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Preparation and Standardisation of Poly-Nutraceuticals Formulation from Seeds and Comparison of its *In Vivo* Anti-Obesity Activity with the Marketed Product

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Research Article

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ABSTRACT

Background: Obesity-associated is rapidly increasing throughout the world. It is generally recognized that natural products with a long history of safety can modulate obesity.

Aim: To investigate the development of obesity in response to High fat cafeteria diet and to estimate the effect of prepared poly-nutraceutical formulation from seeds on bodyweight, anthropometric parameters, organ and fat pad weights, serum lipid profiles, hepatic assay in wistar rats.

Method: Seeds were collected & authenticated from authorised centres. Preliminary Pharmacognostical evaluation parameters of seeds and their powders like LOD, microscopy of seeds powder were carried out. Evaluation of their standardisation parameters like Bulk density, tapped density, Carr's (compressibility)index, Hauser's ratio, angle of repose were carried out which were finally used in preparation of nutraceutical powder formulation.

Experimental study was carried out using adult Female Wistar rats weighing between 150-170 g. Obesity was induced by feeding with an high fat cafeteria diet for over a period of 40 days. Animals were divided into 5 groups, 6 rats in each group: Group 01: Negative Control (fed with normal pellets chows), Group 02: Positive control group (fed with normal pellets 50%+HFCD 50%), Group 03: Pellet chew, Cafeteria diet(HFCD)+High dose of prepared formulation (400 mg), Group 04: Pellet chew, HFCD+Low dose of prepared formulation (200 mg), Group 05: Normal chew pellets+HFCD +marketed formulation (300 mg) as Standard, The prepared poly-nutraceutical formulation powder and marketed formulation was

administered orally in 3% acacia suspension with the aid of oral gavage every day for about 6 weeks (40 days), 400 mg of diet is high dose, 200 mg of diet is low dose of prepared formulation and 300 mg of marketed formulation powder as standard. Pre and Post analysis of Body weight, anthropometric parameters, organ and fat pad weights, lipid profile, serum biochemical analysis liver homogenation (LPO assays), liver histopathology of experimental animals were carried out.

Results: Data showed that feeding with cafeteria diet significantly increased final body weight, fat pad weights, glucose, Triglycerides (TG), LDL, total cholesterol levels in serum, concentration compared with controls, meanwhile treatment with prepared poly-nutraceutical formulation significantly normalized the body weight, anthropometric parameters, glucose and lipid profile. Serum ALT, AST were significantly higher in a Cafeteria diet group compared with normal controls; and administration of prepared poly-nutraceutical formulation significantly lessened the effect of the Cafeteria diet.

Anthropometric parameters were found to be increased in a Cafeteria diet group in comparison with the control group. The treatment with prepared poly-nutraceutical formulation normalized the condition.

Conclusion: Cafeteria diet induced obesity associated with a disturbed lipid profile (TGs, TC, LDL, HDL), liver (AST, ALT), this may have implications for the progress of obesity related problems. Treatment with prepared poly-nutraceutical formulation of flax, cucumber, chia, black seeds improved obesity and its associated metabolic problems in different degrees. Moreover poly-nutraceutical formulation of flax, cucumber, chia, black seeds might be a safe nutraceutical formulation on the organs whose functions were examined, as a way to surmount the obesity state; and it has a distinct anti-obesity effect.

INTRODUCTION

The Obesity is a complex condition in which increase in body weight beyond skeletal and physical standards as the result of an excessive accumulation of fat in body. Obesity associated with increased risk scope numerous Diseases, including type 2 diabetes, hypertension, cardiovascular disease, respiratory problem, gall bladder disease, osteoarthritis and certain cancer [1]. Obesity also has serious social and psychological consequences and affect all ages. Obesity is defined as Body Mass Index (BMI) of 30 Kg per m^2. Obesity is abnormal or excessive fat accumulation that presents risk to health. The Body Mass Index (BMI) of a person is 25-30 kg/m^2 indicates overweight and above 30 kg/m^2 or more where a person BMI is defined as their weight n Kg divided by the square of their in meter square. Excess body weight and elevated body mass index are strongly correlated with high bone mineral density [2].

Globally, according to the WHO, there are more than one billion overweight adults, of which at least 300 million are clinically obese. Approximately two thirds of US adults are overweight or obese. World Health Organization (WHO) assigns obesity as global epidemic. WHO' latest study indicate that globally in 2005, approximately 1.6 billion adults (15+) were overweight and at least 400 million adults were obese. Further WHO projects that by 2015 approximately 2.3 billion people will be overweight and more than 700 million will be obese [3].

Currently FDA approved only two drugs in US for long-term treatment for obesity. These are Orlistat and sibutramine [4]. In recent year phytochemical constituents of plants with varied pharmacological, physiological and biochemical activity has received attention. Spice and herbs are part of daily food in several parts of the worlds. In India, the dietary inclusions of peppers, ginger coriander, cardamom, dill, turmeric, onions, etc. have been practiced for centuries. Diets rich in bioactive phytochemical reduce the risk of degenerative disorders such as cancer, diabetes,

Cardiovascular and oxidative dysfunction

Food containing these phytochemicals not only can provide our diet with certain antioxidant vitamins like vitamins C, vitamins E, and pro vitamins A but also a complex mixture of other natural substances with antioxidant capacity. A need is felt to assess the diet substituent which have medicinal values and are safe. The flax, cucumber, chia, black seeds of nutraceutical class have been shown to have antioxidant, anti-obesity, anti-inflammatory, antihypertensive and hyperlipidemic activities besides many other pharmacologically important properties [5]. According to the World Health Organization (WHO), obesity is classified as class I for a BMI between 30 and 34.9 kg/m^2, class II for a BMI between 35 and 39.9 kg/m^2, and class III for a BMI ≥ 40 kg/m^2. In turn, class I obesity is associated with (hence, labeled as) a "moderate risk", class II with a "high risk", and class III with a "very high risk" of mortality.

Formulas for the calculation of BMI [6].

$$BMI = \frac{Weight\ in\ Kg}{Height\ in\ m^2}\ OR\left[BMI = \frac{Weight\ in\ \text{pounds}}{Height\ in\ \text{inches}}\right]\times 703 \quad \text{..... Equation 1}$$

From the literature available the flax, cucumber, chia, black seeds has found to show various degrees of antihypertensive, anti-obesity, anti hyperlipidemic activities besides many other beneficial pharmacological properties of interest. It is also known fact that adulthood obesity depicts many co-morbid conditions such as hypertension, diabetes, and hyperlipidemia. Hence, it is envisaged to undertake to study effects of powder nutraceutical formulation of flax, Cucumber, and chia, black seeds on hyperlipidemia and on metabolic indicators of CVS. Several well-recognized risk factors contribute to the development of CHD; include hypertension, smoking, diabetes, hyperlipidemia, current cigarette smoking, and a family history of premature CHD. In the last decade, however, cholesterol has emerged as an independent risk factor for the development of CHD in the elderly population [7].

Nutraceuticals

A nutraceutical or 'bioceutical' is a pharmaceutical alternative that claims physiological benefits. In the US, "nutraceuticals" are largely unregulated, as they exist in the same category as dietary supplements and food additives by the FDA, under the authority of the Federal Food, Drug, and Cosmetic Act.

Nutraceuticals are products derived from food sources that are purported to provide extra health benefits, in addition to the basic nutritional value found in foods. Depending on the jurisdiction, products may claim to prevent chronic

diseases, improve health, delay the aging process, increase life expectancy, or support the structure or function of the body [8].

Nutraceutical formulation

Although it may be easy for a formulator to develop a pharmaceutical form (tablets, powders, capsules, suppositories, etc.) containing bioactive food, it is quite challenging to obtain a satisfactory bioavailability for such nutraceuticals. The bioavailability is often jeopardized by the low solubility, stability, and/or permeability of the bioactive component in the GIT. For example, in the case of curcumin, extremely low serum levels, limited tissue distribution, apparent rapid metabolism, and short circulation half-life are the underlying causes of its low oral bioavailability. Another example is the Probiotics Encapsulation Technology (or PET).

Criteria of selection of poly-nutraceutical formulation: The concept of nutraceuticals as pharma-foods comes from far. This term is made from the two words "nutrient" and "pharmaceutical", was coined by Stephen De-Felice, and is defined as "a food or part of a food that provide medical or health benefits, including the prevention and/or treatment of a disease". This definition leads to a partial overlap with the definition of a food supplement. In fact, both claim beneficial effects for health; however, while nutraceuticals are made from food or part of a food, food supplements are single substances used alone or in mixtures with the scope of adding micronutrients when the body is in need of them.

Nutraceuticals foreseeing their use "beyond the diet, before the drugs" as tools which can be able to prevent or delay the onset of some asymptomatic long term pathological conditions (e.g. hypercholesterolemia, hypertriglyceridemia, etc.). The steps involved in a new nutraceutical formulation should start with the identification of the target pathologic condition, in a way similar to what happens for drugs.

MATERIALS AND METHODS

Plant material and preparation

The seeds was obtained from the Seeds were collected from the Amruth kesari depot, No.18 1st cross belli basavanagudi, street mamulpet, chickpet, Bangalore Karnataka 560053. Polyherbal marketed formulation (Herbal vibes, slim herbs) weight loss powder used as standard was bought from an organic store. Collected seeds were authenticated at Regional Ayurveda Research Institute for Metabolic Disorders, #12 uttarahalli manavarthe kaval uttarahalli (Hobli) Bangalore south, Karnataka, kanakapura main road, thalagattapura post, 560109.

Seeds were tray dried at optimal temperature ranging between 35°C -40°C for removal of excess of moisture and volatile constituents. Seeds were powdered in a suitable blender for powder formulation. Sieving of the individual powder in the #30 mesh sized sieve to obtain fine powder required for nutraceutical powder formulation.

The seeds powder was standardized by determining the proximate values as listed below prior to formulation process, qualitative and quantitative estimations such as Foreign matter, Total Ash, Acid-insoluble ash, Alcohol-soluble extractive, Water-soluble extractive, Moisture content (LOD),Powder microscopy and Powder Evaluation parameters like Bulk density, tapped density, Carr's (compressibility) index, Hauser's ratio and Angle of repose.

RESULTS AND DISCUSSION

Estimation of TBARS in rat liver: Lipid peroxides and their quantitation serves as a direct and valuable index of the oxidative status of polyunsaturated fatty acid-containing tissues (membranes) or bio systems. Oxidatively modified human serum Low Density Lipoprotein (LDL) has recently gained increasing interest in atherosclerosis research. Based

on the reaction of malondialdehyde, a break & product of lipid peroxides, with & Thiobarbituric Acid (TBA), the measurement of so called Thiobarbituric Acid Reactive Substances (TBARS) has been used commonly to check lipoproteins for products of lipid peroxidation. In this assay the lipoprotein is precipitated by trichloroacetic acid, the water-soluble malondialdehyde present in the supernatant is estimated. (Table 1)

Table 1. Effect of *Linum usitatissimum*, *Cucumis sativus, nigella sativa and salvia hispanica* nutraceutical powder formulation and standard formulation on body weight from Day 1-40.

Sl.no	Animal groups	Body weight (g) in	Mean ± SD
		Day 01	Day 40
1	Negative control	141.5 ±4.14	202 ± 3.301
2	Positive control/ cafeteria diet	163.8 ±4.247	202 ± 3.301
3	Cafeteria diet+High dose	274.6 ± 7.23	256.6 ± 6.84
4	Cafeteria diet+Low dose	247 ± 3.728	238.2 ± 3.49
5	Cafeteria+Standard dose	248.2 ± 5.42	234.8 ± 5.13

Lipid peroxidation was assayed in the form of Thiobarbituric Acid-Reactive Substances (TBARS) according to the method described by Ester Bauer and Cheese man (1990). 500 µl of tissue supernatant was added to one ml Trichloro acetic acid (20%) and mixed well. The mixture was centrifuged at 3000 rpm for 10 min. One ml of the supernatant was added to 0.5 ml of 0.7% Thiobarbituric acid and boiled for 10 min in boiling water bath then cooled. The absorbance was read at 532 nm against blank.

Liver homogenation assay: Estimation of tissue LPO parameters in liver

Preparation of tissue homogenate: At the end of HFD induced anti-obesity study models, the animals were weighed and sacrificed, the livers were isolated, weighed and stored in 10% formalin for histopathological studies and at -20°C for the estimation of tissue antioxidant/LPO parameters. % homogenate of the Liver was prepared by slicing them into fine pieces and homogenizing it with pH-7.0, 0.05 M phosphate buffer saline which was ice cold. The supernatant was obtained by subjecting the tissue homogenate for centrifugation at 8000 rpm for 15 minutes at 4°C and it was used for the estimation of tissue antioxidant/LPO parameters in Liver.

LPO (Liver Peroxidation Assay): The manifestations of oxidative damage due to the production of free radicals and reactive oxygen species irrespective to their etiology results in the formation of lipid peroxide and eventually cell death. The hydroxyl radical and hydrogen peroxide are the chief oxidants that target the unsaturated compounds such as lipids leading to the formation of lipid peroxide. Based on the lipid peroxidation intensity and the restoration ability of the antioxidants, the cell can either initiate survival or a programmed cell death–apoptosis. When the cellular antioxidants fail to combat the effects of lipid peroxide, development of various clinical manifestations can be observed due to the cell apoptosis.

A simple and robust method to estimate the intensity of lipid peroxidation is by measuring the quantity of Malondialdehyde (MDA). MDA is a by-product of lipid peroxidation which reacts with Thiobarbituric acid resulting in pink color substance known as Thiobarbituric Acid Reactive Substances (TABRS) at pH 4.0. Hence, the extent of lipid peroxidation can be estimated by measuring the amount of MDA in the tissue.

Docking/Molecular docking: In the field of molecular modeling, docking is a method which predicts the preferred orientation of one molecule to a second when bound to each other to form a stable complex. Knowledge of the preferred orientation in turn may be used to predict the strength of association or binding affinity between two molecules. It is a Computational simulation of a candidate ligand binding to a receptor.

Molecular docking is one of the most frequently used methods in structure-based drug design, due to its ability to predict the binding-conformation of small molecule ligands to the appropriate target binding site. Characterization of the binding behavior plays an important role in rational design of drugs as well as to elucidate fundamental biochemical processes. In the present study, the anti-obesity activity of *Linum usitatissimum, Cucumis sativus, Nigella sativa, Salvia hispanica* seeds nutraceutical powder was studied using High fat cafeteria diet fed animal model of obesity as they have been reported to bear close resemblance to human obesity. Cafeteria diets have been previously reported to increase energy intake and cause obesity in humans as well as animals. Further, the composition and variety of atherogenic diet also exert synergistic effects on the development of obesity.

Obesity are common most health problems that affect millions of people. In recent decades, the association between obesity and co morbid cardiovascular changes has been actively investigated from an epidemiological standpoint and from a basic research standpoint. And common pathogenic links have been proposed. Recent epidemiologic studies show that body fat itself excises risk factor for cardiac function. Accumulating evidence suggests that metabolic syndrome like obesity and the individual components of the metabolic syndrome such as increased triglycerides, and reduced high-density lipoprotein cholesterol are heightened risk factors for cardiovascular properties.

Obesity is considered to be a disorder of energy balance, occurring when energy expenditure is no longer in equilibrium with daily energy intake, so as to ensure body weight homeostasis. Although the etiology of obesity is complex, dietary factors, particularly the consumption of an atherogenic diet, is considered a risk factor for its development. The current results showed that body weight increased significantly ($P<0.001$) in the cafeteria diet group when compared with the control group. Consumption of the cafeteria diet led to obesity because it facilitates the development of a positive energy balance leading to an increase in visceral fat deposition; this led to abdominal obesity in particular. Moreover, it was found that an cafeteria diet (high fat diet) feeding is accompanied by molecular adaptations that favor fat storage in muscle rather than oxidation. The very fact in appreciated in the present study, because of the fact that the experimental animal consumed considerable more food that is the diet than the control group animals throughout the experiment. In the current study, rats fed cafeteria diet consumed considerably more food than the control rats throughout the experiment. So their caloric intake was increased and they showed a large increase in Perirenal visceral adipose tissue mass, suggesting that the excess energy led to the buildup of adiposity.

In animal models of obesity, there will be decrease in diet induced thermogenesis, due to decrease in sympathetic activation of brown adipose tissue. The main factor for this decrease in diet induced thermogenesis is the neurotransmitter NPY synthesized throughout the brain. NPY causes increase in food intake within 10-15 min, combined with a reduction in thermogenesis on its endogenous release. It is well known that, obesity is associated with increased adipose tissue accumulation in the body. It is reported that feeding with a cafeteria diet in wistar rats leads to increase in weight of body organs such as liver, heart, spleen and both kidneys. It's also reported that cafeteria diet induces substantial increase in deposition of fat in the mesenteric, Perirenal and uterine region in wistar rats. experimental animals fed with cafeteria diet showed increase in the weight of the organs namely liver, heart, spleen and kidneys and

also a significant increase in the weight of the mesenteric, Perirenal and uterine fat pads. The study also showed that *Linum usitatissimum*, *Cucumis sativus*, *Nigella sativa*, *Salvia hispanica* seeds nutraceutical powder at the dose levels studied, significantly exert effect on weight gain of the organs *viz*. liver, heart, spleen and kidneys and greatly influenced the fat deposition process in the fat pads and in fact significantly reduced the fat accumulation in mesenteric, Perirenal and uterine fatty tissues. This fact again establishes that the *Linum usitatissimum, Cucumis sativus, Nigella sativa, Salvia hispanica* seeds nutraceutical powder has a definite influence in body fat metabolism.

To explore mechanism of the association of polyunsaturated fatty acids in obesity and obesity associated protein (FTO), molecular docking studies were done for FTO with Polyunsaturated fatty acids with Orlistat (anti-obesity drug) as a control. Auto dock tools were used for docking polyunsaturated fatty acids and Orlistat with FTO. The results were visualized by PyMol and Discovery studio visualizer. Upon docking simulation, it was observed that Polyunsaturated fatty acids alpha-linoleic acid, omega-3 fatty acid showed highest binding affinity (most negative δG), whereas daidzein was least affinity towards FTO. This study concludes that Polyunsaturated fatty acids primarily, alpha-linoleic acid ameliorates obesity by establishing a physical interaction with FTO. Interactions were also observed between FTO and other polyunsaturated fatty acids were of not greater inhibition compared to alpha-linoleic acid. The results of docking concludes that Ligand 1fk6_5280934_uff_E=142.21 show Binding Affinity -6.8, rmsd/ub of 0 and rmsd/lb of 0 and Ligand 2q9s_target-preprocessed_o-CH3_uff_E=165.98 showed Affinity -7.9, rmsd/ub of 0 and rmsd/lb of 0. Such that the alpha-linoleic acid and omega-3 fatty acid have good affinity towards FTO of obesity and proves to cause good lipolytic activity in reducing obesity.

Histopathological examination section studied shows liver parenchyma with partially effaced architecture. Most of the hepatocytes show apoptotic changes (Short-arrow, Equation 1), while some show cytoplasmic vacuolations. Most of the central veins (Long-arrow, Equation 1) and sinusoids are dilated and congested. The experimental animals treated with *Linum usitatissimum, Cucumis sativus, Nigella sativa* and *Salvia hispanica* seeds nutraceutical powder formulation Section studied shows liver parenchyma with intact architecture. Few of the central veins (Short-Arrow, Equation 1) and sinusoids show congestion (Long-Arrow, Equation 1). Some of the hepatocytes show regenerative changes. There are seen few mononuclear inflammatory infiltration within parenchyma.

CONCLUSION

In the present study showed significant results in analyzing the anti-obesity potential of *Linum usitatissimum*, *Cucumis sativus, Nigella sativa* and *Salvia hispanica* seeds nutraceutical powder formulation evaluated by *in vivo* anti-obesity activity. By this we can conclude that α-Linoleic acid, omega-3 fatty acids (PUFA, MUFA's) and various other constituents present in *Linum usitatissimum, Cucumis sativus, Nigella sativa* and *Salvia hispanica* seeds are responsible for significant anti-obesity property. Histopathology of arteries showed in cafeteria diet group as the layers of artery appear intact except for focal disruption of the endothelium. Within the tunica media and beneath the intima are seen few closely packed foam cells and scattered lipid containing elongated smooth muscle cells.

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AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

AUTHORS' CONTRIBUTIONS

POE and WD performed the experiments, analyzed, interpreted the data and wrote the manuscript. WM and IP conceived and designed the study, supervised the study, interpreted the data and drafted the manuscript. ST designed and supervised the study, interpreted the data, discussed the results, wrote and revised the manuscript. All authors read and approved the final manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

The experiment was conducted is strict compliance according to ethical principles and guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).The institution animal ethics committee approved the study (Ref no. GCP/IAEC/DOP/20/E.C/ADM/2020-21/45).

PATIENT CONSENT FOR PUBLICATION

Not applicable.

CONFLICTS OF INTEREST

There are no conflicts to declare.

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