Development of novel protein tyrosine phosphatase - 1B (PTP1B) inhibitors to enhance insulin cell signaling

Origin of the proposal

Diabetes mellitus is a primary cause for large number of pathophysiologic co-morbidities including obesity, hypertension, hyperlipidemia, atherosclerosis. Obesity is intimately associated with diabetes, which is not only a major but also a costly healthcare problem for the individual and to the society. The World Health Organization (WHO) estimates that approximately 135 million people worldwide currently have diabetes and in that type II diabetes accounts for around 90% and it will rise up to two fold by the year 2025. To manage diabetes and diabetes linked abnormalities wide variety of therapeutic agents including combination therapy are now being used. Targeting blood glucose level without disturbing lipid metabolism becomes more difficult to maintain with monotherapy. Even worse the current anti-diabetic therapy promotes weight gain i.e. obesity, while reducing blood glucose (eg. Thiazolidinediones and Sulphonylureas). Botanicals are thought of offering strong potential with minimal side affects particularly against metabolic syndromes as most of their efficacies are from a mixture of active molecules acting at the same time. By understanding the potential of medicinal plants and the expanding diabetic population it is highly desirable to find anti diabetic agents that regulate glucose metabolism without disturbing lipid metabolism with complete mechanism of action, safety and quality profile.

Multiple imperfections in the pathophysiology of diabetes and obesity are mostly imprecisely understood, and therefore targeting a single target (gene/ protein) by a single drug is not adequate to the reversal of all or majority of aspects of the disease. Recently, there has been an increasing interest on several nature based medicinal ingredients, to prevent and treat metabolic disorders such as obesity through multi-targeting mechanism. Ayurveda and other Indian literature mention several thousands of medicinal plants for diabetes management but their molecular mechanism of action and role in causing/treating obesity has not been carried out since now. In view of literatures and the facts
the current need is a drug that controls glucose metabolism but without disturbing lipid metabolism and it can be achieved by screening anti diabetic medicinal plants for obesity.

**Objective of the study**

The main impact of the proposed study is aimed to illustrate the effect of anti-diabetic herbals on lipid metabolism and their molecular mechanism in modulating lipid metabolism.

**Objective 1**

Screening of selected medicinal plants for
- PTP1B enzyme inhibition
- Lipase inhibition (Pancreatic, Hormone sensitive and Lipoprotein lipases)
- Anti oxidant potential

**Objective 2**

Isolation of active principle by bioassay guided fractionation through column chromatography purification. Characterisation of the isolated molecule by UV, FT-IR, Mass spectroscopy, $^1$H-NMR, $^{13}$C-NMR and CHN analysis.

**Objective 3**

Evaluation of the effect of crude extracts and lead molecule(s) on various targets involved in lipid metabolism (PPAR$\gamma$, SREBP1c, C/EBP$\alpha$, LPL, TNF$\alpha$, NF$\kappa$B, antioxidant enzymes) and establish a mechanism of action for the same.

**Methodology**

1) Extraction of plant materials using different solvents from non-polar to polar gradient (Hexane, Ethyl acetate, Methanol and Water) and TLC fingerprinting for the same.

2) Screening of crude extracts using cell based (3T3-L1 adipocytes) *in vitro* assays like
   - Adipogenesis differentiation inhibition assay – Adipored staining
   - PTP1B enzyme inhibition assay
   - Pancreatic lipase inhibition assay
• Hormone sensitive lipase inhibition assay
• Lipoprotein lipase assay

3) Bioassay guided fractionation of active extracts for the lead molecule(s) with dual activity.

4) Structure elucidation of lead molecule(s) by UV, FT-IR, Mass spectroscopy, \(^1\)H-NMR, \(^{13}\)C-NMR and CHN analysis.

5) Screening of lead molecules using cell based *in vitro* assays and establishment of mechanism of action by targeting the specific markers involved in obesity
   Validation by:
   a) RT-PCR – mRNA level
   b) Western blotting – protein level

6) *In vivo* evaluation of selected plant extracts and lead molecules in an appropriate animal model

**Definition of the problem**

Metabolic disorders, including diabetes and obesity provide tremendous challenge to current drug discoverers. The precise causes of these diseases are not known, but they, in general, can be considered as ‘gene expression diseases’ in which a battery of gene program is aberrantly activated or blocked. The rapid improvement in molecular biology over the last decade has led to the identification of the bio markers of many diseases, thereby providing plenty of novel targets for therapeutic intervention. Polygenic diseases like diabetes and obesity affects various tissues or cell types and is not completely controlled by the drugs devised to act on single molecular target.

Increasing fat burning, appetite control and controlling fat absorption are the recent strategies applied to manage obesity. The drugs like serotonin reuptake inhibitors (Sibutramine), HMG CoA inhibitors (Statins), cannabinoid receptor agonists (Rimonabant) and Pancreastic lipase inhibitor (Orlistat) are the current choice of medicine for treating obesity but the usage also limited due to the neuronal side effects, myopathy and serious liver problems. In parallel, the association of oxidative stress,
lipase enzymes and PTP1B enzyme are also well reported for diabetes and obesity related disorders. Hence the targets were selected for evaluating the role of anti diabetic medicinal plants in obesity.

Free radicals are the main cause of many deleterious reactions in the pathogenesis of autoimmune diseases. It has been reported that genes involved in redox modification is a key mechanism in mediating pathological process such as diabetes, atherosclerosis, aging, etc. and stressed the importance of antioxidant therapy with existing monotherapies. Nowadays, plant-based products are widely commercialised as a nutrition supplement by rationalizing its antioxidant potential. Tannins, polyphenols and flavonoids are the main antioxidants present in most of the plants and their significance in treating diabetes is well documented. Since, most of the plants exhibit anti oxidant activity it can be used as one of the target in multi targeting approach of metabolic disorders.

Lipases are fat-digesting enzymes that play major role in the management of obesity. The major human lipases include the gastric, pancreatic and bile-salt-stimulated lipase that aid in the digestion and assimilation of dietary fats, and the hepatic, lipoprotein and endothelial lipase that function in the metabolism of lipoproteins. The triacylglycerol and phospholipase activities of these enzymes enable these varied functions. In fact, there have been various kinds of health foods like grape seed, pomegranate, etc., introduced in commercial market, which contain dietary fibers, lipase inhibitors allegedly being effective in treating obesity.

Current researchers have demonstrated the possible association of PTP1B in diabetes and obesity. Insulin mediated glucose uptake is negatively regulated by Protein Tyrosine Phosphatases (PTPs). Among several PTPs, PTP1B has been implicated to be a key modulator of insulin signal transduction by acting at downstream signaling components, such as IRS1 and PI3K. Over expression of PTP1B protein has been reported in insulin-resistant states associated with obesity. Since, PTP1B plays pivotal role in insulin resistance and adipogenesis, it can be used as a target for controlling abnormalities in
lipid metabolism. Tannin present in *Cichorium intybus* are reported for PTP1B inhibition (Muthusamy *et al.*, 2008 and 2010)

Natural products from medicinal plants provide crucial, unmatched chemical diversity to modern drug discovery programs. But the usages of herbals are limited to neutraceutical level because of the defects like complex nature, herbal – drug interaction and quality assurance. The rapid growth of robust biotechnological and analytical techniques gives a ray of hope in ethnobotanical approach. Accordingly, for overcoming the shortcomings of the current therapy described above a detailed study on herbals and herbal products are needed. We anticipate using our knowledge in traditional medicine and to develop a potent and safer plant based product/ molecule.

**Importance of the proposed project in the context of current status**

In the past 10 years there has been unprecedented growth in the arena of pharmaceutical biotechnology field that yields increasing attention on several plant-based medicinal ingredients for preventing and treating metabolic disorders such as diabetes and obesity. Developing new drugs from medicinal plants has been one of the oldest routes that are currently in use. Natural product based drug discovery from past to current status had resulted in the discovery of several successful drugs from Digoxin to Prostratin (a drug candidate for treatment of HIV). The approach however has been changed due to the advancements in biological sciences. Using *in vitro* tools to identify drug it is now possible to pull out molecules based on specificity to cellular targets. This has helped to hasten the process of drug discovery. Integration of ancient traditional knowledge in to the modern biotechnological sciences – a modern initiative may help to understand the profound mechanism of herbals and phytochemicals.

**Expected outcome and prospects of this work:**

The causes of diabetes and obesity have proved to be multifactorial and inconsistent. Although obesity has a strong genetic component, environmental factors, such as oxidative stress, sedentariness and excessive food intake are commonly considered to be
the underlying cause of the increase in obesity. So it becomes necessary to regularise the food habits for maintaining ideal BMI. Since the food valuable plants are also having medicinal value and non toxic, it will be rational to evaluate the role of them on managing obesity. So that it can be applied as a diet or neutraceutical food to Defence Organisations.

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To summarise, the social impact of the study on the herbal therapy

- may help in understanding the complex nature of medicinal plants and its significance in obesity treatment – so that we can ensure the biologically and chemically standardized products to the society.

The purified active compound (s) isolated from selected medicinal plants

- may be used as a chemical/ biological marker for the standardisation of herbal extract
- may be a potent and novel lead that strengthens the intellectual value of our Nation
- may be used as a drug candidate for obesity treatment