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International Journal of Innovative Research in Technology, Science & Engineering (IJIRTSE)
ISSN: 2395-5619, Volume – 4, Issue – 3. May 2018

Modulatory effect of *Betula Alnoides* bark extract on free radicals and antioxidants in tissues of high fructose-fed rats

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ABSTRACT

Objective: Increased fructose consumption is strongly associated with metabolic syndrome (MS) including insulin resistance, diabetes mellitus and cardiovascular disease. A diet high in fructose (> 60/100 g) induces IR in animals, and rats that are fed a high dose of fructose are considered in forming a nutritional model for insulin resistance (IR). Oxidative stress plays a vital role in the pathology associated with insulin resistance. The present study aimed to investigate the effects of *Betula alnoides* bark extract on the oxidant-antioxidant status in liver, kidney and heart of high fructose-fed diet (HFFD) rats. **Materials and Methods**: Male albino Wistar rats (160-180 g) were divided into six groups. The control group received the control diet containing starch. The fructose group was given a high-fructose diet (>60% of total calories). The Third group received High fructose diet. The fourth group was given fructose diet and administered *Betula alnoides* bark extract. The total experimental protocol was carried out for 45 days. Lipid peroxidation indices and antioxidant status in liver, kidney and heart were quantitated. **Results**: The HFFD fed rats showed increased levels of thiobarbituric acid reactive substances (TBARS), conjugated dienes (CD), lipid hydroperoxides (HP) and impaired antioxidant defense as evidenced by decreased in the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) glutathione reductase (GR) and the levels of reduced glutathione (GSH). Treatment with *Betula alnoides* bark extract to the fructose-fed rats mitigated these alterations **Conclusion:** *Betula alnoides* bark extract administration for 15 days decreased lipid peroxidation and restored the antioxidant potential. These findings support and strengthen the utility of *Betula alnoides* bark extract in the management of IR and associated pathology during diabetes.

Key Words: Antioxidants, *Betula alnoides* bark extract**,** fructose diet, insulin resistance, lipid peroxidation, liver.

Abbreviations:

CAT- catalase; GSH- reduced glutathione; SOD- superoxide dismutase; TBARS - thiobarbituric acid reactive substances; HFFD-High fructose fed diet

INTRODUCTION

Type 2 diabetes mellitus is one of the most common diseases in occidental society and it is associated with a high cardiovascular risk, not only due to the classical factors but also to a chronic low-grade inflammation.[1] It has been estimated that the global burden of type 2 diabetes mellitus will increase to 438 million in 2030. Similarly, for India this increase is estimated to be 58%, from 51 million people in 2010 to 87 million in 2030.[2] Insulin resistance (IR), defined as the reduced response of tissues to circulating insulin, is the core pathogenic factor in type 2 diabetes and obesity.[3] IR is the core pathogenic factor in the evolution of common human diseases like type 2 diabetes, obesity

and coronary heart disease. Oxidative stress plays a major role in the development of IR and is known to be an instigator of IR. [4] A high-fructose diet (60 g/100 g diet) induces IR associated with hyperinsulinemia and hyperglycemia in animals.[5]

Studies involving supplementation of antioxidants to fructose-fed rats have witnessed improved insulin sensitivity,[6] suggesting that oxidative stress is an instigator of IR in this model. Diet can have an impact on the balance between pro- and antioxidants and also between pro- and anti-inflammatory cytokines. For example, chronic consumption of fructose has been shown to stimulate reactive oxygen species (ROS) production, lower antioxidant power and initiate proinflammatory processes, and cause dysregulation of adipokines.[7] Administration of fructose can trigger free radical production, thereby decreasing antioxidant status and causing oxidative damage to proteins and lipids in the liver.[8] Therefore, antioxidant status in the liver is a major concern when evaluating fructose-induced metabolic syndrome.

Oxidative stress is defined as the persistent imbalance between the production of ROS and antioxidant defense culminating in irreversible cellular alterations.[9] This redox imbalance is associated with various pathological conditions such as diabetes mellitus, obesity, and cardiovascular disease.[10] Recent evidence shows that the increased flux of free fatty acids, glucose or hexosamine, and NADPH oxidase in diabetes leads to enhanced production of mitochondrial ROS resulting in oxidative damage.[11] ROS, such as superoxide anion ($O_2^{\bullet -}$), hydroxyl radical (OH), and hydrogen peroxide (H_2O_2), which are produced during normal metabolic processes, are constantly buffered by endogenous antioxidants like reduced glutathione (GSH), superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase.[12] Overproduction of ROS or a reduced level of antioxidants, or both, lead to oxidative damage of membrane proteins, lipids, and DNA.

In the modern era also most of the people believe the plants and phytoconstituents are a better choice to treat diseases than the allopathic drugs, even most of the drugs used in primitive medicine were instigated from plants.[13] *Betula alnoides* Buch. (Betulaceae), locally called the "Saur" tree, is indigenous to the Himalayan region of India.[14] In the traditional system of Indian medicine, *Betula alnoides* is used to treat microfractures or dislocated bones, to cure post-natal pain and bleeding, wounds, joint pains, and sprains. The bark is aromatic and is chewed to improve digestion and to cure diabetes. Furthermore, *Betula alnoides* has been shown to possess biological activity, rubefacient, analgesic, and phosphodiesterase inhibitory activity. Although *Betula alnoides* is widely used in local traditional medicinal treatment for a several of diseases. This plant has not been investigated for its possible antioxidant, antimicrobial activity and α-glucosidase inhibitory effect.[15] Betula bark extract showed a wide spectrum of *in vitro* and *in vivo* pharmacological activities like immunomodulatory, anti-inflammatory, antimicrobial, antiviral, antioxidant, antidiabetic, dermatological, gastroprotective and hepatoprotective. Antiarthritic and anticancer are the two major areas of research conducted on these species. The anticarcinogenic effects of Betula bark, betulin as well as betulinic acid have been extensively studied by modern researchers [16]. The plant has been used as an antidote in the treatment of snake bites. A decoction of the bark is used to treat dislocated bones. The bark also used as an antifungal activity, heamatonic, inflammation, strength nourishing, treatment fatigue, antimalaise and rheumatic alienments. Preliminary phytochemical screening of bark showed the presence of polyphenol, flavonoids, amino acids, fatty acids, β- sitosterol terpenoids and structurally similar to glycoside.[14] Therefore, the aim of this study was to effect *betula alnoids* bark extract in antioxidants and lipid peroxidation status in HFFD rats.

MATERIALS AND METHODS

Animals

Male albino rats approximately weighing 160-180 g 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 experimental animals were fed with standard pellet diet; HFFD 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.

Preparation of alcoholic extract

The bark of *Betula alnoides* was first washed well and dust was removed from the bark. Barks was 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 was stored in a refrigerator until used.

Chemicals

Fructose, bovine serum albumin, and other fine chemicals were obtained from Sigma Chemical Company, ST. Louis, MO, USA. All other chemicals and reagents used were of highest purity and of analytical grade marketed by Glaxo Laboratories, Mumbai, SD Fine Chemicals, Mumbai and Sisco Research Laboratories, Pvt. Ltd., India.

After one week of acclimatization the animals were divided into two batches. One batch was provided with a control diet containing starch as the source of carbohydrate (groups I and II) and the other was fed a fructose-enriched diet for 45 days (Groups III-IV). Different composition of diet (Table. 1) given to all the rats for 45 days. Group IV HFFD rats were received *Betula alnoides* bark extract was given orally for 15 days. Blood samples from all the groups of animals were collected from the tail vein on the 10th, 20th and 30th days and estimated glucose levels to ensure diabetic status.

Experimental Design

The rats were divided into six groups, each group consisting of six rats. *Betula alnoides* bark extract dissolved in water and given to rats twice daily for a period of 15 days, after 45 days of control and HFFD.

Group I : Normal control rats (for 45 days)

Group II : Control rats treated with *Betula alnoides* bark extract (1000 mg/kg) twice daily for 15 days

Group III : High Fructose fed rats (>60% fructose for 45 days)

Group IV : HFFD + *Betula alnoides* bark extract (1000 mg/kg) twice daily for 15 days

Collection of Samples

At the end of the 45th day, all the rats were fasted overnight and sacrificed by cervical decapitation under mild ether anesthesia. Blood was collected tube with heparin and plasma was separated by centrifugation. The liver, heart and kidney tissues were immediately removed and washed in ice- cold saline to remove blood. The tissues were sliced and homogenized in 0.1 M Tris- HCl buffer (pH 7.0). The homogenates were centrifuged at 1000 rpm for 10 min at 4ºC in a cold centrifuge.

Table 1: Composition of diets fed to rats for the determination of insulin resistance

Ingredient (g/100 g)	Control diet	High-fructose diet
Corn starch	60	-
Fructose	-	60
Casein	20	20
Methionine	0.7	0.7

Groundnut oil	5	5
Wheat bran	10.6	10.6
Salt mixture†	3.5	3.5
Vitamin mixture‡	0.2	0.2

†Composition of the mineral mix (g/kg): $MgSO_4.7H_2O$, 30.5; NaCl, 65.2; KCl, 105.7; KH_2PO_4, 200.2; $3MgCO_3.Mg(OH)_{2.3}H_2O$, 38.8; $FeC_6H_5O_7.5H_2O$, 40.0; $CaCO_3$, 512.4; KI, 0.8; NaF, 0.9; $CuSO_4.5H_2O$, 1.4; $MnSO_4$, 0.4; and $CONH_3$, 0.05.

‡One kilogram of vitamin mix contained: thiamine mononitrate, 3 g; riboflavin, 3 g; pyridoxine HCl, 3.5; nicotinamide, 15 g; d-calcium pantothenate, 8 g; folic acid, 1 g; d-biotin, 0.1 g; cyanocobalamin, 5 mg; vitamin A acetate, 0.6 g; α-tocopherol acetate, 25 g; and choline chloride, 10 g.

Analytical Method

An estimation plasma glucose level was assayed by the method of Sasaki *et al*., [17]. The levels of TBARS[18] and HP[19] were determined in tissue homogenates. CD was measured by the method of Rao and Recknagel [20]. The clear supernatant thus obtained was used for the assay of SOD[21], CAT[22], GPx[23], GSH[24] and GR[25].

Statistical analysis

Values or mean ± SD for six rats in the each group and statistically significant differences between mean values were determined by one way analysis of variance (ANOVA) followed by DMRT values of $P < 0.05$ was considered to be significant. Statistical Package for Social Studies (SPSS Inc., Chicago, IL)) 19.0 versions were used for this analysis.

RESULTS

Figure 1 shows the level of plasma glucose in control and experimental rats. The increased level of plasma glucose in fructose-fed rats as compared to control rats has been observed. Administration of *M. Betula alnoides* bark extract (1000 mg/kg) twice daily for a period of last 15 days were significantly decreased the level of glucose in HFFD rats as compared to group diabetic rats.

Table 2 presents the levels of TBARS, CD and HP in plasma, liver, kidney and heart of normal and experimental rats. Rats induced with HFFD, showed a significant increase in the levels of TBARS, CD and HP in plasma and tissues (liver, kidney and heart) when compared to normal control rats. Treatment with *Betula alnoides* bark extract (1000 mg/kg) to HFFD-induced rats significantly decreased the levels of TBARS, CD and HP in plasma and tissues when compared with HFFD-alone induced rats.

The activities of SOD, CAT, GPx, in the liver, kidney and heart were significantly lowered in fructose fed rats than normal control rats shown in Table 3. In High fructose rats treated with *Betula alnoides* bark extract (1000 mg/kg) were significantly increased the activities of these enzymatic antioxidants as compared to high fructose fed rats.

Table 4 illustrates the effect of *Betula alnoides* bark extract (1000 mg/kg) on the activities of GR and the levels of GSH in plasma, liver, kidney and heart in normal and HFFD-induced rats. Rats induced with HFFD, showed a significant decrease in the activity of GR and the levels of GSH in comparison with normal control rats. Oral

administration of *Betula alnoides* bark extract (1000 mg/kg) to HFFD-induced rats significantly increased the activities of these antioxidant enzymes.

Betula alnoides bark extract (1000 mg/kg) is an effective dose for all parameters significant effect in HFFD rats as compared to control rats. *Betula alnoides* bark extract (1000 mg/kg) in normal control rats didn't show any significant.

DISCUSSION

IR and/or Hyperinsulinemia are closely associated with oxidative stress and liver damage. It has been shown that chronic intake of high fructose diet can cause hyperinsulinemia and oxidative stress in the liver of rats.[26] IR is not only an early and major feature in development of noninsulin-dependent DM, but also associated with hyperlipidemia, hypertension, obesity, enhanced oxidative stress, endothelial dysfunction and cardiovascular disease.[27] It has been shown that rats fed with 60% fructose diet for 60 days exhibit higher insulin and glucose levels.[28]

In our study, the level of plasma glucose significantly increased in HFFD rats (group III) as compared to control rats (group I). High levels of dietary fructose and severe hyperglycemia may have interactive effects, which contribute to the progression and development of pathology. HFFD can certainly cause IR. Lavau *et al.*,[29] pointed that high-fructose diet could lower the activity of the intracellular enzymes associated with fatty acid synthesis and decrease the intra cellular capacity to utilize glucose, which in turn resulted in a blunted glucose metabolism response to insulin. Treatment with *Betula alnoides* bark extract (1000 mg/kg) twice daily for 15 days to HFFD rats significantly reduced the glucose levels, could be due to positively alter the enzymes involved in glucose metabolism.

In our study, the lipid peroxidation markers such as TBARS, CD, and HP were significantly increased in the plasma, liver, kidney and heart of HFFD rats. Enhanced lipid peroxidation in fructose-fed rats could be associated with high circulating glucose, which enhances free radical production from glucose autoxidation and protein glycation. Fructose feeding can induce free radical formation by down regulation of HMP shunt enzymes that generate a reduced environment in the form of NADPH and NADH.[30] The measurement of TBARS contents is an index of lipid peroxidation. Very high positive correlation show that fasting glucose and insulin levels were significant determinants of TBARS levels, suggesting a role of IR increased lipid peroxidation. CD is also linked to several steps of lipid peroxide degeneration. Around 30-35% of lipid peroxidation is actually detected by diene measurements.[31] LHP oxidize ferrous ion to ferric ion, which depends not only on the rate of initiation of peroxidation but also their decomposition to other products. Although the TBA test and CD measurement are very nonspecific, they can offer an empirical window on the complex process of lipid peroxidation.[32]

Moreover, administration of *Betula alnoides* bark extract (1000 mg/kg) to the HFFD rats reduced the oxidative stress and enhanced the insulin sensitivity. The phytochemical constituents of *Betula alnoides* bark extract (1000 mg/kg) were also established for their potent effect to inhibit/decrease the generation of ROS by reducing the oxidative stress. From the results of the present study, it could be observed that supplementation of *Betula alnoides* bark extract (1000 mg/kg) to HFFD rats had effectively decreased the formation of $O^{2\bullet -}$ in the liver, kidney as well as in heart which is reflected through scavenging of lipid peroxidative products like TBARS, CD, and HP. *Betula alnoides* bark extract compounds have been ascribed to the scavenging of free radicals, reducing oxidative stress and preventing the oxidation of biomolecules that can break reaction chains of pathogens in the deterioration of physiological functions.

The present study shows that decreased activities of SOD, CAT in the liver, kidney and heart were shown in HFFD rats. High levels of free radicals and the simultaneous decline in endogenous antioxidants can lead to damage of cellular organelles, and development of IR.[33] ROS can themselves reduce the activity of antioxidant enzymes such as SOD, CAT and GSH. SOD is a ubiquitous chain breaking antioxidant and a metalloprotein, plays an important protective role against oxidative damage induced by ROS. As an antioxidant enzyme, SOD scavenges the $O_2^{\bullet -}$ into H_2O_2, which was decreased with fructose feeding in our study. The major reason for SOD reduction could be an

increase in $O_2^{\bullet -}$ production and/or glycation of the active site of SOD under hyperglycemic conditions.[8] Under these circumstances, liver cells are more prone to oxidative damage or necrosis and lose their original function. Besides, normalizing the $O_2^{\bullet -}$ production has been shown to prevent hyperglycemic damage.[34]
Liver CAT activity was increased after fructose feeding, which might be part of the defensive response against fructose-induced oxidative stress. CAT detoxifies hydrogen peroxide into molecular oxygen and water, due to this conversion the toxic hydrogen peroxide is converted in to non-toxic one. Moreover this herbal powders may serve as an effective free radical scavenger and/or neutralizes the free radicals and increase the activities of SOD and CAT, which is due to the phytoactive constituents (polyphenols) of *Betula alnoides* bark extract treatment. Restored SOD and CAT activity by *Betula alnoides* bark extract treatment indicates that excessive $O_2^{\bullet -}$ radicals may be effectively eliminated, and hyperglycemia-mediated enzyme inactivation alleviated.
GSH is an important reducing agent in the cell, where it protects against the toxic effects of free radicals, peroxides and other toxic components. The increase in ROS and the decrease of GR activity lead to depletion of GSH concentration.[35] The decreased GSH levels in HFFD could be due to increased utilization to trap free radicals, and/or decreased regeneration as evident with the lower activity of GR.[36] GSH, being a potent free radical scavenger, is also a cofactor of GPx and plays the essential role in the antioxidant defense of the body. The decrease in the activity of hepatic GPx might also be due to increased turnover of the enzyme. GR is a protective mechanism to reduce the peroxide toxicity where in GSSG formed is actively reduced to GSH.[37]

Glutathione levels and activities of glutathione dependent enzymes were increased in rats treated with *Betula alnoides* bark extract. GPx and GR are essential for maintaining the constant ratio of GSH to GSSG in cells. Decreased glutathione levels in fructose fed rats may be due to increased utilization for protecting sulfhydryl group of proteins from lipid peroxides. *Betula alnoides* bark extract feeding restores the glutathione level and increased the activities of GPx and GR. In our studies administration of *Betula alnoides* bark extract to rats with high fructose diet induced oxidative stress showed improvement in antioxidant enzymes such as SOD, CAT. Hence, all the medicnal properties of *Betula alnoides* bark extract could be responsible for reducing glucose, lipd peroxidation and increasing antioxidant levels in HFFD rats.

The results of the present study showed that the *Betula alnoides* bark extract contains flavonoids and polyphenols. These phytochemicals exhibited greatest antioxidant activity DPPH, superoxide anion scavenging, nitric oxide scavenging and hydroxyl radical scavenging activities and metal chelating acyivity [iron chelator and iron reducing power] which participate in the various pathophysiology of diseases including cancer, diabetic and aging.

CONCLUSION

Our results show that HFFD rats results in development of oxidative stress in plasma, liver, kidney and heart. This oxidative stress may play a role in pathology associated with fructose feeding such as IR. In uncontrolled diabetes, oxidative stress results from increased free radical production and depletion of antioxidants like SOD, CAT, GPx, GR and GSH. Administration of *Betula alnoides* bark extract significantly reduced the free radical mediated lipid peroxidation, preserved the activities of antioxidant enzymes and maintained the levels of non-enzymic antioxidant. The finding of the present study shows that utility of *Betula alnoides* bark extract could be considered as therapeutic tool for the management of diabetic complications in which induction of oxidative stress is the major contributing mechanism.

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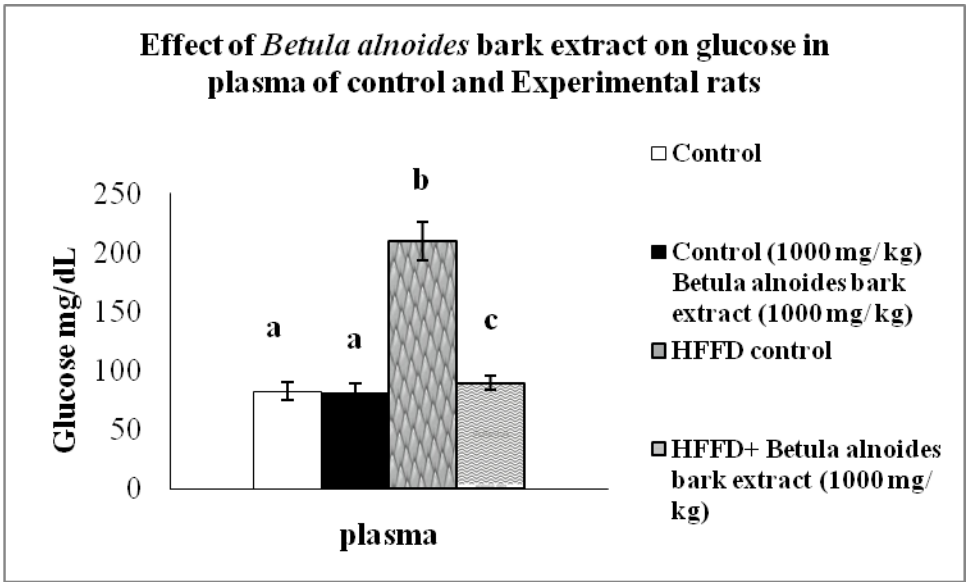
Figure 1: Effect of *Betula alnoides* bark extract on glucose in plasma of control and Experimental rats



Columns are mean ± S.D. for six rats in each group.

Columns not sharing common superscript are significant with each other at P<0.05 (Duncan's multiple range test).

Table 2: Effect of *Betula alnoides* bark extract on TBARS, CD and HP in plasma and tissues of control and Experimental rats

Parameters	Control	Control (1000 mg/ kg) *Betula alnoides* bark extract (1000 mg/ kg)	HFFD control	HFFD+ *Betula alnoides* bark extract (1000 mg/ kg)
TBARS Plasma (m moles/L)	0.70±0.05[a]	0.69±0.05[a]	2.32±0.14[b]	0.79±0.05[c]
Liver	1.42± 0.15 [a]	1.41±0.14[a]	2.54 ±0.20[b]	1.53±0.15[c]
Kidney	1.74±0.16[a]	1.72±0.15[a]	2.44±0.22[b]	1.79±0.16[c]
Heart	0.95±0.06[a]	0.94±0.06[a]	1.64±0.13[b]	1.01±0.07[c]
CD (A 233) Plasma (m moles/L)	0.90±0.07[a]	0.88±0.06 [a]	1.57±0.13[b]	1.00±0.07[c]
Liver	0.70±0.06[a]	0.68±0.05[a]	0.97±0.06[b]	0.74±0.06[c]
Kidney	0.66±0.05[a]	0.64±0.04[a]	0.93±0.06[b]	0.69±0.05[c]
Heart	0.58 ±0.04[a]	0.56 ±0.04[a]	0.75 ±0.04[b]	0.59 ± 0.04[c]
HP Plasma (m moles/L)	0.97±0.06[a]	0.96±0.07[a]	2.89±0.25[b]	1.09±0.09[c]
Liver	1.53±0.12[a]	1.52±0.11[a]	2.23±0. 17[b]	1.61±0.11[c]
kidney	1.65±0.15[a]	1.64±0.14[a]	2.29±0.24[b]	1.71±0.12[c]
Heart	1.31±.0.11[a]	1.29±0. 11[a]	1.92±0. 10[b]	1.37 ±0.12[c]

Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscripts (a, b, c) differ significantly at $P < 0.05$ (DMRT).
Tissue TBARS: µmol/mg protein
Tissue HP: µmol/mg protein

Table 3: Activities of *Betula alnoides* bark extract on antioxidant enzymes in the liver, kidney and heart of control and experimental animals.

Parameters	Control	Control (1000 mg/ kg) *Betula alnoides* bark extract (1000 mg/ kg)	HFFD control	HFFD+ *Betula alnoides* bark extract (1000 mg/ kg)
SOD (Units) Liver	3.94±0.29[a]	3.96±0.30[a]	2.42±0.22[b]	3.88±0.31[c]
kidney	4. 14±0.39[a]	4. 16±0.38[a]	3.05±0.26[b]	3.99±0.34[c]
Heart	3.54 ± 0.28[a]	3.59 ±0.29[a]	2.76 ±0.20[b]	3.50 ± 0.28[c]
CAT Liver	56.21±4.62[a]	57.1±3.29[a]	30.5±2.18[b]	53.82±5.42[c]
Kidney	54.1±4.83[a]	56.5±4.82[a]	34.99±3.2[b]	51.48±4.81[c]
Heart	53.15 ± 5.1[a]	54.1 ± 4.9[a]	40.8 ±4.31[b]	51.6 ± 4.9[c]
GPx Liver	6.04 ± 0.48[a]	6. 09±0.47[a]	4.26 ±0.40[b]	5.92±0.49[c]
Kidney	5. 14±0. 40[a]	5. 15±0.40[a]	4.01±0.39[b]	5.06±0.39[c]
Heart	5.23 ± 0.48[a]	5.26 ± 0.45[a]	4.06 ± 0.43[b]	5.16 ± 0.45[c]

Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscripts (a, b, c) differ significantly at $P < 0.05$ DMRT).
SOD: Amount of enzyme, which gave 50% inhibition of NBT reduction/mg protein
CAT: µmol of H_2O_2 consumed/min/mg protein
GPx: µmol of GSH consumed/min/mg protein

Table 4: Activities of *Betula alnoides* bark extract on antioxidant enzymes GSH and GR in the liver, kidney and heart of control and experimental animals.

Parameters	Control	Control + *Betula alnoides* bark extract(1000 mg/ kg)	HFFD control	HFFD+ *Betula alnoides* bark extract (1000 mg/ kg)
GSH liver	159.1±14.6[a]	161.2±15.4[a]	99.2±8.7[b]	151.5±12.6[d]
kidney	102.4±9.75 [a]	105.8±9.27 [a]	60.1±6.4 [b]	99.5±9. 17 [a]
Heart	103±9.5[a]	105.3±9.8[a]	54.1±6.8[b]	98.1±9.2[d]
GR Liver	24.8±1.92[a]	25.74±2.2[a]	11.29±1.56[b]	22.2±2.2[a]
Kidney	24.9±1.5[a]	25.7±1.6[a]	14.12±1.6[b]	22.58±2. 1[a]
Heart	20.5±1.4[a]	21. 1±1.40[a]	11.8±1.2[b]	18.9±1.6[a]

Each value is mean ± S.D. for six rats in each group.
Values not sharing a common superscripts (a, b, c) differ significantly at $P < 0.05$ DMRT).
GSH: µmol/mg protein

GR: µmoles/h/mg protein