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RESTORATIVE EFFECT OF *BETULA ALNOIDES* BARK ON HEPATIC METABOLISM IN HIGH FAT DIET FED *WISTAR* RATS

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ABSTRACT

Liver has been shown to play a central role in the maintenance of lipids and glucose homeostasis. The objective of this study was to evaluate regulatory effect of *Betula alnoides* bark extract (BABE) in a rat model of high fat diet (HFD)-induced obesity. The carbohydrate metabolic enzymes Glycogen phosphorylase, Hexokinase, Fru, 1,6-bis-phosphatase, Phosphoglucose isomerase, Glu-6-phosphatase, Glucose-6-phosphate dehydrogenase and the content of pyruvate and glycogen were measured in experimental rats. The lipid metabolic enzymes HMG co A reductase, Total lipase, lecithin cholesterol acyl transferase (LCAT) and lipoprotein lipase (LPL) were measured in experimental rats. Supplementation of BABE to obesity rats for 8 weeks restored the altered carbohydrate and lipid metabolizing enzymes. The results of the present study indicate that BABE supplementation could be helpful in overcoming the deregulations of glucose and lipid metabolism in high fat fed rats.

KEYWORDS: Metabolism, *Betula alnoides,* Obesity, High fat diet, Lipase

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INTRODUCTION

Obesity and its associated conditions such as insulin resistance, type 2 diabetes, dyslipidemia, and steatosis hepatis represent major challenges for basic science and clinical research. The coincidence of obesity, insulin resistance, hypertension and dyslipidemia is commonly referred to as the 'metabolic syndrome'. This condition affects approximately 20-40% of the population in the industrialized nations, and its prevalence is expected to rise further in the next decades[1]. It is obvious that appropriate animal models are crucial for studies on the pathogenesis and therapy of this complex metabolic disorder. Central obesity and alterations of adipokine secretion, together with a concomitant fat accumulation in different metabolically active tissues such as liver, muscle and pancreas, build the pathophysiologic basis of the metabolic syndrome [2,3,4], and hepatic steatosis is now often added to the classical components [5]. It is generally agreed that individual genetic background and lifestyle factors contribute to the pathogenesis of this disorder. The first description of a 'high-fat diet' to induce obesity by a nutritional intervention was in 1959 [6]. Subsequent studies have revealed that high-fat diets promote hyperglycemia and whole-body insulin resistance, and numerous researchers have examined their effects on muscle and liver physiology as well as insulin signal transduction. From this experience, it is generally accepted that high-fat diets can be used to generate a valid rodent model for the metabolic syndrome with insulin resistance and compromised beta-cell function [7, 8]. Natural products/ dietary photochemicals have aroused considerable interest in recent years as potential therapeutic agents to counteract obesity. These compounds exert the anti-obesity effects mainly through regulation of various pathways, including lipid absorption, intake and expenditure of energy, increasing lipolysis, and decrease lipogenesis, and differentiation and proliferation of preadipocytes [9]. In the present study to investigate the regulatory effect of *Betula alnoides* bark on carbohydrate and lipid metabolic enzymes in high fat diet fed *Wistar* rats.

MATERIALS AND METHODS

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

Nitroblue tetrazolium (NBT), ethylenediaminetetra acetic acid (EDTA), Trichloro acetic acid (TCA), Thiobarbituric acid (TBA), Casein, Sucrose, 1-chloro-2,4-dintiro benzene (CDNB), 5,5'-dithio-bis (2-nitrobenzoic acid), glutathione (reduced), glutathione (oxidized), Nicotinamide adenine dinucleotide phosphate ($NADP^{+/}NADPH$) and L-ascorbic acid were purchased from Sigma Chemical Company (St. Louis, MO, USA). All other chemicals used were of analytical grade and were obtained from Glaxo Laboratories, Mumbai, India, and Sisco Research Laboratories, Mumbai, India.

Plant material

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, (Rtd.) Department of Botany, H.H. Rajahs College (Autonomous), Pudukkottai, Tamil Nadu, India. Currently, He was working at J. J. College, Pudukkottai. A Voucher specimen (RJOBS/JJC/2013) has been deposited at the

Herbarium, J. J. College, Pudukkottai, Tamil Nadu, India.

Preparation of plant extract

The collected plant materials were washed, sliced and completely dried in a hot-air oven at 37°C. The dried materials were ground into make a fine powder and used for extraction. Three hundred grams (300g) of the powered plants were extracted with ethanol (70%) using "Soxhlet Apparatus" for 48 hours. A semi solid extract was obtained after complete elimination of alcohol under reduced pressure. The extract was stored in the refrigerator until used. The extract contains both polar and non-polar phytocomponents. For experiments 500mg/kg body weight of *Betula alnoides* bark extract (BABE) was used. This effective dose was selected based on dose dependent studies of BABE carried out in our laboratory.

Preparation of control and high fructose diet

The control and high fat diet were prepared by the method of Arcari *et al.,* [10]. Table 1 represents the composition of the experimental diets.

Table 1

Shows the composition of the experimental diets (g/kg diet

Ingredients	Control diet	High-fat (HF) diet
Casein	200	115.5
Corn starch	397.5	200
Sucrose	100	100
Dextrinated starch	132	132
Lard	--	312
Soybean oil	70	40
Cellulose	50	50
Mineral mixture	35	35
Vitamin mixture	10	10
Choline	2.5	2.5
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 served as a control. Group 2: Fat-fed animals received fat-enriched diet for a period of 8 weeks. Group 3: Fat-fed animals co-administrated 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 8 weeks. Group 4: Fat-fed animals treated with standard drug Orilstat at a dose of 9 mg/kg body weight for 8 weeks.

Tissue homogenate

The animals were sacrificed by cervical dislocation and the liver was dissected out, washed with ice-cold physiological saline. The required amount was weighed and homogenized using a Teflon homogenizer. Tissue homogenate was prepared in 0.1 M Tris Hcl buffer (pH 7.4) and used for the estimation of various biochemical parameters.

Biochemical estimation

Glucose-6-phosphate dehydrogenase was assayed by the method of Korenberg and Horecker [11]. Fructose-1,6-bisphosphatase activity was measured by the method of Gancedo and Gancedo[12]. Phosphoglucoisomerase activity was measured by the method of Horrocks et al., [3]. Glucose-6-phosphatase was assayed by the method of Koide and Oda[14]. Hepatic hexokinase activity was assayed by the method of Brandstrup *et al*

[15]. Hepatic glycogen content was estimated by the method of Morales *et al.* [16]. The activity of liver HMG CoA reductase was determined by the method of Rao and Ramakrishnan [17] by determining the ratio of HMG CoA: Mevalonic acid. The activity of total lipase was determined by the method of Bier [18]. Activity of lecithin cholesterol acyl transferase (LCAT) was assayed by the method of Hitz *et al.*[19]. Lipoprotein lipase (LPL) activity was assayed by the method of Korn [20]. The level of pyruvate was estimated by the method of Frledemann and Haugen [21].

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 was used and $p< 0.01$ was considered to be significant.

RESULTS

The activities of enzymes hexokinase and the content of glycogen were significantly ($p< 0.01$) decreased while that of Phosphoglucoisomerase, glucose 6-phosphatase, Fru, 1,6-bis-phosphatase and glycogen phosphorylase were increased in fat-fed rats as compared to control rats. BABE treatment prevented these alterations and the enzyme activities were near normal in BABE treated fat-diet fed rats (Table 2). The effect of BABE on the circulating levels of gluconeogenic substrates such as pyruvate in control and experimental animals were investigated. Fat-fed rats showed a significant increase in the concentrations of pyruvate as compared to the control rats. Administration of BABE to high fat fed rats significantly ($p< 0.01$) reduced the levels as compared to the untreated high fat fed rats. The alteration observed in lipid concentrations was accompanied by changes in enzyme activities. The activities of lipo protein lipase (LPL), Lecithin cholesterol Aclytransferase (LCAT), HMG co A reductase and total lipase in liver were given in the Tables 3, respectively. LPL, LCAT, HMG co A reductase and total lipase activities were lowered in liver of HF fed rats ($p<0.01$). The activities of these enzymes were restored to normal when rats were treated with BABE. Standard atrovastin supplementation to HF fed rats also enhanced the LPL activity in liver.

Table 2

Effect of BABE on glucose metabolizing enzymes of control and experimental diets in rats

Parameters	Group I	Group II	Group III	Group IV
Glycogen	36.41 ± 2.54	24.32 ± 1.70 [a]	34.32 ± 2.40 [b]	32.76 ± 2.29 [b]
Glycogen phosphorylase	4.49 ± 0.31	5.21 ± 0.36 [a]	4.31 ± 0.30 [b]	3.98 ± 0.27 [b]
Hexokinase	0.24±0.01	0.17±0.01 [a]	0.25±0.01 [b]	0.24±0.01 [b]
Fru, 1,6-bis-phosphatase	2.42 ± 0.16	10.78 ± 0.75 [a]	3.12 ± 0.21 [b]	3.84 ± 0.26 [b]
Phosphoglucose isomerase	12.42 ± 0.86	21.42 ± 1.41 [a]	13.82 ± 0.96 [b]	15.43 ± 1.08 [b]
Glu-6-phosphatase	8.21 ± 0.57	9.82 ± 0.68 [a]	8.64 ± 0.60 [b]	8.95 ± 0.62 [b]
G6PD	3.36 ± 0.19	4.21 ± 0.24[a]	3.42 ± 0.21 [b]	3.13 ± 0.20[b]
Pyruvate	0.37 ± 0.02	1.27 ± 0.08 [a]	0.52 ± 0.36 [b]	0.67 ± 0.04 [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 III. *$p<0.01$.*
Glycogen- mg per g tissue; Hexokinase = µ moles of glucose phosphorylated/min / g protein; Phosphoglucoisomerase = nmole of fructose formed/min/mg protein; Glucose-6-phosphate dehydrogenase Units/min/mg protein;Glucose 6 phosphatase and Fructose-1,6-bis phosphatase = nmole Pi liberated/min/mg protein; Pyruvate = µmol/g tissue. Glycogen phosphorylase = mg of Pi liberated/min/mg protein

Table 3
Effect of BABE on lipids metabolizing enzymes of control and experimental diets in rats

Parameters	Group I	Group II	Group III	Group IV
HMG co A reductase	1.98 ± 0.13	1.22 ± 0.08 [a]	1.72 ± 0.11[b]	1.42 ± 0.09[b]
Total lipase	20.42 ± 1.42	14.62 ± 1.02 [a]	21.42 ± 1.49[b]	20.12 ± 1.40[b]
LCAT	23.24±1.39	18.21±1.09 [a]	22.45±1.34[b]	21.56±1.29[b]
LPL	10.78±0.64	8.34±0.50 [a]	10.56±0.63[b]	9.89±0.59[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 III. *p<0.01.
HMG Co A Reductase-HMG CoA: mevalonate ratio; Total lipase- nmomles of p- nitrophenol liberated hr^{-1} mg of protein $^{-1}$. LCAT -µmoles of cholesterol/hr/mg protein; LPL-µmoles of glycerolliberated/hr/50 mg acetone powder

DISCUSSION

Fat-enriched diets have been used for decades to model obesity, dyslipidemia and insulin intolerance in rodents. It has been observed that the disorders achieved by high-fat feeding resemble the human metabolic syndrome closely, and this also may extend to the cardiovascular complications [22, 23].

Glucose metabolism

The liver has been shown to play a central role in the maintenance of glucose homeostasis. It is the primary site of endogenous glucose output which produces glucose either *de novo* from 3-carbon precursors such as glycerol, lactate and alanine (gluconeogenesis) or via the breakdown of glycogen stores (glycogenolysis). At the cellular level, regulation of these pathways is mainly mediated by hormonal and nutritional signals. Several key enzymes integrate these signals to manipulate glucose production in the liver [24]. Insulin is the dominant hormone which influences the regulation of glucose metabolism. One of the major effects of insulin is to enhance overall glucose disposal by stimulation of glucose uptake into the target tissues [25]. Obesity is associated with insulin resistance and thereby an impaired ability of tissue to respond to insulin and effectively store glucose. Further, in response to insulin resistance production is increased, leading to hyperinsulinemia, hyperglycemia and ultimately type 2 diabetes [26].Depletion of tissue glycogen content in HF diet rats could be associated with the breakdown of hepatic autoregulation of glycogen synthesis and degradation. While relative impairment of insulin-stimulated glycogen synthesis could be linked with lipid accumulation [27] and decreased activation of the enzyme glycogen synthase, an excessive breakdown could be related to reduced insulin function to suppress glycogenolysis. Decreased response to insulin of glycogen synthase has been observed in soleus muscle in fat rich-diet fed rats [28]. Elevated FFA causes an allosteric stimulatory effect on glucose 6-phosphatase, which in turn increases hepatic glucose production by catalyzing glycogenolysis [29]. In this study decreased glycogen content and increased the activity of glycogen phospharylase activity in HF diet supplemented rats. Administration of BABE to obesed rats restored the content of glycogen and glycogen phosphorylase activities were observed.The enzyme glucose 6-phosphatase catalyses the terminal steps in both the gluconeogenic and glycogenolytic pathways and is opposed by the enzyme glucokinase that catalyzes glucose phosphorylation as the first step of glucose utilization / storage [24, 30]. Fructose 1,6-bis phosphatase, glycogen phosphorylase and glucose 6-phosphatase along with phosphoenolpyruvate carboxykinase, phosphoglucose isomerase work together to generate glucose, whereas glucokinase, hexokinase, phosphofructokinase-1, pyruvate kinase and glycogen synthase work together in a coordinated fashion for glucose disposal in the liver. The increased in the two-gluconeogenic enzyme activities (glucose 6-phosphatase and fructose 1,6-bis phosphatase) in fat-fed rats observed in this study are indicative of the liver being in the gluconeogenic state. Accelerated

gluconeogenesis in fat-diet fed rats has been reported earlier and has been attributed to insulin resistance observed in these rats [31]. Insulin can inhibit gluconeogenesis by repressing the activities of one or both these enzymes. It has been reported that glucose 6-phosphatase mRNA levels increase in liver after exposure to fat even in the presence of physiological concentrations of glucose [32,33]. Studies have shown that elevation of fructose 1,6-bis phosphatase alone in the obese rat, a model of obesity and type 2 diabetes is responsible for increased gluconeogenesis from glycerol [34,35,36]. The increased activity of G-6-PDH, the first and rate limiting enzyme of the pentose hexose monophosphate shunt, may provide an additional liver mechanism against oxidative [37]. The simultaneous increase in triglyceride content suggests that these rats have channeled the exceeding substrates towards lipid synthesis. All these changes might represent an adaptive mechanism to the carbohydrate load by which the liver switches its metabolism from the oxidative to the non-oxidative pathway, resulting in a lower fuel provision to the mitochondria with the consequent decrease in ROS production. Supplementation of BABE to obese rats regulated the glucose metabolism might be due to the increased the insulin sensitivity and decreased the insulin resistance to the target tissues.

Lipid metabolism

Lipoprotein lipase (LPL) is a central enzyme in overall lipid metabolism and transport, being responsible for catalysing the hydrolysis of triglycerides transported in the bloodstream by chylomicrons and VLDL, thereby providing non-esterified fatty acids and 2-monoacylglycerols for tissue utilization. The activity of LPL in the muscle and the adipose tissue is regulated by nutritional status and a plethora of factors, including hormones, growth factors and lipid metabolite products [38]. The lowered HDL-C concentration was observed in high fat fed rats can be attributed to the decreased LPL and LCAT activities in the liver. This result corroborated with Menaka *et al* [39] studies. 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 HF fed rats. The effect of HF feeding on LCAT and LPL produces changes in lipid components, mainly in the concentrations of triglyceride, HDL-C and VLDL-C. BABE supplementation is known to increase the HDL-C concentration without any change in total cholesterol concentrations in rats. It was observed that BABE supplemented HF fed rats showed restored the activity of LPL and LCAT and thereby marked increase in HDL-C concentrations. This results agreement with Menaka *et al.* [39] studies. The rise in plasma HDL-C concentrations in BABE treated rats may be due to clearance of plasma lipids and increased synthesis of HDL constituents. Stimulation of LPL leads to a rise in HDL production and a reduction in VLDL constituents. Elevated LPL activity in hepatic tissues could be responsible for clearance of tissue lipid load.HMG Co A reductase is the key metabolic enzyme for synthesis of cholesterol in hepatocytes [40]. Low activity of HMG CoA reductase in liver of HF rats indicates decreased *de novo* synthesis reported by Menaka *et al.* [39]. Reduced HMG CoA reductase activity resulting in augmented LDL-R expression has been reported earlier [41,42]. In this context, it is hypothesized that, reduced hepatic cholesterol content in HFD and BABE rats facilitates LDL uptake from circulation due to increased expression of LDL receptors. Decreased plasma LDL levels record in HFD and BABE rats corroborate this hypothesis. In conclusion, BABE supplementation could be helpful in overcoming the dysregulation of glucose and lipid metabolism in high fat fed rats. Reduction in hepatic gluconeogenesis, increased oxidative glucose utilization and restored the lipid metabolizing enzymes by the hepatocytes may explain regulatory effect of BABE action in this model. The regulatory effects of supplementary BABE on hepatic metabolism indicate antiobesity activity.

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