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Anti-obesity and hypolipidemic activity of *Pithecellobium dulce* against high-fat diet-induced obesity in experimental animals

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ABSTRACT

Pithecellobium dulce (Leguminosae) is conventionally used for many diseases. The objective of this present study intended to assess the anti-obese and hypolipidemic properties of petroleum ether (PEPD), ethyl acetate (EAPD), and methanolic extract (MAPD) of leaves of aerial parts of PD in high-fat diet (HFD)-induced obesity and hypolipidemic effect in obese animals. The present investigation, PD at the doses of 100 and 200 mg/kg, was given along with HFD for 40 days. Our findings such as lipid profiles, serum glutamate oxaloacetate transaminase, serum glutamate pyruvate transaminase, glucose, body weight, food intake, body temperature, atherogenic index, coronary index, weight of the organs (liver and kidney), and anti-antioxidants were estimated. The chronic administration of HFD in rats produced hypercholesterolemia (230.66 ± 12.26 mg/dL), which led to an increase in the body weight gain (106.29 g), total cholesterol, triglycerides (192.84 ± 11.09 mg/dL), and reduction in the levels of high-density lipoprotein (35.67 ± 5.31 mg/dL) as well as changes in body temperature of animals. Further, rats treated with MAPD (100 and 200 mg/kg) show reduced atherogenic index (0.98 ± 0.09 and 0.68 ± 0.04), coronary index (3.29 ± 0.42 and 2.93 ± 0.52), retroperitoneal fat (0.84 ± 0.05), epididymal fat (1.24 ± 0.02), and mesenteric fat (1.08 ± 0.03) and show comparatively good inhibition in the thiobarbituric acid reactive substances, glutathione, glutathione peroxidase, glutathione reductase, superoxide dismutase, and catalase. From the results, it might be expected that PD used significant anti-obese and hypolipidemic effect in rats fed with HFD.

Keywords: Body temperature, body weight, high-fat diet, lipid profiles, obesity, Pithecellobium dulce

INTRODUCTION

Nowadays, obesity is one of the serious health concern in the world and has been connected with the increased morbidity, mortality rate, and reduced life span.[1] It has become an eventual outcome after certain age of the human due to their lifestyle and food habits. Obesity increases the risk of many diseases such as hyperlipidemia, diabetes, atherosclerosis, liver damage, and cancers.[2] Moreover, obesity increases financial burden on the individual and eventually on the government also.[3] The major concern with the western food is either it consists of too much of energized materials or high-fat compounds.[4] This too induces the free radical generation with increased possibility of cardiac related complications.[5] The currently available lipid lowering drugs are showing various side effects. Therefore, researchers and nutritionists are continuing their research of investigating other anti-obesity treatments with nutritional prescriptions or useful components. Despite of the pressing requirement for proficient and safe therapeutics and the likely size of the market for anti-obesity drugs, the current endeavors for development of such medications are still under process.[5]

At present, possible use of natural agents for the management of obesity is not fully explored and might be wonderful alternate approach for developing safe and effective...
anti-obesity drugs. Many studies have revealed that bioactive compounds such as steroids, flavonoids, alkaloids, saponins, and tannins have promising impacts in handling stoutness by a few components.

*Pithecellobium dulce* (PD) (Roxb) Benth belongs to family **Leguminosae**, it is extensively distributed green tree in India and is also found in Southeast Asia. Several parts of plant are used for diverse purposes such as leaves must be conveyed to have abortifacient, emollient, anticonvulsant, antiulcer, antidiabetic, smeared as bandages for pain, and venereal sores.[6] The leaf of the plant possessed as an astringent in dysentery, febrifuge, and it is also beneficial in dermatitis and inflammation of eye. Oxidative destruction instigated due to allowed free radicals is associated through numerous diseases. Numerous studies exist successful on universal diseases. Numerous studies exist successful on universal diseases.

**Materials and Methods**

**Animals**

Both sexes of Wistar strain rats (150–200 g) were chosen and adapted to the assessment center for week for this study. The designated animals were preserved under standard research laboratory environments temperature at 25 ± 1°C, relative humidity 55 ± 10%, and with 12 h light/dark cycle and provide pelleted diet and water *ad libitum*. The experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC) (Committee for the Purpose of Control and Supervision of Experiments on Animals [CPCSEA]/IAEC/ JIPS/19/8/4) and conducted according to the guidelines of the CPCSEA, New Delhi, India.

**Chemicals**

Orlistat purchased from Surety Healthcare, Gujarat, India. Triglycerides (TG: PBTG-2328), total cholesterol (TC: PBCHO-5652), high-density lipoprotein (HDL: PBHDL-2194), serum glutamate oxaloacetate transaminase (SGOT: PBGOT-7811), and serum glutamate pyruvate transaminase (SGPT: PBALT-2218) were done using kits purchased from proton biological India, Bangalore and the other chemicals are analytical grade.

**Herbal Extract Preparation**

PD leaves were collected, accurate identification, and certification was done by the Botanist, Kakatiya University, Warangal, and Telangana. The leaves were shade dried out and made small particles by grounding with electronic mixer. The powder was through sieve (2 mm) and subjected for Soxhlet extraction with PEPD, EAPD, and MAPD solvents. The solvent was entirely detached below condensed vacuum until the dehydrated extract was obtained. The residue was kept in dryer and a 1 g quantity adds in sodium carboxyl methyl cellulose (superoxide dismutase [SOD]. CMC) (2%) which was used as a vehicle.

**Dose Selection**

For the current work, multiple dosages of PD leaf extract were chosen, that is, 100 and 200 mg/kg p.o. These dosages were chosen on the base of earlier observations that it is accomplished by single oral administration of concentrations 100, 500, 1000, and 2000 mg/kg of PEPD, EAPD, and MAPD extracts which display no marks of harmfulness to animals.[11]

**Composition of High-Fat Diet (HFD)**

Casein-20%, D, L methionine-0.3%, corn starch-15%, sucrose-27.5%, cellulose powder-5%, mineral mixture-3.5%, vitamin mixture-1%, choline bitartrate-0.2%, corn oil-9.9%, and lard oil-17.6%. The HFD was ready, dehydrated, crushed, and given throughout the treatment period.[12]

**Experimental Design**

Wistar rats allocated randomly separated into nine sets of six each then treatment such as follows:

Group I treated as normal control (NC) and no treatment received 2% SOD. CMC (NC) and Group II treated as negative control that it was fed with HFD and received standard drug orlistat (50 mg/kg) (Standard Diet); Groups IV, V, VI, VII, VIII, and IX (treatment groups) were fed with HFD and received PEPD, EAPD, and MAPD extracts at dosages of 100 and 200 mg/kg, respectively, for 40 days.[13]

**Estimation of Lipid Profile**

On 40th day, animals made for overnight fasting and animals were sacrificed by cervical decapitation on the same day and collect 2 mL of blood by cardiac puncture for estimation of serum TC, HDL (CHOD-PAP), and TG (GPO-PAP) were estimated by typical commercial kits. Low-density lipoprotein cholesterol (LDL-C) and very low-density lipoprotein cholesterol (VLDL-C) were intended by Friedewald formula: VLDL-C = TG/5; LDL-C = TC−(HDL-C+VLDL-C).[14]

**Estimation of Antioxidants**

**Estimation of lipid peroxidation (LPO)**

The thiobarbituric acid reactive substances (TBARS) levels in the serum and other tissues were measured by the method of Ohkawa et al., (1979) as a marker for LPO. The serum or live homogenate sample were treated with 0.8% of thiobarbituric acid solution at pH 3.5 maintained by SDS-acetate buffer and heated for 1 h at 95°C. After cooling, centrifuged for 15 min at
3000 rpm by adding n-butanol-pyridine (5.0 mL) and later the organic layer was measured the absorbance at 532 nm. From the standard curve of malondialdehyde (MDA) equivalents generated, the sample results were expressed in nmoles/mL for serum and nmoles/mg protein for tissues.\textsuperscript{[15]}

**Assay of glutathione (GSH)**

Estimation of GSH reduction was performed by the procedure described by Ellman \textit{et al.}, briefly, the serum sample was precipitated with trichloroacetic acid (TCA) (25%,125 µL), centrifuged, and cooled in ice for 5 min. Later, the sample mixture was diluted with 5% TCA (0.6 mL) and centrifuged for 10 min at 5000 rpm and from the supernatant (0.3 mL) of was separated and added to phosphate buffer (0.7 mL) and Ellman’s reagent (2 mL). The developed yellow color was read at 412 nm in a colorimeter. The amount of GSH was expressed in µg/mL for serum and µg/mg protein for tissues.\textsuperscript{[16]}

**Assay of glutathione peroxidase (GPx)**

Rotruck \textit{et al.}, (1973) method was used for the estimation of GPx activity of the test samples.\textsuperscript{[17]} The serum samples (0.2 mL) were taken into Tris-HCl buffer (0.2 mL), ethylenediaminetetraacetic acid (EDTA) (0.2 mL), and sodium azide (0.1 mL) was added and mixed well. To this mixture, GSH (0.2 mL) followed by H$_2$O$_2$ (0.1 mL) was added. All these contents were and incubated for 10 min at 37°C along with a control sample, after 10 min, the reaction was ceased by the adding of TCA (10%, 0.5 mL). After centrifuged, the supernatant layer was collected and assayed for GSH inhibition at 340 nm by colorimetric method.

**Glutathione reductase (GR)**

The liver collected from the experimental animals was minced into small pieces and homogenized in ice-cold sucrose solution (0.25M, 9 mL/g) in a blender for 45 min at 14,000 rpm. The supernatant layer pH was adjusted to 5.5 with 0.2 M acetic acid and subjected for centrifugation for another 45 min at 45, and 60 s by adding dichromate acetic acid mixture. The test tubes were heated on water bath for 10 min, cooled and the color developed was read at 590 nm.\textsuperscript{[20]}

**Effect on Liver Enzymes**

1 mL of the plant extract was taken into a test tube and heated on water-bath (40°C) for 10 min and 0.2 mL of serum was added and after proper mixing incubated for 60 min for SGOT, 30 min for SGPT. To the above solutions, 1 mL of the 2, 4-dinitrophenylhydrazine reagent is added immediately to stop the reaction. Then cooled to room temperature and added 0.4 N sodium hydroxide (0.4 N, 10 mL), fixed with rubber stopper, and mixed the contents by inversion. After 30 min, the optical density was measured at 505 nm, using water as the blank.\textsuperscript{[21]}

**Effect of on Blood Glucose Levels**

The blood samples were collected by puncture retro-orbital plexus from the anaesthetized animals. Basal reading was taken at day of treatment and later during the treatment. Blood was centrifuged at 3500 rpm for 20 min and serum was separated and estimates the blood glucose level by GOD-POD method.\textsuperscript{[22]}

**Improvement of Weight and Consumption of Food**

Weight improvement (g) was recorded on 1\textsuperscript{st} day and then weekly on day 40 using a weighing apparatus. In addition to this, the eating pattern was observed among all the groups.\textsuperscript{[23]}

**Atherogenic and Coronary Risk Index Assessment**

The atherogenic and coronary risk indexes continued to measure by formulae LDL-C/HDL-C and TC/HDL-C, respectively.\textsuperscript{[24]}

**Body Temperature**

Basal rectal temperature was measured before, by inserting digital clinical thermometer to a depth of 2 cm into the rectum. The rise in rectal temperature was recorded. Rectal temperature of animals was noted at regular intervals following the respective treatments.\textsuperscript{[21]}

**Organ and Fat Pad Weights**

The liver, kidney, and fat pads (retroperitoneal, epididymal, and mesenteric fat pads) separated, washed in frozen salt water, and weighed.\textsuperscript{[23]}

**Statistical Analysis**

The results were stated as mean ± standard deviation. Results were analyzed by one-way analysis of variance followed by Tukey’s post hoc test and $P < 0.05$ value was considered as statistically significant.
RESULTS

Plasma Lipid Levels Estimation

The plasma lipid level results are shown in Figure 1, the increased levels of TC, TG, LDL, VLDL ($P < 0.001$), and decreased HDL ($P < 0.001$) in HFD animals while compared to NC. Treatment with MAPD at 100 and 200 mg/kg for 5 weeks significantly altered the TG, HDL ($P < 0.01$, $P < 0.001$), TC ($P < 0.05$, $P < 0.01$), LDL ($P < 0.001$, $P < 0.001$), and VLDL, ($P < 0.001$), respectively, to the normal but shows more significant effect and PEPD also shows lipid lowering effect but less significant than MAPD.

Liver Enzymes Estimation

Liver enzymes were significantly elevated in HFD animals ($P < 0.001$), as shown in Figure 2. All the treatments except EAPD, have shown significant hepatoprotective effect. Treatment with PEPD 100 mg/kg ($P < 0.05$) and 200 mg/kg ($P < 0.01$) significantly reduces SGPT and SGOT whereas MAPD 100 mg/kg ($P < 0.01$) and 200 mg/kg ($P < 0.001$) shows more significantly reduce when compared with disease control.

Blood Glucose Estimation

In HFD animals, the blood glucose outstandingly ($P < 0.001$) raised when compared with NC. Treatment with MAPD with 100 mg/kg ($P < 0.01$) and 200 mg/kg ($P < 0.001$) had outstandingly decreased, but PEPD 100 mg/kg ($P < 0.05$) and 200 mg/kg ($P < 0.01$) also significantly reduces glucose levels when matched with animals which received only HFD [Figure 3].

Improvement of Weight and Consumption of food:

The results of improvement of weight and consumption of food were represented in Figures 4 and 5. During the 5-week trial, weight of animals with HFD exhibited rapid rises. The weight gain and food intake in regular diet was observed low when compared with HFD animals ($P < 0.001$). After the treatment with MAPD with 100 mg/kg (b$P < 0.01$) and 200 mg/kg (c$P < 0.001$); PEPD 200 mg/kg (b$P < 0.01$) EAPD and 200 mg/kg (a$P < 0.05$) had considerably reduced the body weight gain and food intake dose dependently when associated with HFD control group.

Atherogenic and Coronary Risk Index

The atherogenic and coronary risk index in HFD were considerably ($P < 0.001$) increased when compared with regular diet animals. The increased atherogenic indexes were significantly reduced by PEPD and MAPD with 100 mg/kg ([a$P < 0.05$, c$P < 0.001$]) and 200 mg/kg ([b$P < 0.01$, c$P < 0.001$]), respectively, but coronary
index also reduced by both the doses, that is, ([P < 0.05],
[P < 0.01]), respectively [Figures 6 and 7].

**Body Temperature**

The rectal body temperature was greatly reduced in HFD animals when compared with regular diet animals on 0 and 120 min. The reduced rectal temperature was not significantly restored by treatment with PEPD, EAPD, and MAPD with 100 and 200 mg/kg [Figure 8].

**Organ Weight**

The weight of the liver and kidney were considerably ([P < 0.001]) improved in HFD animals while matched with regular diet animals. Daily management with standard Orlistat ([P < 0.001]), PEPD, EAPD, and MAPD and 200 mg/kg extracts has increased significantly (aP < 0.05).

**Fat Pad Weights**

In HFD animals, the fat pad weights (Epididymal, Mesenteric, and Retroperitoneal) significantly ([P < 0.001]) increased while matched to NC. Daily management with standard Orlistat ([P < 0.001]), PEPD, EAPD, and MAPD at 200 mg/kg extracts has reduced the fat pad weights significantly ([P < 0.05]).

**Antioxidant Status**

The results of TBARS, GSH, GPx, GR, SOD, and CAT content showed in Table 1. The antioxidants TBARS, Gpx ([P < 0.001]), GSH, GR, SOD, and CAT ([P < 0.001]) significantly restore with MAPD 200 mg/kg, whereas PEPD with 200 mg/kg, TBARS, CAT, Gpx ([P < 0.05]), GSH, GR, and SOD ([P < 0.01]) significantly alters when compared with the HFD fed group.

**DISCUSSION**

Hypolipidemia and obesity ensure remained fast important care from community health officials in emerging nations as they can affect in surprising rise in the risk features of diabetes, hepatic adipose infiltration, and cardiovascular disease. This obesity associated illnesses are the chief source of mortality worldwide,[25] prominent to the expansion of numerous treatments for obesity to stop its difficulties. For treatment of obesity conventional drugs ensure limitations, outcomes show numerous adverse events and individually used for patients with a body mass index over 30 or of 27–29.9 kg/m² through multiple medical conditions who are incapable to attain loss of weight. In this background, it would be greatest applicable to assess the herbal extracts, especially phytochemicals with hypolipidemic and antioxidant properties.
HFD results into deposition of fat in liver and to rise in mass, intuitive adipose tissue, TG, and TC. HFD persuaded fat method is a perfect tool to know the relationship of HFD and progress of obesity. The present work assessed the lipid lowering properties of PD in HFD produced fat. An outcome, management through PD led to in lipid-lowering effects on weight, inhibited lipid and sugar intensities.

Total body weight decrease is a main area in considering obesity, as investigation consumes with decreased weight in substantial or obese is connected through lessening death level. In the present study, the treatment with PD was accompanying through a substantial decrease in complete body weight and food intake. Lipid production, acetyl-CoA is changed toward TG intended to loading in fat materials, creates together free fatty acids and TG. TG associates in the fluid connective tissue, surrounded in a hydrophobic chylomicron through enzyme. In HDL-C, elevation intensities consistently display opposite link with coronary actions. However, excessive amount of TC and LDL-C raise the risk of atherosclerotic coronary diseases. In these studies, the levels of free fatty acids, TG, TC, and LDL-C remained reduced in PD contrasted with HFD group. The PD-treated groups presented an enhanced HDL-C likened with HFD group. Our results specify that PD hypothetically beneficial reduces the elevated lipid levels and associated complications. Extreme HFD diet can lead to the non-alcoholic fatty liver disease which can induce the liver problems such as non-alcoholic steatohepatitis and cirrhosis of liver. Excess eating of a diet rich in fatty acids deteriorates the normal functioning of hepatocytes. HFD induces changes in the normal physiology of hepatocytes, by depositing fat inside them, as it exerts pressure on cell linings and makes them leaky. While SGOT and SGPT are in large quantities establish enzymes in kidney and liver only, their high serum concentrations occur when cellular gateways are open or are made open by physiological or toxicological changes in normal cell functions. This evidence helps understand our findings of the high serum SGOT and SGPT levels in the HFD group that excess feeding of a fat-rich diet led to ample amounts of saturated fat inside the hepatocytes, acquiring enormous space, and making cell membranes leaky by pushing cellular material to the peripheries. High serum concentrations of these localized enzymes illustrate HFD-induced insult and damage to cell membranes. PD has improved the serum profiles of SGOT and SGPT by healing hepatocyte membranes, as PD is known to be a highly hepatoprotective nutraceutical agent.
The atherogenic and coronary index is measured as an indicator on the way to cardiovascular diseases. In this study, the animals were fed with a HFD caused in increased atherogenic and coronary index in the atherogenic diet group whereas treatment with PD displayed significant lessening, so given that cardioprotection.[34]

In specific, the retroperitoneal fat, epididymal fat, and mesenteric fat were greater in the HFD group than in the normal group.[35] In the current assessment, the anti-obesity evaluation of PD observed in HFD animals by measuring the markers of obesity [Table 2]. The retroperitoneal fat, epididymal fat, and mesenteric fat were found to be significantly lesser in the PD treatment. In the present study, weight of the liver significantly increased in the HFD animals whereas weight of the kidney not altered.. These results may suggest that PD extracts have no adverse effects on the liver. In HFD fed, animals found to be possessing increased oxidative stress [Table1]. In the same way, the present study shows that in high LPO and lesser levels of CAT, GSH, Gpx, GR, and SOD, indicating the induction of oxidative stress in HFD-induced obese group. However, management with PD improved the levels of CAT, GSH, Gpx, GR, and SOD and decreased MDA by obstructing the lipid accumulation in the liver. However, the previous studies stated on PD for its polyphenolic, flavonoid contents, and coumarins illustrate auspicious source of antioxidant with various pharmacological activities.[8,9]

**CONCLUSION**

From the observations of the study performed, it could be predicted that PD extract exerted significant anti-obese activity due to its hypoglycemic and hypolipidemic effect in rats fed on HFD. The long history of use of PD may have therapeutic and protective applications in the treatment of these disorders.

Further, investigation involving measure of enzymes in lipid pathways and hormones would ascertain the exact mechanism of anti-obese effect and to figure out the therapeutic potential of PD in the treatment of obesity.

This ensures an understanding of the mechanism involved in the treatment of these disorders. Further, there is need to identify exact phytoconstituents responsible for the activity at brain level and to formulate poly herbal anti-obese preparation containing PD extract as main ingredient along with other novel weight reducing and hypolipidemic herbal drugs.
REFERENCES


