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PREVENTIVE EFFECT OF *DALBERGIA SISSOO* AGAINST HIGH FRUCTOSE-INDUCED INSULIN RESISTANCE AND OXIDATIVE STRESS IN MALE WISTAR RATS

Kiran V. Pund[1*], Neeraj S. Vyawahare[1], Sandeep P. Sarode[1], Mahesh A. Kahane[1], Rajendra T. Gadakh[2]

[1]Department of Pharmacology, AISSMS College of Pharmacy, Near R.T.O., Pune-411 001, India.
[2]Department of Pharmacology, MGV's College of Pharmacy, Panchavati, Nashik- 422003, India.

ABSTRACT

High intake of dietary fructose exerts a number of adverse metabolic effects. The aim of the present study was to investigate whether ethanolic extract of *Dalbergia sissoo* bark (DS) ameliorate high-fructose diet-induced insulin resistance and oxidative stress in rats. High-fructose (60% of fructose) and DS (250 and 500 mg/kg/day) were given simultaneously from 30 days onwards. Fructose fed rats showed significant activity against hyperglycemia, hyperinsulinemia, hypertriglyceridemia, impaired glucose tolerance and impaired insulin sensitivity. In the oral glucose tolerance test, rats fed a high-fructose and supplemented with DS had a significantly reduced plasma glucose after 30 min of glucose loading, indicating that DS 500mg/kg significantly improved glucose intolerance in male wistar rats. In addition, rats fed a high-fructose and supplemented with DS markedly increased activity of hepatic superoxide dismutase, catalase, and suppressed lipid peroxidation when compared to fructose control groups. However, rats fed a high-fructose supplemented with DS 250mg/kg were found to have a less significant change in the activity of hepatic glutathione peroxidase. In conclusion, intake of DS may be a useful therapeutic strategy for prevention of a high-fructose diet-induced insulin resistance and oxidative stress.

Keywords: *Dalbergia sissoo*, High fructose, Oxidative stress, Insulin resistance.

INTRODUCTION

Fructose is a naturally occurring monosaccharide, an epimer of glucose, found in fruits and vegetables, and it makes up approximately 50% of honey and sucrose [1, 2] The last 25 years have witnessed a marked increase in total per capita fructose intake as a sweetener in the food industry, primarily in the form of sucrose (a disaccharide consisting of 50% fructose) and high-fructose corn syrup (HFCS; 55–90% fructose content) [3].

It is well known that fructose, at elevated concentrations, can promote metabolic changes that are actually or potentially deleterious, e.g., hyperlipidemia, hyperinsulinemia, insulin resistance, hyperuricemia, hypertension, glucose intolerance and non-enzymatic fructosylation of proteins [4, 5, 6, 7]. In addition, excessive fructose consumption may be responsible in part for the increasing prevalence of obesity, diabetes mellitus, non-alcoholic fatty liver disease and cardiovascular diseases [8, 9].

Rats fed with a high-fructose diet form a model of diet-induced insulin resistance, associated with hyperinsulinemia, hypertriglyceridemia and glucose intolerance [4] as well as affect on liver enzymes level. Recently, antioxidants are found to be effective in preventing a majority of the abnormalities induced by high-fructose diet [10].

Dalbergia sissoo (Roxb.) also called Indian Rosewood, belongs to the legume family (Fabaceae). It is a large deciduous perennial tree found in the lowland region Throughout India and is also indigenous to Pakistan, Bangladesh, Afghanistan and Nepal. It is used as timber or fire wood and for the treatment of a variety of ailments by different ethnic groups[11, 12, 13]. *Dalbergia sissoo* has also been used in folk medicine as an aphrodisiac, abortifacient, expectorant, antihelminthic, antipyretic, and in the treatment of various digestive disorders and skin diseases [11, 14]. *Dalbergia sissoo* leaves have been reported to have antidiabetic, anti-inflammatory activity, analgesic and antipyretic activities [15, 16, 17, 18].

Corresponding Author: **Kiran V. Pun Email:-** kvpund@gmail.com

Its barks reported as antioxidant activity, anti-Inflammatory activity, anti-spermatogenic activity [19, 15, 20].

Although, the antidiabetic and antioxidant activities of this plant in experimentally diabetic rats has been well documented in scientific literature [18, 19], studies regarding its efficacy in preventing insulin resistance which plays a role in Pathophysiology of type 2 diabetes mellitus have not been studied.

The preliminary phytochemical analysis of the ethanolic extract of the bark of the *Dalbergia sissoo* showed the presence of carbohydrates, flavonoids and tannins. Earlier report have been shown that flavonoids and tannins are chief phytochemical responsible for both antiobesity and antidiabetic activity [18, 21]. In our study *Dalbergia sissoo* was found to be safe upto 5000mg/kg of dose after 14 day observation.

MATERIALS AND METHODS

Fructose (New Neeta Chemicals, Pune), Trichloroacetic acid (TCA), Ethylene diamine tetra acetic acid (EDTA), Ferrous sulphate ($FeSO_4$) and other chemicals were purchased from local chemical supplier. Biochemical kits for estimation of serum glucose, total cholesterol and triglyceride were purchased from Biolab diagnostic (I) Pvt. Ltd., Tarapur Boisar, India.

Plant extract

The barks of *Dalbergia sissoo* Syn. Shisav (Family: Fabaceae) were collected in the month of November-December from local areas near Pune. The *Dalbergia sissoo* sample was authenticated and certified by Dr. P. G. Diwakar from Botanical Survey of India, Pune. Voucher specimen number (BSI/2011/KIVPDAS1) was deposited. The shade dried barks of *Dalbergia. sissoo* were subjected for size reduction to coarse powder. The powder was defatted with petroleum ether (60-80°C) and then extracted with 90% ethanol using solvent in soxhlet apparatus at 80^0 C under vacuum. The ethanolic extract was concentrated to dryness under reduced pressure and controlled temperature (48°C–50°C) with a rota vapour. The extract was dried in order to produce a dark brown solid extract. The ethanolic extract of *Dalbergia. sissoo* was then subjected to phytochemical screening.

Animals

Wistar albino male rats were used. They were maintained at 25± 2°C and relative humidity of 45 to 55% and under standard environmental conditions (12 hour light: 12 hour dark cycle). Animals were allowed to take specified amount of standard laboratory feed (VRK Nutrition, Pune) and water ad libitum. All the experimental procedures and protocols used in this study were reviewed and approved by the Institutional Animal Ethical Committee (IAEC) of AISSMS College of Pharmacy, Pune which is constituted under Committee for Purpose of Control and Supervision of Experiments on Animals (CPCSEA), approval no. CPCSEA/IAEC/PC-06/05-2K11. Ethical guidelines were strictly followed during all the experiments.

Experimental design

All the animals were weighing around 200±15 g at the time of dietary manipulation. Animals were randomly assigned into four groups of six each (Table 1).

Table 1. Experimental design of fructose and *Dalbergia sissoo* treatment

Group	Treatment	Dose and route
1 (Control)	NPD + distilled water	Oral + 1 ml/kg orally
2(Fructose control)	60% Fructose + saline	Oral + 1ml/kg orally
3 (Test 1)	60% Fructose + DS	Oral + 250 mg/kg, orally
4 (Test 2)	60% Fructose + DS	Oral +500 mg/kg, orally

Vehicle (tap water for Control and Fructose control group) and DS (dissolved in distilled water) were administered orally by gastric intubation. The animals were maintained in their respective groups for 60 days. The body weight and fructose intake were at each 15 days, fasting plasma glucose, total cholesterol and triglycerides of all animals were measured on 30, 45 and 60th day of experiment, while insulin on last day of treatment.

Oral glucose tolerance test (OGTT)

At the end of experimental period (60 days), the 12-h fasted animals were subjected to oral glucose tolerance test. For this, a glucose solution was introduced directly into the stomach through a fine gastric catheter at a dose of 2 g/kg body. A plasma glucose level was determined at 0 (before glucose administration), 30, 60 and 120 min after glucose administration.

Sample collection

Blood was collected from 12-h fasted rats with capillary tube from retino-orbital plexus of the animals in fresh vials containing EDTA as anticoagulant. The samples were centrifuged at 3000 rpm for 5 min (BioLab, Mumbai, India) and the plasma obtained was aliquoted and frozen for insulin assay. Plasma glucose and triglycerides were determined immediately. The blood sample collected during oral glucose tolerance test was from the retro orbital plexus of animals. After the experimental period the animals were fasted overnight and killed by cervical decapitation. The body was cut open and liver was dissected out into ice-cold saline and then thoroughly rinsed.

Biochemical measurements

The concentration of plasma glucose was measured by the glucose oxidase method, Plasma triglyceride level was estimated by GPO–POD enzymatic method using Diagnostic kit (BiolAB, Mumbai, India). Insulin was determined by radioimmunoassay kit (RIAK-1).

Hepatic antioxidant enzymes assay (estimation of MDA, GSH, SOD, and CAT)

Liver samples were dissected out and washed immediately with ice cold saline to remove as much blood as possible. Liver homogenates (10%, w/v) were prepared in ice- cold 0.15 M KCl using homogenizer. The unbroken cells and cell debris were removed by centrifugation at 5000 rpm for 10 min using a Remi refrigerated centrifuge. The supernatant was used for the estimation of reduced glutathione (GSH) [22], malondialdehyde (MDA) [23], superoxide dismutase (SOD) [24] and catalase levels [25, 26].

Statistical analysis

Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between two groups whereas multiple comparisons using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*. P values of less than 0.05 were regarded as significant. #p<0.05, ##p<0.001, ###p<0.0001 compared with control; *p<0.05 **p<0.01 compared with fructose control.

RESULTS

Effect of *Dalbergia sissoo* treatment on body weight

Graph 1. shows the effect of fructose on rats. A statistically very significantly (p<0.0001) increase in the body weight of vehicle treated fructose groups was observed as compared to the normal group from 30 days onwards. Interestingly, oral supplementation of the DS for 30 days at 500 mg/kg/day of body weight showed significant (p<0.05) decreased the weight gain as compared to the body weight gain vehicle treated fructose group animals.

Effect of DS treatment on food and fructose intake

After starting 60% fructose solution as a drinking fluid, food intake in the vehicle treated and DS treated groups slightly decreased compared to that in control rats, but fluid intake decreased significantly (p<0.01)from 15 and 30 days but increase intake 30 days onwards till the end of experimental period. There was no significant difference in food or fluid intake between the vehicle-treated group and DS-treated fructose group upto 60 day of treatment (Data not showed).

Effect of DS treatment on blood glucose levels

After 60 days of fructose fading, vehicle treated fructose group showed very significant (p<0.0001) increase in plasma glucose level as compare to control animals. DS 250 mg/kg and 500 mg/kg treated animals showed significant (p<0.01) reduction in plasma blood glucose level after30 days of treatment (Graph 2).

Effect of DS treatment on oral glucose tolerance (OGTT)

The administration of glucose in vehicle treated fructose group rats showed very significantly (p<0.0001) increase in plasma glucose level as compare to control animals. After 120 minute of DS 250 mg/kg, 500 mg/kg treated animals showed significant (p<0.01) reduction in plasma glucose level compare to vehicle treated fructose group rats (Graph 3).

Effect of DS treatment on cholesterol and triglyceride levels

After 60 days of fructose fed, vehicle treated fructose group showed significant (p<0.0001) increase in serum TC and TG level as compare to control animals. DS 250 mg/kg and 500 mg/kg treated animals showed significant (p<0.01) reduction in serum TC and TG level after 30 days of treatment as compare to vehicle treated fructose group rats (Graph 4 and 5).

Effect of DS treatment on plasma insulin levels

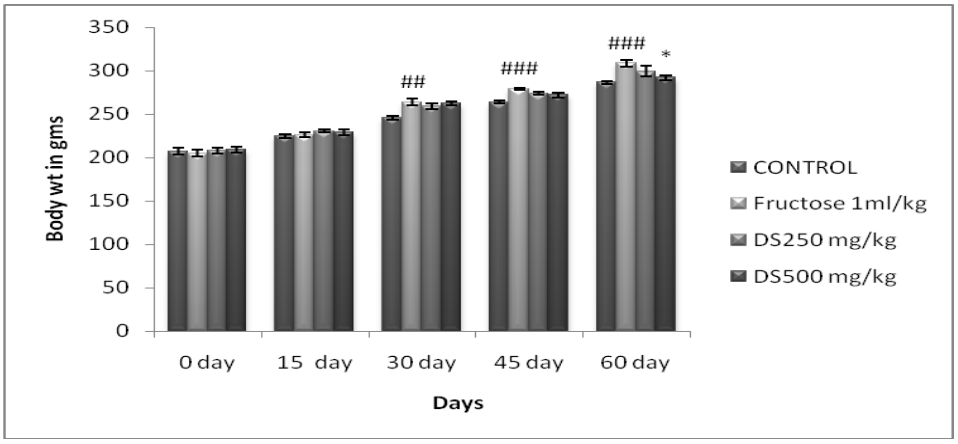
After 60 days of fructose fading, vehicle treated fructose group showed very significant (p<0.0001) increase in plasma insulin level as compare to control animals. DS 250, and 500 mg/kg treated animals showed significant (p<0.01) reduction in plasma insulin level after 30 days of treatment as compare to vehicle treated fructose group rats (Graph 6).

Table 2. Effect of *Dalbergia sissoo* treatment on antioxidant levels against high-fructose-induced insulin resistance and oxidative stress in male wistar rats

GROUPS	ANTIOXIDANT ENZYMES LEVEL			
	LPO (nM of MDA/ g of tissue)	GSH (µg of GSH/g of tissue)	Catalase (µM of H_2O_2/g of tissue/min)	SOD (units/ mg of tissue)
CONTROL	20.06 ±3.00	14.65 ±1.89	17.89 ± 2.72	109.69 ± 3.53
FRUCTOSE DW 1 ml/kg	35.54 ± 4.3##	7.59 ±0.79###	9.39 ±0.56##	55.01 ±1.16###
DS 250 mg/kg	23.11 ±3.35*	9.77 ±1.43	16.02 ±1.40	81.64 ± 2.73**
DS 500 mg/kg	23.33 ±2.72*	13.38 ± 1.95*	17.35 ±2.51*	93.85 ± 4.47**

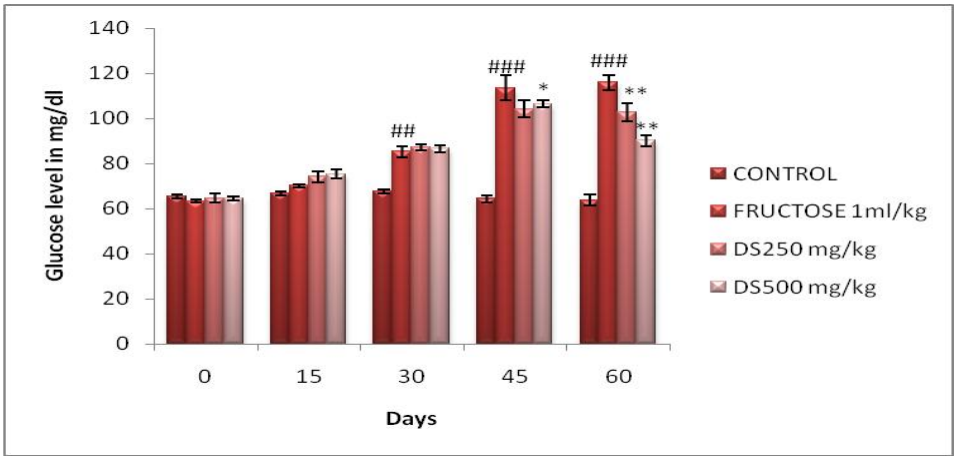
Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*.
##p<0.01 compared with control; *p<0.05, **p<0.01 compared with fructose control

Graph 1. Effect of *Dalbergia sissoo* treatment on body weight against high-fructose-induced insulin resistance and oxidative stress in male wistar rats



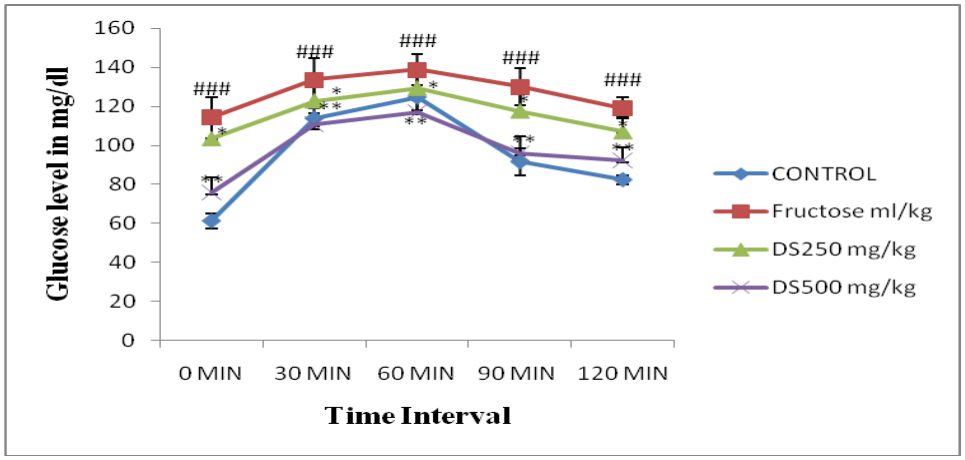
Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*.
##p<0.01, ###p<0.0001 compared with control; *p<0.05, compared with fructose control

Graph 2. Effect of *Dalbergia sissoo* treatment on plasma glucose levels against high-fructose-induced insulin resistance and oxidative stress in male wistar rats



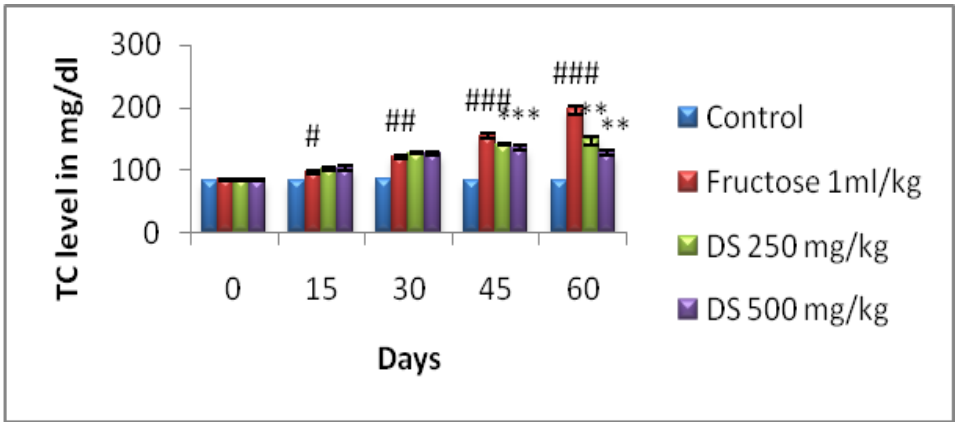
Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*.
##p<0.01 compared with control; *p<0.05, **p<0.01 compared with fructose control

Graph 3. Effect of *Dalbergia sissoo* on plasma glucose levels after a single oral administration of 2 g/kg of glucose in fructose treated male Wistar rats



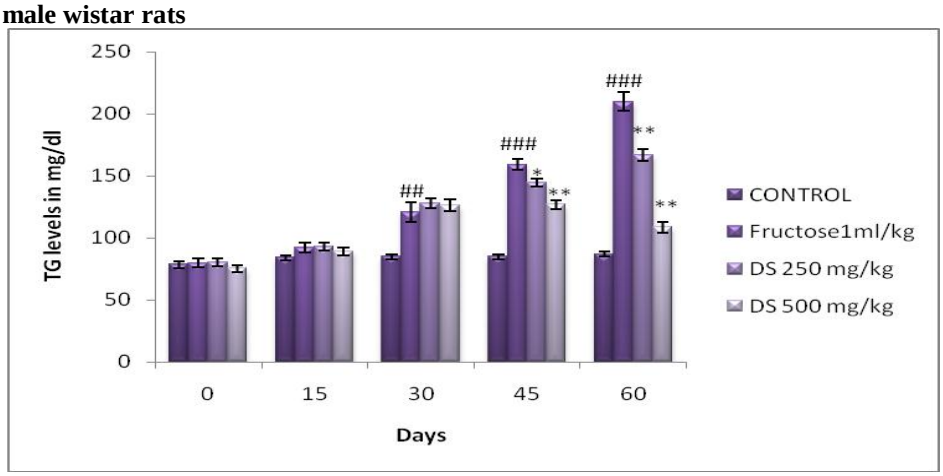
Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test*.
###p< 0.0001 compared with control; *p<0.05, **p<0.01 compared with fructose control

Graph 4. Effect of *Dalbergia sissoo* treatment on cholesterol levels against high-fructose-induced insulin resistance and oxidative stress in male wistar rats



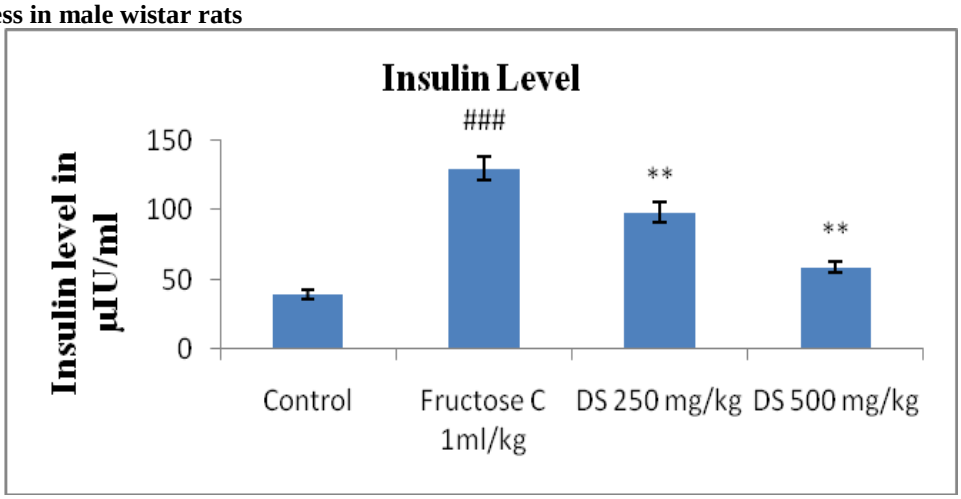
Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test.*
##*p*<0.01, ###*p*<0.0001 compared with control; **p*<0.05, ***p*<0.01 compared with fructose control

Graph 5. Effect of *Dalbergia sissoo* treatment on triglyceride levels against high-fructose-induced insulin resistance and oxidative stress in male wistar rats



Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test.*
##*p*<0.001, ###*p*<0.0001 compared with control; **p*<0.05, ***p*<0.01 compared with fructose control

Graph 6. Effect of *Dalbergia sissoo* treatment on plasma insulin level against high-fructose-induced insulin resistance and oxidative stress in male wistar rats



Results are expressed as Mean ± SEM (n=6). The unpaired Student's t-test was used for analyzing the data between two groups where as multiple comparisons using One-way Analysis of Variance (ANOVA) followed by *Dunnett's- test.*
###*p*<0.0001 compared with control;***p*<0.01 compared with fructose control

Effect of DS treatment on antioxidant enzyme levels

Graph 7.shows the effect of administration of DS on MDA, CAT, GSH and SOD in liver tissue of different groups of rats. There was significant (p<0.01) elevation in tissue MDA and significant (p<0.0001) decreased in GSH and CAT levels in vehicle treated fructose group rats as compared to normal rats. Treatment with DS 250 and 500 mg/kg for 30 days resulted in less significant (p<0.05) decrease in liver tissue MDA level. Treatment with DS 500 mg/kg for 30 days resulted in less significant (p<0.05) increased in liver tissue GSH and CAT levels. DS 250 and 500 mg/kg for 30 days resulted in significant (p<0.01) restored in liver tissue SOD levels (Table 2).

DISCUSSION

Dietary fructose (or sucrose) is a monosaccharide which can induce metabolic disorders [27, 28, 29] including insulin resistance, hyperinsulinemia, hypertension and dyslipidemia which is of pathophysiologic importance for the development of diabetes, obesity, non alcoholic fatty liver disease and atherosclerosis [30]. There are many reports in the literature describing an increase in body weight, glycemia, and insulinemia with the consumption of high-fructose diets in both humans and animal models [31, 32].

Fructose intake is known to be responsible factor for development of insulin resistance [33, 34], hence in this study high fructose feeding in rats for 60 days resulted in fasting hyperglycemia, hypertriglyceridemia, hyperinsulinemia, glucose intolerance and impaired antioxidant potential leading to the development of insulin resistance [6].

On treatment with DS 250 and 500 mg/kg to fructose fed animal showed slight change in food and fructose intake as compared to fructose control group. There is now much emerging evidence that chronic consumption of high-fructose diets contributes to excessive formation of reactive oxygen species (ROS). This leads to induced oxidative stress, and mediated insulin resistance [35]. Moreover, an increase in cellular ROS accumulation directly triggers the activation of serine/threonine kinase cascades such as c-Jun N-terminal kinase, and nuclear factor-kappa B that, in turn, phosphorylate multiple targets, including the insulin receptor and the insulin receptor substrate (IRS) [36]. Increased serine phosphorylation of IRS directly decreases its ability to undergo tyrosine phosphorylation and accelerates the degradation of IRS-1, causing impaired glucose uptake in muscle, liver and adipose tissues [36, 37].

In this study, both the doses were found to be effectively improved glucose tolerance, antioxidant activity and decrease in insulin level along with decrease TC, TG levels, but DS 500 mg/kg showed more activity, thereby suggested possible use in related human disorders. The earlier reports documented prominent role of flavonoids and tannins may responsible for its therapeutic activity [38, 39, 40].

CONCLUSION

The present study indicates that the supplementation of *Dalbergia sissoo* at doses 250 and 500 mg/kg significantly showed improvements on hyperglycemia, hypertriglyceridemia, hyperinsulinemia, glucose intolerance and impaired antioxidant potential. These favorable effects might be due to presence of tannins and flavonoids acting individually or synergistically each with a single or a diverse range of biological activities. The present study also provides additional evidence in support of the use of DS for prevention and/or management of diabetes, the pre-diabetic state of insulin resistance.

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