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Antioxidant Activity of *Betula Alnoides* Bark Extract in High Fat Diet Fed *Wistar* Rats

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Abstract: Obesity and hyperlipidemia synergistically promote systemic oxidative stress-imbalance between tissue free radicals, reactive oxygen species (ROS) and antioxidants. Antioxidants are the substances that may protect cells from the damage caused by free radicals. The antioxidants may be exogenous or endogenous in nature. The endogenous antioxidants can be classified as enzymatic and non enzymatic. In the present study to investigate the antioxidant activity of *Betula alnoides* bark extract (BABE) on oxidative stress induced by high fat diet (HFD) fed rats. The results of the present study indicate that the antioxidant activity of BABE may be related to a counteraction of free radicals by its antioxidant activity of BABE. Antioxidant activity of BABE is mainly attributed to the presence of enriched therapeutic phytochemical constituents such as flavonoids, polyphenol etc.
Keywords : *Betula alnoides,* Antioxidants, Reactive oxygen species, Oxidative stress.

Introduction

Currently, more than 1 billion adults worldwide are overweight, at least 300 million are clinically obese, and nearly 43 million children younger than 5 y were overweight in 2015[1]. According to estimates of the International Obesity Task Force, 1,7 billion people are exposed to health risks related to body weight, while the increase in Body Mass Index (BMI) is responsible for more than 2.5 million deaths annually, which is expected to double by 2030 [2].

Obesity and hyperlipidemia synergistically promote systemic oxidative stress-imbalance between tissue free radicals, reactive oxygen species (ROS) and antioxidants [3]. Redox metabolism corresponds to a complex interacting network involving the generation of reactive oxygen species and enzymatic and non-enzymatic cellular antioxidant defenses. Any small and transient disturbance of this balance induces redox signaling, which can act on several transduction pathways or enzyme and transcription factor activities. In contrast, when antioxidant defenses are chronically overwhelmed, the result is an oxidative stress in which free radicals may exert their deleterious effects[4]. Oxidative stress has been implicated in the pathogenesis of several metabolic diseases as well as in the co-morbidity of diabetes mellitus, obesity and atherosclerosis. In such studies, redox metabolism was evaluated by the pattern of various parameters such as manganese dependent superoxide dismutase, glutathione peroxidase or catalase activities, and glutathione or α-tocopherol content in blood and liver[5,6]. A similar pattern was observed in obesity and seemed to indicate that such pathology was related to pro-oxidative context. Indeed, obesity prevalence is correlated with decreased concentrations of plasma

antioxidants[7] (Reitman *et al.,* 2002). An increase in the markers of systemic oxidative stress has therefore been associated with obesity and metabolic syndrome[8]. High fat-induced hyperglycemia is one of the important factors to increase ROS, lipid peroxidation causing the depletion of the antioxidant defense status in various tissues including liver[9].

Antioxidants are the substances that may protect cells from the damage caused by free radicals. Antioxidants interact with and stabilize free radicals and may prevent some of the damage free radicals might otherwise cause. The antioxidants may be exogenous or endogenous in nature. The endogenous antioxidants can be classified as enzymatic and nonenzymatic. The antioxidant enzymes include Superoxide dismutase (SOD), Catalase (CAT), glutathione peroxidase (GPx), glutathione reducates (GRx)[10]. The non-enzymatic antioxidants are also divided into metabolic antioxidants and nutrient antioxidants. Metabolic includes lipoic acid, glutathione, L-arginine, uric acid, bilirubin etc. While nutrient antioxidant belonging to exogenous antioxidants are compounds which can not be produced in the body and must be provided through foods such as vitamin E, vitamin C, carotenoids, trace elements (Se,Cu,Zn,Mn)[11]. In the present study to investigate the antioxidant activity of *Betula alnoides* bark extract (BABE) on oxidative stress induced by high fat diet (HFD) fed 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 was 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 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 SRBE 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.,*[12]. 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.

Collection of sample

On completion of the experimental period, animals were anaesthetized with thiopentone sodium (50mg/kg). The blood was collected without EDTA. Serum was separated for the estimation of various biochemical parameters.

Tissue homogenate

Immediately after blood collecting, 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

Malondialdehyde was estimated by the thiobarbituric acid assay method of Beuge and Aust [13]. Superoxide dismutase activity was determined by the procedure of Kakkar et al. [14]. The activity of catalase was assayed by the method of Beers and Sizer[15]. The activity of glutathione peroxidase was assayed by the method of Rotruck et al[16]. The activity of glutathione reductase was measured by the method of Staal et al.[17]. Glutathione-s-transferase was assayed by the method of Habig et al [18]. Reduced glutathione was estimated by method of Moron et al[19]. The level of ascorbic acid was estimated by the method of Omaye *et al* [20]. α-tocopherol was estimated by the method of Baker *et al* [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

Plasma oxidative stress markers and antioxidants

Effects of BABE on MDA and GSH

An increase ($p < 0.01$) in the mean MDA level was found in plasma of Group II (HFD fed rats) rats relative to Group I (normal) rats. In Group II rats (HFD fed rats but treated with the BABE) the mean MDA concentrations in plasma approximated those of normal rats (Table 2). There is no significant changes were observed in Group III and IV standard treated rats.

In Group II rats, a significant decrease ($p < 0.01$) (relative to normal rats) in GSH levels was observed in the plasma. Treatment with BABE in Group III rats resulted in GSH levels that were similar to the levels in normal rats (Table 2). Group IV standard treated rat has no significant changes were observed.

Effects of BABE on antioxidant enzymes

Significantly ($p < 0.01$) lower activities of CAT, SOD and GPx enzymes were observed in the plasma of HFD fed rats (Group II) rats when compared to the values in normal (Group I) rats. In Group III rats that had been administered the BABE, the activities of these enzymes were maintained at near normal levels, that is, there were no significant differences compared with the values noted in Group I rats (Table 2). Group IV standard treated has significant increase were observed.

Effects of BABE on vitamins C and E

Significantly ($p < 0.01$) lower levels of vitamin C were observed in the plasma of HFD fed (Group II) rats, when compared to levels in normal (Group I) rats. Similar significantly ($p < 0.01$) lower concentration of vitamin E were noted in plasma of Group III rats compared to levels in Group I rats. However, the levels of vitamins C and E in plasma of Group III rats were found to be comparable to the levels found in normal (Group I) rats (Table 2). Group IV standard treated rat has no significant changes were observed as compared to group I.

Hepatic oxidative stress markers and antioxidants

Table 3 summarizes the levels of MDA, GSH and activities of enzymatic antioxidants SOD, CAT and GPx in the liver of control and experimental animals. Group-II showed significantly higher levels of MDA and depleted hepatic GSH as compared to group-I rats. Treatment with BABE to group III rats significantly decreased MDA and increased in GSH levels when compared to group-II rats. The activities of enzymatic antioxidants SOD, CAT and GPx were significantly lower in group-II rats than in group-I rats. In group-III rats treated with BABE, the activities were significantly higher as compared to group-II. Orilstat treated rats also observed similar effects.

Table 2 Effect of BABE on plasma antioxidant defence of control and experimental diets in rats

Parameters	Group I	Group II	Group III	Group IV
MDA	10.43±0.69	18.63±1.24 [a]	11.81±0.79 [b]	12.72±0.85 [b]
GSH	8.12±0.54	5.36±0.35 [a]	7.66±0.51 [b]	7.36±0.49 [b]
SOD	4.57±0.30	2.66±0.17 [a]	4.38±0.29 [b]	3.93±0.26 [b]
Cat	8.45±0.56	5.85±0.39 [a]	7.80±0.52 [b]	7.15±0.47 [b]
GPX	7.13±0.47	5.66±0.37 [a]	6.86±0.45 [b]	6.6±0.44 [b]
Vit C	3.47±0.23	1.20±0.08 [a]	3.50±0.23 [b]	3.15±0.21 [b]
Vit E	2.86±0.19	1.13±0.07 [a]	2.57±0.17 [b]	2.26±0.15 [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. [*]$p<0.01$.
MDA: nmol of MDA formed/L; SOD, CAT, GPx U/ml; GSH, Vit C, Vit E mg/dl.

Table 3 Effect of BABE on liver antioxidant defence of control and experimental diets in rats

Parameters	Group I	Group II	Group III	Group IV
MDA	8.37±0.54	13.54±0.88 [a]	9.12±0.59 [b]	10.54±0.68 [b]
GSH	3.25±0.21	2.07±0.13 [a]	3.39±0.22 [b]	2.96±0.19 [b]
SOD	9.64±0.62	6.47±0.42 [a]	9.33±0.60 [b]	9.01±0.58 [b]
Cat	5.62±0.36	4.03±0.26 [a]	5.72±0.37 [b]	5.46±0.35 [b]
GPX	7.61±0.46	4.13±0.26 [a]	6.86±0.44 [b]	6.33±0.41 [b]
GR	6.87±0.44	4.37±0.28 [a]	6.25±0.40 [b]	5±0.32 [b]
GST	0.51±0.03	0.37±0.02 [a]	0.50±0.03 [b]	0.48±0.03 [b]
Vit C	2.72 ±0.17	1.27±0.08 [a]	5.45±0.35 [b]	5.18±0.33 [b]
Vit E	4.17±0.27	2.85±0.18 [a]	1.52±0.09 [b]	3.85±0.25 [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. [*]$p<0.01$.
MDA: nmol MDA formed/mg protein; MnSOD : 50% reduction of NBT/min/mg protein; Catalase :µmol H_2O_2 consumed/min/mg protein. Glutathione peroxidase :µmole GSH utilized/min/mg protein; Glutathione reductase: nmol NADPH oxidized/min/mg protein; Glutathione-s-transferase: µmoles CDNB conjugated/min/mg protein; Reduced Glutathione :µg/mg protein; Vitamin C: µg/mg protein ; Vitamin E: µg/mg protein.

Discussion

Effect of BABE on plasma antioxidant defence

Obesity and hyperlipidemia synergistically promote systemic oxidative stress-imbalance between tissue free radicals, reactive oxygen species (ROS) and antioxidants. ROS could react with polyunsaturated fatty acids, which lead to lipid peroxidation[22]. Malondialdehyde is a by-product of lipid peroxidation and reflect the degree of oxidation in the body. Possible mechanisms that generate oxidative stress in obesity include hyperglycemia, elevated lipid levels, inadequate antioxidant defenses and hyperleptinemia[23]. In the present study, plasma TBARS levels, determined by evaluating malondialdehyde content were decreased after BABE treatment in HFD-fed rats.

Plasma is endowed with innate antioxidant defense mechanisms, including the enzymes catalase, superoxide dismutase, glutathione peroxidase and glutathione-S-transferase. CAT is responsible for the detoxification of significant amounts of H_2O_2. SOD catalyses the removal of superoxide radical (O_2•-), which would otherwise damage the membrane and biological structures. GST is actually composed of a group of isoenzymes capable of detoxifying various exogenous and endogenous substances by conjugation with glutathione. A reduction in the activity of these enzymes is associated with the accumulation of highly reactive free radicals, leading to deleterious effects such as loss of integrity and function of cell membranes. A decrease has been observed in the activity of CAT, SOD and GPx enzymes in plasma [24] of rats that had received HFD. Noeman *et al*. [9] suggested that the reduced activities of these enzymes might reflect a feed-back inhibition or oxidative inactivation of protein caused by excess generation of ROS. So also, in the present study, significantly lower activities of these enzymes were noted in rats that had received HFD, when compared to the levels in normal rats. Administration of BABE markedly elevated the levels of these antioxidant enzymes so that they approximated the levels seen in normal rats. Increased activity of SOD enzyme in BABE treated rats divulges that BABE may act as a potent scavenger of superoxide radicals and metal chelator reported in our study. The BABE treated group showing increase in CAT activity may be explained by the free radical scavenging action of components present in the BABE [25]. Similar elevation of these enzyme levels by supplementation of antioxidant source as *Embelia ribes* extract has already been reported[24].

Circulating antioxidants such as vitamin C (ascorbic acid) and vitamin E (α-tocopherol) are non-enzymatic scavengers of free radicals. Vitamin E reacts with lipid peroxy radicals, acting as a chain terminator of lipid peroxidation, and protects the cellular structures from attack by free radicals [26] . Vitamin C facilitates the maintenance of vitamin E levels at optimum concentrations. A decrease in ascorbic acid levels in plasma of rats that had been administered HFD fed rats has been reported. In the present study, significantly lower (relative to normal rats) levels of vitamins C and E were recorded in HFD fed rats; however, in rats that had been treated with BABE and had then received HFD, the levels of these vitamins approximated the levels seen

in normal rats. A similar increase in plasma levels of vitamins C and E has been reported in rats administered with HFD and then treated with the potent antioxidants [27].

Effect of BABE on Hepatic antioxidant defence

Animal studies have shown that high fat diet-fed rats display hepatic oxidative damage and altered lipid metabolism due to hepatic stress as a result of the burden of fat metabolism[28]. The development of oxidative stress, an imbalance between pro- and antioxidant status, has been shown to play an important role in mediating insulin resistance, and therefore, we studied the antioxidant potential. Decreased hepatic GSH, vitamin C and vitamin E levels, decreased activities of antioxidant enzymes and increased lipid peroxidation intermediates in HF fed rats clearly indicates the development of oxidative stress in these animals. Peroxidative deterioration of lipids is evident from the increased levels of malondialdehyde (MDA), while the increased protein carbonyl groups signify protein damage. The increase in catabolism of lipids could be associated with the cellular energy depletion that can increase the susceptibility of cells to lipid peroxidation[29]. Reactive oxygen species (ROS) can themselves reduce the activity of hepatic antioxidant enzymes such as CAT, GPx, GR and GST[30]. The decreased SOD activity in high fat fed rats may be due to inactivation of enzymes. The findings of this study are in agreement with other investigations that reported a significant increase in lipid peroxidation and a significant decrease of hepatic antioxidant enzyme activities in high fat induced rats[9].

The decreased GSH, vitamin C and vitamin E levels in HF fed rats could be due to increased utilization to trap free radicals, and/or decreased regeneration as evident with the lower activity of glutathione reductase enzyme. The antioxidant potential of BABE against high fat diet-induced oxidative stress is evident with lower levels of MDA, higher GSH, vitamin C and vitamin E levels and increased activities of antioxidant enzymes seen in HF fed rats treated with BABE when compared with HF fed rats. Present finding is similar to the Noeman *et al.* [9] The *in vitro* antioxidant potential of this plant was well documented[25].

As oxidative stress has been suggested as one mechanism for the detrimental effects of fat, the antioxidant potential of BABE may be one among several mechanisms by which this plant prevented insulin resistance. More recently, studies have linked ROS production and oxidative stress to insulin resistance[31,32]. Through *in vitro* studies and in animal models, it has been found that antioxidants improve insulin sensitivity[33, 34]. Several clinical trials, have also demonstrated that treatment with vitamin E, vitamin C, or glutathione improves insulin sensitivity in insulin-resistant individuals and/or patients with type 2 diabetes[35,36]. Our data clearly demonstrate that the beneficial effect of BABE against high fat-induced hepatic oxidative stress. These favorable effects might be due to different types of active principles acting individually or synergistically each with a single or a diverse range of biological activities

The results of the present study indicate that the protective role of BABE on oxidative stress induced by HFD fed rats may be related to a counteraction of free radicals by its antioxidant activity of BABE, to strengthen endogenous antioxidant defense by its ability to increase the non enzymatic antioxidants like GSH, vitamin C and vitamin E decreased content of lipid peroxide which is used as a marker for oxidative stress. This protective activity of BABE is mainly attributed to the presence of enriched therapeutic phytochemical constituents, which act synergistically to detoxify the free radicals produced by HFD and thereby decrease oxidative stress.

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