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RESEARCH ARTICLE

Anti-Obesity Effect of *Betula Alnoides* Bark Extract (Babe) on Plasma Lipids in Male Wistar Rats Fed a Fructose-Rich Diet

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ABSTRACT

The anti-obesity effect of *Betula alnoides* bark extract (BABE) on plasma lipids in male Wistar rats fed a fructose-rich diet (63%w/w) was investigated. Fructose feeding caused significant elevations in the concentrations of plasma triglycerides, phospholipids and free fatty acids. High-density lipoprotein cholesterol (HDL-C) was significantly reduced and very low-density lipoprotein cholesterol (VLDL-C) and low-density lipoprotein cholesterol (LDL –C) were significantly elevated. Activities of lipoprotein lipase (LPL) and lecithin cholesterol acyl transferase (LCAT) in plasma were reduced significantly ($p<0.01$) as compared to animals fed control diet. Simultaneous oral administration of BABE along with fructose diet mitigated the effects of fructose and these rats showed near-normal levels of the parameters studied. We conclude that BABE normalizes the enzyme activities and plasma lipid alterations in this experimental model. Anti-obesity activity of BABE might be due to phytochemicals as polyphenols.

KEYWORDS

Fructose, Betula alnoides, Obesity, Hyperlipidemia, Lipid Profile

INTRODUCTION

Worldwide health of human being is progressively threatened by an imbalance between increased energy intake and decreased energy expenditure through physical activity resulting to obesity, a serious and chronic disease. Obesity has increased at a striking rate over the three decades. It is projected by WHO (World Health Organization) is that approximately 2.3 billion adults will be overweight and more than 700 million will be obese by the year 2015.[1] The increasing global prevalence of obesity demonstrates that neither diet and exercise nor pharmacological approaches to this health problem are well addressed till the date. This negative trend has dramatically impact on physical health and on the relative cardiovascular risk. In fact, obesity alone is strongly associated with an increased risk of life-threatening conditions such as diabetes, arterial hypertension, dyslipidemia and cardiovascular diseases.[2] Hyperlipidemia is metabolic complication of both clinical and experimental obesity.[3] Its prevalence is growing not only in developed countries but also in developing countries.[4] Treatment of

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dyslipidemia reduces cardiovascular events. [5] The modern pharmacological therapy for abnormal lipids is effective but is costly and associated with side-effects [6] leading to patient incompliance. Therefore, alternative therapies particularly, herbal based are being explored.

More than thirteen thousand plants have been studied for various pharmacological properties. An herbal treatment for hypercholesterolemia has no side effects and is relatively cheap, locally available. The chosen medicinal plant namely as *Betula alnoides* bark L belongs to the *Betulaceae* family. A survey of literature revealed that no systematic approach has been made to study antiobesity activity of this plant. Therefore, the present study was to investigate the antiobesity properties of ethanolic extract of *Betula alnoides* bark in high fructose induced rats.

MATERIALS AND METHOD

Animals

Male albino rats of Wistar strain approximately weighing 180-190g were used in this study. They were healthy animals purchased from the Indian Institute of Science, Bangalore. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27 ± 2º C and 12 hour light/dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet and water were provided *ad libitum*. They were acclimatized to the environment for one week prior to experimental use. The experiment was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Chemicals

Fructose, Ethylene diamine tetra acetic acid (EDTA), sodium nitroprusside (SNP), Trichloro acetic acid (TCA) was purchased from Sisco Research Laboratories Pvt. Ltd., India. All other chemicals and solvents used were of analytical grade available commercially.

Plant Materials

The mature *Betula alnoides* barks were collected in May 2012 from Kodaikanal, Dindugal district, Tamil Nadu, India. The barks were identified and authenticated by Botanist, Prof. S. Palaniappan, Department of Botany, H.H. Rajahs College (Autonomous), Pudukkottai, Tamil Nadu, India. A Voucher specimen (RJOBS/JJC/2013) has been deposited at the Herbarium, J. J. College, Pudukkottai, Tamil Nadu, India.

Preparation of Alcoholic Extract

The bark of *Betula alnoides* were first washed well and dust was removed from the bark. Barks were washed several times with distilled water to remove the traces of impurities from the bark. The barks were dried at room temperature and coarsely powdered. The powder was extracted with 70% ethanol for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The *Betula alnoides* bark extract (BABE) was stored in refrigerator until used.

Dosage Fixation

The effect of different doses of BABE on content of plasma lipid parameters in triton induced hyperlipidemia was evaluated. Different doses of *Betula alnoides* bark extract (BABE) (250mg, 500mg and 750mg/kg body weight) were treated for one week after Triton WR-1339 injection. Triton WR-1339 is well known to induce hyperlipidemia in short term period. The effective dose of BABE was assessed based on the contents of plasma lipids. Supplementation of BABE at doses of 500mg and 750mg/kg body weight for one week was found to be effective in Triton WR-1339 induced hyperlipidemic rats. Among these doses, the minimal effective dose 500mg was fixed as therapeutic dosage for the subsequent studies.[7]

Preparation of Control and High Fructose Diet

The control and high fructose diet were prepared by the method of Suwannaphet *et al*.[8]

Table 1 represents the composition of the experimental rats.

Table 1: Shows the composition of the experimental diets (g/kg diet)

Ingredients	Control diet	High-fructose (HF) diet
Casein	200	200
Corn starch	530	----
Sucrose	100	----
Fructose	---	630
Soybean oil	70	70
Mineral mixture	35	35
Vitamin mixture	10	10
Cellulose powder	50	50
L-Cystine	3	3

Experimental Design

Body weights of the animals were recorded and they were divided into 4 groups of 6 animals each as follows. Group 1: Normal control rats fed with control diet and served as a control. Group 2: Fructose-fed animals received fructose-enriched diet for a period of 6 weeks. Group 3: Fructose-fed animals treated with *Betula alnoides* bark extract (BABE) by oral gavage daily at a dose of 500 mg/kg body weight (based on effective dosage fixation studies) for 6 weeks. Group 4: Fructose-fed animals treated with standard drug as Orilstat at a dose of 9 mg/kg body weight for 6 weeks.

Collection of Samples

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected with EDTA as anticoagulant. Plasma was separated for the estimation of various biochemical parameters.

Biochemical Estimation

Biochemical Analysis

The total cholesterol was estimated by the method of Allain et al.[9] Triglycerides was estimated by the method of Werner et al.[10] HDL cholesterol was separated by adding phosphotungstic acid and magnesium chloride to the fresh samples to precipitate other lipoproteins and the HDL cholesterol was estimated by the method of Allain et al.[9] The concentration of LDL cholesterol was calculated by using the Friedewald et al.[11] formula and VLDL cholesterol was calculated by dividing the triglycerides value (in mg/dl) by 5. The phospholipids were estimated by the method of Zilversmit *et al.*,[12] and liberated phosphorous was estimated by using Fiske and Subbarrow method.[13] Atherogenic Index (AI) [14] and Coronary Risk Index (CRI)[15] were calculated by the following formula;.

$$AI = LDL\text{-}C/HDL\text{-}C$$

$$CRI = \text{Total cholesterol}/HDL - \text{Cholesterol}$$

Statistical Analysis

Values were expressed as mean ± SD for six rats in the each group and statistical significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by the Tukey's test for multiple comparisons. The results were statistically analyzed by Graphpad Instat Software (Graphpad Software, San Diego, CA, USA) version 3 and $p < 0.01$ was considered to be significant.

RESULTS

Effect of BABE on Plasma Lipid Profile

The plasma lipid concentrations in control and experimental animals are given in Table 2. There was a significant increase in cholesterol, triglycerides, LDL-C and VLDL-C concentrations whereas a decrease in HDL-C was observed in fructose- fed rats. These alterations were reversed and the values were near normal in BABE treated rats. Also, treatment with BABE to fructose supplemented rats caused significant ($p < 0.01$) reductions in the atherogenic and coronary artery risk indices. Lipid profile was also restored in Orilstat treated animals. The reduction of cholesterol was 20.33%, triglycerides were 26.15%, LDL-C was 86.40%, VLDL-C was 73.85% and increment of HDL-C was 132.03% was observed in BABE treated rats.

Table 2: Effect of BABE on lipid profile of control and experimental diets in rats

Plasma	Group I	Group II	Group III	Group IV
TC	74.54±5.06	240.69±16.36[a]	73.63±5.08 [b]	78.78±5.35 [b]
TG	83.33±5.66	361.11±24.55 [a]	94.44±6.42 [b]	105.55±7.17 [b]
HDL	31.25±2.12	14.58±0.99 [a]	33.83±2.30 [b]	27.08±1.84 [b]
VLDL	16.66 ± 1.13	72.22 ± 4.91 [a]	18.88 ± 1.28 [b]	21.11 ± 1.43 [b]
LDL	26.63 ± 1.81	153.89 ± 10.46 [a]	20.92 ± 1.44 [b]	30.59 ± 2.08 [b]

Each value is expressed as mean ± SD for six rats in each group.
[a]As compared with group I, [b]As compared with group II. $p<0.01$.
TC, TG, HDL, VLDL and LDL = mg/dl

Table 3: Effect of BABE on phospholipids, free fatty acid, LACT, LPL, AI and CRI of control and experimental diets in rats

Plasma	Group I	Group II	Group III	Group IV
AI	0.85 ± 0.05	10.55 ± 0.73	1.61 ± 0.11	1.12 ± 0.07
CRI	2.38 ± 0.16	16.50 ± 1.15	2.17 ± 0.14	2.90 ± 0.20
Phospholipids (PL)	84.21±5.72	145.61±9.90 [a]	92.98±6.32 [b]	100±6.8 [b]
Free Fatty acid (FFA)	119.45±8.12	194.23±13.20 [a]	121.54±8.26 [b]	125.78±8.55 [b]
LCAT	70.56±4.23	55.65±3.33 [a]	68.89±4.13 [b]	69.56±4.17 [b]
LPL	6.32±0.06	5.21±0.31 [a]	6.54±0.39 [b]	6.12±0.36 [b]

Each value is expressed as mean ± SD for six rats in each group.
[a]As compared with group I, [b]As compared with group II. $p<0.01$.
PL, FFA = mg/dl; LCAT= µmoles of cholesterol/hr/L ; LPL = µmoles of glycerol liberated/hr/L

Fructose supplemented rats had elevated concentrations of free fatty acid and phospholipids in plasma as compared to control rats. BABE supplementation normalizes the levels of free fatty acid and phospholipids in plasma of the fructose-fed rats. The atherogenic index and coronary artery index were also increased in HF fed rats. Supplementation of BABE to HF fed rats reduced the atherogenic index and coronary artery index (Table 3). The alteration observed in lipid concentrations was accompanied by changes in enzyme activities. The activities of lipoprotein lipase (LPL) and lecithin cholesterol acyl transferase (LCAT) in plasma were given in the Table 3. LPL and LCAT activities were lowered in plasma of fructose fed rats ($p<0.01$). The activities of these enzymes were restored to normal when rats were treated with BABE. BABE supplementation to normal rats also enhanced the LPL activity in liver. The reduction of phospholipids was 52.63%, free fatty acid was 37.42% and increase the activity of LCAT was 23.79% and LPL was 25.52% were observed in BABE treated rats. Lipid profile and enzyme activities were also near normal in Orilstat treated animals.

DISCUSSION

As dietary exposure to fructose has increased over the past 40 years, there is growing concern that high fructose consumption in humans may be in part responsible for the rising incidence of obesity worldwide.[16] Rats fed a high-fructose diet develop a cluster of abnormalities including hyperinsulinemia, hyperlipidemia, glucose intolerance and hypertension.[17] In addition to this, prolonged fructose treatment affects lipid metabolism and causes alterations in the plasma lipid profile.[18]. An increased intake of fructose causes atherogenic changes in the aorta. It has been documented that all these alterations are secondary to the development of insulin resistance.[19] It is well-established that rats and mice become obese when offered free access to concentrated solutions of sugars.[20]

A mechanism for the hyperlipidemic effects of fructose has been suggested previously.[21] Once absorbed, fructose is primarily metabolized by the liver. Fructose metabolism is unique in that it enters glycolysis or gluconeogenesis at the triphosphate level, bypassing the need for insulin and the action of phosphofructokinase. After fructokinase catalyzes phosphorylation of fructose to fructose 1-phosphate, the resulting compound is split by hepatic aldolase B into glyceraldehyde and dihydroxyacetone phosphate. The activities of fructokinase and hepatic aldolase B are increased when the amount of fructose in the diet is increased, leading to enhanced hepatic lipogenesis. This process is likely to increase VLDL production and secretion, thus elevating both blood triglycerides and cholesterol. Furthermore, fructose does not appear to stimulate lipoprotein lipase, which may result in reduced clearance of triglycerides from the plasma. It is important to note that chronic fructose feeding may result in adaptation by healthy animals without developing metabolic disturbances,[22] and that shorter test periods as used in this study could produce adverse results that may be transient.

Hypertriglyceridemia may be due to a defect in removal of VLDL from plasma or increased secretion of VLDL. Lipoprotein lipase is an important enzyme responsible for the hydrolysis of triglyceride chylomicrons and VLDL. Significant reduction in the activity of LPL as seen in the present study can cause hypertriglyceridemia and accumulation of VLDL in plasma of the fructose-fed rats. Hypertriglyceridemia found in fructose-fed rats was reversed when the rats were supplemented simultaneously with BABE. The triglyceride lowering effect of BABE is attributed to both enhanced peripheral tissue clearance of plasma triglycerides and increased LPL activity.

Insulin has a regulatory effect on FFA metabolism. A defect in the ability of insulin to regulate the FFA metabolism could contribute to increase FFA levels. Free fatty acid concentrations remain higher in fructose-fed rats than in control rats during hyperglycemia induced hyperinsulinemia.[23] Elevated FFA concentration is associated with hypertriglyceridemia and has been reported in fructose induced hypertensive rats.[24] FFAs are important substrates for hepatic triglyceride synthesis and a diminished insulin suppression of plasma FFA leads to higher plasma triglyceride concentrations. Furthermore the acute inhibitory effect of insulin on hepatic very low-density lipoprotein (VLDL) secretion is modified by the ambient plasma FFA concentration.

High plasma FFA concentrations impaired the action of insulin on glucose disposal via substrate competition in the glucose–FFA cycle.[25] Conversely diminished insulin-stimulated glucose disposal could lead to impaired FFA reesterification and thereby, to higher circulating FFA concentrations. Elevated concentrations of plasma FFAs may play a key role in the pathogenesis of NIDDM by impairing peripheral glucose utilization and by promoting hepatic glucose overproduction.[25] these data suggest that the action of insulin on FFA metabolism is impaired in hyperinsulinemia/insulin resistance state that could explain higher triglyceride concentrations in fructose fed rats in the present study. The fatty acids in phospholipids undergo changes during the process of injury, repair and cell

growth. Abnormalities in fatty acids and in phospholipid metabolism are important in the pathogenesis of cell membrane dysfunction.[26] BABE supplemented fructose fed rats showed marked decrease in FFAs and phospholipids concentrations.

The lowered HDL-C concentration in fructose-fed rats can be attributed to the decreased LPL and LCAT activities in plasma. LCAT, the enzyme that catalyzes esterification of cholesterol with fatty acids, along with LPL is responsible for HDL-C synthesis. It plays an important role in cholesterol and triglyceride transport and metabolism. The decreased activity of LCAT indicates impairment in HDL-C synthesis as well as triglyceride metabolism in fructose-fed rats. The effect of fructose feeding on LCAT and LPL produces changes in lipid components, mainly in the concentrations of triglyceride, HDL-C and VLDL-C.

The rise in plasma HDL-C concentrations in BABE-treated rats (FRU+BABE) may be due to delayed clearance and increased synthesis of HDL constituents. Stimulation of LPL leads to a rise in HDL production and reduction in VLDL constituents. BABE supplementation is known to increase the HDL-C concentration in a dose dependent manner.[7] It was observed that BABE supplemented fructose fed rats showed marked increase in HDL-C concentrations.

The effect of BABE treatment on the atherogenic and coronary artery risk indices is also notable. The ratio of total cholesterol to HDL-C (also known as the atherogenic index) and the ratio of LDL-C to HDL-C (equally known as coronary artery index) are strong and reliable indicators of whether or not cholesterol is deposited into tissues or metabolized and excreted.[27] In humans, the normal reference values for atherogenic index and coronary artery index should not be higher than 4 and 2.5, respectively.[28] Thus, patients with cardiac risk indices higher than these reference values are predisposed to developing ischaemic heart disease and thrombotic cardiovascular accident.[29] In this present study, results showed that treatment with BABE caused profound reductions in the atherogenic and coronary indices in both control and experimental rats and these strongly suggest that BABE possesses cardioprotective potential. Orilstat is a well known anti-obesity drug which treated with high fructose diet rats significant positive response was observed in this study.

Betula has high levels of phenolic compounds such as flavonoids, myricetin, quercetin derivatives, chloregenic acid, hydroxyl cinnamic acids and condensed tannins,[30] which could be attributed to the hypolypidaemic activity of *B. alnoides.* Saponin, Flavonoids and polyphenolic compounds reported to possess hypolipidemic property.[31] Our earlier studies reported that BABE contains flavonoids, saponin, terpenoids, steroids, alkaloids, polyphenols and tannin.[32]

In conclusion, the results of this study showed that BABE has therapeutic potentials in the management of obesity, hyperlipidaemia and in the prevention of atherogenic cardiovascular diseases and these effects were may be mediated via inhibition of intestinal lipid uptake and de novo triglyceride and cholesterol biosyntheses. The potential anti-obesity activity of BABE might be due to the phytochemicals polyphenols, flavonoids, terpenoids, saponin present in the extract.

CONCLUSION

The present investigation reveals that the administration of BABE in HF fed rats restored the altered plasma lipids to near normal levels. The results of this study demonstrated that BABE has therapeutic potentials in the hyperlipidaemia, management of obesity and in the prevention of atherogenic cardiovascular diseases and these effects were may be mediated via inhibition of intestinal lipid uptake and de novo triglyceride and cholesterol biosyntheses. The potential anti-obesity activity of BABE might be due to the phytochemicals polyphenols, flavonoids, terpenoids, saponin present in the extract.

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