ORIGINAL ARTICLE

Effect of aqueous enriched fraction of *Premna integrifolia* root against cafeteria diet induced obesity in Swiss albino mice

Prashant Y. Mali
Department of Pharmacology, Radharaman College of Pharmacy, Ratibad, Bhopal, Madhya Pradesh, India

Objective: The objective of the present study was to evaluate the effect of aqueous enriched fraction of *Premna integrifolia* root (AEFPIR) against cafeteria diet induced obesity in Swiss albino mice. **Materials and Methods:** Female Swiss albino mice were divided into four groups, which received cafeteria diet, standard drug simvastatin (10 mg/kg) and AEFPIR (200 and 400 mg/kg) daily for 40 days. The body weight, body mass index (BMI), food consumption, serum glucose, triglyceride, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were studied along with histopathological assessments for screening the effect of AEFPIR against cafeteria diet induced obesity. High performance liquid chromatography (HPLC) fingerprint profile of AEFPIR was also studied using quercetin as the reference standard. **Results:** The results of present study revealed that, there was a significant decrease in body weight, BMI, food consumption and in the levels of serum glucose, triglyceride, total cholesterol, LDL and VLDL with a significant increase in the level of HDL in mice treated with simvastatin and AEFPIR groups compared with cafeteria diet group. Mice treated with AEFPIR shows dose dependent effect. The AEFPIR (400 mg/kg) supplementation attenuated all the above alterations, which indicates the protective effect against cafeteria diet induced obesity that was further confirmed by histopathological analysis. The solvent system was used for HPLC fingerprint profile of AEFPIR, 50 Mm potassium diphosphate (pH-3 with ortho phosphoric acid): Methanol (50:50 v/v) at 360 nm. The chromatograph showed three peaks at retention times 3.835, 5.649 and 11.106. **Conclusion:** The present study suggests that AEFPIR possess protective effect against cafeteria diet induced obesity that was substantiated its ethno-medicinal use in the treatment of obesity. The exploration of chemical constituents and further pharmacological evaluation will give us basis for its therapeutic use. Further series of studies are required to prove its clinical reliability, safety and efficacy.

Key words: Aqueous enriched fraction, cafeteria diet, obesity, *Premna integrifolia*

INTRODUCTION

Premna integrifolia Linn. (*Verbenaceae*), commonly known as Arni or Agnimantha[1,2] have been widely used for obesity and other obesity associated disorders.[3] *P. integrifolia* root extract is an active ingredient of many ayurvedic preparations such as *Arishtam*, *Avaleham*, *Kvatham*, *Ghritam* and *Tailum*.[4] Roots are used in the treatment of diabetes, chyluria, inflammations, swellings, bronchitis, dyspepsia, liver disorders, piles, constipation and fever.[1] The root of *P. integrifolia* mainly contains premnine, ganiarine,[5] ganikarine,[6] premnazole,[7] clerodendrin-A,[8,9] glycerine, 2,5-furandione, 3-methyl-, 2-furancarboxaldehyde, 5-(hydroxymethyl)-, benzofuran, 2,3-dihydro-, 2-hydroxy-3-methylbenzaldehyde, seychellene, dodecanoic acid, 1H-cycloprop[e] azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1aà,4aà,7á,7aá,7bà)]-, 2-propenoic acid, 3-(4-methoxyphenyl)-, tetramethyltricyclo[5.2.2.0 (1,6)] undecan-2-ol, 3,7,11,15-tetramethyl-2-hexadecen-1-ol, n-hexadecanoic acid, phytol, octadecanoic acid, ethyl ester, 2-phenanthrenol, 4b, 5,6,7,8,8a, 9,10-octahydro-4b, 8,8-trimethyl-1-(1-methylethyl)-,[10] luteolin,[11] 1β,3α,8β-trihydroxy-pimara-15-ene, 6α,11,12,16-tetrahydroxy-7 -oxo-abieta-8,11,13-triene, 2α,19-dihydroxy-pimara-7, 15-diene[12,13] and acteoside (verbacoside).[14] Moreover, alkaloids, carbohydrates, amino acids, steroids, flavonoids, glycosides, tannins and phenolic compounds were found in preliminary phytochemical screening of the aqueous enriched fraction of *P. integrifolia* root (AEFPIR, aqueous extract).[15] *P. integrifolia* has been reported for its potential actions such as antidiabetic, hypoglycemic and antihyperglycaemic,[16-18] anti-inflammatory,[7,19,20] immunomodulatory,[21] cardiac stimulant and cardioprotective,[22,23] analgesic and antibacterial,[24] antimicrobial,[25-27] anti-arthritic,[19,28] hepatoprotective,[29-31] *in-vitro* cytotoxic and tumor suppression,[31,32] hypolipidemic or anti-hyperlipidemic and anti-obesity,[3,33-36] neuroprotective,[37] antiparasitic,[38]

Access this article online	
Quick Response Code:	Website: www.greenpharmacy.info
	DOI: 10.4103/0973-8258.122068

Address for correspondence: Asst. Prof. Prashant Y. Mali, Department of Pharmacology, Radharaman College of Pharmacy, Ratibad, Bhopal - 462 044, Madhya Pradesh, India. E-mail: pymali2008@rediffmail.com
Received: 07-07-2013; **Accepted:** 26-08-2013

antioxidant,[14,20,29,30,32,39,40] anticoagulation,[41] antitumor,[42] gastroprotective and antiulcer[43] and longevity-promoting.[44] The herbal formulations containing *P. integrifolia* were also evaluated for their memory enhancing,[45] hypolipidemic,[46] and *in-vitro* bioactivity effects.[47]

Obesity is a multifactorial, chronic disorder that has reached a pandemic proportion world-wide.[48,49] It is rapidly turning into an epidemic afflicting much of the industrialized world, resulting in a prohibitive health and economic burden on society.[50-52] Its prevalence is on continuous rise in all age groups of many of the developed countries in the world.[53] Nearly, one third of the world's adult population (1.3 billion people) was overweight or obese in 2005 and if recent trends continue, by 2030 nearly two-third of the world's adult population (3.3 billion people) could be either overweight or obese.[54] Moreover, obese and overweight patients are at higher risk from coronary artery disease, hypertension, hyperlipidemia, diabetes mellitus, cancers, gall bladder disorders, cerebrovascular accidents, osteoarthritis, restrictive pulmonary disease and sleep apnoea.[55,56] Now a day's natural medicine are gaining prominence because they are economical, easily available and relatively free from side-effects. It is evident from the present scenario that herbal cure is gaining world-wide acceptance and has emphasised on modern scientific exploration, extraction and evaluation of foil medicines from plants. These are either used directly as a plant extract or modified through further synthesis.[57] Therefore, based on the ethno-medicinal use of *P. integrifolia* roots in the treatment of obesity, author has screened its effect against cafeteria diet induced obesity in Swiss albino mice.

MATERIALS AND METHODS

Chemicals

The quercetin (purity ≥ 98%, high performance liquid chromatography [HPLC]) was procured from Sigma Aldrich, Bangalore. Glucose, triglyceride, total cholesterol, high density lipoprotein (HDL) kits were obtained from ERBA Diagnostics, Mannheim GmbH, Germany. Cafeteria diet was purchased from local market of Bhopal, Madhya Pradesh. Simvastatin was obtained from USV Ltd., Baddi, India. All other chemicals and reagents used were of analytical grade.

Plant Authentification

Fresh, well-developed *P. integrifolia* plants and their roots were collected from the region of North Karnataka, India in the month of September 2008 and it was authenticated by a taxonomist, Department of Botany, Basaveshwar Science College, B.V.V.S. Campus, Bagalkot, Karnataka, India. Voucher specimen (No. B.Sc/Bot/13/08) was deposited in the same.

Preparation of Aqueous Enriched Fraction

Roots were dried in the shade and powdered. The root powder was extracted with petroleum ether (40-60°C) and subsequently with 1:1 ratio of chloroform-methanol (55-60°C) for 24 h by using soxhlet apparatus. The above residue successively was used for the preparation of aqueous enriched fraction (aqueous extract), the powder was heated at 20-22°C in distilled water. After filtration through Whatmann filter paper no. 40, the extract was evaporated to dry by slow heating and continuous stirring on water bath. After the residue extractions, the excess water were completely removed by using a rotatory flash evaporator to get concentrated, then completely dried in freeze drier and preserved in an airtight container under refrigeration. Percentage yield of AEFPIR (aqueous extract) was found to be 5.43% based on the initial plant material used for the extraction and then it was used for screening the effect of cafeteria diet induced obesity in Swiss albino mice

HPLC Fingerprint Profile of Aqueous Enriched Fraction

HPLC fingerprint profile of AEFPIR was studied using quercetin as the reference standard. The mobile was used, 50 Mm potassium diphosphate (pH-3 with ortho phosphoric acid): Methanol (50:50 v/v) at wavelength 360 nm; however, the HPLC column was used C18 (250 mm × 4.6 mm, 5u), flow rate 1 ml/min. The flavonoids were separated and quantified by using refurbished water isocratic reversed phase-HPLC system with chromatography software was used for data analysis and data processing. The HPLC system consists of water pump, Rehodyne injector and waters 486 ultraviolet visible detector using HPLC grade water. The samples were analysed at 80°C on a C18 (250 mm × 4.6 mm, 5u) column.

Animals

All the experimental protocols used in the study were approved by Institutional Animal Ethics Committee, Radharaman College of Pharmacy , Bhopal. Inbreed female Swiss albino mice (22-26 g) were used for the study and housed at room temperature (25°C ± 1°C) with 50% ± 5% relative humidity and given standard laboratory feed (Hindustan Lever, India) and water *ad libitum* throughout the experimental period.

Acute Toxicity Study

Acute toxicity study of AEFPIR was performed as per the Organisation for Economic Co-operation and Development guideline No. 425 and 420 followed by up and down and fixed dose method.[58,59] The dose range of 200 and 400 mg/kg was selected for the further evaluation of study.

Experimental Protocol for Screening of Effect of AEFPIR

Inbreed female Swiss albino mice (22-26 g) were randomly divided into four groups of six mice in each and treated

are as follows:

- Group I: Received cafeteria diet in pellets forms
- Group II: Cafeteria diet + simvastatin (10 mg/kg, orally) was administered daily
- Group III: Cafeteria diet + AEFPIR (200 mg/kg, orally) was administered daily
- Group IV: Cafeteria diet + AEFPIR (400 mg/kg, orally) was administered daily.

Preparation and Presentation of Cafeteria Diet for Induction of Obesity

The method described by Harris and Kulkarni was followed with some modifications.[60,61] Cafeteria diet is highly palatable, energy rich animal diet that includes a variety of human snack foods that consists of three diets, which includes (condensed milk 48 g + bread 48 g), (chocolate 18 g + biscuits 36 g + dried coconut 36 g), (cheese 48 g + boiled potatoes 60 g). The cafeteria diet was presented in the form of pellets to five groups for 40 days. However, the effect of AEFPIR against cafeteria diet induced obesity was evaluated using following parameters.

Body Weight

Body weights of mice (g) were recorded on 1, 10, 20, 30 and 40 day in each group.[60]

Body Mass Index

Body mass index (BMI) of mice were recorded on 1 and 40 day of study (i.e., initial and final body weight and body height) and was determined by using formula:[62]

BMI = Body weight (in g)/height (in cm^2).

Food Consumption

Food consumption study was carried out on 1, 10, 20, 30 and 40 day and recorded at 1 h, 2 h and 3 h of time intervals. The food consumption was estimated by subtracting the amount of food left on the grid from initial food weight.[63]

Serum Lipid Profile

On 41 day, the blood samples were taken by penetrating the retro-orbital plexus with a fine glass capillary. The blood samples were centrifuged at 2500 rpm for 15 min to separate the serum and preserved (−20°C) for analysis of glucose (Trinder's Method), triglycerides (GPO-Trinder Method, End Point), total cholesterol (Modified Roeschau's Method), HDL-cholesterol (HDL-c) (Phosphotungstic Acid Method), low density lipoprotein-cholesterol (LDL-c) (calculated using Friedewald's equation, LDL-c = Total cholesterol – Very low density lipoprotein-cholesterol (VLDL-c) – HDL-c) and VLDL-c (VLDL-c = Triglyceride/5) were estimated.[60,64]

Histopathological Assessments

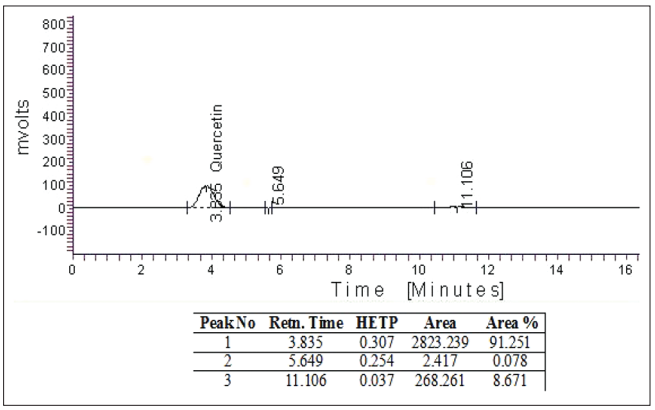
Liver from the cafeteria diet, simvastatin and AEFPIR treated groups was removed. It was fixed in 10% formalin and embedded in paraffin wax and cut into a sagittal plane section of 5 µ of thickness. The sections were stained with haemotoxylin and eosin dye for histopathological assessments under a light microscope.

Statistical Analysis

The data were statistically analysed and expressed as means ± standard error of mean (SEM). The significance of difference was determined by using one-way analysis of variance (ANOVA) followed by multiple comparisons Dunnett's test. $P < 0.05$ value was considered as statistically significant.

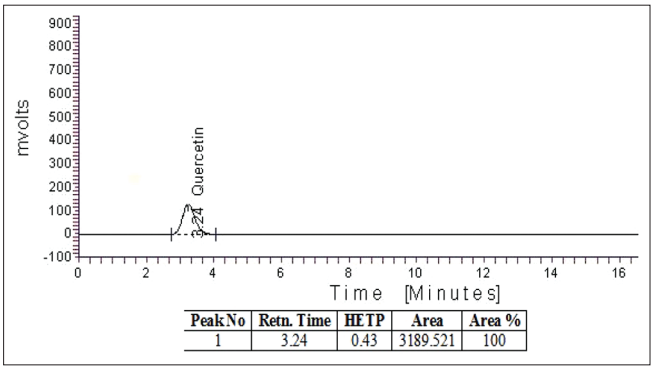
RESULTS AND DISCUSSION

HPLC fingerprint chromatograph showed three peaks at retention times of 3.835, 5.649 and 11.106 as in Figure 1. The reference standard was used as quercetin, which showed a peak at retention time 3.24 as in Figure 2. A cafeteria diet induced obesity model is the simplest obesity induction model and possibly the one that most closely resembles the reality of obesity in humans.[65] Cafeteria diet has been previously reported to increase energy intake and cause obesity in humans[66] as well as in animals.[67] The cafeteria diet has been reported to



Peak No	Retn. Time	HETP	Area	Area %
1	3.835	0.307	2823.239	91.251
2	5.649	0.254	2.417	0.078
3	11.106	0.037	268.261	8.671

Figure 1: High performance liquid chromatography chromatogram of aqueous enriched fraction of *Premna integrifolia* root



Peak No	Retn. Time	HETP	Area	Area %
1	3.24	0.43	3189.521	100

Figure 2: High performance liquid chromatography chromatogram of quercetin as reference standard

induce hyperphagia in rats,[68] which results in higher fats stores resulting in increased body weight and organ weight.[69] The results of present findings was in line with the above studies as author have observed, the cafeteria diet fed mice treated with simvastatin showed significant ($P < 0.05$-$P < 0.001$) decrease in body weight on 10, 20, 30 and 40 day as compared with cafeteria diet group. However, AEFPIR group (400 mg/kg) showed significant ($P < 0.001$) reduction in body weight at 30 and 40 day when compared with cafeteria diet group as shown in Table 1. Effect of AEFPIR on BMI is shown in Table 2, cafeteria diet fed mice treated with simvastatin, AEFPIR (400 mg/kg) groups showed a significant ($P < 0.001$) decrease in the final BMI when compared with cafeteria diet group. Table 3 shows the effect of AEFPIR on food consumption in the experimental group of mice. Simvastatin administered mice showed a significant ($P < 0.01$, $P < 0.001$) decrease in food consumption on 30 and 40 day as compared with cafeteria diet group. The AEFPIR (400 mg/kg) treated mice showed significantly ($P < 0.05$, $P < 0.001$) decreased food consumption on 30 and 40 day as compared with cafeteria diet group. High fat diet induced obesity can lead to insulin resistance. Obesity is associated with a decreased capacity of insulin to regulate glucose metabolism in the peripheral tissues.[70] Reports have strongly suggested that obesity is strong associated with the imbalance in glucose and insulin homeostasis.[71] In the present study, cafeteria diet fed mice showed abnormally increased blood glucose levels. Treatment with simvastatin and AEFPIR markedly brought down the levels of blood glucose suggesting its anti-hyperglycemic effect. Obesity is associated with an unfavourable lipid profile. Lipid abnormalities related to obesity include an elevated serum concentration of triglycerides, cholesterol, LDL and VLDL as well as a reduction in serum HDL.[72] It is shown that cafeteria diet elevates serum triglyceride levels essentially by preventing its uptake and clearance by inhibiting catabolising enzymes like lipoprotein lipase (LPL) and lecithin cholesterol acetyl transferase.[73] Cafeteria diet induced hypercholesterolemia has been related to its ability to alter the physico-chemical properties of lipoproteins and thereby prevent their uptake by the liver for clearance.[74] High fat diet increases both LDL-cholesterol and oxidative stress that results in increased oxidized LDL levels leading to atherosclerotic plaque formation.[75] Moreover, cafeteria diet decreases the levels of HDL, which is considered to be the good cholesterol that is anti-atherogenic in nature.[76] The above findings were in supportive with present results as we have observed, the cafeteria diet fed mice treated with simvastatin, AEFPIR (400 mg/kg) groups showed a significant ($P < 0.001$) decrease in the levels of serum glucose, triglycerides, total cholesterol, LDL, VLDL and significant ($P < 0.001$) increase in the levels of HDL when compared with cafeteria diet group is shown in Table 4. The reduction in triglycerides level is due to an increase in activity of endothelium bound LPL, which hydrolyzes the triglyceride into fatty acids or may be due to an inhibition of peripheral lipolysis so that fatty acids are not released and get converted into triglyceride.[77] The decline in VLDL levels in treated groups could be directly correlated to a decline in triglyceride levels as it is well-established that VLDL particles are the main transporters of triglycerides in plasma.[78] A decrease in serum cholesterol and LDL level is due to decreased cholesterol absorption from the intestine by binding with bile acids within the intestine and increasing faecal bile acids excretion.[79] Tannins present in *P. integrifolia* have been reported to increase faecal bile acid excretion, thereby leading to reduction in cholesterol levels.[80] Elevated levels of HDL helps to carry cholesterol back to the liver, where it gets excreted as bile salts.[81] In our present study, mice fed with AEFPIR were found to significantly increase serum HDL levels suggesting its cardioprotective nature. The increased level of HDL observed in AEFPIR compare to the cafeteria diet group

Table 2: Effect of aqueous enriched fraction of *P. integrifolia* root on BMI

Treatment groups	Initial BMI (g/cm²)	Final BMI (g/cm²)
Cafeteria diet	0.471±0.047	0.675±0.588
Cafeteria diet+simvastatin	0.428±0.042	0.408±0.355[c]
Cafeteria diet+AEFPIR (200 mg/kg)	0.467±0.046	0.511±0.445[b]
Cafeteria diet+AEFPIR (400 mg/kg)	0.465±0.046	0.451±0.393[c]

All values are expressed as a mean±SEM, n=6. AEFPIR – Aqueous enriched fraction of *P. integrifolia* root; SEM – Standard error of the mean; BMI – Body mass index; *P. integrifolia* – *Premna integrifolia*. [b]P<0.01; [c]P<0.001 compared with cafeteria diet group

Table 1: Effect of aqueous enriched fraction of *P. integrifolia* root on body weight

Treatment groups	Days and body weight (g)				
	1 day	10 days	20 days	30 days	40 days
Cafeteria diet	26.51±2.63	28.77±2.85	29.16±2.88	31.92±3.17	33.12±3.29
Cafeteria diet+Simvastatin	25.78±2.55	25.23±2.50[a]	24.19±2.38[b]	22.12±2.19[c]	20.01±1.96[c]
Cafeteria diet+AEFPIR (200 mg/kg)	26.28±2.59	26.78±2.64	26.03±2.57[a]	25.80±2.55[b]	25.05±2.42[c]
Cafeteria diet+AEFPIR (400 mg/kg)	26.19±2.58	26.99±2.66	25.49±2.51[a]	24.39±2.41[c]	22.12±2.18[c]

All values are expressed as a mean±SEM, n=6. AEFPIR – Aqueous enriched fraction of *P. integrifolia* root; SEM – Standard error of the mean; *P. integrifolia* – *Premna integrifolia*. [a]P<0.05; [b]P<0.01; [c]P<0.001 compared with cafeteria diet group

Table 3: Effect of aqueous enriched fraction of *P. integrifolia* root on food consumption

Treatment groups	Days and food consumption (g)				
	1 day	10 day	20 day	30 day	40 day
Cafeteria diet	17.3±1.69	14.1±1.34	12.9±1.25	10.8±1.05	10.1±0.98
Cafeteria diet+simvastatin	18.4±1.79	13.7±1.28	11.8±1.13	8.92±0.89[b]	5.23±0.49[c]
Cafeteria diet+AEFPIR (200 mg/kg)	17.9±1.74	14.2±1.38	13.9±1.34	12.1±1.19[a]	8.36±0.83[b]
Cafeteria diet+AEFPIR (400 mg/kg)	18.2±1.78	13.9±1.29	12.1±1.17	10.2±1.02[a]	7.31±0.70[c]

All values are expressed as a mean±SEM, *n*=6. AEFPIR – Aqueous enriched fraction of *P. integrifolia* root; *P. integrifolia* – *Premna integrifolia*; SEM – Standard error of the mean. [a]*P*<0.05; [b]*P*<0.01; [c]*P*<0.001 compared with cafeteria diet group

Table 4: Effect of aqueous enriched fraction of *P. integrifolia* root on serum lipid profile

Treatment groups	Glucose (mg/dl)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Cafeteria diet	95.3±9.1	99.8±9.4	193.9±19.1	26.8±2.4	147.2±14.4	19.9±1.8
Cafeteria diet+simvastatin	74.3±6.9[c]	71.1±6.9[c]	171.4±16.9[c]	36.6±3.2[c]	120.6±11.9[c]	14.2±1.3[c]
Cafeteria diet+AEFPIR (200 mg/kg)	79.5±7.6[b]	76.6±7.4[b]	178.3±17.6[c]	30.6±2.9[b]	132.4±13.1[b]	15.3±1.5[b]
Cafeteria diet+AEFPIR (400 mg/kg)	75.9±7.1[c]	73.3±7.1[c]	176.2±17.4[c]	35.2±3.3[c]	126.4±12.3[c]	14.6±1.4[c]

All values are expressed as a mean±SEM, *n*=6. AEFPIR – Aqueous enriched fraction of *P. integrifolia* root; HDL – High density lipoprotein; LDL – Low density lipoprotein; VLDL – Very low density lipoprotein; *P. integrifolia* – *Premna integrifolia*; SEM – Standard error of the mean. [a]*P*<0.05; [b]*P*<0.01; [c]*P*<0.001 compared with cafeteria diet group

may be due to increase the activity of lecithin cholesterol acyl transferase (An enzyme which incorporates free cholesterol from LDL into HDL and transfer it back to VLDL and intermediate density lipoprotein).[77] Figure 3 shows the histopathological changes in liver of cafeteria diet, simvastatin and AEFPIR (200 and 400 mg/kg) treated groups of mice. The liver section of the cafeteria diet mice showed fatty changes, i.e., predominantly intracellular fats deposition [Figure 3a]. Simvastatin treated mice liver showed sparse microvesicular fatty changes [Figure 3b]. Mice treated with 200 mg/kg AEFPIR showed few irregular intracellular fat depositions [Figure 3c] and 400 mg/kg treated mice showed thin microvesicular fatty changes along with restoration of normal architecture [Figure 3d].

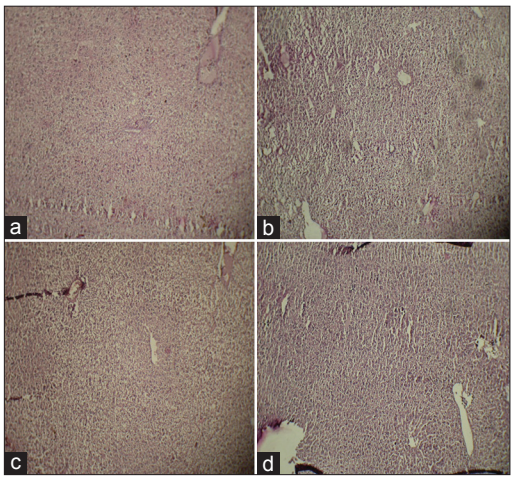


Figure 3: Histopathological assessment of aqueous enriched fraction of *Premna integrifolia* root in experimental group of mice; (a) Cafeteria diet treated mice liver showing fatty changes, i.e., predominantly intracellular fats deposition (×40); (b) Simvastatin treated mice liver showing sparse micro-vesicular fatty changes (×40); (c) Mice treated with aqueous enriched fractions of *Premna integrifolia* root at dose 200 mg/kg showed few irregular intracellular fat depositions (×40); (d) 400 mg/kg showed thin micro-vesicular fatty changes along with restoration of normal architecture (×40)

CONCLUSION

Hence, it can be concluded that these observations are evidence for marked protective effect of AEFPIR against cafeteria diet induced obesity. Most of the isolated compounds of *P. integrifolia* need to explore its reported pharmacological activities, which provide scope for further pharmacological studies. Exploration of chemical constituents and further pharmacological evaluation will give us basis for its therapeutic use. However, further series of studies are required to prove its clinical reliability, safety and efficacy.

ACKNOWLEDGMENT

The author wish to thank the Principal, Radharaman College of Pharmacy, Ratibad, Bhopal, Madhya Pradesh, India for encouragement and availing of the laboratory facilities during the course of investigation.

REFERENCES

1. Chunekar KC. Illustrated Dravyaguna Vijnana. 2nd ed. Vol. 2. Varanasi: Chaukhambha Orientalia; 2005. p. 418-21.
2. Warrier PK, Nambiar VP. Indian Medicinal Plants. Madras: Orient Longman; 1997. p. 348-52.
3. Mali PY, Bigoniya P, Panchal SS, Muchhandi IS. Anti-obesity activity of chloroform-methanol extract of *Premna integrifolia* in mice fed with cafeteria diet. J Pharm Bioallied Sci 2013;5:229-36.
4. George KV, Samuel KA, Abraham J, George BP. Investigations on bioactive compounds of *Premna integrifolia* Linn, 2008. [About 8 pages]. Available from: http://www.forestry.caf.wvu.edu/wvu_woodscience/bdawson/web_bibu/Premna.pdf. [Last cited on 2008 Feb 20].
5. Basu NK, Dandiya PC. Chemical investigation of *Premna integrifolia* Linn. J Am Pharm Assoc Am Pharm Assoc 1947;36:389-91.

6. Basu NK, Joneja AN. Chemical investigation of *Premna integrifolia*. Indian J Pharm 1949;11:191-3.
7. Barik BR, Bhowmik AK, Dey AK, Patra A, Chatterjee A, Joy S, *et al*. Premnazole, an isoxazole alkaloid of *Premna integrifolia* and *Gmelina arborea* with anti-inflammatory activity. Fitoterapia 1992;63:295-9.
8. Gokani RH, Kapadiya NS, Shah MB. Comparative pharmacognostic study of *Clerodendrum phlomidis* and *Premna integrifolia*. J Nat Remed 2008;8:222-31.
9. Gokani RH, Shah MB. Isolation and estimation of *Clerodendrum phlomidis* and *Premna integrifolia* root. J Pharm Res 2009;8:9-11.
10. Singh CR, Nelson P. Muthu K, Pargavi B. Identification of volatile constituents from *Premna serratifolia* L. through GC-MS. Int J Pharm Tech Res 2011;3:1050-8.
11. Singh CR, Nelson P, Boopathy S. *In-vitro* conservation and protective effect of *Premna serratifolia* Linn.: An important medicinal tree. Int J Pharm Appl 2012;3:332-43.
12. Yadav D, Tiwari N, Gupta MM. Diterpenoids from *Premna integrifolia*. Phytochem Lett 2010;3:143-7.
13. Yadav D, Tiwari N, Gupta MM. Simultaneous quantification of diterpenoids in *Premna integrifolia* using a validated HPTLC method. J Sep Sci 2011;34:286-91.
14. Bose LV, Varghese GK, Habtemariam S. Identification of acteoside as the active antioxidant principle of *Premna serratifolia* root wood tissues. Phytopharmacology 2013;4:228-36.
15. Mali PY, Bhadane VV. Comparative account of screening of bioactive ingredients of *Premna integrifolia* Linn. with special reference to root by using various solvents. J Pharm Res 2010;3:1677-9.
16. Alamgir M, Rokeya B, Hannan JM, Choudhuri MS. The effect of *Premna integrifolia* Linn. (*Verbenaceae*) on blood glucose in streptozotocin induced type 1 and type 2 diabetic rats. Pharmazie 2001;56:903-4.
17. Kar A, Choudhary BK, Bandyopadhyay NG. Comparative evaluation of hypoglycaemic activity of some Indian medicinal plants in alloxan diabetic rats. J Ethnopharmacol 2003;84:105-8.
18. Dash GK, Patro CP, Maiti AK. A study on the antihyperglycaemic effect of *Premna corymbosa* Rottl. roots. J Nat Remed 2005;5:31-4.
19. Rathore RS, Prakash A, Singh PP. Preliminary study of anti-inflammatory and anti-arthritic activity. Rheumatism 1997;12:130.
20. Sasidharan N. Qualitative and quantitative analysis of biologically active principles, baicalein, luteolin and psoralen from *Oroxylum indicum, Premna serratifolia, Aegle marmelos* and their allied species. KFRI Research Report No. 350 (KFRI/486/05). Peechi: Kerala Forest Research Institute; 2010. p. 1-57.
21. Gokani RH, Lahiri SK, Santani DD, Shah MB. Evaluation of immunomodulatory activity of *Clerodendrum phlomidis* and *Premna integrifolia* root. Int J Pharmacol 2007;3:352-6.
22. Rajendran R, Suseela L, Meenakshi SR, Saleem BN. Cardiac stimulant activity of bark and wood of *Premna integrifolia*. Bangladesh J Pharmacol 2008;3:107-13.
23. Rajendran R, Basha NS. Cardioprotective effect of ethanol extract of stem-bark and stem-wood of *Premna serratifolia* Linn. (*Verbenaceae*). Res J Pharm Technol 2008;1:487-91.
24. Kumar KU, Soma P, Kumar SS, Chandra SM, Kumar BS. Assessment of analgesic and antibacterial activity of *Premna integrifolia* Linn. (Family: *Verbenaceae*) leaves. Int J Pharm Sci Res 2011;2:1430-5.
25. Singh CR. Antimicrobial effect of callus and natural plant extracts of *Premna serratifolia* L. Int J Pharma Biomed Res 2011;2:17-20.
26. Rajendran R, Basha NS. Antimicrobial activity of crude extracts and fractions of *Premna serratifolia* Linn. root. Med Plants 2010;2:33-8.
27. Rajendran R. Antimicrobial activity of different bark and wood of *Premna serratifolia* Linn. Int J Pharma Biosci 2010;6:1-9.
28. Rajendran R, Krishnakumar E. Anti-arthritic activity of *Premna serratifolia* Linn., wood against adjuvant induced arthritis. Avicenna J Med Biotechnol 2010;2:101-6.
29. Singh CR, Nelson R, Krishnan PM, Mahesh K. Hepatoprotective and anti-oxidant effect of root and root callus extract of *Premna serratifolia* L. in paracetamol induced liver damage in male albino rats. Int J Pharma Biosci 2011;2:244-52.
30. Selvam NT, Vengatakrishnan V, Damodar KS, Murugesan S. Evaluation of tissue level antioxidant activity of *Premna serratifolia* leaf in paracetamol intoxicated Wistar albino rats. Int J Pharm Life Sci 2010;1:86-90.
31. Vadivu R, Jerad S, Girinath K, Kannan PB, Vimala R, Kumar NM. Evaluation of hepatoprotective and *in-vitro* cytotoxic activity of leaves of *Premna serratifolia* Linn. J Sci Res 2009;1:145-52.
32. Selvam TN, Venkatakrishnan V, Damodar KS, Elumalai P. Antioxidant and tumor cell suppression potential of *Premna serratifolia* linn leaf. Toxicol Int 2012;19:31-4.
33. Bagchi C, Tripathi SK, Hazra A, Bhattacharya D. Evaluation of hypolipidemic activity of *Premna integrifolia* Linn. Bark in rabbit model. Pharmbit 2008;18:149-53.
34. Karthikeyan M, Deepa MK. Anti-hyperlipidemic activity of *Premna corymbosa* (Burm.f.) Rottl. and Willd. in liver damaged Wistar albino rats. J Pharm Res 2008;1:61-4.
35. Patel MJ, Patel JK, Evaluation of the anti-hyperlipidemic activity of *Premna integrifolia* using experimental animal model. Int J Res Phytochem Pharmacol 2011;1:146-9.
36. Patel MJ, Patel JK. Evaluation of the anti-hyperlipidaemic activity of *Premna integrifolia* on nicotine induced hyperlipidaemia in rats. Int J Pharma Biosci 2012;3:226-32.
37. Banu UK. Evaluation of neuroprotective effect in the experimental model of febrile seizure of the roots of *Premna serratifolia* Linn. International Herbal Conference. Banglore, 2009. (Poster No. SNPS IHCB-09781). Available from: http://www.herbalconference2009.com. [Last cited on 2013 Aug 29].
38. Desrivot J, Waikedre J, Cabalion P, Herrenknecht C, Bories C, Hocquemiller R, *et al*. Antiparasitic activity of some New Caledonian medicinal plants. J Ethnopharmacol 2007;112:7-12.
39. Yadav D, Masood N, Luqman S, Brindha P, Gupta MM. Antioxidant furofuran lignans from *Premna integrifolia*. Industrial Crops Prod 2013;41:397-02.
40. Gokani RH, Lahiri SK, Santani DD, Shah MB. Evaluation of *in-vitro* anti-oxidant activity of *Premna integrifolia* Linn. root. Res J Pharmacog Phytochem 2010;2:196-9.
41. Gopal RH, Purushothaman KK. Effects of plant isolates on coagulation of blood: An *in-vitro* study. Bull Med Ethnobot Res 1984;5:171-7.
42. Sridharan G, Brindha P, Jaiganesh C, Sivasubramanian S. Anti tumor potential of *Premna integrifolia* Linn. against Ehrlich ascites carcinoma cell lines. Pharmacologyonline 2011;2:438-50.
43. Jothi ET, Karthikeyan R, Suryalakshmi PV, Srinivasababu P. Gastroprotective potential of *Premna serratifolia* Linn. leaves against aspirin induced ulcer in albino rats. Pharmacologyonline 2010;3:189-98.
44. Shukla V, Yadav D, Phulara SC, Gupta MM, Saikia SK, Pandey R. Longevity-promoting effects of 4-hydroxy-E-globularinin in *Caenorhabditis elegans*. Free Radic Biol Med 2012;53:1848-56.
45. Parle M, Vasudevan M. Memory enhancing activity of Abana: An Indian ayurvedic poly herbal formulation. J Health Sci 2007;53:43-52.
46. Jabbar S, Khan MT, Choudhuri MS, Sil BK. Bioactivity studies of the individual ingredients of the *Dashamularishta*. Pak J Pharm Sci 2004;17:9-17.
47. Khanna AK, Chander R, Kapoor NK. Hypolipidaemic activity of Abana in rats. Fitoterapia 1991;3:271.
48. Flier JS. Obesity wars: Molecular progress confronts an expanding epidemic. Cell 2004;116:337-50.

49. Haslam DW, James WP. Obesity. Lancet 2005;366:1197-209.
50. Hall JE, Crook ED, Jones DW, Wofford MR, Dubbert PM. Mechanisms of obesity-associated cardiovascular and renal disease. Am J Med Sci 2002;324:127-37.
51. Sowers JR. Update on the cardiometabolic syndrome. Clin Cornerstone 2001;4:17-23.
52. World Health Organization. Obesity: Preventing and managing the global epidemic. Report of a WHO Consultation on Obesity. Geneva: WHO; 1998.
53. Nammi S, Koka S, Chinnala KM, Boini KM. Obesity: An overview on its current perspectives and treatment options. Nutr J 2004;3:3.
54. Jindal V, Dhingra D, Sharma S, Parle M, Harna RK. Hypolipidemic and weight reducing activity of the ethanolic extract of *Tamarindus indica* fruit pulp in cafeteria diet- and sulpiride-induced obese rats. J Pharmacol Pharmacother 2011;2:80-4.
55. Flegal KM, Graubard BI, Williamson DF, Gail MH. Cause-specific excess deaths associated with underweight, overweight, and obesity. JAMA 2007;298:2028-37.
56. Eckel RH. Clinical practice. Nonsurgical management of obesity in adults. N Engl J Med 2008;358:1941-50.
57. Phani KG, Kumar R, Badere R, Singh SB. Antibacterial and antioxidant activities of ethanol extracts from trans Himalayan medicinal plants. Pharmacog J 2010;2:66-9.
58. Organization for Economic Co-operation and Development (OECD) Guideline for Testing of Chemicals. Acute oral toxicity-Up and down procedure. OECD Test Guideline No. 425., 2008. p. 1-27. Available from, http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECDtg425.pdf. [Last cited on 2013 Sep 10].
59. Organization for Economic Co-operation and Development (OECD) Guideline for Testing of Chemicals. Acute Oral Toxicity – Fixed Dose Procedure. OECD Test Guideline No. 420. 2001. p. 1-14. Available from, http://iccvam.niehs.nih.gov/SuppDocs/FedDocs/OECD/OECD_GL420.pdf. [Last cited on 2013 Sep 10].
60. Kaur G, Kulkarni SK. Anti-obesity effect of a polyherbal formulation, OB200G in female rats fed on cafeteria and atherogenic diets. Indian J Pharmacol 2000;32:294-9.
61. Harris RB. The impact of high- or low-fat cafeteria foods on nutrient intake and growth of rats consuming a diet containing 30% energy as fat. Int J Obes Relat Metab Disord 1993;17:307-15.
62. Gallou-Kabani C, Vigé A, Gross MS, Boileau C, Rabes JP, Fruchart-Najib J, *et al*. Resistance to high-fat diet in the female progeny of obese mice fed a control diet during the periconceptual, gestation, and lactation periods. Am J Physiol Endocrinol Metab 2007;292:E1095-100.
63. Auzi AR, Hawisa NT, Sherif FM, Sarker SD. Neuropharmacological properties of *Launaea resedifolia*. Braz J Pharmacol 2007;17:160-5.
64. Basarkar PW, Nath N. Hypocholesterolemic and hypolipidemic activity of quercetin: A vitamin P-like compound in rats. Indian J Med Res 1983;77:122-6.
65. Sclafani A, Springer D. Dietary obesity in adult rats: Similarities to hypothalamic and human obesity syndromes. Physiol Behav 1976;17:461-71.
66. Bull NL. Studies of the dietary habits, food consumption and nutrient intakes of adolescents and young adults. World Rev Nutr Diet 1988;57:24-74.
67. Rothwell NJ, Stock MJ, Warwick BP. The effect of high fat and high carbohydrate cafeteria diets on diet-induced thermogenesis in the rat. Int J Obes 1983;7:263-70.
68. Naim M, Brand JG, Kare MR, Carpenter RG. Energy intake, weight gain and fat deposition in rats fed flavored, nutritionally controlled diets in a multichoice ("cafeteria") design. J Nutr 1985;115:1447-58.
69. Barr HG, McCraken KJ. High efficiency of energy utilization in "cafeteria-" and force-fed rats kept at 29 degrees. Br J Nutr 1984;51:379-87.
70. Boden G. Role of fatty acids in the pathogenesis of insulin resistance and NIDDM. Diabetes 1997;46:3-10.
71. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: Direct role in obesity-linked insulin resistance. Science 1993;259:87-91.
72. Grundy SM, Barnett JP. Metabolic and health complications of obesity. Dis Mon 1990;36:641-731.
73. Campillo JE, Torres MD, Dominguez E, Romeroa A, Perrez C. *Ficus carica* lead administration reduces hypertriglyceridemia in streptozotocin diabetic rats. Diabetologia 1994;37:213.
74. Byers SO, Fiedman M, Sugiyama T. Triton hypercholesteremia: Cause or consequence of augmented cholesterol synthesis. Am J Physiol 1963;204:1100-2.
75. Warnholtz A, Mollnau H, Oelze M, Wendt M, Münzel T. Antioxidants and endothelial dysfunction in hyperlipidemia. Curr Hypertens Rep 2001;3:53-60.
76. Muthu AK, Sethupathy S, Manavalan R, Karar PK. Effect of methanolic extract of *Dolichos biflorus* Linn. on lipid profile of rabbits fed with high fat diet. Adv Pharmacol Toxicol 2007;8:19-24.
77. Tenpe CR, Thakare AB, Upaganlawar AB, Yeole PG. Hypolipidemic and weight-controlling activity of *Terminalia catappa* Linn. in rats on sucrose-high fat diet. Indian Drugs 2007;44:16-20.
78. Howell TJ, MacDougall DE, Jones PJ. Phytosterols partially explain differences in cholesterol metabolism caused by corn or olive oil feeding. J Lipid Res 1998;39:892-900.
79. Lemhadri A, Hajji L, Michel JB, Eddouks M. Cholesterol and triglycerides lowering activities of caraway fruits in normal and streptozotocin diabetic rats. J Ethnopharmacol 2006;106:321-6.
80. Johns T, Chapman LI, Amason JT, Mata R, Romeo JT. Phytochemistry of Medicinal Plants. 3rd ed. New York: Kluwer Academic Publishers; 1995.
81. Tan MH, Johns D, Glazer NB. Pioglitazone reduces atherogenic index of plasma in patients with type 2 diabetes. Clin Chem 2004;50:1184-8.

How to cite this article: Mali PY. Effect of aqueous enriched fraction of *Premna integrifolia* root against cafeteria diet induced obesity in Swiss albino mice. Int J Green Pharm 2013;7:315-21.

Source of Support: Nil, **Conflict of Interest:** None declared.