseases. However, during the last few decades, modern science itself has become suspect because of overuse or abuse of several chemicals.

In recent years, as scientific methods of proof were perfected, scientists have come to accept the fact that what people eat greatly affects their risk of acquiring certain diseases. As the role of food in the etiology and alleviation of various clinical conditions continues to expand, the distinction between food and medicine is becoming less and less defined. Hence, today's generation is requesting the good old cures of yesteryear and the "back to nature" movement is in obvious juxtaposition with the high-tech segment of population.

Historical backdrop

While labels like "designer foods" make these seem like a modern innovation, in reality the use of foods in the treatment of diseases and injuries predates recorded history. In fact before World War II, medicine was basically herbal.

The 1950s and 1960s saw the rise of the Pharmaceutical companies, and medicine drifted away from functional foods to clinically quantifiable doses of regulated drugs. This shift highlighted some of the problems with food as medicine. Not only their use requires a sophisticated knowledge of plants, but also dosage amounts were difficult to determine.

However, by the 1980s, public interest in and demand for functional foods was growing dramatically. As scientists have studied more and more about the nutrients in the foods we eat, they have discovered that these foods may be very powerful components in the fight against diseases. There is a trend toward using food as preventive medicine. And the reason for this

trend could be that "people are tired of the side effects of other medicinal modalities and "want to get back to nature and back to the basics".

By 1996, 'phytochemicals' were identified as one of the most important health-promoting ingredients in the food industry. More recently, a survey reported in Food Processing magazine found that functional foods / nutraceuticals is one of the most important areas in new product development (223).

What are medical foods?

A 'medical food' is a food that is formulated to be consumed or administered enterally under medical supervision. It is intended for use in the specific dietary management of a disease or condition for which distinctive nutritional requirements, based on recognized scientific principles, are established (227).

Because medical foods focus on improving function and facilitating 'dietary environmental control', they present an ideal first line approach that allows clearer case assessment for further intervention, if necessary. Integrating the use of medical foods into a treatment program offers numerous advantages to both the practitioner and patient.

A physician prescribes a medical food when a patient has special nutrient needs in order to manage a disease or health condition, and the patient is under the physician's ongoing care. The label must clearly state that the product is intended to be used to manage a specific medical disorder or condition. For example, the consumption of garlic and onion in high amounts can result in antimicrobial, cholesterol lowering, and blood thinning effects. These should be appropriately described in some publication or labeling where they have been put into forms intended for non- food use.

Medical foods are not meant to be used by the general public and may not be available in stores or supermarkets. Medical foods are not those foods included within a healthy diet intended to decrease the risk of disease, such as reduced-fat foods or low-sodium foods, nor are they products for reducing weight.

Medical foods are foods that are formulated to aid in the dietary management of a specific disease or health-related condition that causes distinctive nutritional requirements that are different from the nutritional requirements of healthy people. Foods for special dietary use, on the other hand, are foods that are specially formulated to meet a special dietary need, such as a food allergy or difficulty in swallowing, but that provide nutrients intended to meet ordinary nutritional requirements.

All in all, it can be summed up that for a product to qualify as a medical food product, the product should meet with the following important criteria.

- A product marketed for use as a medical food in the dietary management of a disease or condition should have characteristics that are based on scientifically validated distinctive nutritional requirements of the disease or condition.
- There should be a scientific basis for the formulation of the product and the claims made for the product.
- There should be sound, scientifically defensible evidence that the product does what it claims to do.
- There is a statutory requirement that a medical food should be consumed or administered enterally under the supervision of a physician.
- There is the statutory requirement that a medical food be intended for the specific dietary management of a disease or condition.

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- There is the statutory requirement that a medical food be intended for the specific dietary management of a disease or condition.

In simple words, the objectives of incorporating medical foods for disease management are to ameliorate clinical manifestations of the disease, favourably influence the disease process and positively influence morbidity and mortality of patients.

These medical foods are also referred to as by other names like health foods, pharma foods, functional foods, nutraceuticals, and designer food products. Henceforth, the term designer food products shall be used.

Benefits of designer food products

- Simplicity of preparation.
- Better palatability.
- Proven safety and nutritional efficacy.
- Cost saving.
- Better compliance than conventional drug therapy.

Regulations governing designer foods: A troubling paradox (228)

One of the first medical foods to be developed was the infant formula Lofenalac, a product that was designed for use in the dietary management of a rare genetic condition known as phenylketonuria (PKU). This product contains only a very limited amount of the essential amino acid phenylalanine because the individuals suffering from PKU have an impaired ability to metabolize this amino acid.

Before 1972, FDA regulated products like Lofenalac as drugs because of their role in mitigating serious adverse effects of the underlying diseases. The FDA was interested in fostering innovation in the development of these products. At the same time, it recognized that use of these products for feeding healthy individuals could be hazardous. For example, an infant formula that was purposely formulated to be suitable for an infant with PKU

would be nutritionally inadequate for a normal infant. Thus, the FDA saw that it was important to differentiate these products from foods for general use. As a result, in 1972, FDA stated that the PKU product described above would no longer be regulated as a drug but rather as a 'food for special dietary use'.

Foods for special dietary use are subject to the same requirements with regards to nutritional labeling, nutrient content claims and health claims as applicable to most other foods by the 1990 amendments. Thus, foods for special dietary use, like ordinary foods, must be labeled with certain nutrition information in a prescribed format to ensure that such information is presented in an informative and understandable fashion. Moreover, any nutrient content claims or health claims on the label or in the labeling of a food for special dietary use must have been authorized by FDA to ensure that the claim is scientifically valid and is presented in such a way that it is truthful and not misleading.

However, medical foods are exempt from such labeling requirements. Thus, a medical food that is intended for the specific dietary management of a disease or condition for which distinctive nutritional requirements have been established may be sold without any nutrition information on its label, and it may bear claims that have not been evaluated under the 1990 amendments to ensure that they are scientifically valid. Moreover, there is no assurance that the formulation of a medical food has been evaluated prior to sale to ensure that it is suitable for the intended patient population. The exemption from the requirements of the 1990 amendments, therefore, creates a troubling paradox. Medical foods intended for use by sick people are subject to much less scrutiny than virtually all other foods, which are intended for the healthy general population. This lack of scrutiny creates a situation that could have adverse public health consequences if these

products bear claims that are not scientifically valid, or if their labeling does not disclose nutrition or other information that is necessary for the safe and effective use of the food.

With the increased use of nutrients in food products, a change has come in food labeling regulations. First, the Nutrition Labeling and Education Act of 1990 (NLEA) was enacted by the US Food and Drug Administration (FDA). The NLEA mandates that all processed foods bear a more comprehensive and easier to understand nutritional label.

Because the fine line between a food and a dietary supplement is sometimes blurred, the Dietary Supplement Health Education Act of 1994 (DSHEA) was passed. Briefly, a dietary supplement is any of a number of products used to supplement the diet by increasing the total dietary intake of important nutrients.

Herbal ingredients have begun to appear more frequently in food products since the passage of the DSHEA. Earlier, if an ingredient was not Generally Regarded as Safe (GRAS), the FDA could pull the product that contained it from the market. With the DSHEA, this is no longer an issue because this Act defines phytochemicals as dietary supplements and products can now be sold and marketed under this category. The DSHEA has opened a door for the food industry. In the future, FDA may require the quantities of active ingredients to be listed on the label or it could even agree to allow certain health claims pertaining to the structure or function of the body to be made for an herbal ingredient.

Market scenario of Designer food products (229)

Designer food, this industry buzzword, highlights the application of Pharmaceutical research tools in the field of nutrition. At the core of the new nutrition in the nutraceutical era is the blurring of the boundaries

between food, Pharmaceutical, Chemistry and biotech industries. Many new dietary substances have been discovered since the 1990s that have tremendous health-promoting potential. **Consumers do not mind paying a bit more for foodstuffs that pack optimal health in a convenient instant-delivery vehicle.**

In the past, foods were commonly fortified with nutrients to help prevent specific nutritional deficiencies. Technically, any fortified food could be considered a functional food. Some examples of conventional functional food that are available in the market are iron-fortified cereals, vitamin D-fortified milk, and iodized salt. Today, many functional foods are aimed at boosting intakes of phytochemicals to reduce risk for chronic diseases like heart disease, diabetes, stress, arthritis etc. (230 - 33).

Examples of functional foods found at the US and European stores include: cereals and breads with added isoflavones fruit juices with herbs that have alleged immune-enhancing properties, such as echinacea margarine with added phytosterols, which help to reduce cholesterol.

In 1974, a limited survey of pharmaceutical and food manufacturers revealed that fewer than three-dozen products were being sold as medical foods. As of 1989, however, well over 200 products were being sold as medical foods.

The nutraceutical market is expanding at three times the rate of the conventional food industries. The US nutraceutical market was valued at $19.9 billion in 1998 and is estimated to hit $27.5 billion in 2003 according to a recent market report. The European market parallels the growth trends projected for the US.

Marketers have soared to refreshing heights with innovative brand names - Ginseng Rush, Whoopass Energy drink, and Red Bull Energy drink - suggestive of the flavourful performance-enhancing qualities of these

nutraceutical beverages. An exciting breakthrough for those interested in reducing their cholesterol levels without having to pop pills or engage in lots of physical exercises, has come from plant stanol esters. Stanol esters are phytochemicals found in a variety of plants and vegetable oils such as soy, corn, pine trees and wheat. An added value is their incorporation as colourless and tasteless ingredients into a variety of foods. In other words, consumers can enjoy health-hearty benefits through a range of products like cereal snack bars, yoghurts, cheeses, salad dressings and margarine.

Currently marketed functional foods include snack and meal items, but in the US it is only snack items at this stage, namely diabetic bars and energy / nutrition bars. Functional foods not manufactured or marketed in the US, but successfully marketed overseas, are margarine and yogurt.

Functional foods could be just about any type of food, including breakfast foods, baked goods, dairy products, prepared meal items, condiments, snacks / desserts, confections, reduced fat / sugar / caloric items, and spreads (234 - 35).

Currently marketed functional beverages include ready-to-drink (RTD) cultured milks / drinkable yogurts, powders to mix into beverages, and bags for tea. Potential functional beverages could include RTD milk shakes, soft drinks, new age drinks, juices, and infant formulas, as well as powders to brew for coffee and cocoa.

New-age beverages fortified with antioxidant vitamins have splashed onto the market. There are candies, power bars and even chewing gums with added natural herbal ingredients like ginseng. Five of the most frequently consumed herbal dietary supplements include echinacea, garlic, ginkgo biloba, St. John's wort and ginseng.

Some production considerations

As any Pharmaceutical preparation, the designer foods should also be prepared in accordance with GMP regulations. Some of the aspects relevant to the production of designer foods are considered below (234).

i) Phytochemical's quality considerations

There is currently little information on testing phytochemicals. Most companies have spent years developing their own protocols in-house. Plant materials are natural agricultural commodities with very complex chemistries. Because the products are all natural, there will be variation in the amount of active compounds or phytochemicals present in the herbs.

Testing herbals involves two stages: identification and quantitation. Identification involves taking a combination approach and using several tests together to ensure that the product is identified correctly.

The various tests that are employed include the following:

- Organoleptic evaluation
- Thin layer chromatography
- Microscopic image analysis for checking adulteration, and for identifying the taxonomic features of the plant.
- Ultraviolet / visible spectroscopy
- Fourier transform infrared spectroscopy
- High-pressure liquid chromatography
- Gas chromatography / mass spectrometry

Testing becomes much more complicated with a food matrix. More extraction procedures are required to isolate the vitamin or herbal component from interfering macroingredients such as fibre, fat or protein. Even during analysis, precautions must be taken to ensure accurate quantification of an ingredient.

While the US Pharmacopoeia (USP) is the "Bible" for testing purposes, there are currently no test procedures or tolerance limits dealing with quality control parameters for phytochemicals.

ii) Formulation development considerations

The presence of complexing substances, solubility, reactivity, stability and other factors can hamper a nutrient's effectiveness. The correct marketable dosage form of the food should be chosen carefully in order to be compatible with the product and its processing parameters.

Some tips to be considered for formulation development include:

- The concept of bioavailability is very important in formulating products requiring the delivery of optimal nutrition.
- Increasing the sweetness can help to overcome unpleasant tastes.
- Adding furaneol, a flavourant that occurs naturally in fruits, works well as a flavourant in these products. It imparts a creamy, sweet taste that can mask both the grassy, earthy off-notes as for instance from the soy protein as well as those from the vitamins.
- "Light" flavours like lemon, lime, strawberry or vanilla are not always effective for masking the medicinal taste of some nutrients. However, the sweet profiles of honey or vanilla can mask the astringent aftertaste of herbal ingredients. The "heavier" flavours such as grape, raspberry, maple and chocolate more effectively cover up the unpleasant taste of nutrients. They tend to be less volatile and hold up better under heat treatments.
- Sequestering agents that bind minerals, such as EDTA, citric acid or a polyphosphoric acid, also help prevent degradative changes in these preparations.

The technology used to formulate these products can be quite complex. The following points during preparation of designer foods may be considered.

- The heating time should be minimized so that the thermolabile constituents are not destroyed.
- The use of encapsulated micronutrients is vital in overcoming negative characteristics such as "off-flavours".
- Encapsulating ingredients affords protection during baking process.

iii) Packaging and storage considerations (236)

The packaging material plays a vital role in the stability of a product during its shelf life. A wide variety of packaging materials are available from which appropriate material can be selected. As the concern for environmental protection is increasing the packaging material so selected should be environment friendly.

There is a need to properly handle and store designer foods to assure their maximum safety, food quality, freshness and flavour acceptability. Some tips on storage are given below:

- Designer foods should be stored at a proper temperature.
- Reducing exposure of designer foods to air; light; moisture; and heat can minimize food quality losses.
- Containers should be kept tightly closed to limit exposure to air and humidity.
- Following of the instructions: "use by date" stated on labels is necessary as extended storage may cause flavours to fade and change and reduce nutrient value.

iv) Labeling considerations

The medical foods are specifically exempted from the requirements for nutrition labeling, nutrient content claims, and health claims. As long as the word "cure" is not used, companies can make a carefully worded health claim.

v) Evaluation considerations

The designer foods must be evaluated just like any other Pharmaceutical preparation. Some of the important evaluation parameters that may be considered include:

- Organoleptic evaluation: In order to evaluate a flavour or blend of flavours used in the formulation of designer foods, a panel of experts tastes the designer food prepared with the flavour under test. The decision is then arrived at collectively as to the flavour that is most appealing to the taste buds. Also, special consideration should be given to the mouth feel of the designer food, its viscosity in mouth, appropriateness of colour used, etc.
- *In-vitro* evaluation: For determination of physico-mechanical characteristics like hardness, density, disintegration, viscosity, release pattern etc.
- *In-vivo* evaluation: For determination of desired therapeutic activity in animals.
- Clinical evaluation: This is to be carried out in healthy volunteers and in diseased individuals in a phased manner.

Summary

Foods show so much promise in the field of medicine that Pharmaceutical scientists have begun attempts to improve certain foods by increasing the amount of naturally occurring nutrients or adding nutrients. Called 'functional foods', 'designer foods' or 'nutraceuticals', these foods are under a tremendous amount of research. Golden rice, enriched with beta-carotene to improve eyesight, is one of these designer foods, and shows promise in improving eye health in developing countries. But as promising as this seems, there is controversy over whether too much of a good thing might be harmful, and studies will continue to shed light on the future of food as medicine.

Among the 42 clinical trials currently listed under National Institute of Health (NIH) alternative medicine program, about one third focus on researching health benefits of potential nutraceuticals. This testifies to their gradual acceptance by the scientific community as promising medicinal foods or supplements.

Functional foods are one of the most important and exciting developments in the food industry, opening up a huge new market and transforming the relationship between food, nutrition and health.

The nutraceutical industry is poised for tremendous growth according to a report by the research firm Frost and Sullivan. More research needs to be done on understanding their mechanisms of action in health and disease and on how best to formulate with Mother Nature's Pharmaceuticals.

There is a need for additional regulation of designer foods concerning their composition, current good manufacturing practice, quality control procedures, labeling requirements, safety and effectiveness, standards governing claims that the product will be safe for its intended use and that any claims made for the product are supported by sound science.

If medical foods are to be ever adopted as a legitimate class of agents, the standards in the above concerned areas must be as rigorous as those for drugs, if they are to perform as intended.

Food product designers are busy, not only trying to improve the stability and functionality of these 'new' phytopharmaceuticals, but also formulating innovative foods that will entice consumers. **Manufacturers face major challenges in product development, and in substantiating and marketing health claims for the new generation of designer foods.**

The challenge is to develop designer foods and market them within the current regulatory and technical environments, so that consumer demand for products with disease-fighting properties can be satisfied.

Also a food product designer tries to identify those foods and beverages, now in conventional forms, that have the potential to be marketed as functional products.

Manufactured functional foods cannot duplicate all of the benefits of whole plant foods, some of which are not yet known. For example, plant foods like leafy green vegetables, which are naturally rich in calcium, also provide vitamin K and vitamin C, both of which are needed for healthy bones, as well as numerous nutrients and phytochemicals that protect against chronic disease. A diet based on whole plant foods is likely to be more healthful than one based on functional foods.

However, in authors' opinion the best approach is to include manufactured functional foods in moderate amounts to a healthy planned diet. This may help to reduce disease risk in general and one may live a healthy life. Nutraceuticals can be useful adjuncts to conventional therapies for enhancing general wellness.

2.12: DEVELOPMENT OF ANTIDIABETIC, ANTIHYPERLIPIDIMIC DESIGNER FIBRE FOODS

Ingestion of such a large quantity of guar gum of consistency akin to wallpaper paste and beanie odour has resulted in poor patient compliance.

Introduction

One of the objectives of the treatment of diabetes is to establish and maintain good metabolic control, which involves not only improving glycemic control but also lowering plasma cholesterol and triglyceride levels. Currently the treatment of diabetes involves using diet alone, or diet and oral hypoglycemic, or diet and exogenous supply of insulin. Diet is the cornerstone of treatment. One of the main dietary recommendations for people with diabetes is to increase the amount of complex carbohydrate, which includes dietary fibre, particularly the water - soluble fibre.

Whole grain i.e. the cereals and legumes along with fruits and vegetables have been the traditional food of persons in the East. Leguminous foods such as guar gum and other galactomannan containing seeds provide the required daily amount of food fibre (25-30g), while a diet composed of meat and other non-vegetarian products is low in food fibre. Compared to a home cooked meal, in processed and packaged food some fibre is lost.

Guar gum is one of the most widely prescribed food-fibre for controlling sugar metabolism in diabetic patients and those having high serum lipid levels and high blood pressure. Rheological behavior of guar gum and other polysaccharides is the key factor in determining its acceptability for the patient. Clinical dose of guar gum when used as food

fibre can be as much as 10-15 gm., once or more in a day. Ingestion of such a large quantity of guar gum of consistency akin to wallpaper paste and beanie odour has resulted in poor patient compliance (237 - 39).

The physiological activity of gums depends on their capacity to increases digesta viscosity in the stomach and small intestine. Dietary supplements of polysaccharide gums in solution are extremely unpalatable. In an attempt to improve the palatability of gums, pharmaceutical preparations were designed so that when mixed with water or fruit juice and taken before meals they would hydrate slowly and thus reduce the viscosity in the mouth. No formal sensory analytical studies have been published to substantiate the claim that these preparations are palatable. Moreover, many of these preparations, administered in doses of 15 gm. per day, were found to be clinically ineffective when administered as a pre-meal drink. A number of reports have shown that the palatability and clinical efficacy of guar gum can be greatly improved by incorporating it into foods. The concept of designer or 'new generation' food products which involves incorporation of gums into foods and formulating such preparations is a development of past two decades (240 - 45).

Market scenario of antidiabetic, antihyperlipidemic designer fibre foods

A wide range of high - fibre foods including crispbread (246), a variety of wheat breads, pasta, a snack bar, biscuits and a breakfast cereal made with wheat flakes, mainly containing commercially available food grades of guar gum, have been developed and clinically tested for use in the treatment of diabetes. Wheat bread is considered to be a suitable vehicle for guar gum, mainly on the ground that bread could be regarded as a normal ingredient of any meal of the day and that it is classified as a high glycemic

index food. This indicates that bread produces a large rise in the post parandial blood glucose level relative to other foods. Thus, one of the benefits of adding a polysaccharide gum to wheat bread is to transform the bread into a food with low or medium glycemic index food.

Two Japanese food and pharmaceutical companies developed food fibres based on enzymatic depolymerization of guar gum, which have now found worldwide acceptability. The product of Dannippon Pharmaceutical Co. has the trade name 'Fibreon', while that from Taiyo Kagaku Co. is called 'Novartis' or 'Benefibre' in USA marketed by Sandoz Nutrition. Both these products have FDA clearance for use in food and are also recommended clinically. Low viscosity grades (viscosity of 10% = 80-100 cps as compared to 5-6 thousand cps at 1% concentration for starting material) can also be produced by thermal-catalytic depolymerization. On clinical prescription these can be taken with water or any beverage.

Besides guar gum based food fibres, corn and wheat husk hemicellulose are also low viscosity polysaccharides not requiring any further modification like guar and are recommended as additives for food. Purified tamarind polysaccharide, introduced by Dannippon Co. of Japan, under the trade name 'Gellose' is also an acceptable food fibre.

Optimum dose

It is difficult to recommend a precise dose for polysaccharide gums since this will depend on many factors including the patients' characteristics and background diet. It is probably more helpful to clinicians and patients to define an upper and lower dose range, although in practice the daily intake of gum incorporated into a food product will depend on how much of the food, patients normally eat. Gums being obtained from natural source vary with regards to fibre contents, fine structure, etc. and as such are not

standardized or regulated like drugs. Therefore, a question arises as to how much and how long the designer food product containing galactomannan should be taken. It would seem appropriate to define the lower and upper doses of guar gum as being 6 and 15 gm. per day. This estimate is made on the basis that there may be problems of transient side effects at doses above 15 gm. per day and there is lack of reliable long - term studies of guar gum at doses below 6 gm. per day.

Adverse effects

No adverse effects have been observed in diabetic patients who had been administered guar gum for periods of up to 12 months at doses of up to 30gm. per day and no significant changes in hematological, hepatic or renal function have been reported. Although guar gum is likely to reduce the absorption of minerals, electrolytes, fats, vitamins A and E and some drugs, probably by the similar mechanism as is applicable to the reduced rate of absorption of glucose (247 - 249).

In general, side effects in some individuals appear to be associated with gastrointestinal events. These adverse reactions, which appear to be transient and diminish with regular consumption of guar gum, include flatulence, gastrointestinal pain or discomfort, nausea and diarrhea. The non-starch polysaccharides are known to be degraded by bacterial enzymes in the large intestine, leading to the formation of gas, which in some cases can result in severe abdominal pain. Increased stool frequency and bulking has also been reported in some, although this may be considered to be beneficial in patients suffering from constipation. Adverse gastrointestinal effects appear to occur only when the guar gum is administered to patients in large doses i.e. 20 - 40 gm. per day. Guar gum has little or no side effects when intimately mixed with food and administered in moderate doses.

Summary

There is an increasing occurrence of diabetes in developing countries. The standard treatment for people with diabetes in these areas is likely to be expensive and therefore, not widely available. If dietary supplements of polysaccharide gums prove to be an acceptable form of treatment in the developing countries, then it may be desirable to utilize fibre rich plant foods that are indigenous to such areas. However, whatever the mode of administration of non - starch polysaccharides, they will have little value if they are unpalatable and therefore not consumed by diabetic patients on a regular basis. Much work is needed to improve the sensory properties of polysaccharide gums if they are to be incorporated into foods at pharmacological doses. Concentration, molecular mass, hydration kinetics, rheology and interactions of food fibres with constituents in a diet are critical factors influencing their therapeutic applications and development of designer food products.

Currently, none of the 'new generation' designer food fibre products are being manufactured in India. Clinical prescription of food fibre is, occasionally made by doctors and dietitians in India. Food fibres based on wheat bran (Vitacel) and those based on orange and apple pectin and microcrystalline cellulose of German origin (Siber Hegner) are being marketed in India as food additives. This certainly is an indication of increasing demand for designer food fibres products in India.

CHAPTER 3
MATERIALS

Apparatus

measuring cylinders, beakers, pestle mortar, test tubes, funnel, desiccator, scale, round bottom flask, reflux condenser, cotton, counting chamber, RBC pipette, cover slip, sieves # 10, 22, 30, 44, 60, 80, China dish, vials for sample collection or storage of blood, Soxhelet apparatus, thermometer, water bath, TLC Kit, tube of D.T. apparatus, rabbit holder, gag, centrifuge tubes, catheter, infant's feeding tube no.8, syringes 5 ml. and 20 ml., needles of gauge 22, cotton, pasture pipette

Chemicals

Fenugreek seeds, Guar gum (GG), Locust Bean Gum (LBG), Depolymerized Guar gum (DGG) (gifted by Sunita Mine Chems, Jodhpur), Fenugreek Galactomannan (FGM), petroleum ether, menthol, ethanol, conc. sulfuric acid, glycerin, acetic acid, benzene, methanol (SD fine chemicals), silica gel, sodium metabisulfite, potassium bromide – I.R. grade, glucose, Benedict's quantitative reagent, Fehling's solution, sodium bicarbonate, sodium taurocholate, cholesterol (Benzo Chem, Bombay), gentian violet, xylol, liquid paraffin, platelet diluting fluid

Equipments

FTIR 8300 Schimadzu, hydraulic press, Synchrolectric Brookfield viscometer, Spindle no.4, Auto analyzer - AMES SEAC CH – 100, I.R. moisture balance, Mechanical and magnetic stirrers, stalagmometre, balance, centrifuge, microscope, vernier calipers, oven, Monsanto hardness tester, heating element, stage and occulo micrometers

Animals

Albino rats, rabbits

Food ingredients

Vanaspati (saturated fat), jaggery, sodium bicarbonate, fennel powder, cardamom menthol, sugar, chocolate powder, milk powder, wheat flour, ghee, gum, rose petals

CHAPTER 4
EXPERIMENTAL

4.1: EXTRACTION, IDENTIFICATION AND CHARACTERIZATION OF FENUGREEK GALACTOMANNAN

4.1.1: Extraction of galactomannans from fenugreek seeds.

The fenugreek seeds were ground mechanically using pestle mortar. The coarse sized seed powder was moistened with petroleum ether (60 - 80), packed in soxhelet apparatus and extraction was carried out. Further, the extraction was carried out using alcohol. Finally, the material from the extraction chamber was collected and macerated with hot water (50 - 55°C). The mucilaginous solution was filtered. Alcohol was added to the filtrate to precipitate the galactomannans. The solution was filtered and the precipitate of galactomannans was collected on muslin. The collected galactomannans were lastly washed with acetone and dried. The dried gummy mass was ground to fine powder. The fine powder that passed through sieve # 200 was collected and used for further study.

4.1.2: Acid hydrolysis of fenugreek galactomannan (FGM).

1 % w/v dispersions of both guar and fenugreek galactomannans were prepared using mechanical stirrer. The dispersions were transferred to a test tube. Concentrated sulfuric acid was added to the dispersions to achieve a final concentration of 2 % v / v. The test tubes were placed in a water bath maintained at a temperature of 70°C. The heating was continued for 30 - 40 minutes. The solution was filtered.

The filtrate so obtained was tested for reducing sugars using Fehling's solution.

4.1.3: Infrared spectroscopic study of FGM.

Potassium bromide was dried at a temperature of 150°C for 2 hrs. The sample of FGM was kept in the desiccator. A pellet of the sample with Kbr was compressed, using hydraulic press. The spectrum was obtained. Similarly, the spectrum of GG was obtained.

4.1.4: Performance of qualitative test for proteins, alkaloids, starch and saponins on the extracted FGM powder.

Qualitative tests were performed on the extracted FGM powder to ensure freedom from impurities. The tests are described below in Table–11.

Table - 11: Qualitative tests of FGM powder

S. no.	Method	Observation	Inference
1.	A small amount of the sample was stirred with few drops of dil. HCl and filtered. The filtrate was tested with the following reagents. a) Hager's reagent b)Wagner's regent	no yellow ppt. no reddish brown ppt.	No alkaloids present
2.	A 1 % w/v dispersion of the extracted FGM was prepared in water and subjected to biuret test.	no black ppt.	No proteins present
3.	A 1 % w/v dispersion of the FGM was prepared. 1ml of this was diluted to 20 ml. with water in a graduated cylinder. The cylinder was shaken for 20 min.	Slight foam observed; but did not measure 1cm.	No saponins (to an appreciable extent)

4.	A 1 % w/v dispersion of FGM was prepared. To the dispersion alcohol and acetone were added.	A white ppt was obtained	The ppt is the gum having a property to absorb water.
5.	Mounted a small quantity of FGM with 0.02 N Iodine and observed under the microscope.	No bluish particles observed.	No starch.

Acid insoluble matter: The acid insoluble matter was found to be not more than 3 %. This matter was obtained by hydrolyzing a 1 % w/v dispersion of FGM with 2 % v/v conc. sulfuric acid.

Moisture content: The moisture content of FGM was found to be 10 % on dry weight basis as determined on the I. R. moisture balance.

4.1.5: Chromatographic study of acid hydrolyzed FGM.

The acid hydrolyzed solution, as obtained above, was spotted on the prepared TLC plate. The experimental details are compiled below.

Table - 12: Thin Layer Chromatogram of FGM

Date	:24 / 09 / 2001.
Compound class	:Sugars
Literature	:Egon Stahl, Thin Layer Chromatography - A handbook.
Plate size	:7.5 x 2.5 cm.
Adsorbent layer:	:Silica gel impregnated with 0.1 N Sodium metabisulfite
Preparation and Drying	:Silica gel .....1gm 0.1 N Sod metabisulfite aq. Soln....qs.2.5ml. The slurry, prepared, according to the above formula, sufficient for one plate, was poured on to the plate and uniformly spread. The silica gel plate was activated by heating at a temperature of 110° C for1 hour.
Chamber type	:Jar 8 x 4 x 4 cm
Chamber saturation	:Normal saturation.
Separation technique	:Ascending Chromatography
Solvent composition	:Benzene: Acetic acid: Methanol. 20:20:60.
Total volume	:2.5 ml.
Solvent and concentration of solution used for application	:Approximately 1 % acid hydrolyzed soln.

Margin between start point and plate edge	:1cm
Amount applied	:2 drops using a syringe
Length of run	:4.5 ± 0.06 cm.*
Time of run	:20 min.
Spray reagent	:concentrated sulfuric acid.
Treatment after spraying	:Heated at a temp. of 110°C for 10 min.
Spot 1	:Rf value: 1.6±0.06 / 4.5±0.06 = 0.36±0.01*
Spot 2	:Rf value: 2.8±0.1 / 4.5±0.06 = 0.62±0.02*

* Data indicates mean ± S.D. of triplicate experiments.

4.1.6: Rheological study of the extracted FGM and comparison with that of GG, LBG, and depolymerized guar gum (DGG)

A 2 % w/v dispersion of the galactomannans was prepared using mechanical stirrer. Warm water was used for preparing the dispersion of LBG; while the other gums are dispersible in cold water. The dispersions were diluted to give concentrations of 1.5, 1 and 0.5 % w/v. Dispersions were brought to a temperature of 25° C. The viscosity was then determined on Synchrolectric Brookfield viscometer, using spindle no. 4 at an rpm of 20. The obtained viscosity values were plotted to obtain a graph.

4.2: DEPOLYMERIZATION OF GALACTOMANNANS

4.2.1: Depolymerization of galactomannans by gamma irradiation.

Samples of galactomannan, GG and FGM, were weighed and transferred to Borosil glass tubes. The material in these glass tubes was exposed to gamma rays for different time intervals, based on which the dose of radiation in kilo greys is calculated. The facility of gamma radiation was provided by the Defense Laboratory, Jodhpur.

After exposure to gamma rays, dispersions of the samples were made. Their viscosity was determined using Synchrolectric Brookfield Viscometer at 20 rpm, using spindle no. 4 at a temperature of 25° C.

The effect of different doses of gamma radiation on viscosity was determined and tabulated.

Out of the various test doses, ranging from 5 to 50 kgrays, one of the doses i.e. 5 kgrays was selected for depolymerizing the high viscosity galactomannan.

4.2.2: The effect of gamma radiation at a fixed dose on depolymerization of galactomannans having different galactose mannose ratios.

The three galactomannans, guar gum (GG), fenugreek galactomannan (FGM) and locust bean gum (LBG), were exposed to a fixed gamma radiation dose of 5 kgrays. 1.5 % w / v aqueous dispersions of the galactomannans, before and after depolymerization, were prepared using a mechanical stirrer at 1500 rpm. Their viscosities were determined using Brookfield's viscometer spindle no. 4 at 25°C.

4.3: *IN-VITRO* INTERACTIVE STUDIES

4.3.1: The interactive binding effect of different galactomannans with glucose.

Control: A 10 % w / v aqueous solution of glucose was made. A glass tube was taken open at both the ends. One of its ends was covered with a cellophane sheet and the tube clamped on to a stand. The prepared solution was poured into the glass tube. The tube was lowered to bring in contact its cellophane - covered end by just dipping its surface with the water kept in a beaker below. Thus, two compartments were set up: one containing glucose solution and the other water; both being separated by a semi permeable cellophane membrane. Both the compartments were also magnetically stirred in order to prevent the formation of a stagnant layer.

At intervals of 30 minutes, samples from the beaker were collected (equal volume was also added to the beaker). These samples were analyzed for the concentration of glucose, using the Benedict's quantitative reagent. This was considered to be the control.

Different galactomannans at a concentration of 0.1 % w / v: Next, an aqueous dispersion of 0.1 % w / v GG (considered as the standard) was made and to this glucose was added in a concentration of 10 % w / v. In a similar manner, this solution was poured into the tube, cellophane sheet fixed in position, clamped, brought in contact with water below and samples collected at half hour intervals for two hours. Likewise, the experiment was performed using similar concentration of 0.1 % w / v of FGM, LBG, and DGG.

Different galactomannans at a viscosity of 75 cps: Next, the experiment was performed using dispersions of the above galactomannans having a similar viscosity of 75 cps. The different concentrations of the galactomannans used for preparing dispersions of a similar viscosity of 75 cps are given below in Table - 13.

Table - 13: Concentrations of galactomannans used for preparing dispersions having a similar viscosity of 75 cp.

S. No.	Galactomannan	Conc. % w / v
1.	Guar	0.1
2.	Fenugreek	0.12
3.	Locust bean	0.16
4.	Depolymerized guar	0.45

In the above similar manner, the samples were collected and glucose concentration determined. Graphs were plotted between the glucose concentration and time.

4.3.2: Effect of fenugreek galactomannan (FGM) on the surface activity of a bile salt: Sodium taurocholate.

Aqueous solutions of Sodium taurocholate of different concentrations, from 0.05 % to 3.0 % w/v, were prepared. Surface tensions of these solutions were determined using stalagmometre. Next, an aqueous dispersion of 0.05 % w/v FGM was prepared and divided into seven portions. To these seven portions, similar concentrations of Sodium taurocholate, as above (namely: 0.0, 0.05, 0.1, 0.15, 0.2, 0.25, 0.3 % w/v) was added. The surface tensions of these dispersions were again determined using stalagmometre. A graph was plotted between surface tension and the concentration of Sodium taurocholate with and without 0.05 % w/v FGM.

Another experiment to assess the influence of FGM on surface activity was performed which is based on the production of foam on vigorous shaking of a solution of a surfactant. For this experiment, a 0.2 % w/v solution of Sodium taurocholate was prepared and transferred into a measuring cylinder. The cylinder was vigorously shaken. Foam was produced, as expected; its height was measured. Next aqueous dispersions of FGM of different concentrations namely 0.0, 0.05, 0.1, 0.15, 0.2, 0.25 % w/v were prepared. To these, Sodium taurocholate was added in a concentration of 0.2 % w/v. The solutions were transferred to the same measuring cylinder and vigorously shaken. The height of the produced foam was noted. A graph was plotted between concentration of FGM and height of foam.

4.4: *IN-VIVO* STUDY IN RABBITS.

4.4.1: Preparation of crude aqueous extract of fenugreek seeds.

100gm fenugreek seeds were coarse ground. The coarse powder was taken in round bottom flask; to it 800ml water was added. The contents were refluxed for 18 hours. Then the contents were filtered. The collected filtrate is the aqueous extract.

This aqueous extract was evaporated slowly at a temperature of 40°C to dryness. The powder so obtained was further dried in a hot air oven and weighed.

4.4.2: Determination of lethal dose and physiological dose of fenugreek, using the crude aqueous extract.

A 20 % w / v aqueous dispersion of the powder obtained from the dried aqueous extract, as described above, was prepared.

Albino rats weighing about 200 gm. were caged and fasted 24 hours prior to commencement of the study. Each rat was fed 2ml (containing 400mg of powdered extract) of the prepared dispersion i. e to say a dose of 2-gm/kg body weight. The rats were fed using a catheter, introduced into the stomach. Prior to feeding, it is necessary to confirm that the tube has actually been introduced into the stomach and not the lungs. This is confirmed by introducing the external end of the tube in a beaker full of water and observing for non-appearance of bubbles.

The extract was administered to rats for a period of 14 days. The rats in this test group were observed for lethality, as against animals in the standard group.

4.4.3: Induction of hyperlipidemia by cholesterol feeding and performance of animal experiments for determining the effect of:

a. Fenugreek galactomannan (FGM)

b. Guar gum (GG) as standard and

c. Depolymerized fenugreek galactomannan (DFGM)

on cholesterol absorption in rabbits.

Rabbits weighing 1.75 - 1.9 kg were divided into four groups and marked using gentian violet. Animals were fasted for 18 hours prior to commencement of study. Thereafter, they had free access to food and water. Blood samples were collected on day 1 of study prior to feeding of cholesterol and drug.

The method selected for producing hyperlipidemia was cholesterol feeding by gastric intubation. All the four groups were administered cholesterol in a dose of 500-mg/kg-body weight per day. Cholesterol was administered as dispersion prepared in *vanaspati*. The first group, which served as control, was fed cholesterol alone. The second group, which served as standard, was administered GG in a dose of 250 mg/kg body weight, in addition to cholesterol as mentioned above. The animals in third and fourth groups were fed test drugs, FGM and DFGM as aqueous dispersion in a similar dose as standard plus cholesterol.

For feeding cholesterol and drugs, infant's feeding tube no. 8, lubricated with liquid paraffin, was passed through the gag fitted in the rabbit's mouth (jaw) (see Plate – 1, on the next page). While fixing the gag precaution has to be exercised that the tongue is below the gag. Having introduced the tube, the external end of the tube was dipped in beakerful of water. If no bubbles appeared, then it was confirmed that the tube has passed to the stomach and not to the lungs.

Plate - 1: Feeding tube passed through a gag into rabbit's stomach for gavage

The study was conducted for a period of 18 days. Blood samples were collected on day 1, 9 and 18. For collecting the blood samples, xylol was applied to the rabbits' marginal ear veins for prominent dilation of the vein. Blood was collected in centrifuge tubes using needle of gauge 22 by a technique what is referred to as 'milking' i.e. drop wise collection of blood through a needle pierced in the vein. The collected blood was left for an hour and allowed to clot. Then it was centrifuged for 20 minutes. The separated serum was transferred to a sample vial using pasture pipette.

The serum so obtained was analyzed for total lipid profile using autoanlyzer. The results were tested for statistical significance using student's *t* - test.

4.4.4: Performance of animal experiments to determine the effect of:

a. Fenugreek galactomannan (FGM) and

b. Depolymerized FGM (DFGM)

on glucose absorption using guar gum (GG) as standard.

Glucose loaded rabbit model was selected in order to study the effect of galactomannans on the absorption of glucose. Rabbits, weighing 1.8 kg to 2.3 kg, were caged and fasted for 12 hours before the study. Food and water were withheld till the last blood specimen was collected. Rabbits were divided in four groups: group 1: control, group 2: standard (GG), group 3: test 1 (FGM), and group 4: test 2 (DFGM).

For administration of galactomannans and glucose, a rabbit was put into rabbit holder. A gag was fit between its jaws. A lubricated tube was inserted through the gag hole down to the stomach (see Plate - 1). It was then tested whether the tube had actually passed down to the stomach and not to the lungs. This was done by dipping the tube in beaker full of water and no air bubbles were observed.

A syringe filled with a 3.3 % w/v aqueous dispersion of a galactomannan (in a concentration sufficient to provide a dose of 250 mg/kg body weight) was attached to the external end of the tube. The dispersion was pushed in. Then after a time gap of 15 minutes, a 50% w/v solution of glucose (sufficient to provide 2 gm/kg body weight) was similarly pushed in.

Blood samples were collected in EDTA vials at various time intervals namely, 0 (i.e. before glucose administration), 1, 2, 4, 6, 8, and 24 hours. Glucose concentration in blood was determined using autoanlyzer. A graph was plotted between time and glucose concentration for the four groups of animals. The results so obtained were statistically tested using student's *t*-test.

4.4.5: Assessment of the effect of depolymerized fenugreek galactomannan (DFGM) on blood platelets.

To assess the toxicity potential of DFGM, platelet counts were performed prior to commencement of study on day 1 and on day 18 of the animals being fed DFGM. The platelet count was done by the direct method. For this, platelet-diluting fluid (having Brilliant crysylblue as one of its components), was sucked in the RBC pipette up to the 0.5 mark. Into this pipette, blood was collected from the marginal ear vein of animals being fed DFGM up to the 1.0 mark. Then, blood was diluted to 101 mark using the platelet diluting fluid. It was thoroughly mixed in the pipette's chamber. Initial first two drops were discarded. Then one drop was placed on the counting chamber and after a minute the platelets were counted under the microscope. The counts were made in the 5 big squares as for RBC count. The numbers of platelets counted were multiplied by the appropriate factor (i.e. x 10,000) to obtain the total number of blood platelets per cubic mm.

4.5: DOSAGE FORM DEVELOPMENT

4.5.1: Preformulation study of fenugreek galactomannan (FGM) powder.

For determination of physico-mechanical characteristics, FGM was extracted from Fenugreek seeds by solvent extraction process, as per the procedure described earlier. The galactomannan so obtained was air-dried and then size reduced and passed through sieve # 200. The so obtained powder was used to determine the physico-mechanical characteristics by the established methods as specified in the official compendia and standard reference books.

4.5.2: Preparation and evaluation of Pharmaceutical preparation (granules) of fenugreek galactomannan (FGM).

About 10 gm. FGM powder was taken in a mortar. The granulating agent used was a mixture of alcohol and water in 11 different concentrations, namely 0, 10, 20, 30, 40, 50, 60, 70, 80 90, and 99.5 % of alcohol in water. A quantity sufficient of the above alcohol-water mixture was added so as to make the mass pliable and damp. The volume of the granulating agent used is given in Table - 14 on the following page.

Table - 14: Volume of alcohol-water mixture required to make a damp pliable mass.

Percentage of alcohol	ml/gm required to make a pliable mass	Percentage of alcohol	ml/gm required to make a pliable mass
0	5.8 ± 0.5	60	2.0 ± 0.2
10	5.5 ± 0.5	70	1.75 ± 0.2
20	5.0 ± 0.5	80	1.45 ± 0.2
30	4.6 ± 0.4	90	1.2 ± 0.1
40	3.3 ± 0.4	99.5	1.0 ± 0.1
50	2.5 ± 0.3	-	-

The data indicates mean ± S.D. of triplicate experiments.

The resulting pliable mass was passed through sieve # 10. The granules so obtained were air-dried and evaluated for different physico-mechanical parameters.

4.5.3: Preparation and evaluation of designer food products of fenugreek galactomannan (FGM).

One of the food formulations of FGM that was prepared was FGM compound fennel flavoured balls. The balls were prepared using the formula given in Table – 15 below.

Table - 15: Formula for FGM compound fennel flavoured balls

S. No.	Ingredients	Quantity taken (gm)	Quantity per ball (gm)
1	FGM granules	10.0	2.0gm.
2	Jaggery	20.0	4.0gm.
3	Sodium bicarbonate	1.0	0.2gm.
4	Fennel powder	2.25	0.45gm.
5	Cardamom powder	0.25	0.05gm.
6	Menthol powder	0.25	0.05gm.

Fenugreek powder was taken in a mortar. To this, 40% v/v alcohol was added in sufficient quantity as granulating agent to convert the powder into a soft pliable mass. This was passed through sieve # 8 to form granules. The so formed granules were air-dried. The granules after drying were passed through sieve # 10, below which sieve # 30 was kept. The granules, which were retained on sieve # 30, were used in the formulation.

Chopped jaggery pieces were taken in a China dish. A little amount of water was added to it just to moisten it and the contents were heated. When the mass was molten, a drop of it was put into water to cool it and tasted to check whether it sticks in the mouth. Heating was continued till jaggery did

not stick in mouth. This ensures good keeping quality of the finished product. Then, it was removed from flame and sodium bicarbonate was added to it.

To the above hot molten mass, sufficient FGM granules and the powder mixture of fennel, cardamom, and menthol were added to make a cohesive semi solid mass. Out of this mass, smaller balls of approximately the same size were prepared.

The so prepared flavoured FGM balls were evaluated for weight variation, average diameter and hydration in water. These were finally packed in airtight glass container (see Plate –2).

Another, food formulation of FGM that was prepared was FGM nutritive designer cubes as per the formula given in Table –16 below.

Table - 16: Formula for FGM nutritive designer cubes

S. No.	Ingredients	Quantity taken (gm)	Quantity per cube (gm)
1	FGM granules	10.00	2.0
2	Wheat flour	7.5	1.5
3	Ghee	7.5	1.5
4	Gum	0.625	0.125
5	Sugar	7.5	1.5

FGM powder was converted into granules using 40% v/v alcohol, as described previously in experiment 4.5.2 above. Wheat flour, suitably size reduced sugar and the previously fried gum were first rubbed down with a little of base i.e. molten ghee using a large spatula in a dish over water bath.

Plate - 2: FGM compound fennel flavoured balls

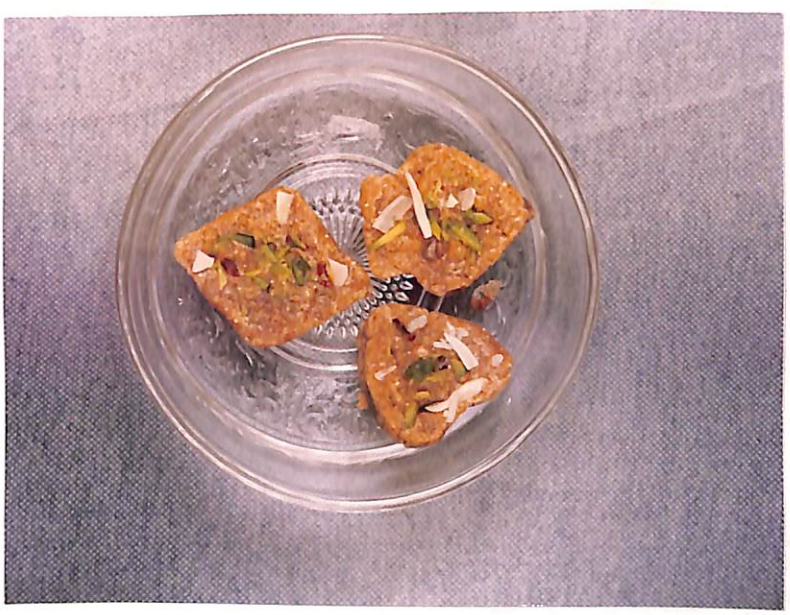


Plate - 3: FGM nutritive designer cubes

The rest of the base was added gradually with thorough mixing. Once, uniformity of mass was achieved, it was removed from the flame and to it, FGM granules were incorporated immediately. The resulting stiff mass was placed and pressed down into plastic designer moulds having different shapes. Care was taken to fill each hole completely and smooth off the excess. It was left at room temperature for two hours to form nutritive designer cubes.

The so formed designer cubes were evaluated for weight variation, density, hardness and organoleptic acceptability. These were finally packaged in airtight glass containers (see Plate- 3).

The third food formulation of FGM, i.e. FGM chocolate sticks, was prepared using the formula given in Table – 17 below.

Table - 17:Formula for FGM chocolate stick

S. No.	Ingredients	Quantity taken (gm)	Quantity per stick (gm)
1	FGM granules	20	2.0
2	Cocoa powder	10	1.0
3	Milk powder	20	2.0
4	Sugar	20	2.0
5	*Ghee*	20	2.0

Ghee was heated in a shallow, curved, metal dish till molten. Cocoa powder, milk powder and size-reduced sugar were added to the hot molten *Ghee*. Contents were vigorously stirred and heating was continued for a minute till a soft, homogenous product resulted. FGM granules were added to the preparation, after removing it from the flame. The preparation was

Plate - 4: Chocolate stick

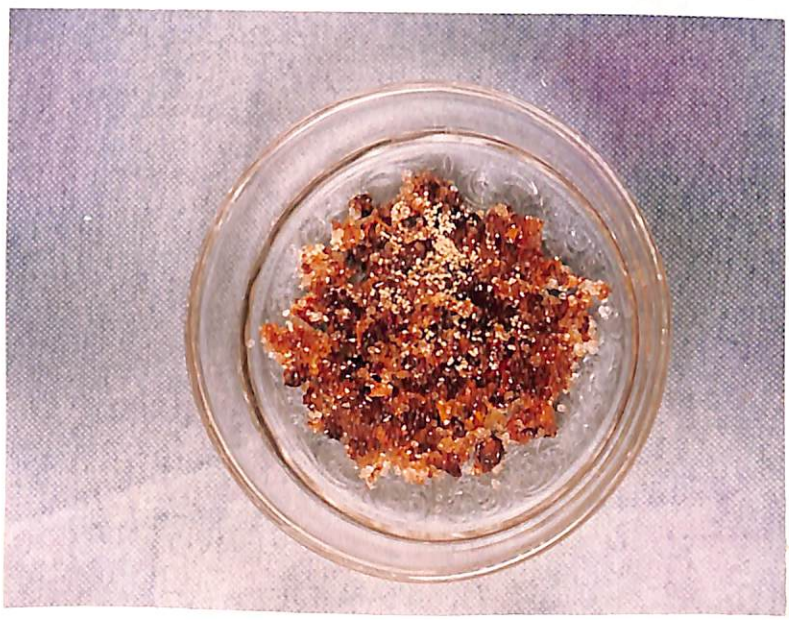


Plate - 5: FGM *gulukhand*

stirred gently till it was viscous enough to hold the granules but not too thick to pour.

The chocolate preparation was then poured into, previously lubricated, straight-sided tray with gentle stirring. When set, it was cut into long, slender sticks using a sharp knife. It was then kept in refrigerator to solidify.

Lastly, the sticks were evaluated and individually wrapped in butter paper and aluminum foil (see Plate - 4).

The formula for the fourth food formulation of FGM, i.e. FGM gulukhand is given in Table – 18 below.

Table - 18: Formula for FGM gulukhand

S. No.	Ingredients	Quantity taken (gm)	Quantity per portion (gm)
1	FGM granules	10	2
2	Rose petals	10	2
3	Sugar	5	1

For this preparation, the gulukhand base was prepared first. This consisted of rose petals suitably size reduced (by cutting into small pieces) and granular sugar both mixed together lightly. The mixture was kept in an airtight glass container and exposed to sunlight for two weeks with occasional shaking of contents. After two weeks time, it had turned into the semisolid base. In this base, FGM granules were uniformly dispersed using a spatula. This preparation is named FGM gulukhand (see Plate -5).

The preparation was evaluated for density and organoleptic acceptability. Finally, it was packed in airtight glass container.

CHAPTER 5
RESULT AND DISCUSSION

5.1: EXTRACTION, IDENTIFICATION AND CHARACTERIZATION OF FENUGREEK GALACTOMANNAN

5.1.1: Extraction of galactomannan from fenugreek seeds.

The yield of fenugreek galactomannans (FGM) obtained was found to be 10 % w/w.

For the extraction of FGM by soxhelation, various solvents used for the following reasons: Petroleum ether is a non - polar solvent which solutes out the lipophillic ingredients like the oils. Alcohol is a semi polar solvent which solutes out the small molecular weight compound. Alcohol, also, is a solvent for the saponins. Lastly, the galactomannans are obtained in the hot water. Any proteins, if present, shall be coagulated during the heating. Finally, the wash with acetone dehydrates the galactomannans and extends its universal solvent action to remove any remaining impurity. Thus, it can be expected that the galactomannans obtained using the above extraction procedure is likely to be free of impurities.

5.1.2: Acid hydrolysis of FGM.

The hydrolysis of fenugreek galactomannan was achieved by treating its aqueous dispersion with 2% v/v sulfuric acid. This was confirmed by treating the resulting solution with Fehling's solution for the two reducing sugars galactose and mannose. The test was positive, giving a brick red precipitate.

Here, GG has been used as the standard since it is the most widely used and studied galactomannan. It shall also be appropriate to mention here that acid hydrolysis depends on factors like acid concentration, time and temperature of heating.

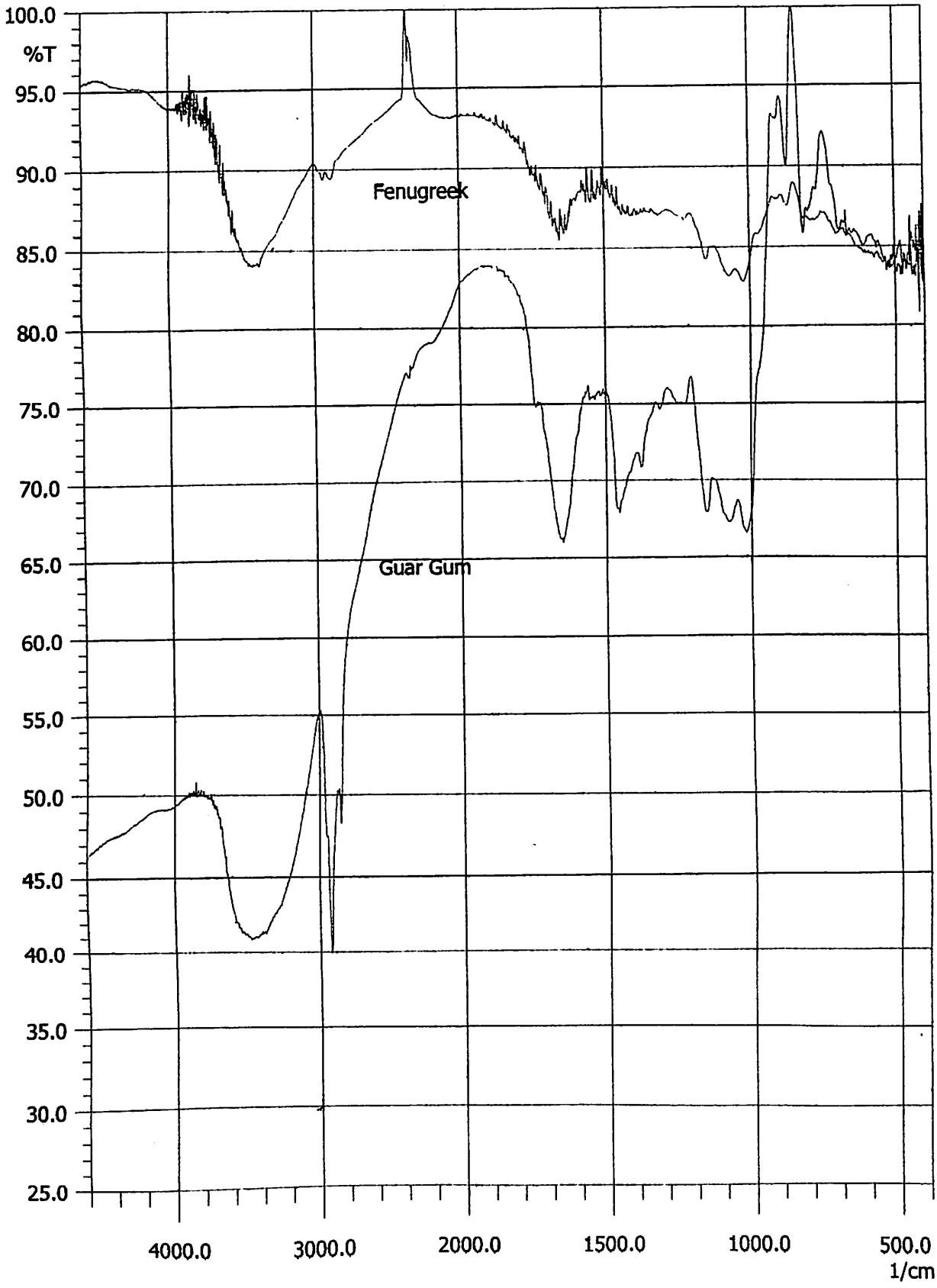


Figure 9: Merged spectra of guar gum and fenugreek galactomannan.

5.1.3: FTIR spectrum of FGM.

The principal application of infrared spectroscopy in Pharmacy is for structural identification and characterization of molecules. For this purpose, I.R. radiation in the region of 4000 - 400 cm^{-1} is employed. The region of spectra from 1300 - 400 cm^{-1} is referred to as the finger print region.

Since the compounds being analyzed are galactomannans, having predominance of -OH groups, it is expected that these shall show absorption peaks in the region of 3200 - 3700, which is actually the case; as seen from the FTIR spectra shown in Figure – 9, on the previous page. In the finger print region, peaks at 407 cm^{-1} and 1020 cm^{-1} are similar. The other matching peak is at 2860 cm^{-1}.

GG has been considered as the standard with which the spectrum of fenugreek has been compared. The GG exported from the companies here at Jodhpur claim a galactomannan content of above 95 % in their products. Hence, the extracted FGM can also be expected to have similar galactomannan content i.e. free from impurities.

5.1.4: Qualitative tests for extracted FGM.

The FGM obtained by extraction in the laboratory can satisfactorily be considered to be pure enough, based on the results of the qualitative tests as described in the experimental chapter 4.1.4. The product obtained is water dispersible, which gets precipitated by the addition of alcohol and is free of proteins and starch. This is the minimum requirement and definition of gums / galactomannans as per the Association of Official Analytical Chemists (AOAC).

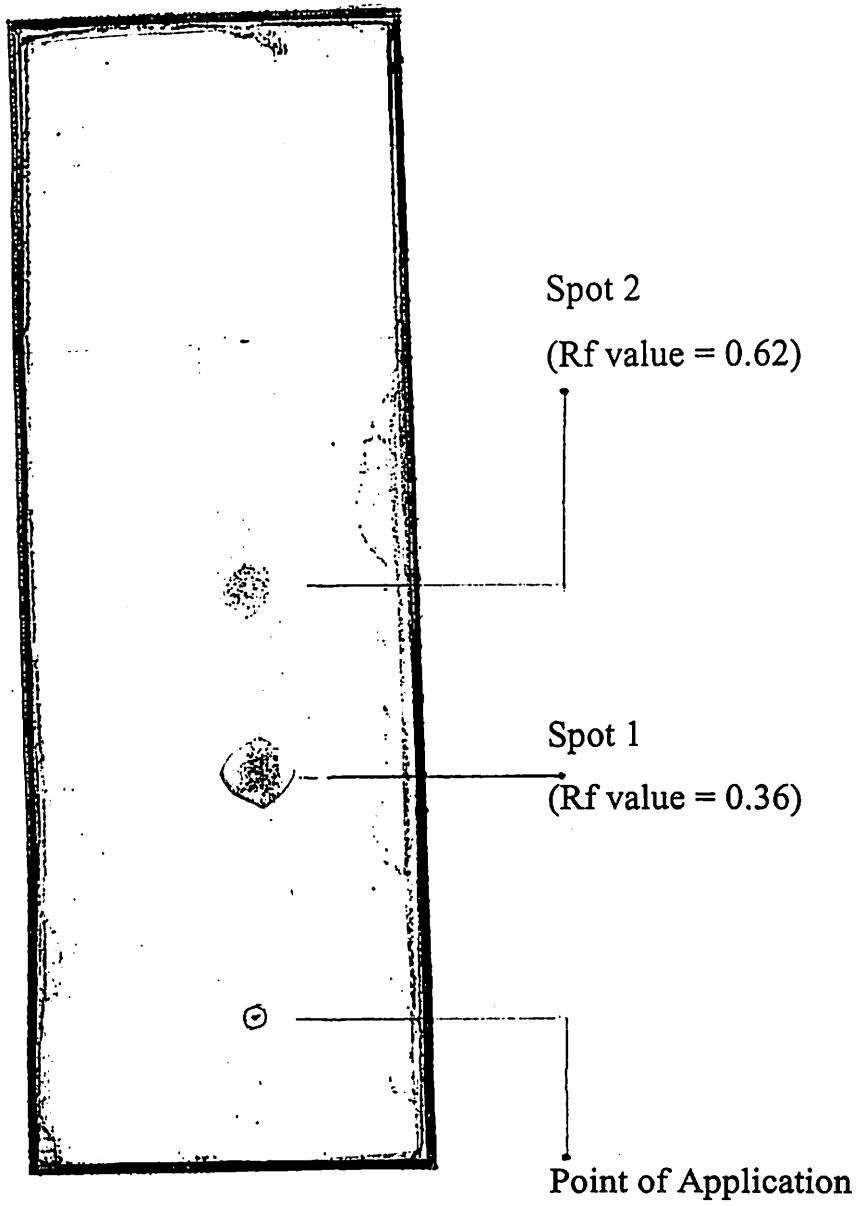


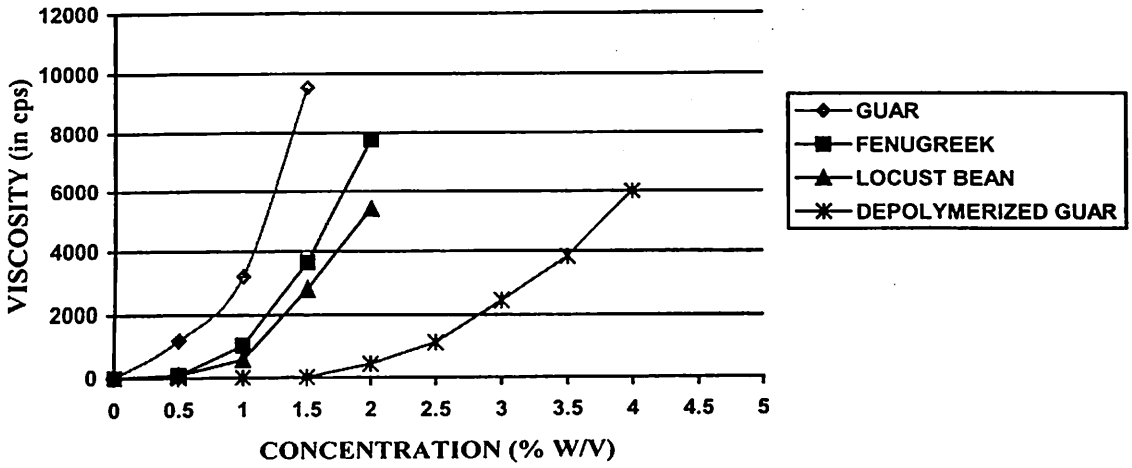
Figure 10: Chromatogram depicting spots of constituents of acid hydrolyzed fenugreek galactomannan

5.1.5: Chromatogram of acid hydrolyzed FGM.

The copy of the chromatogram, in Figure – 10 on the previous page, beautifully exhibits two spots. By the position (Rf values), of the two spots, it can be deducted that the lower spot represents galactose and the top one mannose. These are the two sugars resulting from the hydrolysis of the extracted FGM.

5.1.6: Rheology of FGM in comparison to other galactomannans.

Graph 1: Viscosity of different galactomannans at different concentrations



The graph is plotted using mean values of triplicate determinations.

The viscosity of the galactomannans, as seen from Graph – 1, exhibits the characteristic non - Newtonian pseudoplastic flow that is typical of polymers in general.

The viscosity of GG is the highest among all the galactomannans. This is the main reason, accompanied with its low cost, for its wide acceptability by the industry at large. Such a high viscosity is a nuisance, if

it is to be used medicinally and in the dairy industry. To overcome this problem, its low viscosity depolymerized form, has been prepared.

The viscosity of LBG is too low to be accepted by the industry. Indeed prior to the introduction of GG, LBG was widely used but now has been entirely replaced by GG. As far as, fenugreek is considered, it offers a potential source of galactomannan that can be preferred medicinally. The great added advantage that it has is that it has been used since ages as food product, which proves its nontoxic nature. However, its, medicinal value has to be compared against guar and other dietary fibres.

5.2: DEPOLYMERIZATION OF GALACTOMANNANS

5.2.1: Depolymerization of galactomannans by gamma irradiation.

When ionizing radiation passes through matter some of the energy of the radiation is absorbed. The rad (radiation absorbed dose) is the traditional unit of absorbed dose equivalent to 10^{-2} joules/kg. The gray (Gy) 1joule/kg (equal to 100 rads) has been assigned as the SI unit of absorbed dose.

Table – 19, depicts the effect exposing a galactomannan to increasing doses of gamma radiation and its effect on resulting viscosity.

Table - 19: Depolymerization of galactomannan by gamma radiation

S. No.	Gamma radiation dose (kilograys)	Viscosity of 1 % w /v dispersion (cps)	% Reduction in viscosity = (Initial viscosity - Final viscosity) X 100 / Initial viscosity
1.	0.0	1790 ± 80	0.0
2.	5.52	170 ± 20	90.5
3.	11.04	70 ± 10	96.0
4.	16.56	50 ± 10	97.2
5.	19.8	30 ± 05	98.3
6.	33.0	25 ± 05	98.6
7.	46.5	20 ± 05	98.8

The data indicates mean ± S.D. of triplicate determinations.

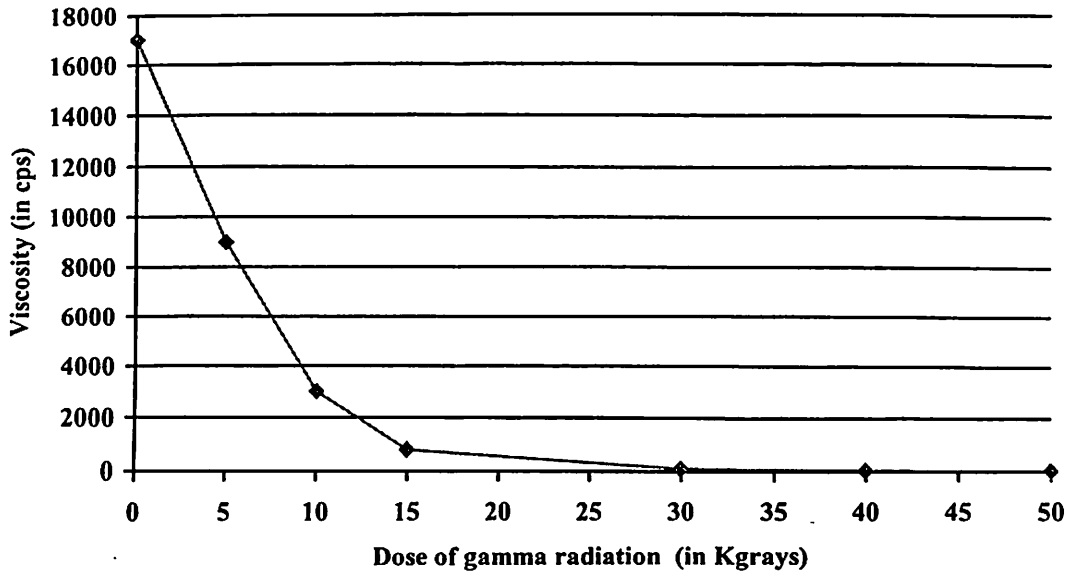
It is seen from the above table that significant reduction in viscosity has taken place on exposure of the sample of galactomannan (Guar gum) to gamma rays for different time intervals. This reduction in viscosity is attributed to depolymerization.

It is appropriate to mention here that existing methods of depolymerization of galactomannans are based on enzymatic cleavage, acid

hydrolysis or thermal hydrolysis. With the above experiment, a novel method for depolymerization using gamma radiation has been developed.

A graph was plotted between different doses of gamma radiation used to depolymerize the FGM sample and the resulting viscosity of the dispersion at a concentration of 2.5 % w / v. The results are depicted in Graph 2.

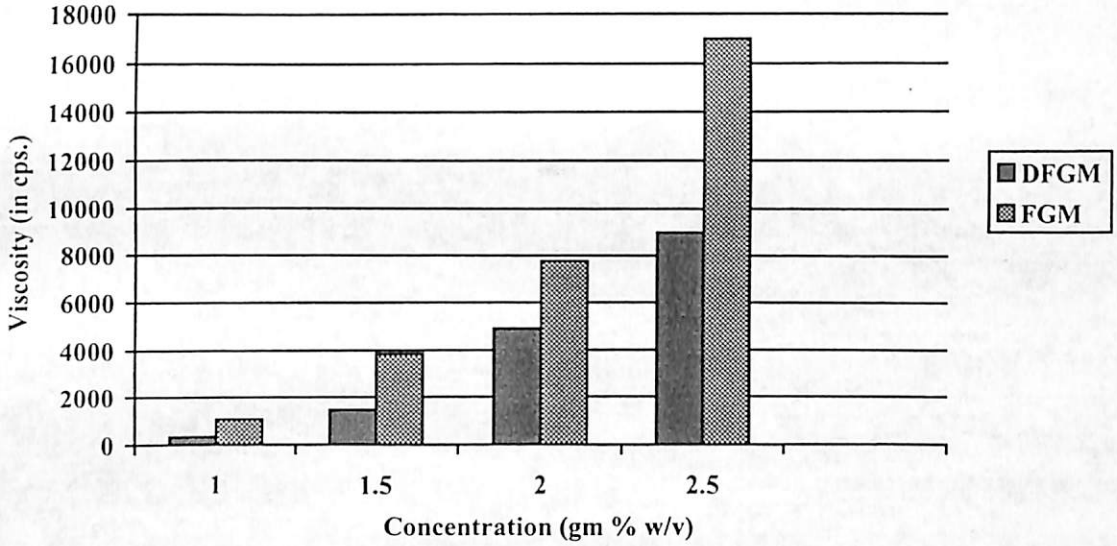
Graph 2: The effect of increasing dose of gamma radiation on the viscosity of a 2.5 % w/v dispersion of fenugreek galactomannan.



The graph is plotted using mean values of triplicate determinations.

Graph 3, depicts the comparative viscosities of non - depolymerized, the so-called high viscosity FGM and the depolymerized FGM (DFGM).

Graph3: Comparitive viscosities of non-depolymerized and depolymerized fenugreek galactomannan.



The graph is plotted using mean values of triplicate determinations.

Fenugreek galactomannan, depolymerized at a gamma radiation of 5kgrays shall be produced and used in further study to determine its medicinal applications: as hypoglycemic and as antihyperlipidemic agent.

5.2.2: Depolymerization of different galactomannans: effect of galactose: mannose ratio.

The comparative viscosities of 1.5 % w/v dispersions of various galactomannans, before and after depolymerization by exposure to gamma radiation at 5kgrays, are tabulated below in Table - 20.

Table - 20: Percentage reduction in viscosity of galactomannans having different galactose mannose ratios

S. no	Galacto-mannan	G : M Ratio	Viscosity before depolymerization (Vi in cps)	Viscosity after depolymerization (V dep in cps)	% reduction in viscosity. $\frac{(Vi - Vdep)}{Vi} \times 100$
1.	Fenugreek	1 : 1	3850 ± 250	1450 ± 150	62 ± 4%
2.	Guar	1 : 2	9200 ± 520	900 ± 180	90 ± 2%
3.	Locust bean	1 : 4	3000 ± 200	150 ± 30	95 ± 1%

The data indicates mean ± S.D. triplicate determinations.

It is evident from the above tabulated results that the percentage reduction in viscosity is least in the case of galactomannan having the highest galactose mannose ratio i.e. namely fenugreek galactomannan. While the reduction in viscosity on depolymerization in case of locust bean is least which co - relates with its lowest galactose mannose ratio. The same corollary is extendable in case of guar.

The above fact is also carbureted by similar observations, when depolymerization was carried out by acid hydrolysis method. It was observed that fenugreek galactomannan took the longest time to depolymerize requiring 30 - 35 minutes. This was followed by guar,

requiring a time of 15 -18 minutes and the least time of 10 - 12 minutes was required by locust bean for acid - hydrolyzed depolymerization.

It can therefore be concluded that a galactomannan with the highest galactose mannose ration resists depolymerization. Based on the percentage reduction in viscosity, the galactose mannose ratios of galactomannans can be deducted.

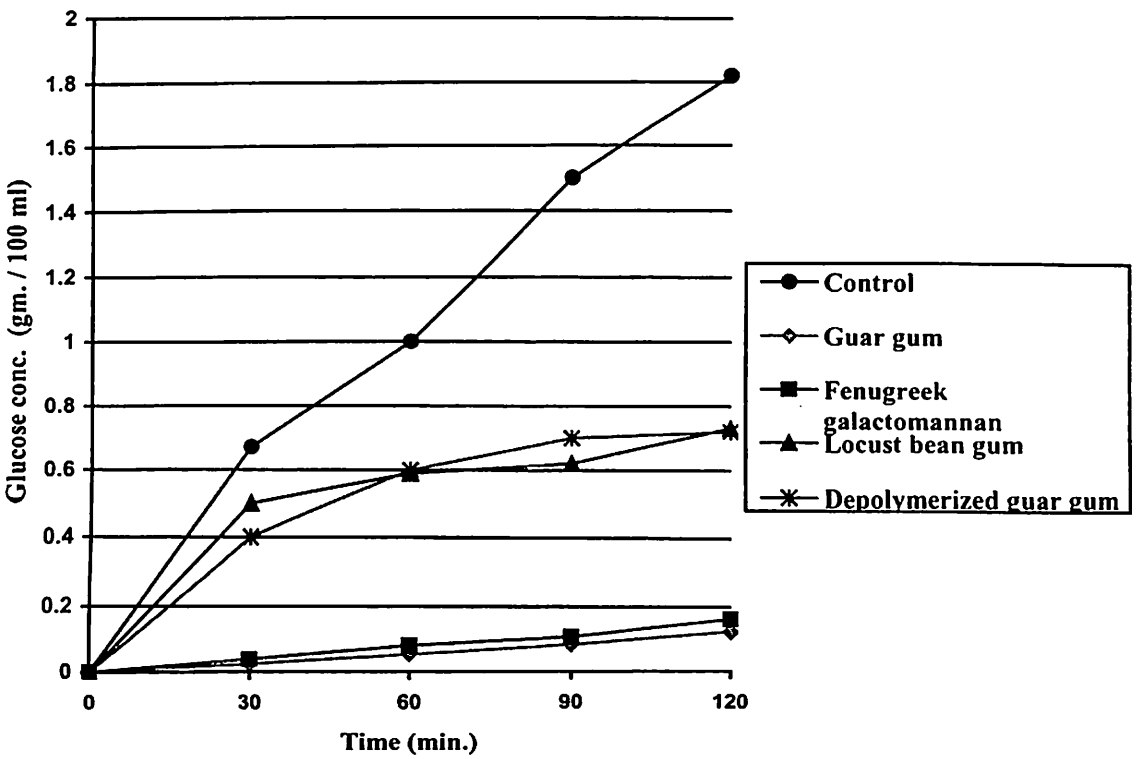
Resistance to depolymerization among galactomannans of different galactose: mannose ratios would also be expected to have physiological importance of its own i.e. a galactomannan having G: M ratio of 1: 1 may exert its effect for a prolonged period of time as it would remain intact in its non-depolymerized form for comparatively a longer period of time.

5.3: *IN-VITRO* INTERACTIVE STUDIES

5.3.1: **The interactive binding effect of different galactomannans with glucose.**

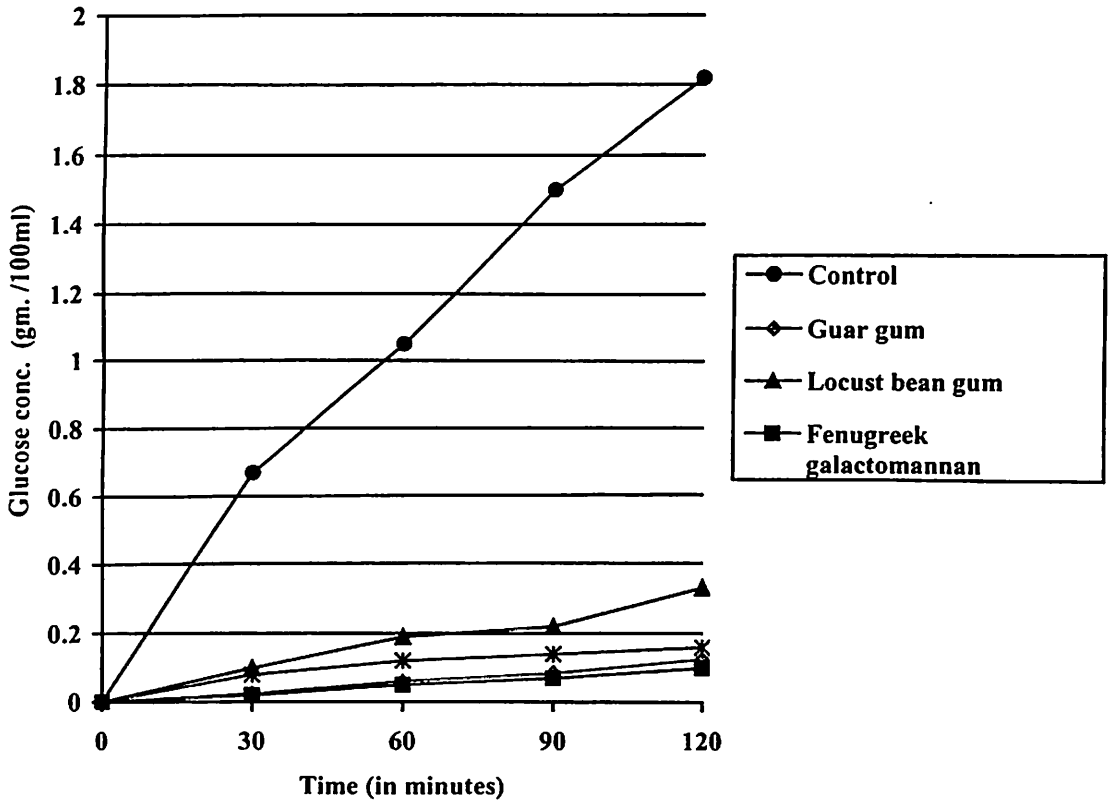
The following graphs were plotted.

Graph 4: Effect of different galactomannans at similar concentration of 0.1 % w/v on glucose diffusion



The graph is plotted using mean values of triplicate experiments.

Graph 5: Effect of different galactomannans at similar viscosity of 75cps on glucose diffusion



The graph is plotted using mean values of triplicate experiments.

From the Graph - 4, above, it is clearly evident that the diffusion of glucose across the cellophane membrane has been reduced / retarded. The quantitative reduction in glucose concentration is tabulated in Table - 21. The percentage reduction in glucose concentration is obtained by taking the difference between the control glucose concentration and the glucose concentration with the galactomannan in question.

Table - 21: Percentage reduction in glucose diffusion due to binding with different galactomannans at a concentration of 0.1 % w/v

S. no.	Galactomannan	Viscosity of 2 % w /v dispersion (cps.)	Quantity of glucose diffused (gm / 100 ml.)	Glucose diffusion (%)
1.	Glucose (control)	1 (for water)	1.82 ± 0.19	100.00
2.	Guar	10,000 <	0.125 ± 0.02	6.9 ± 1.1
3.	Fenugreek	7750 ± 430	0.164 ± 0.02	9.0 ±1.1
4.	Locust Bean	5400 ± 360	0.730 ± 0.15	40.1 ±8.2
5.	Depolymerized guar	450 ± 65	0.720 ± 0.18	39.6 ±9.8

The data indicates mean ± S.D. of triplicate experiments.

Among the galactomannans studied guar gum binds with glucose the most effectively, followed by fenugreek galactomannan, next by locust bean gum and the lastly by depolymerized guar gum. The above effects may be attributed to the relative viscosities of these galactomannans.

Graph - 5, reveals that fenugreek galactomannan affords the greatest binding with glucose followed by guar gum, next its depolymerized form and lastly by locust bean gum. The quantitative reduction in glucose diffusion is tabulated in Table - 22.

Table - 22: Percentage reduction in glucose diffusion due to binding with different galactomannans at a viscosity of 75 cps

S. no.	Galactomannan	Galactose : Mannose ratio	Quantity of glucose diffused (gm / 100 ml)	Glucose diffusion (%)
1.	Glucose (control)	-	1.82 ± 0.19	100.0
2.	Guar	1 : 2	0.12 ± 0.02	6.9 ± 1.1
3.	Fenugreek	1 : 1	0.10 ± 0.02	5.5 ± 1.1
4.	Locust Bean	1 : 4	0.33 ± 0.07	18.1 ± 3.8
5.	Depolymerized Guar	-	0.16 ± 0.05	8.8 ± 2.7

The data indicates mean ± S.D. of triplicate experiments.

The highest binding effect of FGM may possibly be explained due its high galactose: mannose (G: M) ratio of 1:1, which is followed by GG with a ratio of 1:2, next by LBG with a ratio of 1:4

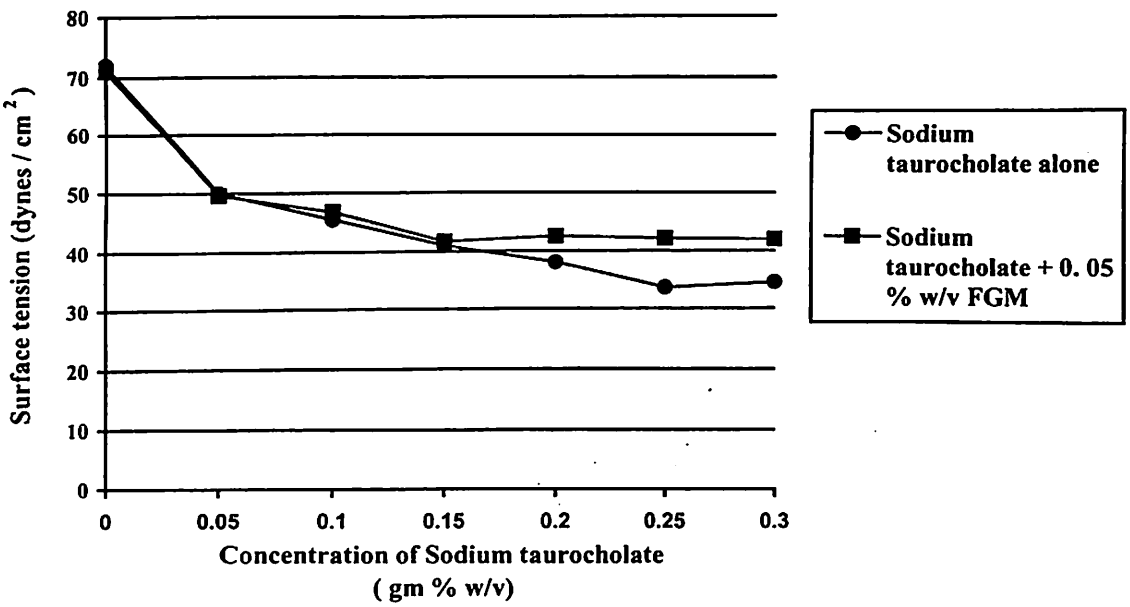
Therefore, from the above it can be concluded that it is not only viscosity that is important but also the G: M ratio needs to be considered while studying the effect of galactomannans influencing glucose absorption. A galactomannan having the highest viscosity and the highest G: M ratio can said to be the most potent. However, a galactomannan with too high a viscosity is difficult to put into formulation.

Therefore, from the above, FGM promises to be best galactomannan in having a high G: M ratio for activity and an appropriate viscosity for easy incorporation into a formulation. Further, the added advantage of fenugreek is that it has been used as a food product since ages hence, there are no doubts regarding its toxicity.

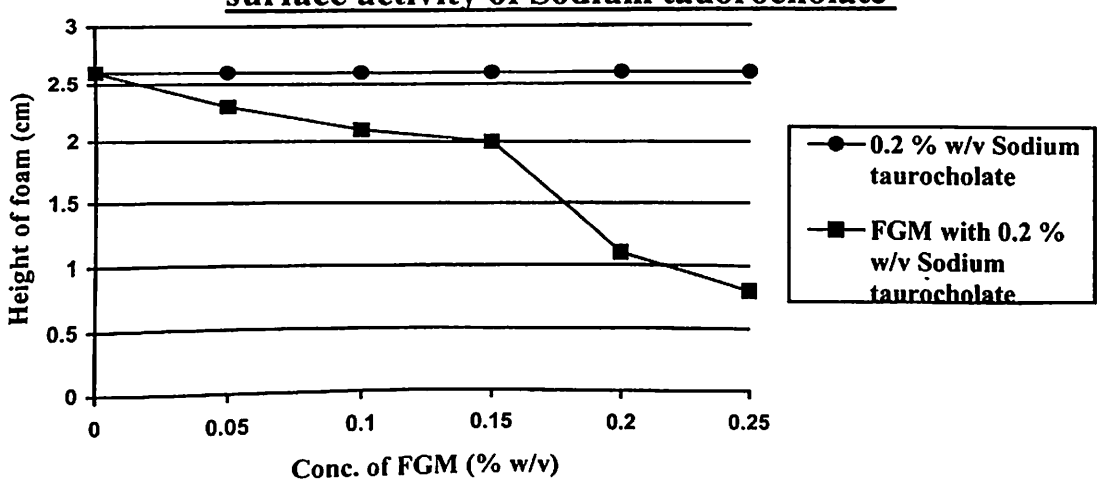
5.3.2: Influence of FGM on surfactant activity.

The effect of FGM on the surface activity of Sodium taurocholate is depicted below in Graphs – 6 and 7.

Graph 6: Effect of fenugreek galactomannan on surface activity of Sodium taurocholate



Graph 7: Effect of fenugreek galactomannan on surface activity of Sodium tauorocholate



The graphs are plotted using mean values of triplicate experiments.

Sodium taurocholate, a surfactant, is a bile salt. The job of bile (or a surfactant in general) is to lower the interfacial tension between two immiscible phases like oil and water. In the gut, bile performs two important functions i.e. emulsification and solubilization which are essential for the absorption of fats, including cholesterol which is itself the precursor of taurocholate.

If these two functions of bile are interfered with than a reduced absorption of fats can be expected due to non - emulsification and non - solubilization and the fats shall pass out as such. Additionally, if cholesterol absorption is reduced, the body shall utilize the cholesterol from its pool of low-density lipids (the so called bad cholesterol), because that is the form in which cholesterol is stored in abundance.

It is evident that FGM has the potential to interfere and lower the surface activity of Sodium taurocholate which is seen from the increased surface tension of water having Sodium taurocholate and 0.05 % w/v FGM (Graph - 6) and also from the reduction in the height of foam with increasing concentrations of FGM (Graph - 7). This also suggests a dose dependent effect.

Now, with the above two set of *in - vitro* experiments, for assessing the effect of FGM on glucose and fat (cholesterol) absorption, the stage is set for performing *in - vivo* experiments to assess its these medicinal applications.

5.4: *IN-VIVO* STUDIES IN RABBITS

5.4.1: Preparation of crude aqueous extract of fenugreek seeds

The yield of the crude aqueous extract was found to be 20 % w/w.

The seed to water ratio used for preparation of the extract was 1: 8, while generally; the recommended ratio is 1: 4. The higher ratio was used since the viscosity of the extract was quite high during its preparation. Extraction at such a high viscosity would have otherwise been incomplete.

5.4.2: Determination of lethal dose of fenugreek

The crude aqueous extract of Fenugreek was found not to be lethal at the dose administered 2-gm/kg-body weight.

Generally, a dose of 2-gm/kg-body weight is considered lethal in most of the drugs of plant origin. Lethality may possibly be seen at higher doses. In the present study, a dose of 2-gm/kg-body weight was administered, since this was the maximum possible dose that could have **practically** been administered to the rats. This is because, the volume of solution that can be administered to the albino rats is very small i.e. 1ml and at the most 2ml that too with difficulty. The dose can be increased beyond 2-gm/kg body-weight in one of the two ways: one by increasing the concentration i. e. more than 200 mg / ml and two by increasing the volume to be administered. If the content of the powder extract for preparing the solution is increased, it becomes highly viscous and even gels. Also, the solution is going to be very concentrated, which practically the gastrointestinal tract may never be exposed to under normal conditions. The second method could not be attempted, as already mentioned above.

Now, as per the WHO guidelines, one fourth to one fifth of the lethal dose is considered to be the physiological dose. In this case, further study on

animals to determine the medicinal applications of fenugreek galactomannan, shall be performed using a test dose calculated in the following manner:

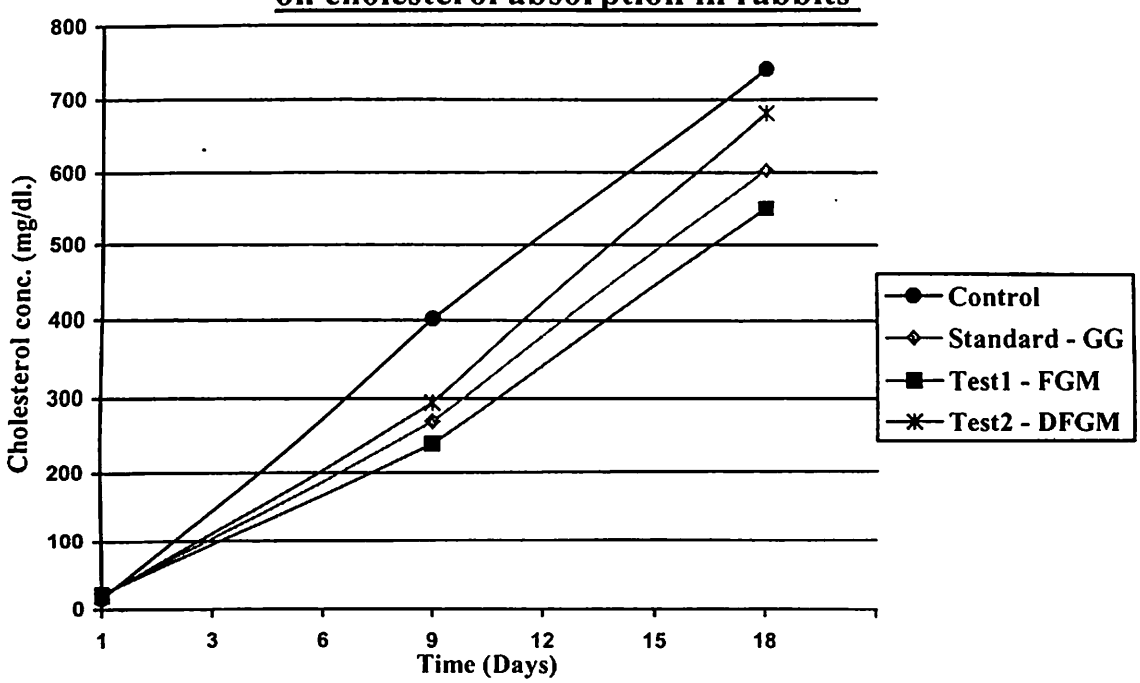
Yield of crude aqueous extract of Fenugreek	= 20 % w/w.
Yield of galactomannan from fenugreek	= 10 % w/w.
Maximum lethal dose (LD $_{50}$) studied	= 2gm/kg body wt.
Physiological dose	= 0.5 gm of crude aqueous extract i.e.one fourth of LD_{50}
Proportion of galactomannan in crude aqueous extract	= 50 %
Test dose i.e. galactomannan to be administered	= 0.25gm/kg body wt.

A number of studies on human volunteers have been performed using guar galactomannan in a dose of 10 - 30 gm. If the above fenugreek galactomannan test dose of 250-mg/kg body-weight is considered for a human volunteer of 70 kg, it works out to be 17.5 gm. This compares well with the dose of guar galactomannan. A higher dose can also be administered safely, since no lethality was observed at a dose of 2-gm/kg-body weight.

5.4.3: Comparative antihyperlipidemic activity of FGM, GG and DFGM

Graph – 8 below depicts the rising concentration of cholesterol on administration of 500mg/kg body weight cholesterol to different animal groups. In Table – 23, serum lipid profile of untreated (control) and galactomannan treated animal groups is tabulated. In Table - 24, the protective effect of galactomannans on the important LDL/HDL ratio and their statistical significance is tabulated.

Graph 8: Effect of various galactomannans on cholesterol absorption in rabbits



The graph is plotted using mean values of five replicate determinations.

Table - 23: Serum lipid profile of different animal groups on day 18

Parameter → Group ↓	Cholesterol	High Density Lipids	Low Density Lipids	Very Low Density Lipids	Triglyceride
Control	741 ± 31	88 ± 16	541 ± 4	111 ± 11	557 ± 44
Standard– GG	603 ± 35	103 ± 18	449 ± 10	51 ± 7	253 ± 35
Test 1 – FGM	550 ± 32	100 ± 21	412 ± 8	38 ± 3	189 ± 14
Test 2- DFGM	681 ± 46	98 ± 14	511 ± 26	72 ± 6	360 ± 30

All values in mg/dl.

The data indicates mean ± S.D. of 5 replicate determinations.

Table - 24: Effect of various galactomannans on serum cholesterol levels as comparable on day 18

Parameter → Group ↓	% Cholesterol	LDL / HDL ratio	Calculated *t* - value
Control	100	6.25	-
Standard – GG	81.4 ± 4.7	4.4	8.49 ^
Test 1 – FGM	74.2 ± 4.3	4.1	11.83 ^
Test 2 - DFGM	91.9 ± 6.2	5.2	2.99 *

The data indicates mean ± S. D. of 5 replicate determinations. Theoretical *t*-value = 3.355 corresponding to p = 0.01 and theoretical *t*-value = 2.896 corresponding to p = 0.02 both for 8 degrees of freedom. The number of animals in each group n = 5.

- for comparison with test values

^ significantly different at p = 0.01

* significantly different at p = 0.02

To study the antihyperlipidemic activity of galactomannans, cholesterol could have been dissolved in any vegetable oil but *vanaspati* was used as the solvent. It was because, *vanaspati*, a saturated fat, was expected to raise serum lipid levels, independent of the amount of cholesterol administered.

From the present study it is concluded that galactomannans from fenugreek possess significant (at $p = 0.01$) antihyperlipidemic activity. FGM with a galactose: mannose ratio of 1: 1 is the most effective galactomannan affecting cholesterol absorption. This effect is observed despite its lower viscosity in comparison to guar gum. DFGM also possess significant (at $p = 0.02$) antihyperlipidemic activity. Comparing the activities of GG and FGM, it is observed that the two are significantly different (at $p = 0.02$) in their action in keeping the cholesterol levels low. Therefore, viscosity alone is not the mechanism by which galactomannans act. The activity depends on the G: M ratio as well.

One of the important parameters considered for evaluation of antihyperlipidemic agents is the ratio between low-density and high-density lipids or LDL / HDL ratio. Normally, the ratio should be between 3 to 4. The ratio increases if there is an increase in LDL (the so called 'bad cholesterol') or if there is a decrease in HDL (The so-called 'good cholesterol'). From the results, it is seen that FGM is the most effective galactomannan in keeping the ratio down, to about 4.

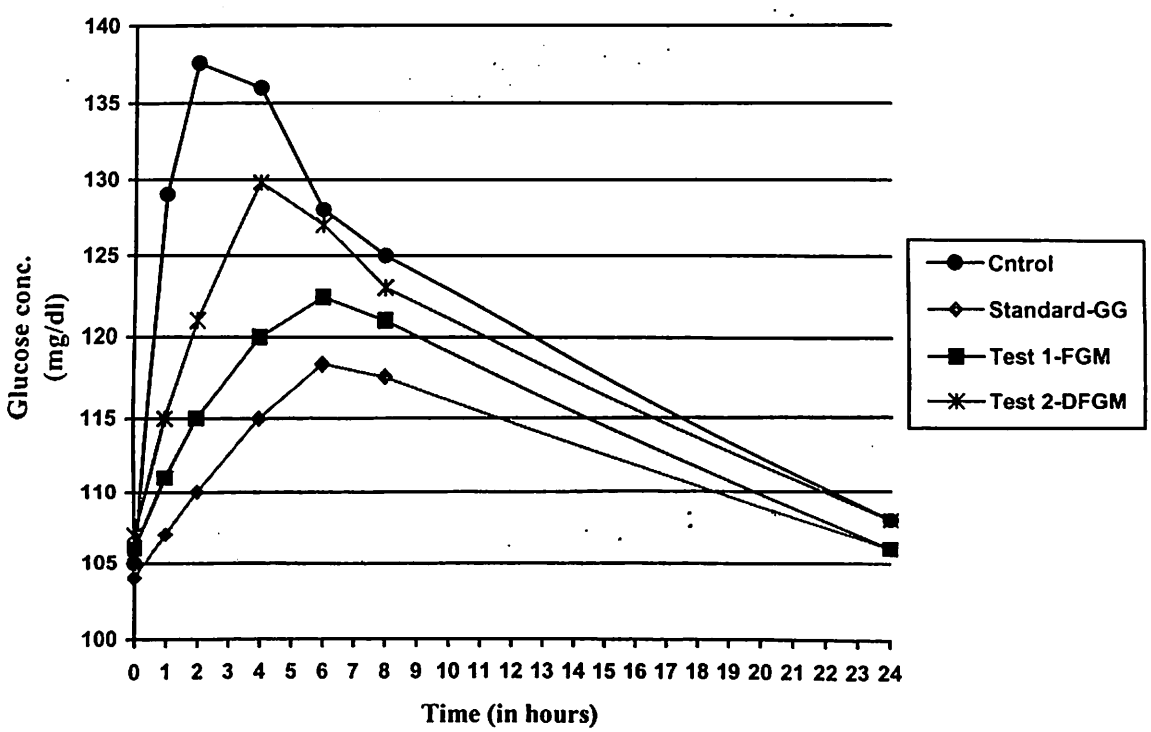
Galactomannans possibly act by forming hydrogen bonds with cholesterol and interfering with its adsorption. The mechanism has to be mechanical since galactomannans are not absorbed through the gut. They also possibly bind with bile salts and interfere with their prime functions of emulsification and solubilization of cholesterol. Interaction with bile salts would also affect enterohepatic cycling of the bile salts resulting in

mobilization of LDL from the lipid pool of the body for synthesis of bile salts to make-up for their loss.

5.4.4: Hypoglycemic activity of FGM.

The following graph was plotted between blood glucose concentration and time to assess the effect of FGM on the absorption of glucose. The hypoglycemic effect of FGM is also compared with that of GG and DFGM.

Graph 9: Effect of different galactomannans on the absorption of glucose



The graph is plotted using mean values of 5 replicate determinations.

Table - 25: Reducing / retarding effect of galactomannans on the absorption of glucose in rabbits

S.No.	Animal group	Duration above PP value (hrs.)	C_{max} (mg/dl)	T_{max} (in hours)	% max rise = $\frac{C_{max}-C_{basal}}{C_{basal}} \times 100$
1	Control	12	137.6 ± 4.78	2	31.0
2	GG	0	118.4 ± 3.76	6	13.5
3	FGM	4.5	122.4 ± 3.67	6	15.4
4	DFGM	9	129.8 ± 1.62	4	20.5

The data indicates mean ± S. D. of 5 replicate determinations.

Table - 26: Statistical significance of the retarding effect of galactomannans on glucose absorption in rabbits

S. no.	Galactomannan	Calculated *t*-value
1.	GG (Standard)	8.56
2.	FGM (Test 1)	7.10
3.	DFGM (Test 2)	4.29
4.	FGM vs. GG	2.09*

Theoretical *t*-value = 3.355 corresponding to p = 0.01 for 8 degrees of freedom. The number of animals in each group i.e. 'n' = 5.

* no significant difference in activities at p= 0.01

Based on the data presented in Graph-9 and Tables -25 & 26 above, it is observed that galactomannans significantly (with 99% confidence at p = 0.01) retard the absorption of glucose. An important inference is that guar gum (GG) and fenugreek galactomannan (FGM) both have a similar effect on the absorption of glucose in rabbits as shown by a lower *t*-value of 2.09 in comparison to the theoretical *t*- value of 3.355.

The retarding effect of FGM is statistically similar to GG, despite the fact that FGM has a lower viscosity than GG. The similar effect of FGM can be attributed to its higher galactose: mannose ratio. However, viscosity is also an important factor in retarding the absorption of glucose. This is evident from the lesser retarding effect on glucose absorption of DFGM, which has considerably a lower viscosity in comparison to either GG or FGM.

The likely mechanism could be the interaction of galactomannans with glucose by way of hydrogen bonding in the gut to reduce / retard the absorption of glucose. All in all, it can be concluded that if FGM is included in food, it could provide a good glycemic control, without the likely occurrence of severe hypoglycemia, in comparison to GG.

5.4.5: Assessment of toxicity potential of DFGM, depolymerized using gamma radiations.

Table - 27: Effect of depolymerized fenugreek galactomannan administration on blood platelets in rabbits

Day	Platelet count*	Calculated *t* - value
1	2.52 ± 0.20	0.99
18	2.64 ± 0.25	

* values in lacs / cubic mm.

The data indicates mean ± S. D. 5 replicate determinations.

Theoretical *t*-value = 3.355 corresponding to p = 0.01 for 8 degrees of freedom. The number of animals in each group i.e. 'n' = 5.

It can be expected that if a material is exposed to gamma radiation, it can have some toxicity. The toxic effect of gamma radiations can be expected to be evident on the haemopoetic system. Hence, the toxicity potential of DFGM was assessed by determining the platelet count in rabbits.

It is observed that there is no significant difference (at p = 0.01) on the platelet levels after DFGM administration. Hence, based on the result of the present study, it can be said that DFGM is safe and effective when the sample has been exposed to a gamma radiation dose of 5 Kgrays and administered for an acute period i.e. for 18 days in a dose of 250mg/kg body weight per day.

It is appropriate to mention here that the advantage of using gamma radiation for depolymerizing would give a sterile product.

5.5: DOSAGE FORM DEVELOPMENT

5.5.1: Physico-mechanical characterization of FGM as part of preformulation study.

The following are the observed values of the physico-mechanical characteristics of fenugreek galactomannan. These values are the average of triplicate readings noted for each characteristic.

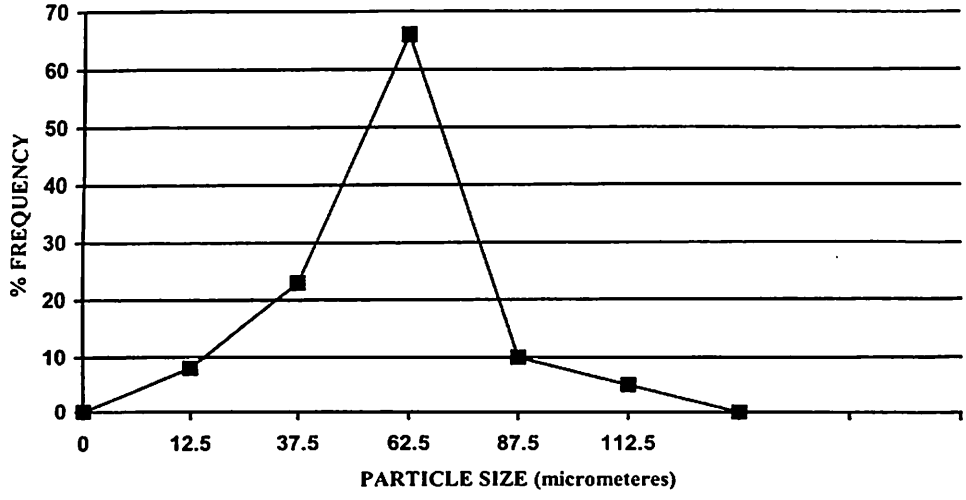
Table - 28: Physico-mechanical characteristics of fenugreek galactomannan powder

S. No.	Characteristic	Value
1.	Particle size (microscopically)	57.74 ± 3 μm
2.	Particle shape (microscopically)	Angular
3.	Particle number/gm.	5,372,044
4.	Particle size distribution	See graph 10
5.	Molecular weight (using Mark Hauwonik's equation)	$4 - 5 \times 10^5$
6.	True density (compressed density) (ρ_t)	1.26 ± 0.1 gm./cc.
7.	Bulk density (ρ_b)	0.63 ± .08 gm/cc.
8.	Tap density (ρ_p)	0.71 ± .08 gm/cc.
9.	Porosity ($1 - \rho_b / \rho_t$)	50%

10.	Carr's index ($1- \rho_b / \rho_p$)	11.3%
11.	Hausener ratio (ρ_p / ρ_b)	1.12
12.	Angle of repose	49.69 ± 4°
13.	Solubility	Gels with 4 parts of water, insoluble in alcohol, acetone.
14.	Swelling power	45 – 50 x
15.	Viscosity	1050 ± 110cps
16.	Stability of solution	Hydrolysis occurs resulting in loss of viscosity considerably over a time interval of 24-72 hours stored at room temperature
17.	Moisture content	Less than 5% on dry wt. basis.
18.	Hygroscopic characteristic (determined by keeping FGM powder in five different constant humidity solutions in a desiccator)	Hygroscopic (see Graph – 11)
19.	Compressibility	Not directly compressible

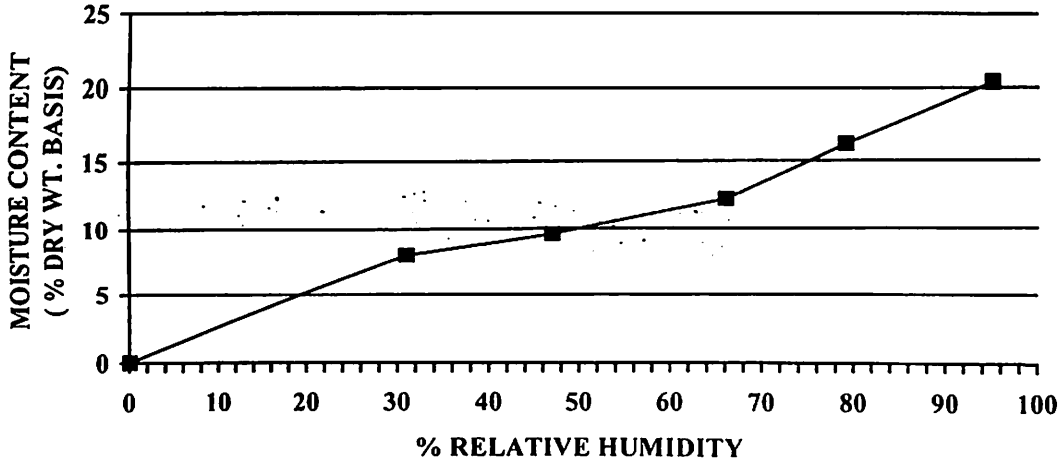
The data indicates mean ± S.D. of triplicate determinations.

Graph 10: Depicting particle size distribution of fenugreek galactomannan powder.



The graph is plotted using mean values of triplicate determinations.

Graph 11: Depicting hygroscopic character of fenugreek galactomannan powder.



The graph is plotted using mean values of triplicate determinations.

The significance of the above physico-mechanical parameters in dosage form development is discussed below:

i) ***Particle size:*** This is one of the factors that affects the dissolution rate, content uniformity, colour, texture, taste and stability of galactomannans. The viscosity of a galactomannan solution is also dependent on the particle size. The medicinal use of galactomannans is due to their ability to increase the digesta viscosity. Therefore, if medicinal activity of two galactomannans is to be compared, than particle size should match closely. Guar which is taken as the standard for the present research work, is commercially available in a size range from sieve 150 # - 300 #. Hence, FGM powder was passed through sieve 200 # and used in the present research work.

ii) ***Particle shape:*** This influences the surface area, flow properties, packing, and compaction properties of the particles. Its influence on surface area is the most important, since according to our hypothesis, a galactomannan interacts (by way of hydrogen bonding) with glucose or cholesterol molecules to inhibit / retard their absorption. Therefore, a large surface area as provided by the angular shaped particles is desirable.

iii) ***Particle number:*** The number of particles present in a dosage form should remain constant in order to maintain dose uniformity. Larger the number of drug particles, smaller the error. This parameter should be maintained constant for the drug and other ingredients present in a dosage form.

iv) ***Particle size distribution:*** It is an important quality control tool in order to obtain powders with reproducible physical, chemical and Pharmacological properties.

The particle size of FGM powder is typically very well distributed around the mean as depicted in Graph - 10 above.

v) ***Molecular weight:*** This is related to the viscosity of a polymer, which in case of galactomannans is very high due to their high molecular weights.

vi) ***Density:*** Bulk density is used to check the uniformity of bulk (raw) powder as part of quality control measure. It helps in selecting the proper size of a container, packing material, mixing apparatus to be employed during production. Normally, the volume of formulation and an excess of 10% of its volume is considered for the selection of container for the mixing process.

vii) ***Porosity:*** Porosity influences the rate of disintegration and dissolution. The higher the porosity, the faster is the rate of dissolution.

viii) ***Flow properties:*** This characteristic is measured by determining angle of repose, Carr's consolidation index, or the Hausner ratio. The last two parameters being simple, fast and reliable are more popular than the former for predicting powder flow characteristics. Flow properties affect content uniformity and dose precision. Flow properties depend on particle shape, size, density and moisture content of bulk powder. The high angle of repose of FGM powder is because of an angular surface and small particle size. However, it has desirably excellent flow characteristics as shown by its low Carr's index and Hausener ratio.

*ix) **Interaction with water:*** The six last mentioned properties namely solubility, swelling index, viscosity, stability of solution, moisture content and hygroscopic characteristic give an idea about the interaction of FGM with water. It is deduced that water can be employed as a solvent for FGM. However, presence of moisture in formulation would result in hydrolysis of FGM resulting in loss of viscosity and making the formulation useless unless certain strong measures are taken to prevent the same. Also if a liquid dosage form is made, the viscosity would be considerable and hence unacceptable to patients. Even for a solid dosage form, the container should be tightly closed so as to warrant any microbial growth, since FGM is a carbohydrate.

From the Graph - 11, it is evident that FGM has a tendency to absorb atmospheric moisture. Adsorption and equilibrium moisture content depend upon the atmospheric humidity, temperature, surface area and mechanism for moisture uptake. As a result of its hygroscopic character, the adsorbed moisture can hydrolyze FGM leading to depolymerization, reduced viscosity and consequently reduced medicinal effect. In addition, microbial contamination would occur and would add to the deterioratory effect of moisture on FGM.

From the above account, it is concluded that a solid dosage form should be developed for FGM powder. Direct compression of FGM is ruled out. However, it cannot be a tablet or capsule, since a dose of 6-16 gm. per day restricts the incorporation of FGM into a tablet or capsule. Therefore, granules of FGM shall be prepared.

Also, atmospheric humidity has to be controlled during handling and processing of FGM. FGM and formulation of FGM should be stored in airtight containers. Extra protection can be achieved by including porous envelopes containing desiccant in container.

5.5.2: Evaluation of a Pharmaceutical preparation of FGM: granules.

The result of evaluation of the prepared FGM granules is given below in the Table - 29.

Table - 29: Evaluation of granules made using different concentrations of alcohol-water mixture.

Alcohol (%)	Angle of Repose (°)	Bulk density (gm/cc)	Moisture content (%)	Colour	Hardness* (kg/m^2)	Particle size (μm)	Hydration in water.
0	12.5±1.4	0.23±0.02	< 5	Off white	5.5 ± 0.5	1426±47	n.i.h.[1]
10	13.5±1.3	0.25±0.02	<5	↑	5.2 ± 0.3	1158±17	"
20	15±1.5	0.3±0.02	<5		5.5 ± 1	753±9	"
30	16±1.5	0.3±0.03	<3		5.0 ± 0	538±16	s.h.[2]
40	**18.7±1.4**	**0.32±0.03**	**<3**		**4.5 ± 0.4**	**417±8.3**	"
50	20.2±1.0	0.32±0.03	<3		4.5 ± 0.4	395±14	"
60	25.8±1.7	0.38±0.03	<3		3.5 ± 0.4	308±43	"
70	30 ±1.2	0.40±0.03	0		1.2 ± 0.2	247±12	i.h.[3]
80	34 ±1.0	0.40±0.03	0		0	210±12	"
90	38 ±1.7	0.40±0.04	0	↓	0	183±8	"
99.5	42 ±1.3	0.42±0.04	0	White	0	176±6	"

*It is the hardness of slug that was compressed out of granules.

[1]no immediate hydration [2]slow hydration [3]immediate hydration

The data indicates mean ± S.D. of triplicate determinations.

From the results in Table – 29 above, it is observed that granules made by using 0 % v/v alcohol (i.e. water) were darker, off-white in colour. The whiteness of granules improved with increasing alcohol concentration from 0 to 99.5%. However, the granulating effect of 99.5% v/v, alcohol was found to be very poor. It was not possible to compress a tablet out of these formed granules. While a tablet of hardness of 5.5±1 kg was compressed, out of the granules made using 0 % alcohol.

The angle of repose was found to be 42° for granules made with 99.5% v/v alcohol. This value is close to the value of angle of repose for the powder indicating small particle size due to insignificant binding. The value for angle of repose varies from 38° to 12.5° for granules made with 90% to 0% v/v alcohol concentration. The desired value of angle of repose is considered to be about 20°. This is seen with granules made with alcohol concentration of 40% and 50% v/v. The slow hydration in water was achieved in granules made with alcohol concentration of 40 % and 50% v/v.

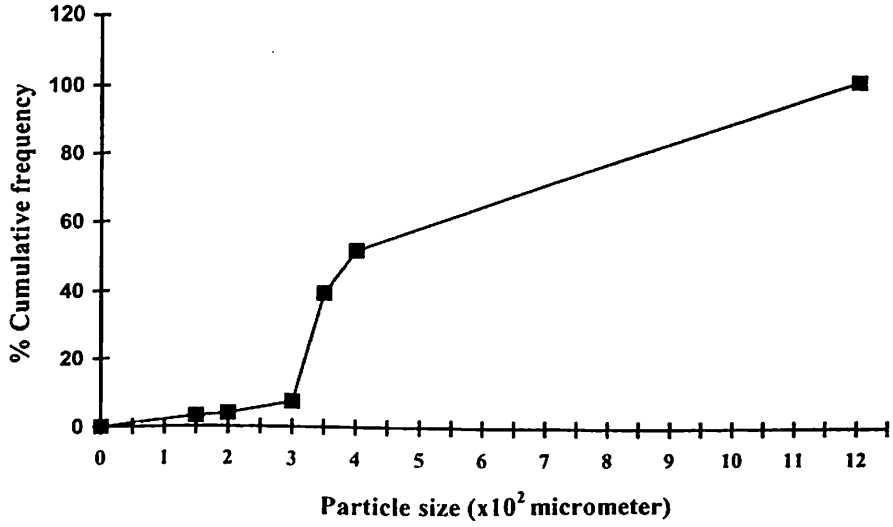
Therefore, a lower alcohol concentration of 40 % v/v was considered to be the best optimum. The results of evaluation for granules made with 40 % v/v alcohol are summarized in Table – 30 on the next page.

Table - 30: Physico- mechanical characteristics of FGM granules made using 40% v/v alcohol as granulating agent

S. No.	Parameter	Value
1.	Particle size (by sieving)	417± 8.3µm (see Graph - 12)
2.	Bulk density	0.32 ± 0.03 gm./cc.
3.	Tap density	0.43 ± 0.04 gm./cc.
4.	True density	1.26 ± 0.1 gm./cc.
5.	Porosity	74.7%
6.	Carr's index	25.6%
7.	Hausner ratio	1.34
8.	Angle of repose	18.7 ± 1.4°
9.	Moisture content	Less than 3%
10.	Compactibility	see Graph - 13

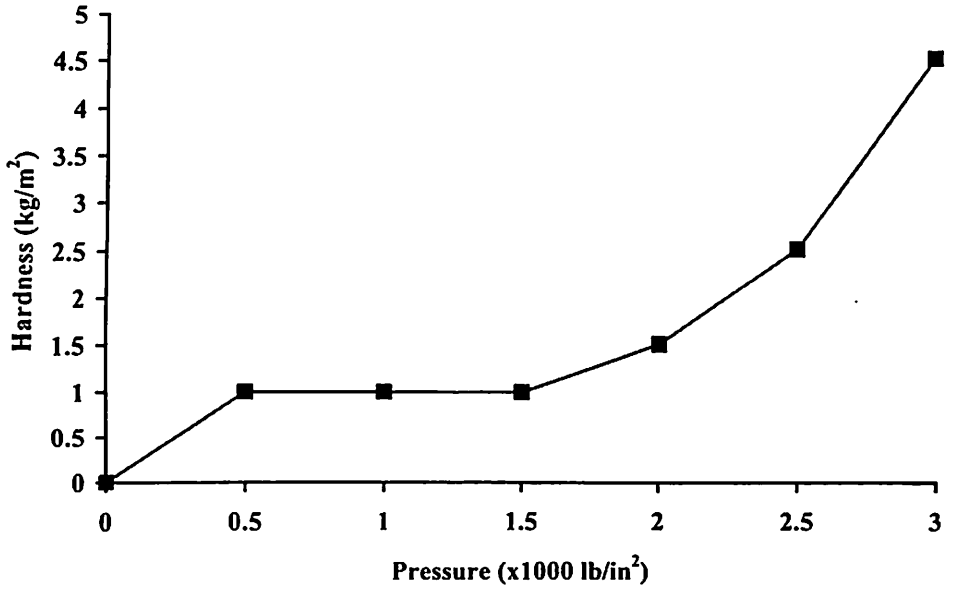
The data indicates mean ± S. D. of triplicate determinations.

Graph 12: Depicting particle size distribution of fenugreek galactomannan granules.



The graph is plotted using mean values of triplicate determinations.

Graph 13: Compactibility of FGM granules.



The graph is plotted using mean values of triplicate determinations.

FGM granules have been developed, taking into account the distinct advantages as offered by this solid dosage form.

i. Less likely chances of formulation and stability difficulties.
ii. Avoidance of problem of stickiness in mouth due to hydration.
iii. Improved patient acceptability.
iv. Better stability due to the reduced surface area.
v. Less likely chances of microbial attack.
vi. Minimized hydrolysis during shelf life.
vii. Uniformity of dosage when packed in sachets.
viii. The granules possess compressibility.

5.5.3: Evaluation of designer FGM food products.

The result of the evaluation carried out on the various designer FGM food products prepared is tabulated below in Table - 31.

Table - 31: Evaluation of fenugreek galactomannan designer foods

S. no.	Formulation	Hardness (kg/m^2)	Density (gm/cc)	Taste	Mouth feel
1	FGM compound fennel flavoured balls	17.8 ± 0.8	1.18 ± 0.2	Agreeable	Acceptable
2	FGM nutritive designer cubes	2.0 ± 0.4	1.88 ± 0.2	"	"
3	FGM chocolate stick	2.5 ± 0.4	1.93 ± 0.2	"	"
4	FGM *gulukhand*	N.A.	1.85 ± 0.2	"	"

The data indicates mean ± S. D. of triplicate determinations.

The hardness of some dry foods is presented in Table - 32 for comparative purpose with the hardness of FGM designer food products.

Table 32: Hardness of some dry fruits / foods

S.No.	Food item	Hardness* (kg/m^2)
1	Cashew nut	2.8 ± 0.23
2	Peanut	3.5 ± 0.41
3	Pista	3.3 ± 0.47
4	Almond	5.8 ± 0.23
5	*Rewari*	15.0 ± 0.41

The data indicates mean ± S. D. of triplicate determinations.

It may seem ironical that in the preparation of designer foods, sugar and ghee have been employed (see Tables – 15 to 18 for formula of designer foods), which are contraindicated for diabetic patients and in patients having high lipid levels. On second thought, a bit of calculation shows that an amount of 2gm jaggery or sugar or ghee contained in single designer food unit works out to 0.029-gm/kg-body weight, considering average adult weight of 70kg. In the in-vivo study performed in rabbits, glucose was used in a dose of 2gm/kg body weight and cholesterol in a dose of 0.5gm/kg body weight was employed to assess the hypoglycemic and antihyperlipidemic activity of FGM. The results showed that FGM has the capacity to reduce / retard the absorption of glucose and cholesterol significantly in comparison to control. Thus additional intake of glucose or cholesterol in a dose of 0.029gm/kg body weight in a unit of designer foods along with FGM is quite insignificant.

On the other hand, if a person does not consume a diet containing 140 gm of glucose (= 2gm/kg x 70kg) or 35gm of cholesterol (0.5 gm/kg x 70kg) or has a very-very low intake of glucose and cholesterol, he may land up with very low blood levels of glucose and cholesterol if FGM is taken in its full recommended dose. Here, in this case, the small amount of glucose or cholesterol may then prevent the occurrence of hypoglycemia or hypolipidemia.

Alternatively, jaggery and sugar may be substituted by non-glucogenetic substances such as sorbitol, mannitol or glycerin or saccharine as sweetening agent. Cholesterol may be substituted by safflower, maize, or olive oil. The use of these oils, because of their high content of glycerides of unsaturated fatty acids, is advocated as a constituent of diets intended to reduce high blood cholesterol levels.

Granules, and not the powder of FGM, have been used in designer food formulations, because granules hydrate slowly in the mouth. It is this immediate hydration of galactomannans in mouth that is responsible for the problem of stickiness of galactomannans in the mouth. Also, the surface area of the granules is lower than the powder. The lower the surface area, the lower would be the moisture attracted from the atmosphere during the shelf life. This is necessary in case of galactomannans, since they hydrolyze in the presence of water, which would lead to reduction in activity. Further, being polysaccharides, microbial contamination in presence of water is likely. Thus granulation is expected to contribute positively to the stability, purity and quality of the formulation.

The designer foods formulated above, except for FGM *gulukhand*, are unit dosage forms, providing accurate dosage. A single unit provides 2 grams of FGM; the daily-recommended dose is 6-16 gm. Thus one may have 1 - 3 units with meals thrice a day. Being solid unit dosage forms, they are stable, easy to carry anywhere and easy to administer.

The administration of medicine in the form of designer foods would have a beneficial psychological effect towards ready acceptability of medicine, which would result in improved patient compliance for dosage schedules.

In the above designer foods, an attempt has been made to have a proper combination of flavour, fragrance and colour, which will contribute to the palatability of the product.

FGM balls were found to be less sticky with improved palatability. Sodium bicarbonate was added in order to make the formulation porous and fluffy and to facilitate release and dispersion of FGM. FGM nutritive designer cubes were formulated using another binder gum (compared with jaggery in formulation 1) and to have a variety of shapes. Chocolate as a

flavour is considered to be the best in masking the bitter taste. The last mentioned formulation, FGM *gulukhand*, was prepared, keeping the geriatric patients in mind and those individuals in general not able to bite hard food.

During the preparation of FGM designer foods, care was taken not to expose FGM to water and heat so as to rule out any possibility of polymer hydrolysis and microbial attack later during shelf life. The flavouring agents were added when the molten mass was removed from the flame so as to avoid loss of aroma. In addition to imparting a pleasant flavour to the preparation, these flavouring agents also act as carminatives, which will counteract the side effect of flatulence accompanied with the administration of galactomannans (food fibres).

The above formulations were tasted and found to have acceptable taste and good mouth feel. It can be said that by incorporating FGM in the above preparations, the problems of stickiness in mouth, unpleasant taste, unpalatability and administration of a large dose have been overcome. All in all a preparation acceptable to individuals has been prepared.

CHAPTER 6
CONCLUSION

6: CONCLUSION

In spite of having only a minor role as energy source, NSPs have certain other important functions as constituents of food.

Fats, proteins, carbohydrates, vitamins and minerals, besides water are the six basic constituents of human food. Among the carbohydrates, sugars namely glucose, fructose, galactose and mannose (all monosaccharides), sucrose, lactose and maltose (disaccharides) and starches (polysaccharide) are metabolized in human system via glucose and serve as energy source.

Food contains many other polysaccharides, which are not metabolized via glucose, but they are either excreted unchanged or are partially fermented by colonic flora into short chain fatty acids (SCFAs), namely, acetate, propionate and butyrate. These SCFAs are good for the colonic health and provide energy to colon, which is in the range of 6-9kJoules/gm i.e. about half that produced by glucose, which 16-17kJoules/gm.

These non-starch polysaccharides (NSPs) are collectively known as food fibres or dietary fibres. Among the NSPs only cellulose is insoluble in water, while all others are soluble. Together these NSPs act as bulking agents or roughage in the food. In spite of having only a minor role as energy source, NSPs have certain other important physiological functions as constituents of food.

Based on this background, this study was taken up to explore the medicinal applications of galactomannans, which occur as reserve food material in seeds of leguminous plants.

The source of galactomannan that was selected for the study was fenugreek seeds. Fenugreek galactomannan (FGM) was extracted from

seeds by soxhelation in hot water. The extracted galactomannan was identified and characterized by acid hydrolysis, qualitative tests for absence of other constituents, chromatography, and FTIR spectrum in comparison with guar gum (GG) as standard. The viscosity and molecular weight of FGM were also determined.

FGM was depolymerized by gamma radiation to obtain depolymerized low viscosity product. The lethal dose of FGM was also determined in rats, up to a maximum daily dose of 2gm/kg body weight. However, neither lethality nor any signs of toxicity were observed.

In-vitro and *in-vivo* experiments were conducted to study two of its medicinal applications as hypoglycemic and antihyperlipidemic agent in rabbits in a daily dose of 250mg/kg body weight. The effect of galactose: mannose (G: M) ratio on the activity of galactomannans was assessed.

From, the *in-vitro* and *in-vivo* experiments conducted, using GG and FGM, it is concluded that these food fibres have the potential to slow down, significantly (at $p = 0.01$ i.e. with 99% confidence), the absorption of sugars in intestines in comparison to the control / untreated group. As for the mechanism, it is believed that they increase the viscosity of digesta in intestine and thus reduce the absorption of food constituents. This effect was indeed observed by performing *in-vitro* experiments involving interaction of sugar with different galactomannans at similar concentrations and similar viscosities. Synergistic increase of viscosity by these galactomannans and other polymeric constituents of food can also contribute to the increased viscosity effect. Alternatively, entrapment of digestive enzymes (amylases and trypsin), as well as, food substrates (starch and proteins), can also slow down starch hydrolysis and formation of absorbable glucose.

Experiments with depolymerized guar gum (DGG) and depolymerized fenugreek galactomannan (DFGM) were also carried out simultaneously. It was found that the depolymerized, low viscosity forms of galactomannans were also effective in lowering glucose absorption, of course not to the same extent as nondepolymerized forms. But from these experiments it is concluded that viscosity alone is not the only factor responsible for reducing sugar absorption.

To confirm the above deduction, galactomannans having different galactose mannose ratios (G: M ratio) were employed in the study. FGM was found to be the most effective in reducing glucose absorption at similar viscosity, followed by guar and than by LBG. These effects are correlated with their G: M ratios; FGM having a G: M ratio of 1: 1, than GG having a ratio of 1: 2, and LBG having a ratio of 1: 4.

The important structural feature of galactose and mannose (galactomannans) is the presence of a pair of *cis-hydroxyl* group on their pyranose ring. This pair of cis -hydroxyl groups can interact more strongly with food constituents via hydrogen bonding.

From the *in-vivo* study, the effect of galactomannans on lowering the absorption of lipids i.e. triglycerides and cholesterol, particularly low-density lipids (LDL) was found to be statistically significant at $p = 0.01$ in comparison to the untreated control group. The last mentioned action has an ultimate effect in controlling blood pressure and reduces the chance of a person having heart attack. Thus for overall improvement of lipid metabolism and reducing the risk of atherosclerosis in persons with hypertension, inclusion of more food fibre in diet is recommended.

It is also appropriate to mention here that the SCFAs produced by the fermentation of food fibres, in general, also have health effects, besides being energy source for colon. These help in maintaining water and

electrolyte balance in the intestines, ensuring smooth bowel motion and in preventing both diarrhea and constipation. They also reduce the frequency of colorectal cancer.

Having determined the medicinal applications of FGM as an agent that significantly reduces / retards the absorption of glucose and cholesterol in rabbit loaded model, the next aim was to put it into a suitable formulation. For the purpose, preformulation studies on the FGM powder were performed. 19 of its physico-mechanical parameters were determined.

The major problems faced in the incorporation of food fibres in formulation include the following:

- Unpalatability –disagreeable taste and unacceptable mouth feel
- Large dose i.e. 6 – 16gm
- Very high viscosity of fine powders – approximately few thousand centipoise of 1% w/v dispersion.

Apart from the above problems, the following points were to be considered while developing a dosage form:

- Prevention of depolymerization by acids, alkali, oxidizing agents, heat, prolonged high shear etc.
- Prevention from microbial contamination
- Flexibility of dosage to suit wide varying dose needs of individuals and
- Relative inefficiency of pre meal or post meal administration of galactomannans.

From the literature surveyed, it was concluded that foods are consumed by humans for two reasons: one for its nutritive value by virtue of its nutritive constituents and two for its medicinal value by virtue of its non-nutritive biologically active ingredients. Foods having nutritive as well as medicinal value are referred to as functional foods. Those plant foods

naturally containing biologically active ingredients in natural amounts are called phytopaharmaceuticals. Foods so designed or especially formulated to contain higher amounts of biologically active compounds than natural amounts are referred to as 'designer foods'. The higher amounts of biologically active compounds can be achieved by one of the two ways: one by genetically engineering the food or two by incorporating it into especially formulated food formulation.

The objectives of incorporating designer foods for disease management are to ameliorate clinical manifestations of the disease, favourably influence the disease process and positively influence the morbidity and mortality of patients. Further, studies with GG have shown that it has little or no side effects when it is intimately mixed with food and administered.

Therefore, designer FGM food products were formulated out of FGM granules and were evaluated. The various designer foods formulated include: FGM compound fennel flavoured balls, FGM designer nutritive cubes, FGM chocolate stick, and FGM *gulukhand*. During preparation of these formulations all the points and precautions as mentioned above, were considered and the desired goals were met.

CHAPTER 7
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