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Original research

Protective effect of *Betula utilis* bark extract on high fat diet induced obesity in Wistar rats

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ABSTRACT

Background: The metabolic complications due to increased incidence of obesity, is now recognized as a major public health problem. In current era herbal medicine has been recognised as adjuvant therapy for the treatment of several clinical implications.

Objectives: The current investigation is aimed to explore the anti-obesity effects pertaining to *Betula utilis* (BU) ethanolic extract in high-fat diet (HFD) induced obesity and hyperlipidemic rat model.

Methods: Experimental obesity was developed in the male rats by administering HFD for 10 weeks. Initial and final body weight, Lee index, Body mass index (BMI), fat pads weight, serum glucose, triglyceride (TG), total cholesterol (TC) and various lipoproteins were estimated in the current study.

Results: After four week of HFD treatment, BU (100–400 mg/kg/day *p.o*) was given along with high fat diet for further 6 weeks which significantly reduced HFD induced body weight gain and increase in adipose tissue mass in a dose dependant manner. Moreover, BU attenuated HFD induced augmented serum glucose, TG and TC. The anti-obesity potential of BU was comparable to a well established marketed drug orlistat.

Conclusion: These results reflect that BU supplementation decreases body weight, improves obesity serum biomarkers (TG, TC, LDL), and the weight reducing activity of BU may be mediated by decreased fat absorption from the GIT.

1. Introduction

Obesity has turn into a major health dilemma, affecting people across all ages, sex, and races and its occurrence has been growing at frightening rate (Verma and Paraidathathu, 2014). It is a pathological state in which surplus fat has been accumulated in the body to the extent it may have deleterious effects on health. Persons are normally contemplated obese if their BMI is more than 30 kg/m^2 and considered overweighed when their BMI ranges 25–30 kg/m^2 (Naila, 2017). Obesity is the chief health concern in terms of type 2 diabetes, cardiac problems, neoplasms and also in financial load to healthcare providers (Haslam and James, 2005). Obesity is the deleterious outcome of imbalance between calorie intake and calorie expenditure, i.e. consumption of extra calories than its utilization, leading to storage of excess fat in the body. Many factors prompt an individual to develop obesity, e.g. easily accessible high-calorie food and inactive life habits and heredity also add to this disparity (Bloom et al., 2008). There are a variety of treatment choices for obese people, that comprise therapeutic agents which can either reduce calorie intake or increase calorie expenditure, in the extreme cases surgical intrusion may be vital. At present, just a few FDA-approved anti-obesity drugs like orlistat, lorcaserin, phentermine-topiramate and naltrexone-bupropion are on hand, but they have remarkable adverse effects (Haslam and James, 2005). Therapeutic interventions given for long-term are largely ineffective as they cause several adverse effects (Adnyana et al., 2014). Most of the anti-obesity drugs had been withdrawn from the market due to their deleterious effects on health in long term therapy (Chandrasekaran et al., 2012). On the contrary, herbal medicines are safer, efficacious and cost effective which make them a drug of choice in today's era for treating various ailments.

Betula utilis (BU) which is mainly known as birch tree and found in great himalayas, at an altitude of 4,500 m, possess broad therapeutic properties and is used in inflammation, cancer, microbial infection, oxidative stress, HIV etc (Safdar et al., 2017). Pentacyclic triterpenoids, betulin, betulic acid, oleanolic acid, lupenone, acetyloleanolic acid, methyl betulate and karachic acid are responsible for its medicinally important activities (Singh et al., 2012). Pentacyclic triterpenoids reduce triglyceride formation through the inhibition of DGAT which is an enzyme that catalyses the final reaction of triglyceride synthesis (Chung et al., 2006) which is a contributing factor in obesity. BU is rich in

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pentacyclic triterpenoids thus it might play a potential role in the management of overweight and associated comorbidities. Based on this assumption the investigator has designed the present study to explore the beneficial effect of BU bark in rat model of experimental obesity.

2. Materials and methods

2.1. Drugs and chemicals

HFD was prepared using casein (Anjan Enterprises, Amritsar, India) and cholesterol (Thomas Baker, Mumbai, India). Orlistat (Sigma Aldrich, India) was used as standard control. The biochemical estimation for serum glucose, TC, TG and HDL was done by commercially available kits, obtained from Reckon Diagnostics Pvt Ltd., Vadodara, India. All other chemicals used in the present study were of analytical quality. All drug solutions were freshly prepared before use.

2.2. Plant extraction

The powdered bark of BU (VHCA herbals, Karnal, India) was defatted with petroleum ether. Thus obtained powder was further extracted in the soxhlet apparatus for 72 h (40 °C) with 95% ethanol. The final extract was vacuum concentrated to get a yellowish brown residue which was placed in desiccator for drying (Kolodziej, 1990; Shuang et al., 1998).

2.3. Phytochemical screening

Various screening methods were used for preliminary phytochemical screening of BU. The identification of important phytochemicals such as triterpenoids carbohydrates, flavonoids, alkaloids, tannins, saponins, glycosides, protein and steroids was done using vacuum dried plant extract (Khandelwal and Sethi, 2014; Kokate, 2014).

2.4. Preparation of high fat diet

Obesity was induced in rats by HFD feeding for 10 weeks and prepared by using following components: powdered normal chow, 365 g; lard, 310 g; casein, 250 g; cholesterol, 10 g; vitamin mix and mineral mix, 60 g; dl-methionine, 03 g; yeast powder, 01 g; NaCl, 01 g for 1.0 kg of diet (Srinivasan et al., 2005). The high fat diet contained 5.33 kcal/g while the normal chow contains 3.80 Kcal/gm.

2.5. Animal treatments

Male Wistar rats having 7–8 weeks of age were used to develop experimental obesity and procured from the central animal house of the Institute. Standard polypropylene cages (two rats/cage) were used to place the animals and these animals were maintained under 25 ± 2 °C temperature with 12:12 h light and dark cycle. The permission was taken from the institutional animal ethics committee for conducting the study with approval number IAEC/CGC/20/11. The prescribed guidelines of committee for the purpose of control and supervision of experiments on animals (CPCSEA), Govt. of India were followed throughout the experimental period. Animals were divided in the beginning of the study as per their body weight and alienated into various groups (n = 6). Normal control (NC) group was fed normal chow, BU *per se* was administered with BU 400 mg/kg/day to normal chow fed rats and high fat diet (HFD) control group was fed high-fat diet for 10 weeks. The low BU, medium BU and high BU groups were administered with BU at a dose of 100, 200 and 400 mg/kg/day *p.o.* respectively, along with high fat diet for 6 weeks to the 4 week treated high fat diet rats. The orlistat group was administered with orlistat at a dose of 30 mg/kg/day *p.o.* along with high fat diet for 6 weeks to the 4 week treated high fat diet rats. The oral route was used for the administration of BU and orlistat. Animals had free access to water throughout the day and were inspected once a day. Body weight and food intake of animals were measured biweekly. Lee index (Bernardis and Bellinger, 1982) and body mass index (Novellie et al., 2007) were calculated using standard formulas on the completion of 10 weeks. At the end of the protocol i.e after 10 weeks, blood was withdrawn by retro-orbital puncture under light ether anaesthesia for estimation of various biochemical parameters and then animals were sacrificed by cervical dislocation for adipose tissue collection. The blood was collected in eppendorf tubes, serum was separated using centrifuge and analyzed using commercially available biochemical kits. The retroperitoneal present near kidneys, mesenteric present in the abdomen and epididymal fat present around testes were dissected, weighed and stored in formalin solution for further histological analysis (Ainslie et al., 2000).

2.6. Histological analysis and morphometry

The stored fat pads which were collected from animals at the end of the study were used for histological analysis. The epididymal fat pad was embedded in paraffin. The microscopic slides were prepared using tissue sections (10 μm). The slides were stained with hematoxylin and eosin and photographed at 100X magnification for further analysis.

2.7. Measurements

Serum glucose, TG, TC and HDL major markers of obesity were analyzed through standard procedures prescribed as per commercially available kits. LDL and VLDL were calculated using formula given by friedewald (Friedewald et al., 1972). Atherogenic index and coronary risk index (CRI) markers of comorbidities associated with obesity were calculated using following formula (Abbott et al., 1988).

$$\text{Atherogenic Index (AI)} = \frac{\text{LDL} - \text{Cholesterol}}{\text{HDL} - \text{cholesterol}}$$

$$\text{Coronary Risk Index} = \frac{\text{Total cholesterol}}{\text{HDL} - \text{cholesterol}}$$

The gravimetrical method was used to determine the fat content in feces. The samples of feces were dried at 105 °C for 12 h and then extracted with petroleum ether under reflux (Alladi and Shanmugasundaram, 1989).

2.8. Statistics

Data are presented as mean ± S.D. The graph pad prism software was used. Each group was compared with other group by one way ANOVA with a Tukey's post hoc test. The p value < 0.05 was considered to be statistically significant.

3. Results

3.1. Phytochemical screening

The ethanolic extract of BU was found to be positive for the presence of triterpenoids, alkaloids, carbohydrates, saponins, and steroids (Table 1).

3.2. Change in body weight, feed intake and fecal fat content

In HFD rats, a significant ($p < 0.05$) increase in body weight, feed intake (Kcal) was observed as compared to the NC rats fed on standard diet. Orlistat which was positive control in the present study decreases body weight and feed intake (Kcal) as compared to HFD rats. Administration of BU in three doses (400, 200 and 100 mg/kg) showed significant ($p < 0.05$) reduction in body weight and feed intake (Kcal) in dose dependant fashion as compared to the HFD rats and the result was comparable to the orlistat group. There was no significant *per se*

Table 1
Preliminary phytochemical analysis of BU.

Phytoconstituents	Ethanolic extract of BU
Triterpenoids	+ve
Alkaloids	+ve
Carbohydrates	+ve
Steroids	+ve
Saponins	+ve
Glycosides	-ve
Tannins	-ve
Protein	-ve

+ve represents present & -ve represents absent.

effect of BU. However, average daily fecal fat content of rats administered with BU were significantly increased compared to rats fed on HFD in a dose dependant manner indicating reduced apparent fat absorption. The fecal fat content of orlistat administered rats were also significantly increased compared to HFD rats (Table 2).

3.3. Change in Lee index and body mass index (BMI)

In HFD rats a significant ($p < 0.05$) rise in Lee index and body mass index (BMI) was observed as compared to NC rats. Administration of BU in three doses (400, 200 and 100 mg/kg) showed significant ($p < 0.05$) reduction in Lee index and BMI in dose dependent fashion compared to the HFD group and the outcome was comparable to the orlistat group. There was no significant *per se* effect of BU (Table 2).

3.4. Change in serum biochemical parameters

High fat diet induces significant ($p < 0.05$) elevation of serum glucose, TC, TG, LDL, VLDL and decrease in HDL levels as compared to NC rats. Orlistat in the present study altered these biochemical parameters. Treatment of HFD rats with BU in three doses (400, 200 and 100 mg/kg/day) showed significant ($p < 0.05$) reduction in serum TC, TG, LDL, VLDL levels and significant increase HDL levels in dose dependent manner as compared to HFD rats. These outcomes were comparable to the orlistat group. There was no *per se* effect of BU (Table 3). Orlistat (30 mg/kg/day) and BU in three doses (100, 200 & 400 mg/kg/day) significantly ($p < 0.05$) decreases atherogenic and coronary risk index as compared to HFD rats (Table 3).

3.5. Change in weight of different fat depots

HFD treated rats exhibited increased fat pad weight: epididymal, retroperitoneal, mesenteric fat pads. Treatment with BU in three doses showed significant ($p < 0.05$) reduction in body fat pad weight in dose dependent manner as compared to HFD control. There was no significant *per se* effect of BU (Table 4).

3.6. Change in adipocytes size

In HFD treated rats significant increase in size of adipocytes (epididymal fat depot) as compared to rats fed on normal diet was seen. Three doses of BU (400, 200 and 100 mg/kg/day) and orlistat significantly decreased ($p < 0.05$) the size of adipocytes (Fig. 1).

4. Discussion

The current research was intended to investigate the effect of BU bark on HFD treated Wistar rats. We experimentally established that BU has encouraging effects in the modification of various parameters of obesity.

Obesity is linked with in augmented morbidity, mortality, impaired quality of life and huge healthcare costs (Rodgers et al., 2012). HFD induced experimental obesity has been regarded as useful model among scientific community due to its high reliability and reproducibility. In this study 10 weeks of HFD administration leads to overweight, impaired lipidemia, increased food intake, BMI, Lee index. These effects are results of accelerated fat accumulation, impaired carbohydrate and fat metabolism.

In the current investigation BU in graded doses positively modulated the outcomes of HFD treatment. It was observed that BU (100 mg/kg, 200 mg/kg and 400 mg/kg) given from the start of fifth week to the end of tenth week along HFD treatment significantly lower the obesity markers in the dose dependent manner in comparison to HFD rats. BU at 400 mg/kg dose has shown better results than at its lower doses. It was observed that the fecal fat content of the BU treated rats increased in a dose dependant manner suggesting reduced apparent fat absorption. Dyslipidemia is an important parameter in the progression of obesity which leads to hyper-triglyceridemia, hyper-cholesterolemia and altered lipoproteinemia. (Buettner et al., 2007; Claudia et al., 2016). Long term dyslipidemia leads to development of cardiovascular comorbidities estimated by AI and CRI (Woods et al., 2003; Storlien et al., 1986). BU (100 mg/kg, 200 mg/kg and 400 mg/kg) as well as orlistat (positive control) administration to HFD rats causes not only reduction in body weight but also significantly reduced the TC, TG, VLDL, LDL and glucose levels in serum. Serum HDL level got enhanced in these groups. HFD consumption in Wistar rats leads to increased AI as well as CRI indicating the higher cardiovascular risk (Jen, 1988; Haley et al., 2013). On the other hand, BU and orlistat treatment leads to cardioprotection as indicated by lower AI and CRI. The present study is in accordance with numerous reports supporting the fact that in experimental animals, HFD consumption causes the impaired glucose metabolism resulting in hyperglycemia and increase in adipose tissue mass (Klop et al., 2013; Martins and Redgrave, 2004). However, in current investigation it was found that BU in graded doses significantly ($p < 0.05$) reduced the elevated blood glucose level and weight of various adipose tissues. The antiobesity activity of BU appears partly to be mediated by decreasing dietary fat absorption from intestine via inhibition of pancreatic lipase activity. Betulinic acid present in BU bark was reported to attenuate lipoprotein lipase activity which hydrolyzes the dietary fats catalyzing the first step in metabolism of lipids (Mbikay, 2012). Betulinic acid also exhibits DGAT inhibitory properties, (De Melo et al., 2009) it catalyzes the final reaction of triglyceride synthesis. Obesity is also characterised by accumulation of triglycerides

Table 2
Effects of BU on the body weight, BMI, Lee index, feed intake (Kcal) and fecal fat (g/day).

Parameters	Initial body weight (g)	Final body weight (g)	BMI (g/cm^2)	Lee index ($g^{1/3}/cm^* 1000$)	Feed Intake (Kcal)	Fecal fat (g/day)
NC	208.33 ± 14.7	264.16 ± 8.61	0.79 ± 0.04	352.05 ± 8.56	96.26 ± 13.30	0.08 ± 0.04
BU *per se*	215 ± 12.64	245 ± 12.64	0.82 ± 0.08	362.19 ± 17.05	80.43 ± 7.60	0.09 ± 0.02
HFD	214.66 ± 19.4[a]	358.5 ± 22.2[a]	1.18 ± 0.03[a]	408.86 ± 10.05[a]	119.32 ± 8.22[a]	0.28 ± 0.02[a]
HFD + Orlistat	210.8 ± 4.9[b]	250.33 ± 6.97[b]	0.75 ± 0.2[b]	350.05 ± 6.96[b]	85.81 ± 1.68[b]	0.56 ± 0.02[b]
HFD + BU (low)	210.83 ± 18.55[b]	305 ± 13.03[b]	0.97 ± 0.04[b]	379.78 ± 7.90[b]	90.78 ± 6.26[b]	0.44 ± 0.03[b]
HFD + BU (medium)	206.66 ± 10.3[b]	258.33 ± 8.16[b]	0.85 ± 0.04[b]	366.20 ± 8.81[b]	85.45 ± 3.85[b]	0.48 ± 0.03[b]
HFD + BU (High)	217.5 ± 6.12[b]	256.33 ± 11.6[b]	0.84 ± 0.07[b]	367.22 ± 14.44[b]	74.26 ± 1.67[b]	0.50 ± 0.02[b]

Data are expressed as Mean ± SD; [a] = $p < 0.05$ vs NC, [b] = $p < 0.05$ vs HFD control. Normal control: NC; high fat diet control: HFD; *Betula utilis:* BU.

Table 3
Effects of BU on the serum glucose and serum lipid profile.

Parameters	NC	BU *per se*	HFD	HFD + Orlistat	HFD + BU (low)	HFD + BU (medium)	HFD + BU (high)
GLU (mg/dl)	93.32 ± 3.08	102.16 ± 1.27	159.87 ± 2.56[a]	101.54 ± 1.06[b]	133.57 ± 1.45[b]	117.34 ± 2.10[b]	100.20 ± 1.32[b]
TC (mg/dl)	99.63 ± 0.82	97.16 ± 0.53	158.42 ± 4.03[a]	108.30 ± 2.15[b]	135.37 ± 1.73[b]	127.68 ± 2.05[b]	100.92 ± 0.84[b]
TG (mg/dl)	68.24 ± 3.67	61.37 ± 1.50	149.29 ± 1.85[a]	76.55 ± 1.22[b]	129.99 ± 1.43[b]	107.06 ± 1.03[b]	89.88 ± 1.58[b]
HDL (mg/dl)	32.27 ± 1.66	30.70 ± 0.91	21.29 ± 0.60[a]	33.29 ± 0.82[b]	23.08 ± 0.88[b]	29.07 ± 0.96[b]	31.29 ± 1.20[b]
VLDL (mg/dl)	13.64 ± 0.73	12.27 ± 0.30	29.85 ± 0.37[a]	15.31 ± 0.24[b]	25.99 ± 0.28[b]	21.30 ± 0.36[b]	17.97 ± 0.31[b]
LDL (mg/dl)	53.7 ± 1.90	54.18 ± 0.84	107.27 ± 3.66[a]	59.69 ± 2.40[b]	85.56 ± 2.12[b]	67.29 ± 2.38[b]	51.60 ± 1.65[b]
AI	1.67 ± 0.15	1.76 ± 0.08	5.04 ± 0.20[a]	1.79 ± 0.10[b]	3.60 ± 1.11[b]	2.31 ± 0.14[b]	1.65 ± 0.12[b]
CRI	3.09 ± 0.18	3.16 ± 0.09	7.44 ± 0.20[a]	3.25 ± 0.12[b]	5.69 ± 0.25[b]	4.05 ± 0.17[b]	3.22 ± 0.14[b]

Data are expressed as Mean ± SD; [a] = $p < 0.05$ vs Normal control, [b] = $p < 0.05$ vs HFD control. Normal control: NC; high fat diet control: HFD; *Betula utilis:* BU; Serum glucose: GLU; total cholesterol: TC; triglyceride: TG; high density lipoproteins: HDL; very low density lipoproteins: VLDL; low density lipoproteins: LDL; atherogenic index: AI; coronary risk index: CRI.

Table 4
Effects of BU on the different fat depots.

Parameters	MES	RET	EPI	TF
NC	2.74 ± 0.29	1.58 ± 0.31	1.92 ± 0.07	6.24 ± 0.4
BU*per se*	2.56 ± 0.22	2.31 ± 0.08	2.15 ± 0.01	7.02 ± 0.16
HFD	6.91 ± 0.38[a]	5.51 ± 0.45[a]	5.89 ± 0.56[a]	18.31 ± 1.3[a]
HFD + Orlistat	2.56 ± 0.54[b]	2.49 ± 0.39[b]	2.79 ± 0.31[b]	7.84 ± 0.12[b]
HFD + BU (low)	4.06 ± 0.21[b]	4.0 ± 0.23[b]	3.96 ± 0.28[b]	12.02 ± 0.04[b]
HFD + BU (medium)	3.51 ± 0.12[b]	3.19 ± 0.67[b]	2.9 ± 0.46[b]	9.6 ± 0.24[b]
HFD + BU (high)	2.04 ± 0.40[b]	2.96 ± 0.45[b]	1.98 ± 0.24[b]	6.98 ± 0.44[b]

Data are expressed as Mean ± SD; [a] = $p < 0.05$ vs NC, [b] = $p < 0.05$ vs HFD control. Normal control: NC; high fat diet control: HFD; *Betula utilis:* BU; mesentric: MES; reteroperitoneal: RET; epididymal: EPI; total fat: TF.

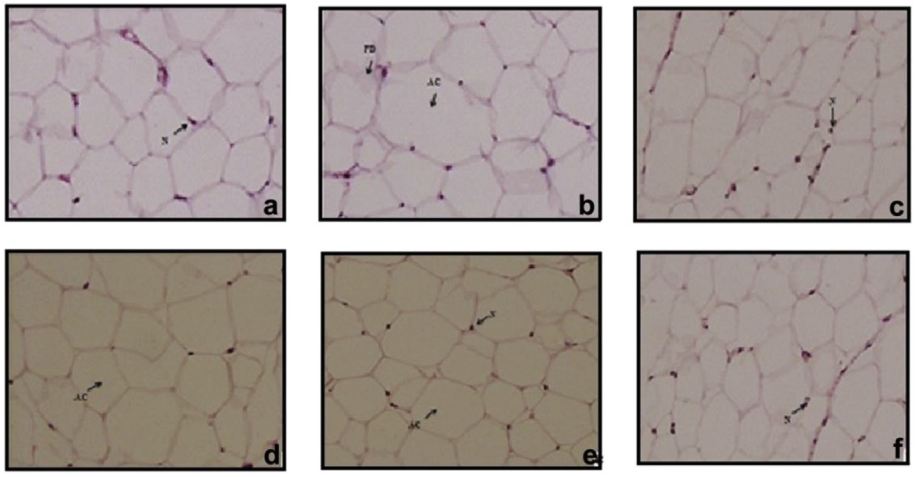

Fig. 1. Histopathology examination of adipose tissue in experimental rats (10X magnifications). (a) Normal control (b) HFD control (c) Orlistat 30 mg/kg (d) BU 100 mg/kg (e) BU 200 mg/kg (f) BU 400 mg/kg. N: Nucleus, FD: Fat deposition, AC: Adipose cell.

in adipocytes, inhibition of DGAT leads to inhibition of synthesis of triglycerides. This may account for the decrease in the TG levels by BU as compared to the TG levels in high fat diet fed rats. Studies have shown that the pentacyclic triterpenoids such as oleanolicacid and lupeol have α-amylase inhibitory activity (Takasaki, 2005). These triterpenoids are also present in BU along with betulinic acid (Altunkaynak, 2005). So inhibition of α-amylase, DGAT (diacylglycerol acetyl transferase) and pancreatic lipase may hamper the digestion and synthesis of carbohydrates and fats respectively, thereby accounting for reduction of body weight gain, adipose tissue weights (fat pads) and hyperlipidemia with the administration of BU. Hence, it has been observed that ethanolic extract of BU bark may be used as herbal alternative to synthetic drugs in the treatment of obesity.

5. Conclusion

The outcome of the current study revealed that supplementation with *Betula utilis* ethanolic extract reverses all the parameters of HFD-induced obesity thus suggesting its weight reducing potential. In conclusion, BU may prove to be a safer, efficacious and cost effective herbal medicine in treatment of obesity.

Conflicts of interest

The Authors declare that they have no conflicts of interest to disclose.

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