CHAPTER 2

2. Literature Review-Gaps in Existing Research

2.1 PL and its crystal structure

The human PL is encoded by the PNLIP gene located at 10q25.3 region of the chromosome and is secreted from the pancreatic exocrine, along with the other pancreatic enzymes[1,2]. The crystal structure of the human PL is composed of 449 amino acids. The larger protein chain constitutes the PL, while the smaller chain constitutes the pancreatic colipase, a small protein (with 85 amino acids) bound to the C-terminal of PL (**Figure 12)**. The colipase is involved in the activation of the PL. The active site of the human PL comprises of a catalytic triad of the amino acids, Ser152 - Asp176 - His263[3]. This triad is highly restricted and is surrounded by the hydrophobic lid domain which consists of the amino acids Gly76-Lys80 and Leu213-Met217[4].

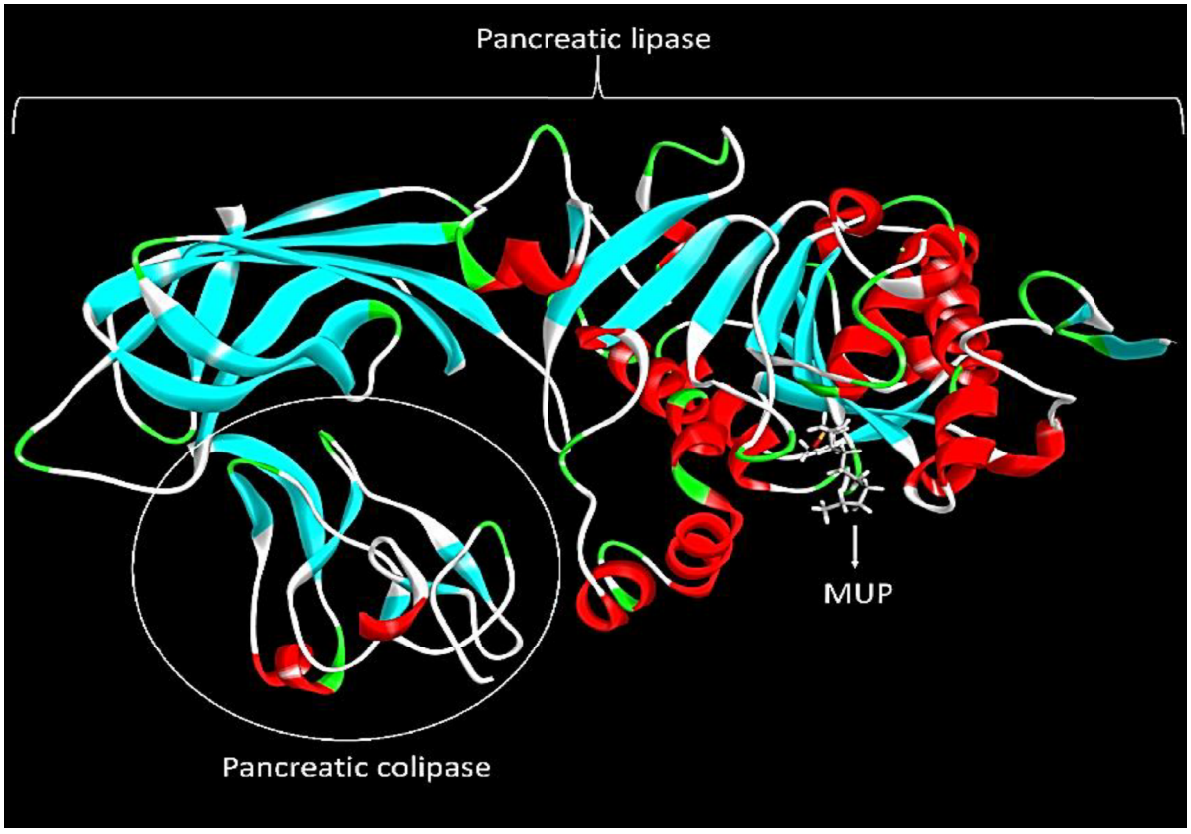

Figure 12: Secondary structure of the human PL-colipase complex, co-crystallised with methoxyundecylphosphinic acid (MUP) at the active site (1LPB)

During the inactivated phase, the triad is inaccessible and enclosed within the lid domain. However, the activation of the PL leads to conformational change in the lid domain, resulting in the opening of the active site. Accordingly, the human PL exists in two conformations; the

closed lid (inactivated) and the open lid (activated) conformations as represented in **Figure 13**. The crystal structures of these two conformations were revealed through X-ray diffractions and are designated by the PDB codes, 1N8S and 1LPB, respectively[3,5].

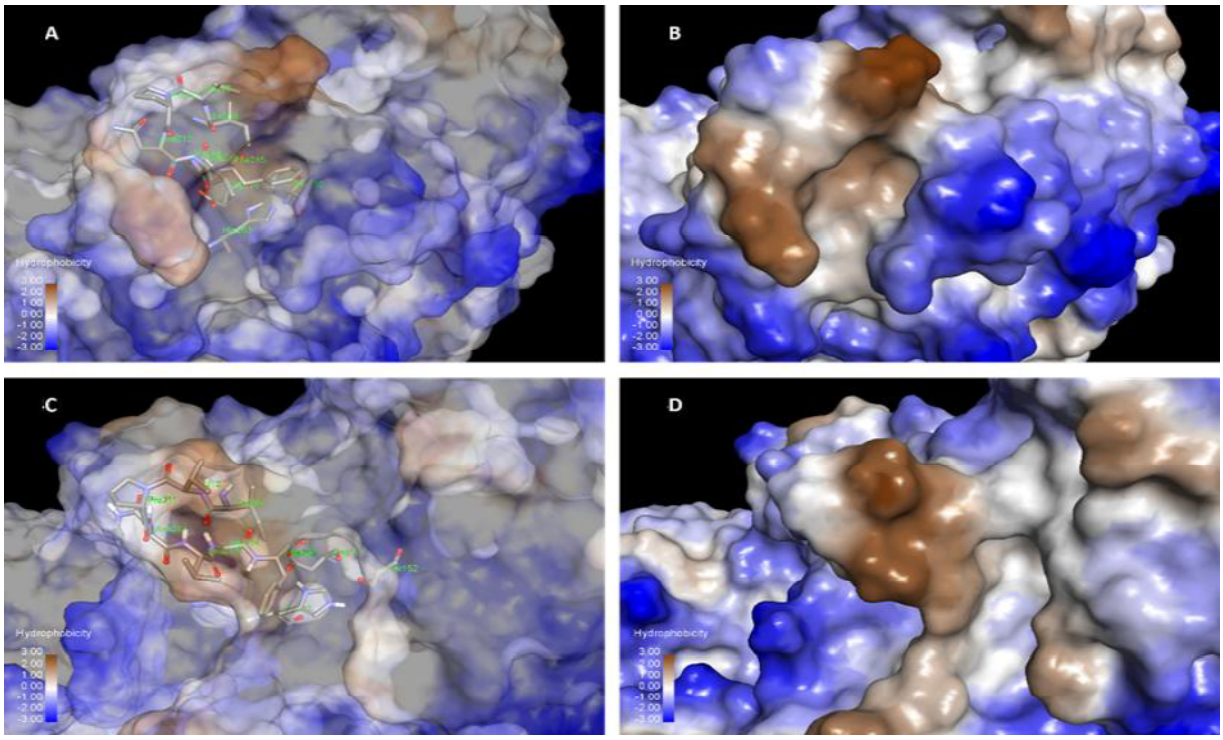

Figure 13: Representation of closed (A, B) and open (C, D) lid forms of human PL. A & C represents transparent hydrophobic surface with active site amino acids; B & D includes only hydrophobic surface clearly representing the closed and open lid conformations

2.1.1 Activation of PL and digestion of lipids

The physiology of lipid digestion involves a series of events, starting with the formation of lipid micelles in the duodenum, in the presence of bile salts and the free fatty acids (released from the gastric lipolysis). This micelle formation allows the interfacial activation of PL[6], facilitating hydrophobic interactions of the long alkyl chains of the lipids with the hydrophobic lid domain of the PL. This phenomenon results in conformational change of PL from the closed lid to the open lid form[7]. The conformational change is further facilitated through a salt bridge formation by Arg256-Asp257 with Tyr267-Lys268[8]. The subsequent steps involve various biochemical reactions between the ester linkage of the TG and the catalytic triad (**Figure 14**), that results in the ester hydrolysis of the triglyceride[9].

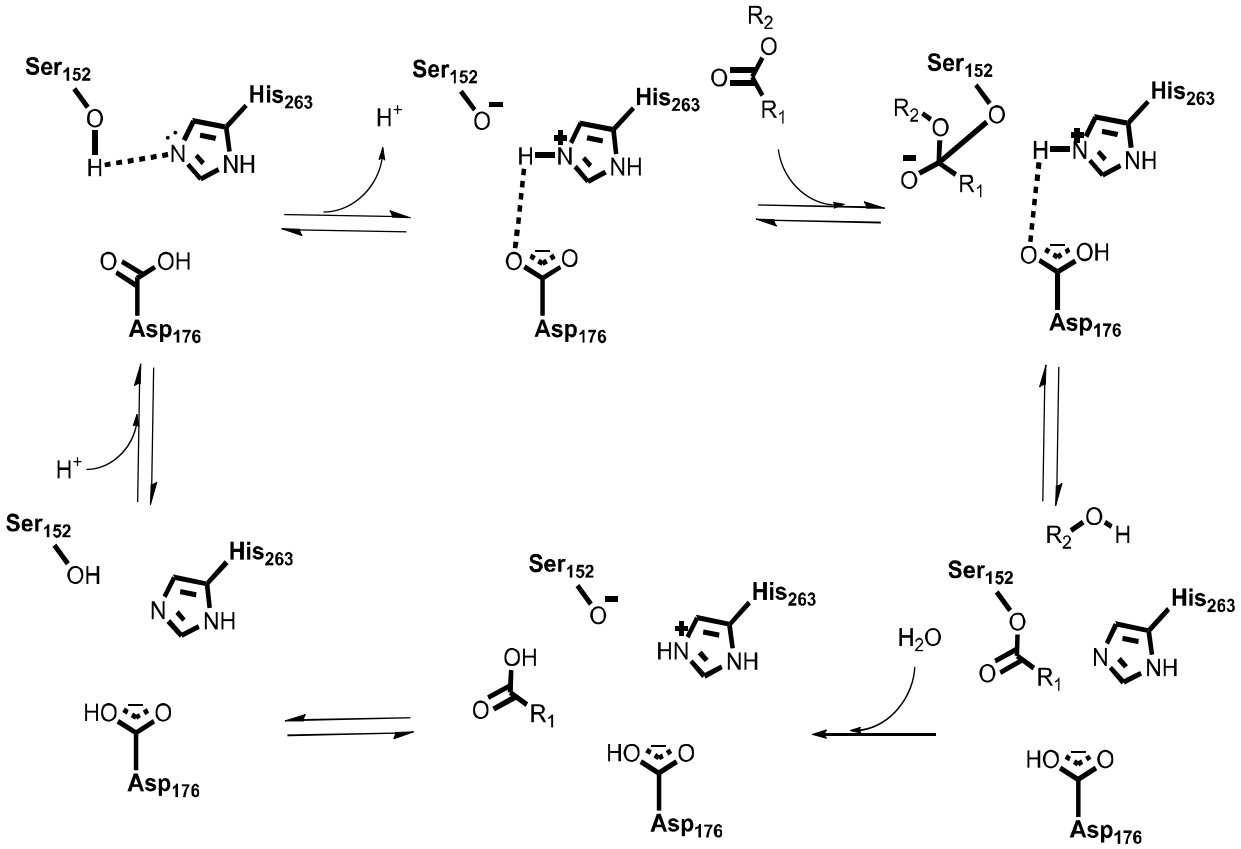

Figure 14: Schematic flow representing series of biochemical reactions at the catalytic triad of PL during ester hydrolysis

2.2 Alternative Systems of Medicine

Alternative medical systems are referred to those health system theories and practice (including traditional chinese medicine, *Ayurved*a, naturopathy, and homeopathy) that are developed separately from conventional medicine. These systems typically use a variety of methods falling under the Complementary and Alternative Medicine (CAM) umbrella that deals with herbal remedies and manipulative practices[10,11]. This type of classification system tries to highlight the differences between therapeutic approaches that may be more useful for the purposes of understanding the various types of CAM therapies[12]. In addition, there are alternative systems of practice that may include a variety of methods such as the use of organic or chemical substances or dietary alterations to promote faster healing. It includes dietary therapy, herbal medicine, and dietary supplements (nutraceuticals); other methods such as aromatherapy, and more controversial methods such as ozone therapy, cell therapy, and many others, including putative cancer therapies. These therapies are classified under two categories

'Western' therapy (homeopathy and naturopathy) and 'non-Western' therapy (*Ayurvedic* medicine and Traditional Chinese Medicine). Literature surveys suggest that scientists now a days are focusing on non-Western therapy for the treatment of obesity and its related diseases.

2.3. Ayurvedic Medicine

Ayurveda is a traditional system of medicine that originated in India more than 3,000 years ago. The term *Ayurveda* is derived from the Sanskrit words *Ayur* (life) and *Veda* (science or knowledge). It states that disease is due to an imbalance or stress in a person's consciousness, *Ayurveda* encourages certain lifestyle interventions and natural therapies to regain a balance between the body, mind, spirit, and the environment. The main classical *Ayurveda* texts is adapted from Sushruta *Samhita* (*Sushruta's Compendium*) and these therapies have varied and evolved over more than two millennia. Ancient *Ayurveda* texts also taught surgical techniques, including rhinoplasty, kidney stone extractions, sutures, and the extraction of foreign objects[13]. The concept of *Ayurveda* is universal interconnectedness between the body's constitution (*Prakriti*) and life forces (*doshas*). There are three main *doshas* (*Kapha*, *Pitta*, and *Vata*). A person is considered healthy if all the three *doshas* are in balance. In the *Ayurvedic* view, the *doshas* are balanced when they are equal to each other, while another view is that each human possesses a unique combination of the *doshas* which define this person's temperament and characteristics. In either case, it says that each person should modulate their behavior or environment to increase or decrease the *doshas* and maintain their natural state.

Ayurveda also explains seven basic tissues (*dhatu*), named as plasma (*rasa*), blood (*rakta*), muscles (*māmsa*), fat (*meda*), bone (*asthi*), marrow (*majja*), and semen (*shukra*). Like the medicine of classical antiquity, *Ayurveda* has historically divided bodily substances into five classical elements known as *panchamahabhuta* in Sanskrit. It includes the earth, water, fire, air, and ether. Combining all *doshas*, *dhatus*, and *panchamahabhuta* together *gunas* (qualities or characteristics) of an individual are considered to be inherent. Almost twenty *gunas* existed that are organized in ten pairs: heavy/light, cold/hot, unctuous/dry, dull/sharp, stable/mobile, soft/hard, non-slimy/slimy, smooth/coarse, minute/gross, and viscous/liquid[13]. In *Ayurveda*, various reports are available that defines obesity. Obesity is defined as "*Medharoga*" that is caused either due to an actual increase in the fat mass or due to malfunctioning of *meda* dhatu. In very few cases it can be an offshoot of other metabolic disorders. According to the type of obesity different approaches have been considered. Thus,

those plants that are described in *Ayurvedic*/traditional texts books played a role in the treatment of obesity. There are various anti-obesity plants that are classified as '*medudhara*" agents. *Berberis aristata* (root, stem and fruit), *Piper longum* (fruits), *Aloe vera* (leaves and roots), *Gymnema sylvestre*(leaves, roots and seeds) are some examples of such plants[14,15].

2.4. Traditional Chinese Medicine (TCM):

Traditional Chinese medicine (TCM) is a 3500-year-old traditional system of medicine of China that includes various forms of herbal medicine, acupuncture, cupping therapy, *guasha*, massage (*tuina*), *bonesetter (die-da*), exercise (*qigong*), and dietary therapy. It is believed that to regain balance using the above techniques, one must achieve the balance between the internal body organs and the external elements of earth, fire, water, wood, and metal[16,17].

TCM defines obesity as phlegm-dampness, a pattern that occurs due to more lipid accumulation for a long time. It is often accompanied with *qi* stagnation and blood stasis that can be characterized as thick fur [18].There are various plants such as *Camellia sinensis*, *Artemesia* sp., *Garcinia cambogia* reported in TCM for anti- obesity effect[19].

2.5. Role of Natural products (NP) in drug discovery

The advent in synthetic organic chemistry led to the systematic studies of plants and micro-organisms that contributed significantly to the development of natural drugs chemistry. The field has been an important source of drugs and drug candidates in the drug discovery process. NP are typically secondary metabolites produced by any living organism in response to external stimuli such as nutritional changes, infection and competition. Moreover, the NPs approach is complementary to the synthetic approach, each providing access to (initially) different lead structures. For e.g., Lovastatin, a secondary metabolite from *Aspergillus niger* that is used as a cholesterol lowering agent. Further, Galegine, derived from *Galega officinalis* was the model for the synthesis of metformin and other bisguanidine-type antidiabetic drugs. Papaverine from *Papaver somniferum* formed the basis for verapamil used in the treatment of hypertension **(Figure 15**)[20].

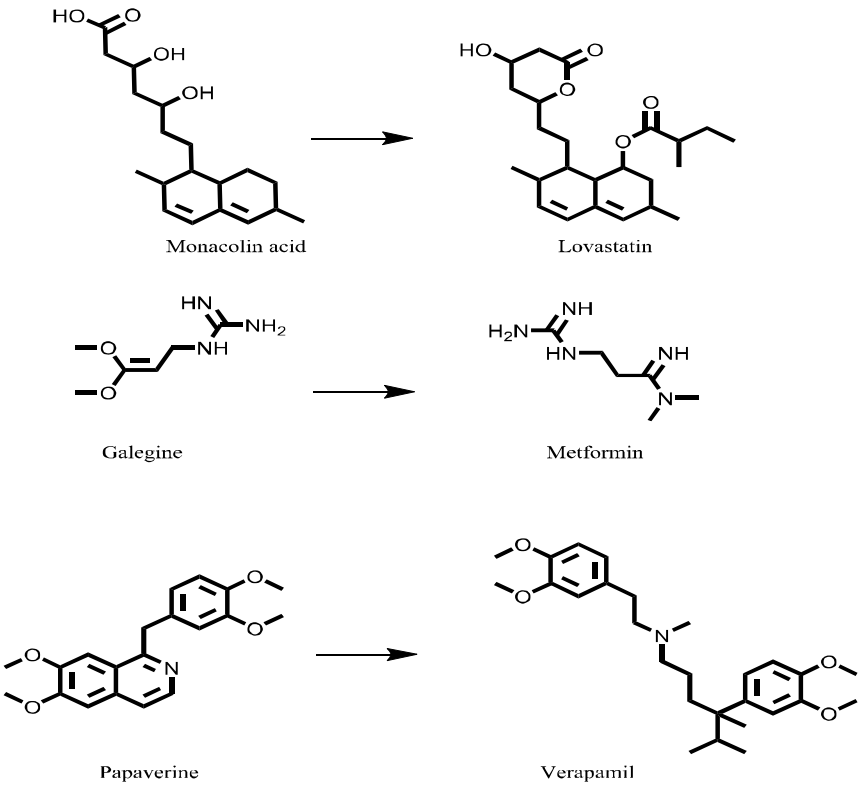

Figure 15: Natural product leads and their synthetic products for treatment of metabolic disorder

2.6. Anti-obesity Natural Products

In search of effective anti-obesity drugs, many phytochemicals from different plant species have been discovered. They exhibit varied mechanisms of action for the treatment of obesity, namely: PL inhibition, DNA methylation, histone modification, inhibition of adipogenesis, etc. Many natural products and bio-active compounds include different classes of compounds such as polyphenols, saponins, terpenes, and alkaloids [21].

Flavonoids constitute the major class of PL inhibitory polyphenols consisting of a C_6-C_3-C_6 structural backbone, in which the two C_6 units (Ring A and Ring B) are phenolic in nature and are linked to a chromone ring. A majority of flavanols are identified from *T. sinensis* (Theaceae), *Bergenia crassifolia* (Saxifragaceae) and *Cassia nomame* (Fabaceae). These included the unsubstituted flavanols, their galloylated esters, procyanidin and various flavanol-based dimers. The unsubstituted flavanols (8-C-ascorbyl (−)-epigallocatechin) and galloylated esters exhibited a potential PL inhibitory activity with IC_{50} values less than 1 μM. These results highlighted the importance of gallate substitution[22]. Procyanidins such as KA-2 also exhibited a potent PL inhibitory activity (IC_{50} = 5.5 μM) [23].

Saponins are another class of natural products, that are nonvolatile, surface-active, structurally diverse and chemically referred to as triterpenes and steroids. Within the area of PL inhibition, saponins contribute to the second most explored class of phytochemicals. Of this, plants such as *Platycodon grandiflorum* (Campanulaceae), *Acanthopanax senticosus* (Araliaceae), *Ilex paraguariensis* (Aquifoliaceae) and *Sapindus rarak* (Sapindaceae) contribute to the major sources of PL inhibitory saponins. For e.g., prosapogenin D isolated from roots of *P. grandiflorum* was found to be the most active with an IC_{50} value of 1.3 mM[24].

Alkaloids have also been explored for a wide range of pharmacological activities, however, only 40 alkaloids have been reported to date for PL inhibition. Further, these alkaloids belonged to various subclasses, *viz.* pyrroles, benzylisoquinolines, carbazoles and bisindoles[25].Various benzylisoquinoline alkaloids from *Nelumbo nucifera* (Nelumbonaceae), *Berberis* sps and *Papaver somniferum* (Papaveraceae) have been studied for their PL inhibitory potential. Few carbazole alkaloids have been reported from the leaves of *Murraya koenigii* (Rutaceae) such as mahanimbine (prenyl substitution) exhibited comparatively greater potential towards PL inhibition. The presence of hydrophobic extension such as prenyl unit (as in mahanimbine), or dimeric extension (as in conophylline) stabilized these molecules through interactions with the lid domain, while the reactive carbonyl group interaction with Ser152 contributed to the potency of these alkaloids against PL[26].

Apart from the isolated NPs, their extracts have also been identified to exhibit PL inhibition. For example, ethanolic extracts of 400 plants were screened using porcine PL assay *in vitro* leading to the identification of several extracts with potential activity against PL. Among the extracts, fruits of *Rubi fructus* and *Cornus officinalis*, cortex bark of *Salicis radicis* and whole grass of *Geranium nepalense* showed the significant PL inhibition when compared to the orlistat (positive control). Acetone extract from flowers, seeds, leaves, pericarps, stems, and rhizomes of *Alpinia zerumbet* also inhibited PL with an IC_{50} of 5 μg/ml [27]. Similarly, many plants from various countries namely Thailand, China, India have also been screened for PL inhibitory potential [28,29].

Due to the huge potential of NPs for management of obesity, more research has been focused on the identification of newer PL inhibitors with less unpleasant adverse effects. So far, many NPs (plant extracts and isolated compounds) have been reported for their PL inhibition property. However, to increase the efficiency with reduced side-effects of these PL inhibitors,

development of patented compositions has also been attempted. These multicomponent compositions can often work in synergism, if explored judiciously. Nevertheless, there are very few scientific studies conducted till date that facilitates proper understanding of the synergism of these herbal drugs and their compositions for PL inhibition. Since 2010, researchers have tried to develop many anti-obesity compositions that act *via* lipase inhibition. In 2010, a United States patent (US7816342B2) disclosed a composition comprising of orlistat (1) and glucomannan namely konjac flour (12) (*refer* **Figure 16**) that reduced the side-effects of orlistat such as oily spotting, fatty/oily stools, fecal urgency, increased defecation, and fecal incontinence. The pharmaceutical composition consisted of about 0.1 - 10% by weight of orlistat and 20 - 75% by weight of glucomannan. "Konjac flour" is a hydrocolloidal polysaccharide obtained from the tubers of species of *Amorphophallus konjac* that is perennial tuber unique to Asia and cultivated in Japan. Konjac flour is a high molecular weight, nonionic glucomannan consisting primarily of mannose and glucose molecules combined in a mole ratio of 1.6:1.0. It is a slightly branched polysaccharide connected by β(1→4) linkages and has an average molecular weight ranging from 200,000 to 2,000,000 daltons[30]. Generally, orlistat and Konjac flour was administered separately *via* oral route within an interval of 2 h for 2 to 3 times a day. Approximately, 0.5 -10 g per day of the flour was administered after consumption of orlistat. Further, the combination was evaluated for *in vitro* and *in vivo* experiments. *In vitro* studies included interaction of konjac with oil and water, that was examined by absorption test. Samples of the compound were brought into contact with either soya oil or simulated intestinal fluid (SIF, phosphate buffer without pancreatin) and incubated for 24 h at 37° C. The SIF and soya oil absorption capacity of konjac was calculated to 4.8 g/g and 0.5 g/g, respectively. SIF showed significant swelling of the polymer, while no swelling occurred in soya oil. The results from the *in vitro* experiments were further supported by studies carried out using an *in vivo* mouse model. The experiment was based on the observation that mice under a high-fat diet (HFD) with orlistat or other lipase inhibitor treatment distribute the excreted free oil over their furs while grooming. Several types and formulations of glucomannan were examined for their ability to reduce or eliminate the production of free oil. Healthy volunteers received orlistat alone or in combination with the test substance during 3 consecutive meals (3-meal test). The modified orlistat formulations used in these 3-meal tests induced 70-80% fat excretion. The most severe side effect was oily spotting. Volunteers treated with glucomannan/orlistat had no decrease in fat excretion (compared to

volunteers treated with orlistat alone) suggesting no interaction of glucomannan with orlistat. No major adverse effect associated with the glucomannan treatment was reported.

In 2013, a World patent WO2013150771A1 disclosed the synergistic activity amongst the three plants namely *Coleus forskohlii* (CF), *Salacia reticulata* (SR), and *Sesamum indicum* (SI). Roots of *C. forskohlii* and *S. reticulata* and seeds of *S. indicum* were used for the combination. *C. forskohlii* contained 1-98% forskolin (13), while *S. indicum* contained 40- 90% sesamin (14) (*refer* **Figure 16**). Water was used as solvent for the extraction of roots of *C. forskohlii* and *S. reticulata,* while ethanol was used for seed of *S. indicum.* The three extracts (CF: SR: SI) were combined in the ratio of 2-30:0.25-3:0.025-0.3. Porcine PL was used to determine the lipase inhibition of the composition. PL was significantly inhibited by when 1 μg/ml sesame extract was added to 10 μg/ml *C. forskohlii* extract. *S. reticulata* also exhibited inhibition against α-glucosidase (50% inhibition of 0.1-200 μg/mg) and α-amylase (50% inhibition of 0.1-100 μg/mg) [31]. The clinical data of 6-week study (15 volunteers) revealed the reduction of visceral fat and blood TG.

A Japanese patent (JP5309292B2), filed in the same year revealed a lipase inhibitory composition consisting of *Polygonum cuspidatum, Phaseolus vulgaris, Caesalpinia pulcherrima, Syzygium samarangense, Ficus microcarpa, Alpinia zerumbet, Heritiera littoralis, Kalanchoe pinnata, Blumea balsamifera, Nandina domestica, Carthamus tinctorius, Cassia glauca, Terminalia catappa,* and *Pinus luchuensis*[32]. These extracts were prepared using 50% ethanol at a temperature of 70-90°C. The extraction was performed under the conditions of 8MPa and extraction time of 10 minutes (min). The extracts were filtered using 0.45μm membrane filters. PL inhibition activity was performed using kit manufactured by Diannipon Pharmaceutical Co. The combinations exhibited PL inhibition in between 48.63 – 98.18%.

In 2013 a United States patent (US8420131B2), filed by CA Smith mentioned about pharmaceutical compositions of extracts of *Rhodiola rosea,* banaba leaf in combination with apple polyphenols,*Gardenia fructus,* and roots of *S. reticulata,* for α-glucosidase and PL inhibitory activity [33]. Apple extracts contained polyphenols such as chlorogenic acid (15), catechin (16), epicatechin (17), procyanidins, phlorodzin (18), rutin (19) etc. (*refer* **Figure 16**), that inhibited more than 70 % of PL enzyme activity. Main components of *G. fructus* were geniposide (20) and crocin (21). Studies demonstrated that crocin and its metabolite crocetin (22) (*refer* **Figure 16**), potently inhibited the effect of PL. In mice fed with HFD, crocetin and crocin

significantly reduced fat deposits, and their potency at a dose of 50 mg/kg, comparable with that of orlistat at a dose of 10 mg/kg. The peripheral energy blockers composition included 250 mg of *G. fructus*, 150 mg of apple polyphenols, and 50 mg of *S. reticulata* that slowed the gastrointestinal transit time and releases of various satiety hormones including CCK, ultimately resulting in reduced appetite. Further trial was conducted, and two groups were considered. Group I was initially administered a placebo and there after a supplement of the three products, while Group II was administered a supplement of the three products for the duration of the trial. The observations indicated that the supplements administered to the subjects caused reduction in the weight, blood cholesterol and blood glucose.

In 2016, a United States patent (US9504725B2) was filed that included obesity-curative and obesity-preventive composition using butanol and ethyl acetate fraction of rhizomes of *P. cuspidatum*. The extract effectively inhibited the activity of PL [34]. The composition provided a functional food for preventing and relieving obesity. The composition contains 0.1 - 99.9 weight % of the fraction of the *P. cuspidatum* extract as active ingredient along with pharmaceutically acceptable carriers, excipients, or diluents such as starch, calcium carbonate, sucrose or lactose, gelatin, etc. One of the major components of the butanol extract of *P. cuspidatum* was resveratrol (23) (*refer* **Figure 16**). All the fractions and resveratrol were tested for PL inhibitory activity using porcine PL and *p*-nitro phenylbutyrate (*p*-NPB) as a substrate. The butanol extract and resveratrol exhibited IC_{50} of 15.8 ± 2.6 µg/ml and 124 ± 6.7 µg/ml, respectively. Further, 4-week-old male Wistar rats were fed with 3 ml of lipid emulsions (3 ml corn oil, 50 mg cholic acid, 3 ml saline and 1 g cholestryloleate). The dose of *P. cuspidatum* butanol extract (POCU-1b) considered was 100 mg/kg and 250 mg/kg. In case of the groups administered 250 mg/kg, the inhibitory effect on fat absorption of the butanol fraction and resveratrol were found to be 140.1±32.4 mg/dl and 228.5 ± 8.4 mg/dl, respectively. Further, 3-week-old male C57BL/6 mice were used and fed a HFD (AIN76A and D12451 diet) to induce obesity. AIN76A contained 45 Kcal % HFD, while D12451 was comprised of fat (45%), carbohydrates (35%) and protein (20%). POCU-1b (1%) when administered was able to decrease the amounts of TC (29%), TG (22%), LDL (32%), and FFA acids (32%) in a significant manner.

In 2017, a Chinese patent (CN106962933A) was filed that stated a anti-obesity composition consisting of extracts of perfume *Flos Nelumbinis* and *Nelumbo nucifera* (leaf), *Camellia sinensis* (leaf), *Cassia obtusifolia* (seed) and *Vitis vinifera* (seed). It showed an anti-obesity effect

by inhibiting PL and thus helped in weight reduction, regulation of lipid and intestinal flora [35]. Eight combinations were prepared, and their PL inhibition activity was evaluated.

Combination 1: Perfume *Flos Nelumbinis* extract alone suppressed PL activity (IC_{50} = 4.02±0.36 mg/ml), pre-preventing obesity and improved of intestines flora effect

Combination 2: Composite formula containing perfume lotus flower extract (65%), green-tea extract (35%) was found to suppress PL activity (IC_{50} = 4.26 ± 0.24mg/ml) and prevent obesity

Combination 3: Composite formula containing perfume lotus flower extract (65%) and lotus leaf extract (35%) was found to suppress PL activity (IC_{50} = 4.67±0.41 mg/ml).

Combination 4: Composite formula containing perfume lotus flower extract (50%) and grape seed extract (25%) was found to suppress PL activity (IC_{50} = 5.03±0.35 mg/ml).

Combination 5: Composite formula containing perfume lotus flower extract (50%), cassia seed extract (15%) and grape seed extract (35%) was found to suppress PL activity (IC_{50} = 5.32±0.39 mg/ml).

Combination 6: Composite formula containing perfume lotus flower extract (50%), green-tea extract (35%) and lotus leaf extract (15%) was found to suppress PL activity (IC_{50} = 4.14±0.31mg/ml).

Combination 7: Composite formula containing perfume lotus flower extract (35%), lotus leafextract (25%), green-tea extract (25%) and grape seed extract (15%) was found to suppress PL activity (IC_{50} = 4.18±0.29 mg/ml).

Combination 8: Composite formula containing perfume *Flos Nelumbinis* extract (30%), Lotus leaf extract (25%), green-tea extract (15%), and Cassia seed extract (15%) was found to suppress PL activity (IC_{50} = 3.81±0.31 mg/ml).

Further, *in vivo* studies were performed using C57BL/6 male mice (8-9 weeks old). Normal group mouse was fed with 10 kcal% low fat food, remaining 4 groups of mice (model control, positive control, implementation group 1, experimental group 2) were feed with 60 kcal% high lipid food. The combinations efficiently controlled mouse body weight, and significantly reduced epididymis peripheral adipose and perinephric fat. It also significantly reduced the TC, TG, LDL and improved HDL level.

In 2018, a Korean patent (KR20180039418A) filed by Daegu Haany University Industry-Academic Cooperation Foundation disclosed an anti-obesity composition using a puckery persimmon (*Diospyros kaki)* and a mandarin peel (*Citrus unshio)* that exhibited reduction of fat

absorption, deduction of TC, TG, LDL and reduction in visceral fat by decreasing the activity of PL[36]. Extracts of both the plants were decomposed using viscoenzyme. The enzyme was further denatured by heating at 90°C for 30 min. After filtration using diatomaceous earth, the extracts were concentrated, and dextrin was added followed by sterilization at 95°C for 30 min. The sterilized extracts (named PCM) were lyophilized that when tested exhibited an increase in the PL inhibitory effect as compared to original extracts. Four-week-old male ICR mice (n = 32) were separated into any four groups (HDF control, orlistat group, two PCM treated groups (50 and 200 mg/kg/ day). The normal group was fed normal diet and the remaining group was fed 60% HDF until the end of the study. PCM lowered the TG, TC and LDL levels in the *in-vivo* study.

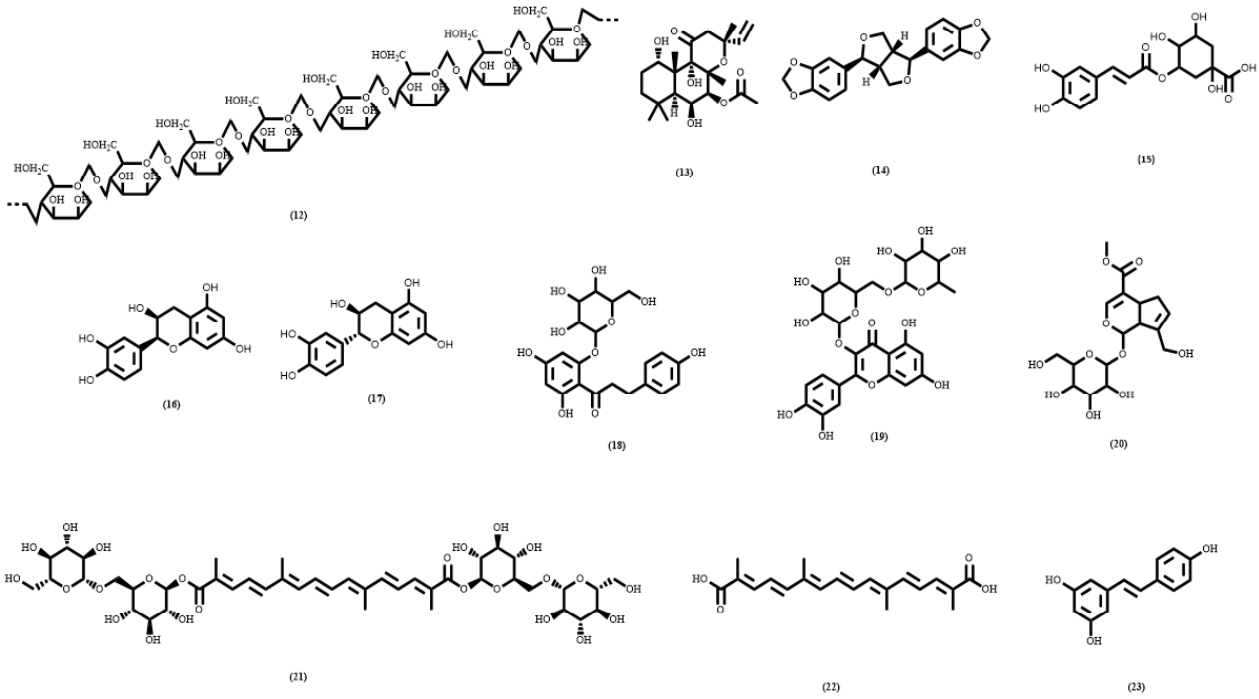

Figure 16: NPs in the patented compositions that target enzymes for anti-obesity effect

2.7. Gaps in existing research

Obesity is one of the major global issue due to which the entire world is being affected. It is a metabolic syndrome that gives rise to various serious co-morbidities such as IR, diabetes mellitus, hypertension, dyslipidemia, and atherosclerosis. As a result, it is referred to as 'slow poison'. There are various synthetic as well as NPs derived anti-obesity agents available in the

market that act *via* various mechanisms such as enzyme inhibition (orlistat, cetlistat) or suppressing appetite (fenfluramine) or suppressing the absorption of nor-epinephrine and serotonin (sibutramine), etc. Despite their potent anti-obesity action, many of these drugs have been withdrawn from the market by FDA due to their toxicities. These events highlight the urgent need for discovery and development of newer anti-obesity therapeutics.

Many plants and their NPs have been studied for PL inhibition. To increase the potency and reduce the adverse effects, researchers are focusing on development of compositions. There are various literature reports that have described the development of natural extracts/NPs based PL inhibitory anti-obesity compositions. Nevertheless, there is a lack of proper systematic approach for the development of compositions. So far the emphasis has been on trial-and-error method for creating these compositions [37]. Therefore, a reliable assessment of such compositions needs to be done using scientific and validated methods such as combination index (CI), isobologram studies, etc.

Moreover, the NPs present in the extracts or the compositions are prone to variations due to climatic conditions, harvesting process, extraction methods etc. Therefore, it becomes utmost important for developing proper standardization protocols using modern analytical techniques such as LC-MS/MS, HPLC, HPTLC-MS *etc* for maintaining a balance between efficacy and quality parameters of such compositions.

Another important parameter that has not been studied during development of reported anti-obesity compositions is the effect of pH on stability of NPs upon oral administration of extracts. There are numerous NPs in extracts that lacks stability in the gastric pH (1.2) and this effect may further potentiate or antagonize the desired *in vivo* results. Therefore, understanding of proper stability studies of the developed composition and especially the probable bio-active NPs in simulated fluids (e.g., simulated gastric juice) is required before performing *in vivo* experiments. There are very few reports that provide comprehensive information about the functional interactions of the bio-active markers with PL using various mechanistic studies such as enzyme kinetics, fluorescence and *in silico* studies with respect to the patented compositions/NPs.

Thus, development of standardized herbal products such as nutraceuticals and phytopharmaceuticals requires investigation of the above gaps in research. Hence, the present study focused on the development of anti-obesity compositions using potential PL inhibitory

extracts along with their assessment of synergistic interactions using combination index and isobologram methods. *In vivo* studies of the best compositions (based on IC_{50}) were performed using HFD induced obesity model in Swiss albino mice. The study also focused on the chromatographic analysis of bio-active markers present in these compositions followed by simulated gastric fluid studies for determination of their stability. Further various mechanistic studies were performed to understand the functional interactions of the bio-active markers with PL using enzyme kinetics, fluorescence and *in silico* experiments.

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