Evaluation of Lipid Lowering Activity and Anti-Oxidant Status of *Pithecellobium dulce* in Obesity Induced Rats

JAGADEESHWAR K*, UMASANKAR KULANDAIVELU, GSN KOTESWARA RAO, RAJASEKHAR REDDY ALAVALA AND SIVA PRASAD PANDA

K L College of Pharmacy, Koneru Lakshmaiah Education Foundation, Vaddeswaram, Guntur, Andhra Pradesh, India

***Pithecellobium dulce* is a plant of numerous utilizations which has a great role in the conventional medicinal system. The present study was planned to assess the hypolipidemic efficacy and anti-oxidant activity of petroleum ether, ethyl acetate and methanolic extracts of the peel of *Pithecellobium dulce* in high fat diet induced obese rats. Petroleum ether, ethyl acetate and methanolic extract of peel were prepared by soxhlet method. Obesity was induced in Wistar rats by giving a high fat diet for 40 d. The lipid lowering activity was assessed by oral administration of extracts at two different dose levels (100 mg/kg and 200 mg/kg) along with a high fat diet for 40 d. The lipid lowering activity was estimated in terms of serum triglycerides, total cholesterol, Low-density lipoprotein-Cholesterol, High-density lipoprotein-Cholesterol, Very Low-density lipoprotein Cholesterol, blood glucose levels, serum glutamate oxaloacetate and serum glutamate pyruvic transaminase. Thiobarbituric acid reactive substances, reduced glutathione, Glutathione peroxidase Glutathione reductase, Superoxide dismutase and Catalase were also estimated to evaluate anti-oxidant status. Administration of a high-fat diet for 40 successive d significantly increased bad cholesterol and glucose levels, decreased good cholesterol levels and also thiobarbituric acid reactive substances level was significantly higher and whereas, reduced Glutathione, Glutathione peroxidise, Glutathione reductase, Superoxide dismutase and Catalase were significantly lower in disease control animals. Animals treated with extracts for 40 successive days along with high fat diet reversed the effects induced by high fat in animals. The present study depicts that the Petroleum ether, ethyl acetate and methanolic extract of the peel of *Pithecellobium dulce* shows potential lipid lowering effect and improvement in antioxidant statuss.**

Key words: *Pithecellobium dulce*, high fat diet, lipid lowering activity and anti-oxidant activity

Pithecellobium dulce is one of the significant products of Indian origin, well known locally as Jangal jalebi in Hindi and Manila Tamarind in English. It is a woody vegetable of little to medium size, evergreen, with a stature of around 18 m. The mash (aril) around the seed has white and pink hues and is exceptionally short-lived, which turns dark colored once the shell is expelled[1]. Pods are 10-15×0.5 cm, the shading winds up ruddy dark colored. Each case contains 5-10 sparkling dark seeds. The firmly wound seed units are the principal trademark highlight of this tree and make it simple to recognize[2]. Hyperlipidemia caused by changes in the lipid metabolic processes together with lipogenesis and lipolysis. It is considered by distended fat mass and lipid concentration in blood[3]. Hyperlipidemia is also linked with many ailments like hypertension and diabetes mellitus[4]. The effectiveness of the peel of this plant for treatment of hyperlipidemia was not been scientifically reported in obese animals. In this study, the attempts made to assess the lipid lowering activity and anti-oxidant status of petroleum ether (PE), ethyl acetate (EA) and methanolic (MA) extract of peel of *Pithecellobium dulce* in high fat diet induced obese rats with Institutional ethical committee approval

***Address for correspondence**
E-mail: rongweiwo108@163.com

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(CPCSEA/IAEC/JIPS/19/1/1). Wistar rats of both sexes weighing 150-200 gm were chosen for the present study and held at 25±1°, relative humidity 55±10 % and 12 h light/dark cycles in normal laboratory conditions. The pellet feeds and water ad libitum have been used for animals. For 1 w animals have been acclimated to lab conditions. Triglycerides (TG), Total Cholesterol (TC), High-Density Lipoprotein (HDL), Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP) and Catalase (CAT) was done using kits, Orlistat was used as standard drug and the other chemicals were analytical grade. Peel of *Pithecellobium dulce* were collected. The collected peel was air dried for 2 w then crushed in a mortar and later pulverized into fine powder using electric blender. The powder was sieved through a 2 mm mesh and used for extraction by Soxhlation presses using petroleum ether (PE), ethyl acetate (EA) and methanolic (MA). Each extract was dried under reduced pressure till semisolid residue was obtained. Extract was stored in desiccators, and the 2 % Carboxyl Methyl Cellulose (2 % CMC) was used as a suspending agent before administration in distilled water in a weighed amount of 1gm. Two doses (100 and 200 mg/kg p.o.) of *Pithecellobium dulce* peel extract have been chosen for the current study on the basis of previous reports of an acute toxicity study conducted by single oral administration of PEPD, EAPD and MAPD at the concentrations of 100, 500, 1000 and 2000 mg/kg which do not display toxicity signs in rats[5]. Rodent feed has been mixed with the following ingredients: casein (20 %), D, L-methionine (0.3 %), maize starch (15 %), sucrose (27.5 %), cellulose powder (5 %), mineral blend (3.5 %), vitamin mixture (1 %), choline bitartrate (0.2 %), corn oil (18.0 %), maize oil (9.9 %), lard oil (17.6 %). During the treatment period the high fat diet was prepared, dried, pulverized and administered[6].Rats were divided into nine groups (n=6): Group-I: Normal control rats fed with standard chow diet and received vehicle for 40 d; Group-II: Obesity control rats fed with high fat diet (HFD) and received vehicle for 40 d; Group-III: Rats fed with HFD and treated with Orlistat (50 mg/kg) orally for 40 d; Group-IV: Rats fed with HFD and treated with petroleum ether of extract *P. dulce* peel 100 mg/kg for 40 d (PEPD); Group-V: Rats fed with HFD and treated with petroleum ether extract of *P. dulce* peel 200 mg/kg for 40 d (PEPD); Group-VI: Rats fed with HFD and treated with ethyl acetate extract of *P. dulce* peel 100 mg/kg for 40 d (EAPD); Group-VII: Rats fed with HFD and treated with ethyl acetate extract of *P. dulce* peel 200 mg/kg for 40 d (EAPD); Group-VIII: Rats fed with HFD and treated with methyl alcohol extract of *P. dulce* peel 100 mg/kg for 40 d (MAPD); Group-IX: Rats fed with HFD and treated with methyl alcohol extract of *P. dulce* peel 200 mg/kg for 40 d (MAPD)[7]. At the end of the experiment i.e., on d 40, rats were fasted overnight and on the following day animals were sacrificed by cervical decapitation. Approximately 2 ml of blood was collected by cardiac puncture. Blood glucose was estimated by Glucose oxidase (GOD)-peroxidase (POD) method[8]. For estimation of lipid profile blood samples were centrifuged at 2500 rpm/min for 20 min to separate serum, which was used for biochemical analysis. Serum total cholesterol and HDL levels (cholesterol oxidase/peroxidase aminophena-zone (CHOD-PAP) method) and triglyceride levels (glycerol phosphate oxidase-peroxidase aminophena-zone (GPO-PAP) method) were estimated using standard commercial kits. Low-density lipoprotein (LDL) cholesterol and Very Low-density lipoprotein (VLDL) cholesterol were calculated by Friedewald formula: VLDL-C=TG/5; LDL-C=TC-(HDL-C+VLDL-C)[9]. By using the same serum, thiobarbituric acid reactive substances (TBARS)[10], reduced Glutathione (GSH)[11], Glutathione peroxidise (GPx)[12], Glutathione reductase (GR)[13], Superoxide dismutase (SOD)[14] and Catalase (CAT)[15] were also estimated. Serum glutamate oxaloacetate transaminase (SGOT) and Serum glutamate pyruvate transaminase (SGPT) estimated by modified IFCC method[16]. The atherogenic index and coronary risk index were calculated by using following formulae LDL-C/ HDL-C and TC/ HDL-C respectively[17].The results were expressed as mean ± SD. Mean values between the different groups were statistically significant $p<0.05$ after evaluating ANOVA and were measured by Tukey's Post Hoc Test. As stated in Table 1, rats feed on a high-fat diet for 40 d, it was noted that lipid level increased markedly, with high levels of Tc, TG, LDL ($p<0.001$) and VLDL ($p<0.001$) being characterized by lower levels of HDL compared to regular feed-in control rats. Hypercholesterolemia is demonstrated by an increased level of LDL. The hyperlipedimic effect produced by high-fat diet ($p<0.001$) was however significantly reverted with PEPD, EAPD and MAPD; however the effect of MAPD is more significant than PEPD and EAPD. The hepatoprotective effect shown in Table 2, treatment with PEPD, EAPD and MAPD extracts exhibits a hepatoprotective effect, indicated by significantly ($p<0.001$) decreases the levels of SGOT and SGPT. The results of blood glucose levels were mentioned in

Table 2. There was a significant elevation in blood glucose levels in HFD control animals. Oral administration of PEPD, EAPD and MAPD had significantly ($p<0.001$) reduced blood glucose levels when compared with animals which received only HFD, but MAPD shows more significant effect than PEPD and EAPD. Administration of PEPD, EAPD and MAPD for 40 d along with HFD significantly ($p<0.001$) decreased atherogenic index and coronary risk index dose dependent manner in animals when compared with HFD control Table 3. In Table 4 shows the liver TBARS, GSH, GPx, GR, SOD and CAT content. At the end of study, TBARS level was significantly higher for the high fat diet fed group when compared to the regular fed group. For PEPD, EAPD and MAPD groups TBARS accumulation was significantly ($p<0.001$) decreased. Also, liver GSH, GPx, GR, SOD and CAT were significantly lower for the high fat diet fed group when compared to the regular fed group (Table 4). Liver GSH level in PEPD, EAPD and MAPD treated groups were markedly increased significantly ($p<0.001$) when compared to the high fat diet control group. Liver GPx level in PEPD, EAPD and MAPD treated groups were markedly increased ($p<0.001$) when compared to the high fat diet control group. On the other hand, Liver GR level in PEPD, EAPD and MAPD treated groups were markedly increased ($p<0.001$) when compared to the high fat diet control group. In addition, liver SOD level in PEPD, EAPD and MAPD treated groups were markedly increased ($p<0.001$) when compared to the high fat diet control group. Finally, Liver CAT level in PEPD, EAPD and MAPD treated groups were markedly increased ($p<0.001$) when compared to the high fat diet control group. The lethal dose of the *P. dulce* indicated that the extract was safe and non-toxic up to 5gm/kg. Previous studies have shown that all parts of *P. dulce* has anti-microbial, anti-inflammatory, cardio protective, anti-bacterial, anti-fungal, antioxidant, anti-diarrheal and antiulcer activity[18]. However, the results of the past studies indicated that crude extract of *P. dulce* possesses anti-diabetic activity in rats[19]. A number of herbal extracts had been used in traditional medicine and scientifically explored for their lipid lowering activities. Still no significant evidences are available for lipid lowering activity of peel extract *P. dulce*. The research was therefore designed to demonstrate the impact of *P. dulce* lipid decrease in high-fat dietary obesity. From the past reports, in current study also rats which received only HFD significantly elevated the serum concentrations of TC, TG, LDL-C and VLDL-C and decrease in HDL-C. The significant elevate in lipid profile may be due to rise in both de-novo triglyceride and cholesterol synthesis and intestinal lipid uptake[20]. Administration of *P. dulce* for 40 d along with HFD decreased the serum concentration of TC, TG, LDL-C and increased the concentration of HDL-C which may be due to lowering lipogenesis, enhancing lipolysis, suppressing appetite and reducing

TABLE 1: EFFECT OF PEPD, EAPD AND MAPD ON LIPID LEVELS OBESE RATS

Parameters	TC	TG	HDL	LDL	VLDL
Normal Control	62.56.±0.73	45.56±1.32	48.59±2.88	4.86±1.72	9.11±0.25
HFD	175.65±0.26¥¥¥	162.29±1.67¥¥¥	33.59±2.19¥¥¥	107.61±2.98¥¥¥	32.45±0.59¥¥¥
Standard	84.99±0.39***	83.78±1.53***	54.16±0.37***	14.08±2.76***	16.75±0.80***
PEPD (100 mg/kg)	97.90±0.48***	95.78±1.54***	34.98±1.28***	38.98±2.51***	23.94±0.77***
PEPD (200 mg/kg)	88.58±0.61***	90.90±1.61***	45.78±2.90***	24.62±2.62***	18.18±0.99***
EAPD (100 mg/kg)	95.06±0.50***	94.78±1.54***	39.42±1.43***	36.69±2.50***	18.95±0.70***
EAPD (200 mg/kg)	85.40±0.70***	89.94±1.60***	47.82±1.30***	19.60±2.56***	17.98±0.65***
MAPD (100 mg/kg)	92.76±0.42***	88.82±1.45***	44.16±0.37***	30.84±2.76***	17.76±0.80***
MAPD (200 mg/kg)	80.54±0.34***	85.88±1.50***	48.28±1.52***	15.09±2.63***	17.17±0.74***

¥¥¥p<0.001 when compared with the normal control group, *p<0.05; **p<0.01; ***p<0.001; when compared with the normal HFD

TABLE 2: EFFECT OF PEPD, EAPD AND MAPD ON LIVER PARAMETERS AND GLUCOSE LEVELS OBESE RATS

Parameters	SGOT	SGPT	Glucose
Normal Control	30.35±0.48	36.62±0.56	77.89±2.99
HFD	66.19±1.81¥¥¥	75.26±2.06¥¥¥	93.96±2.19¥¥¥
Standard	46.79±2.89***	44.56±1.48***	78.28±1.42***
PEPD (100 mg/kg)	59.48±1.96***	57.27±2.40***	84.19±2.92***
PEPD (200 mg/kg)	56.49±3.82***	53.29±2.56***	82.86±3.02***
EAPD (100 mg/kg)	55.20±1.54***	57.27±2.40***	82.16.±1.39***
EAPD (200 mg/kg)	48.69±1.50***	53.29±2.56***	78.66±1.12***
MAPD (100 mg/kg)	49.69±2.76***	48.86±2.44***	80.02±1.46***
MAPD (200 mg/kg)	48.59±2.66***	45.55±2.41***	78.54±1.53***

¥¥¥p<0.001 when compared with the normal control group, *p<0.05; **p<0.01; ***p< 0.001; when compared with the normal HFD

lipid absorption. Several reports showed that long-term exposure to HFD leads to hyperglycemia and hyperlipidemia in laboratory animals. In this experiment, HFD fed rats developed a hyperglycemic status that could be attributed to defective synthesis of insulin and increasing the production of insulin. On the other hand, *P. dulce* supplementation decreases the amount of glucose in the dose based at increasing insulin secretion from the β-cells of pancreatic islets or increasing the insulin output, which promotes blood release into target tissues. The atherogenic and coronary risk indices are tough and dependable indicators of whether or not cholesterol is deposited into tissues or metabolized and excreted[21]. In the present study, outcomes were in excellent agreement with former reports i.e., rats fed with high fat diet, both atherogenic and coronary indices had augmented remarkably higher than the reference value (higher than 4 and 2.5 respectively)[22]. Treatment with *P. dulce* in HFD fed rats cause reflective decrease in the atherogenic and coronary indices and thereby strongly confirmed the hypolipidemic effect. The findings achieved in the current study showed that hepatocellular damage is caused by HFD, as is evident from the significant increase in serum SGOT and SGPT enzymes. Nonetheless, *P. dulce* treatment causes temporary decreased enzyme levels, which indicates *P. dulce's* role in preventing hepatic damage caused by HFD[23]. The liver activities of TBARS, GSH, GPx, GR, SOD and CAT were evaluated and shown. TBARS, GSH, GPx, GR, SOD and CAT liver activities are evaluated and shown. The increased TBARS levels in diseased rats are a strong sign of extreme radical free formation and lipid peroxidation activation. There was a significant decrease in the concentrations of TBARS in rats treated with HFD with *P. dulce* extracts. In the liver at high concentrations, glutathione, an endogenous antioxidant protection is identified. In the high-fat diet rats relative to control, substantial decline in levels of tissue-based oxidative stress biomarkers was seen. From the above results, methanolic extract of peel of *Pithecellobium dulce* exhibits a promising role in management of hyperlipidemia than ethyl acetate and petroleum ether extracts. Further, the present investigation provides scientific evidence for use of peel of *Pithecellobium dulce* in traditional medicine in treating hyperlipidemia.

Conflict of interests:

The authors declared no conflict of interest.

TABLE 3: EFFECT OF PEPD, EAPD AND MAPD ON ATHEROGENIC, CORONARY RISK INDEX IN OBESE RATS

Groups	Atherogenic Index	Coronary risk Index
Normal Control	0.28±0.02	1.28±0.13
HFD	3.93±0.03¥¥¥	5.22±0.35¥¥¥
Standard	0.56±0.12***	1.56±0.12***
PEPD (100 mg/kg)	1.79±0.02***	2.79±0.01***
PEPD (200 mg/kg	0.93±0.11***	1.93±0.09***
EAPD (100 mg/kg)	1.41±0.03***	2.41±0.03***
EAPD (200 mg/kg)	0.78±0.02***	1.78±0.29***
MAPD (100 mg/kg)	1.10±0.12***	2.10±0.19***
MAPD (200 mg/kg)	0.66±0.02***	1.66±0.02***

¥¥¥p<0.001 when compared with the normal control group, *p<0.05; **p<0.01; ***p<0.001; when compared with the normal HFD

TABLE 4: EFFECT OF PEPD, EAPD AND MAPD ON ANTIOXIDANT STATUS IN OBESE RATS

Groups	TBARS (nmol of MDA formed/ gm tissue)	GSH (mg/gm tissue)	GPx (mg of GSH consumed/min /mg of protein	GR (mg of GSH consumed/min /mg protein)	SOD (unit min /mg/protein)	CAT (μmoles H_2O_2 consumed min /mg/protein
Normal Control	24.42±3.02	4.92±1.06	10.36±1.96	2.09±0.06	4.29±0.16	35.99±2.39
HFD	75.28±4.20¥¥¥	1.90±0.09¥¥¥	5.38±1.48¥¥¥	0.80±0.03¥¥¥	1.74±0.09¥¥¥	18.93±2.95¥¥¥
Standard	32.62±3.92***	4.85±0.06***	9.69±1.62***	1.98±0.06***	4.06±1.59***	30.59±3.90***
PEPD (100 mg/kg)	69.49±3.98***	4.40±0.19***	7.32±1.68***	1.20±0.05***	2.40±0.36***	20.60±1.50***
PEPD (200 mg/kg	62.98±3.76***	4.34±0.07***	8.96±1.49***	1.58±0.09***	2.98±0.60***	24.29±1.60***
EAPD (100 mg/kg)	59.42±3.56***	4.28±0.09***	7.56±1.68***	1.26±0.07***	2.56±0.32***	21.19±2.56***
EAPD (200 mg/kg)	50.20±3.62***	4.00±0.12***	9.20±1.28***	1.65±0.04***	3.08±0.20***	25.62±1.68***
MAPD (100 mg/kg)	52.48±3.85***	3.39±0.16***	7.99±1.56***	1.30±0.06***	2.36±0.20***	24.97±2.98***
MAPD (200 mg/kg)	40.67±4.12***	4.16±0.14***	9.39±1.36***	1.76±0.08***	3.19±0.49***	27.99±1.93***

¥¥¥p<0.001 when compared with the normal control group, *p<0.05, **p<0.01; ***p<0.001; when compared with the normal HFD.

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