

# Protective Effect of *Gymnema sylvestre* Ethanol Extract on High Fat Diet-induced Obese Diabetic Wistar Rats

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Kumar, *et al.*: Biochemical and Cellular Alterations by *G. sylvestre*

Obesity is associated with numerous co-morbidities such as cardiovascular diseases, type 2 diabetes, hypertension and others. Therefore, the present study was planned to investigate the effect of water-soluble fraction of *Gymnema sylvestre* ethanol extract on biochemical and molecular alterations in obese diabetic rats. Diabetes was induced by single *i.v.* injection of streptozotocin (45 mg/kg) via tail vein. Obesity was induced by oral feeding of high fat diet for a period of 28 days in diabetic rats. Body weight gain, food intake, water intake, hemodynamic parameters (systolic, diastolic, mean arterial blood pressures and heart rate), serum biochemical parameters (leptin, insulin, lipid levels, apolipoprotein B and glucose), cardiomyocyte apoptosis (cardiac caspase-3, Na<sup>+</sup>/K<sup>+</sup> ATPase activity and DNA fragmentation) organs and visceral fat pad weight and oxidative stress parameters were measured. Oral treatment with water soluble fraction of *Gymnema sylvestre* ethanol extracts (120 mg/kg/p.o.) for a period of 21 days, resulted in significant reduction in heart rate, mean arterial pressure, serum leptin, insulin, apolipoprotein B, lipids, glucose, cardiac caspase-3 levels, Na<sup>+</sup>/K<sup>+</sup> ATPase activity and DNA laddering, visceral fat pad and organ's weight and improved the antioxidant enzymes levels in the high fat diet induced obesity in diabetic rats. The results of present study reveal that water soluble fraction of *Gymnema sylvestre* ethanol extract could be useful intervention in the treatment of obesity and type-2 diabetes mellitus.

**Key words:** *Gymnema sylvestre*, leptin, insulin, blood pressure, caspase-3, dyslipidemia

Obesity has been a common feature in Western Societies, although recent evidence suggests that it becomes also a major problem in Asia and almost all over the world. Obesity is associated with diabetes mellitus, hypertension, dyslipidemia and cardiovascular disease<sup>[1]</sup>. There has been a tragic increase in diabetes across the world, paralleling the overweight and obesity epidemic. Type 2 diabetes mellitus is an increasingly common disorder of carbohydrate and lipid metabolism<sup>[2]</sup>. The high risk of both diabetes and cardiovascular disease associated with obesity in Asians may be due to a predisposition to abdominal obesity, which can lead to the metabolic syndrome and impaired glucose tolerance. Furthermore, approximately 197 million people worldwide have impaired glucose tolerance, most commonly because of obesity and the associated metabolic syndrome<sup>[3]</sup>.

A number of studies have reported that rats treated with streptozotocin (STZ)-induced diabetes show

severe hyperlipidemia after exogenous fat loading<sup>[4]</sup>. One important pathologic phenomenon associated with obesity and its related conditions is cardiomyocyte cell death, and in particular, cardiomyocyte apoptosis<sup>[5]</sup>. It has been reported that high fat diet induces apoptosis<sup>[6]</sup>. During apoptosis, various proteases are activated that lead to the destruction of diverse intracellular proteins and the fragmentation of nuclear chromatin. Caspase-3 is a key player involved in the caspase-dependant apoptotic pathway.

The sodium (Na) and potassium (K)-activated adenosine-triphosphatase (Na<sup>+</sup>/K<sup>+</sup> ATPase) is a membrane enzyme that energizes the Na-pump by hydrolysing adenosine triphosphate and wasting energy as heat, so playing a role in thermogenesis and energy balance. Animal and human obesity is associated with reduction of tissue Na<sup>+</sup>/K<sup>+</sup> ATPase, linked to hyperinsulinemia<sup>[7]</sup>.

*Gymnema sylvestre* R. Br. (family: Asclepidaceae) is a native plant in the south west of India, Australia and tropical Africa. From ancient times,

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*G. sylvestre* has been used in Indian traditional medicine (Ayurvedic medicine) and is considered to be effective in improving urination, and diabetes<sup>[8]</sup>. In an animal study, extract of *Gymnema sylvestre* R. Br leaves improved serum cholesterol and triglyceride levels through influence of lipid metabolism. Bishayee and Chatterjee reported the hypolipidemic and antiatherosclerotic effect of *Gymnema sylvestre* extract in albino rats fed on high fat diet<sup>[9]</sup>. The present study was designed to evaluate the protective effect of water soluble fraction of *G. sylvestre* ethanol extract on blood pressure, hyperleptinemia, hyperinsulinemia, hyperglycemia, dyslipidemia, caspase-3 levels, organs and visceral fat pad weight and oxidative stress on HFD-induced obesity in diabetic albino rats.

## MATERIALS AND METHODS

The present study was approved by the Institutional Animal Ethics Committee (IAEC) of Hamdard University, New Delhi, which is registered with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India, India, (Registration No. 173/CPCSEA, dated Jan 28, 2000). Male Wistar albino rats, weighing 150-200g, were procured from the Central Animal House Facility, Hamdard University, New Delhi and acclimatized under standard laboratory conditions (12 h light and 12 h dark:day:night cycle) at 25±2°, relative humidity (50±15%) and had a free access to water *ad libitum*.

### Diet and chemicals:

High fat diet was purchased from National Centre for Laboratory Animal Sciences (NCLAS), National Institute of Nutrition (NIN), Hyderabad, Andhra Pradesh, India. HFD contains (g/kg): Casein-342 g; cystine-3 g; starch-172 g; sucrose-172; cellulose-50 g; G. N. oil-25 g; tallow-90 g; mineral oil-37 g; vitamin mix-10 g. All other chemicals used were of analytical grade.

### Preparation of water-soluble fraction of the *Gymnema sylvestre* extract:

The leaves of *Gymnema sylvestre* are purchased locally, air dried and authenticated by Raw Materials Herbarium and Museum, NISCAIR, New Delhi, India. The specimen voucher (Ref. NISCAIR/RHMD/Consult/2008-09/980/11) was retained to the Department of Pharmacology, Faculty of Pharmacy Jamia Hamdard, New

Delhi-110 062. Air-dried leaves of *G. sylvestre* extracted with ethanol (70%) in Soxhlet's apparatus. The solvent was removed under reduced pressure. The ethanol extract yield was 10.8% (w/w) in terms of starting material. The ethanol extract was standardized by determination of Ash value, pH, foam index, phytochemical constituent and microbial load according to WHO guidelines<sup>[10]</sup>. Further, the ethanol extract was sub fractionated into water-soluble (W-S) fraction and water-insoluble (W-INS) fractions<sup>[11]</sup>. The yield obtained of W-S and W-INS were 60% and 40% in terms of the total ethanol extract, respectively.

### Experimental design:

After acclimatization, except rats of Group I (normal healthy control), all rats were treated with streptozotocin (STZ, 45 mg/kg, i.v. single dose), those rats having fasting blood glucose level more than 200 mg/dl were included in this study and divided in following groups of 8 animals each and treated as follows: Group I—rats fed with standard rat diet for a period of 28 days; Group II (pathogenic control)—rats fed with HFD for a period of 28 days; Group III—rats fed with HFD for a period of 28 days + from 8th day 120 mg/kg/p.o. water soluble fraction of *G. Sylvestre* ethanol extract.

### Measurement of anthropometric, hemodynamic parameters:

Body weight gain, daily food intake, daily water intake were measured. Hemodynamic parameters (systolic, diastolic, mean arterial blood pressure and heart rate) were measured using non invasive Blood Pressure Recorder using rat tail-cuff method (Kent Scientific Corporation, USA) on 29th day.

### Measurement of serum biochemical parameters:

Blood was collected from the retro-orbital plexus of overnight fasted rats using microcapillary tubes on 29th day. Serum was separated by centrifugation (4000 rpm for 10 min) and transferred to Eppendorf tubes. The concentrations of lactate dehydrogenase (Reckon Diagnostics Pvt. Ltd., Baroda, India) glucose, total cholesterol, triglycerides (all three from Span diagnostics Ltd., Surat, Gujarat, India), HDL-cholesterol (Reckon Diagnostics Pvt. Ltd., vadodara, India), and apolipoprotein- B by immunoturbidimetric immunoassay kit (Randox Laboratories Ltd., Antrim, UK) in serum were measured with commercial kits. Glycohemoglobin was estimated in blood with a

commercial available kit (Asritha *In vitro* Diagnostic Reagents, Hyderabad, India). The concentrations of insulin and leptin in the serum were measured respectively with rat insulin ELISA kit (Alpco Diagnostics, Salem, USA), rat leptin ELISA kit (BioVendor, Brno, Czech Republic). On the final day of experiment the rats were fasted overnight and sacrificed by cervical dislocation and then different organs (heart, liver, Kidney and pancreas) and fat pads (epididymal, perirenal and mesentric) were removed, washed with normal saline and weighed<sup>[12]</sup>.

#### **Cardiomyocyte apoptosis measurement:**

Caspase-3 activity was measured using Caspase-3/ CPP 32 colorimetric assay kit. 50 µl supernatant from homogenized tissue with cooled lysis buffer was taken from each sample and 50 µl of 2X Reaction Buffer (containing 10 mM DTT) was added to each sample. Then 5 µl of the 4 mM DEVD-pNA substrate (200 µM final conc.) was added and incubated at 37° for 1-2 h to allow a dissociation of p-nitroanilide (pNA) from the conjugate DEVD-pNA to form a blue colour. CPP-32 activity was measured spectrophotometrically at 405 nm. Caspase-3 activity was calculated as nmol/h/mg protein<sup>[13]</sup>. Na<sup>+</sup>/K<sup>+</sup> ATPase activity was measured in heart tissue, method reported by Bonting<sup>[14]</sup>.

Apoptosis was evaluated by examining the characteristic pattern of DNA laddering generated in the apoptotic myocardium using gel electrophoresis. Myocardial samples were homogenized in solution containing 50 mmol/l Tris-HCl (pH 8.0), 100 mmol/l EDTA, 100 mmol/l NaCl, and 1% sodium dodecyl sulphate. The tissue homogenate was digested with 5 µl of proteinase K (stock solution 20 mg/ml) at 56° for 2 h and incubated with RNase A (1 µl/ml) at 37° for 1 h. After that phenol/chloroform (1:1) extraction was performed twice. The tubes were shaken and kept at room temperature (5-10 min). Then tissues were precipitated and centrifuged in cooling centrifuge at 4°; 10 000 rpm for 10 min. Supernatant containing DNA were precipitated with 600 µl isopropanol and the resulting DNA pellets after centrifugation were washed with 75% chilled ethanol and dissolved in 100 µl of TE buffer solution (10 mmol/l Tris HCl (pH 8.0), 1 mmol/l EDTA). DNA samples (5 µl DNA+1 µl gel loading dye) were subjected to electrophoresis on 2% agarose gel, stained with ethidium bromide. DNA laddering, an indicator of tissue apoptotic nucleosomal DNA

fragmentation was visualized and photographed under ultraviolet transilluminator<sup>[15]</sup>.

#### **Determination of antioxidant enzymes and oxidative stress in heart tissue:**

All antioxidant enzyme activities were determined after heart tissue was homogenized with phosphate buffered saline (PBS) at a pH of 7.0. Glutathione was measured according to Ellman method<sup>[16]</sup>. Glutathione peroxidase (GPx) activity was determined according to the method of Mohandas *et al*<sup>[17]</sup>. Glutathione reductase (GR) was determined according to the method of Carlberg and Mannerviek<sup>[18]</sup>. GST activity was measured by the method of Habig *et al*<sup>[19]</sup>. Superoxide dismutase (SOD) and catalase activities were estimated according to Marklund and Marklund<sup>[20]</sup> and Claiborne<sup>[21]</sup> methods.

Lipid peroxidation was determined with spectrophotometric measurement of the amount of malondialdehyde equivalents with thiobarbituric acid and was expressed as thiobarbituric acid-reactive substances (TBARS; nmoles malondialdehyde/mg protein), according to the method of Ohkawa *et al*<sup>[22]</sup>.

#### **Histopathological examination:**

The studied animals were killed and heart tissue samples collected, fixed in 10% formalin buffered solution, cut into 5 µm sections and stained with haematoxylin/eosin. The sections of heart tissues were studied to determine the protective effect of water soluble fraction of *G. sylvestre* extract on tissue damage by high fat diet and STZ.

#### **Statistical analysis:**

All values are expressed as mean±standard error of mean (SEM). Comparisons between the treatment groups and pathogenic control group were performed by analysis of variance (ANOVA) followed by Dunnett's *t*- test. *P*<0.05 was assumed to be statistical significant.

## **RESULTS**

#### **Effect of water-soluble fraction of *G. sylvestre* ethanol extract on anthropometric and hemodynamic parameters:**

As shown in Table 1, after STZ (45 mg/kg single i.v.) administration rats fed on HFD for 28 days, water intake and food intake were significantly (*P*<0.05) increased while body weight gain decreased in Group

II (i.e. Pathogenic control group) as compared to the Group I (normal healthy control group). These parameters were reversed in Group III (i.e. STZ 45 mg/kg, i.v. single dose+HFD for 28 days+water soluble fraction of *G. sylvestre* extract 120 mg/kg/p.o. for 21 days) as compared to the Group II.

There was significant ( $P<0.05$ ) increase in hemodynamic parameters (systolic, diastolic, mean BP and heart rate) in Group II as compared to the normal control group (i.e. Group I) while in Group III, these parameters were significantly reduced as compared to the Group II.

#### Effect of Water-soluble fraction of *G. sylvestre* ethanol extract on hyperlipidemia, hyperleptinemia, hyperinsulinemia and hyperglycemia:

As shown in Tables 1 and 2, Group II showed significant ( $P<0.05$ ) increase in TC (3.7 fold), TGs (5.4 fold), LDL-C (5 fold), VLDL-C (5 fold) and atherogenic index as compared to the Group I, while serum HDL-C (1.2 fold) level was significantly reduced in Group II. These parameters were significantly decreased in the Group III (TC- 2.8 fold, TGs- 3 fold, LDL-C- 4.5 fold and VLDL-C- 3 fold) and Group IV (TC- 3.1 fold, TGs- 3.3 fold, LDL-C- 4.8 fold and VLDL-C- 3.2 fold), while HDL-C (1.7 fold in Group III) was increased as compared to the Group II. Serum leptin (3.2 fold), insulin (7.5 fold), apolipoprotein-B (apo-B) (7.8 fold), lactate dehydrogenase (LDH) (12 fold), glucose (4.9 fold) and glycosylated hemoglobin (1.5 fold) were significantly ( $P<0.05$ ) increased in Group II as compared to the Group I. These parameters were significantly ( $P<0.05$ ) decreased in Group III (leptin- 3.3 fold, insulin- 11 fold, apo-B- 5 fold, LDH- 3.5 fold, blood glucose- 4.8 fold and glycosylated hemoglobin- 1.4 fold) as compared to the Group II.

#### Effect of water-soluble fraction of *G. sylvestre* ethanol extract on cardiomyocyte apoptosis:

The mean cardiac caspase -3 levels (4.5 fold) were significantly ( $P<0.05$ ) increased in Group II as compared to the Group I while in Group III (2.3 fold), mean caspase-3 levels were significantly decreased as compared to the Group II. The  $\text{Na}^+/\text{K}^+$  ATPase activity was significantly (1.1 fold) decreased in Group II as compared to the Group I while in Group III (1.4 fold) the activity was significantly increased in heart tissue as compared to the Group II. (Table 2) DNA laddering was more significant in the

group II, while DNA laddering was preserved in the group III (fig. 1).

#### Effect on organs and visceral fat pad weight:

Organs weight (heart, liver, kidney and pancreas) and visceral fat pad weights (mesenteric, perirenal and epididymal) were significantly ( $P<0.05$ ) increased in group II as compared to the group I. The visceral fat pad weights decreased in Group III, while there was no change in organ's weight as compared to the Group II (Table 2).

**TABLE 1: EFFECT OF W-S FRACTION OF GSE ON ANTHROPOMETRIC, HEMODYNAMIC AND SERUM LIPID PARAMETRES**

	Group I	Group II	Group III
Food Intake (g/100 g day)	12.78±0.31	17.27±0.36*	12.07±0.51 <sup>ns#</sup>
Water Intake (ml/100 g/day)	40.34±3.81	49.58±3.41*	42.57±1.26 <sup>#</sup>
Body weight gain (g)	75.13±5.02	46.46±11.61*	59.33±8.44 <sup>ns#</sup>
Systolic BP (mm Hg)	127.83±6.86	161±1.43*	106±1.52 <sup>#</sup>
Diastolic BP (mm Hg)	96±4.65	126.66±1.80*	79.16±2.65 <sup>#</sup>
Mean BP (mm Hg)	106.16±5.00	137.83±0.94*	90.83±1.07 <sup>#</sup>
Heart Rate (BPM)	421.83±15.77	463.66±23.65*	350.33±9.65 <sup>#</sup>
Total cholesterol (mg/dl)	101.69±2.00	373.22±17.51*	132.08±8.61 <sup>#</sup>
HDL-C (mg/dl)	32.26±1.01	28.54±0.86*	48.45±1.04 <sup>#</sup>
Triglycerides (mg/dl)	64.97±2.19	346.57±18.07*	113.23±7.30 <sup>#</sup>
LDL-C (mg/dl)	56.64±0.31	275.37±18.74*	60.99±9.27 <sup>#</sup>
VLDL-C (mg/dl)	12.99±0.43	69.31±3.61*	22.64±1.46 <sup>#</sup>
Atherogenic index	3.25±0.09	13.06±0.38*	2.73±0.19 <sup>#</sup>

All values were expressed as mean±SEM, (n=8 rats/group), \* $P<0.05$  as compared to the Group I; <sup>#</sup> $P<0.05$ , <sup>ns#</sup>: non significant as compared to the Group II (ANOVA followed by Dunnett's test), BP: blood pressure, HDL: high-density lipoprotein, LDL: low-density lipoprotein VLDL: very low-density lipoprotein, GSE: *G. sylvestre* ethanol extract

**TABLE 2: EFFECT OF W-S FRACTION OF GSE ON BIOCHEMICAL PARAMETERS IN SERUM AND HEART TISSUE**

Parameters	Group I	Group II	Group III
Serum leptin (pg/ml)	159.37±6.26	513.5±64.01*	155.5±8.19 <sup>#</sup>
Serum insulin (ng/ml)	0.116±0.02	1.832±0.076*	0.175±0.02 <sup>#</sup>
LDH (IU/L)	23.48±0.64	285.26±11.49*	79.89±9.61 <sup>#</sup>
Apolipoprotein-B (mg/dl)	4.65±0.22	32.78±2.01*	6.18±0.26 <sup>#</sup>
Glucose (mg/dl)	94.14±2.16	430.62±8.78*	95.72±3.30 <sup>#</sup>
Glycated hemoglobin (in %)	7.94±0.11	13.88±0.19*	8.82±0.18 <sup>#</sup>
Caspase-3 activity (nmole/hr/mg protein)	62.34±9.28	288.80±8.36*	124.45±3.77 <sup>#</sup>
Na-K ATPase activity (µmol of Pi liberated/min/mg protein)	0.725±0.04	0.654±0.03*	0.909±0.02 <sup>#</sup>
Organ's weight (g/100gm body weight)	9.60±0.28	12.14±0.42*	12.28±0.39 <sup>ns#</sup>
Visceral fat pad weight (g/100 gm body weight)	1.64±0.04	2.14±0.08*	1.85±0.05 <sup>#</sup>

All values were expressed as mean±SEM, (n=8 rats/group), \* $P<0.05$  as compared to the Group I; <sup>#</sup> $P<0.05$ , <sup>ns#</sup>: non significant as compared to the Group II (ANOVA followed by Dunnett's test), LDH: low-density lipoprotein, GSE: *G. sylvestre* ethanol extract

### Effect of water-soluble fraction of *G. sylvestre* ethanol extract on oxidative stress:

Lipid peroxide levels (i.e. nmol MDA/mg protein) were significantly (5.5 fold) increased in Group II as compared to the Group I, while the levels of lipid peroxides were significantly decreased in Group III (2 fold). Superoxide dismutase levels were significantly decreased in Group II (1.7 fold) as compared to the Group I, while these levels were significantly increased in Group III as compared to the Group II. Catalase levels (5 fold) decreased in Group II as compared to the Group I, while catalase levels in Group III (2.5 fold) were increased as compared to the Group II. Reduced glutathione levels (1.3 fold) were decreased in Group II as compared to the Group I, while these levels were in Group III (1.6 fold) increased as compared to the Group II (Table 3).

Glutathione peroxidase levels (1.5 fold) decreased in Group II as compared to the Group I while these levels were in Group III (1.4 fold) increased as compared to the Group II. Glutathione reductase levels (1.7 fold) were decreased in Group II as compared to the Group I, while in Group III (1.8 fold) increased as compared to the Group II. Glutathione-S-transferase levels (3.1 fold) were decreased in Group II as compared to the Group I; while in Group III (2.2 fold) were increased as compared to the Group II shown in Table 3.

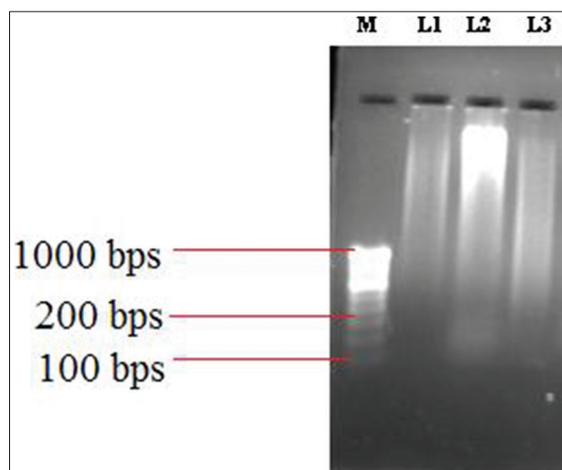


Fig. 1: Effect of water-soluble fraction of *Gymnema sylvestre* ethanol extract on DNA fragmentation.

DNA fragmentation was detected by agarose gel electrophoresis in HFD-induced obesity in diabetic rats. Lane M = Marker, L1 = normal control group, L2 = toxic control group (STZ 45 mg/kg i.v. + HFD group), L3 = water soluble fraction of *Gymnema sylvestre* ethanol extract (120 mg/kg/p.o.) treated group.

### Effect of water-soluble fraction of *G. sylvestre* ethanol extract on histopathological changes:

Water soluble fraction of *G. sylvestre* ethanol extract improved histopathological changes as demonstrated in fig. 2. Photomicrograph of heart of normal control group shows no fatty changes with normal tissue architecture and regular morphology of myocardial cell membrane (fig. 2a). Photomicrograph of heart of STZ+HFD group shows focal fatty

TABLE 3: EFFECT OF W-S FRACTION OF GSE ON OXIDATIVE STRESS AND ANTIOXIDANT ENZYMES

Parameters	Group I	Group II	Group III
TBARS (nmol MDA/mg protein)	0.200±0.003	1.149±0.02*	0.583±0.02#
SOD (IU/mg protein)	1.771±0.01	1.123±0.015*	1.308±0.011#
CAT (nmol H <sub>2</sub> O <sub>2</sub> - consumed/min/mg protein)	55.75±1.88	10.64±0.34*	26.34±0.35#
GSH (μmol of phosphorus liberated/min/mg protein)	26.75±0.97	15.86±0.48*	26.26±0.45#
Glutathione peroxidase (nmol NADPH oxidized/min/mg of protein)	184.81±7.54	121.28±6.46*	173.47±10.46#
Glutathione reductase (nmol NADPH oxidized/min/mg of protein)	29.90±0.43	17.39±1.22*	33.70±1.09#
Glutathione S transferase (nmol CDNB conjugate formed/min/mg protein)	492.31±9.01	155.80±9.71*	399.79±5.68#

All values were expressed as mean±SEM, (n=8 rats/group), \*P<0.05 as compared to the Group I; #P<0.05 as compared to the Group II (ANOVA followed by Dunnett's test), TBARS: thiobarbituric acid-reactive substances, SOD: superoxide dismutase, NADPH: nicotinamide adenine dinucleotide phosphate, CDNB: 1-chloro-2,4-dinitrobenzene, GSH: glutathione, CAT: catalase

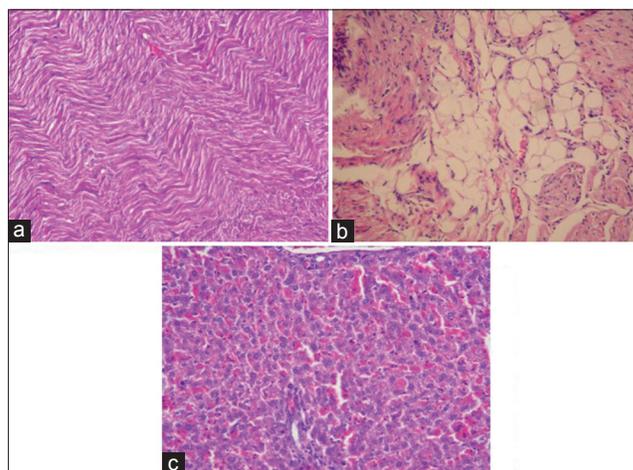


Fig. 2: Histological section of heart tissues.

(a) Photomicrograph of heart of normal control group shows no fatty changes with normal tissue architecture and regular morphology of myocardial cell membrane. (b) Photomicrograph of heart of STZ + HFD group shows focal fatty infiltration in myocardial cells. (c) The heart of STZ +HFD + water-soluble fraction of GSE group shows no pathological changes with normal architecture of myocardium.

infiltration in myocardial cells (fig. 2b). The heart of STZ+HFD+water-soluble fraction of GSE group shows no pathological changes with normal architecture of myocardium (fig. 2c).

## DISCUSSION

There is an increasing amount of data showing that being overweight during childhood and adolescence significantly associated with insulin resistance, dyslipidemia, and elevated blood pressure in young adulthood. Type-2 diabetes is strongly associated with obesity and cardiovascular risk. Diabetic rats when fed with high fat diet produce a metabolic syndrome characterized by insulin resistance, dyslipidemia, type-2 diabetes and central obesity, which is similar with the metabolic syndrome caused by obesity. As carbohydrate and lipid metabolisms are closely linked processes, derangement in the carbohydrate metabolism produces dyslipidemia, hence, STZ+HFD model is one of the ideal model for screening of antiobesity activity in diabetic rats.

This statement is supported by the findings of Ding *et al.* who reported that Wistar rats injected intraperitoneally with low dose of STZ (30 mg/kg) and fed with a high sucrose-fat diet for 8 weeks, develop significant insulin resistance and obesity<sup>[23]</sup>. Similarly, Srinivasan *et al.* reported that high fat diet-fed and low dose of STZ (35 mg/kg, i.p.) treated rats simulate natural disease progression and metabolic characteristics typical of individuals at increased risk of developing type 2 diabetes because of insulin resistance and obesity<sup>[24]</sup>. Further, Zhang *et al.* demonstrated that a combination of HFD and low dose of STZ (45 mg/kg) injection effectively used to generate a rat model that mimics the natural history and metabolic characteristics of type-2 diabetes in humans<sup>[25]</sup>.

In the present study, the protective effect of water-soluble fraction of *G. sylvestre* ethanol extract was determined on STZ diabetic rats in the presence of high fat diet. Previous reports suggested that diabetic animals maintained on a HFD were normophagic relative to diabetic rats maintained on high cholesterol diet<sup>[26]</sup>. In the present study, after STZ administration, there was no significant change in food intake of rats maintained on the high fat diet, as compared to the other groups.

In the present study, blood pressures and heart rate were significantly increased in Group II as

compared to the Group I, while these levels decreased significantly by water soluble fraction of *G. sylvestre* extract treatment. HFD induces BP elevation, could derange the neurohumoral control of the kidney<sup>[27]</sup>. At the same time, diabetes is also associated with alterations in resting heart rate and blood pressure<sup>[28]</sup>.

The serum lipid levels were increased in HFD fed diabetic rats, while the lipid levels were significantly decreased in water-soluble fraction of *G. sylvestre* ethanol extract treated group. Fat-fed, diabetic animals showed abnormalities in lipid metabolism as evidenced from increased serum TC, LDL-C, VLDL-C and TGs levels, as in case of human type 2 diabetic patients, which might contribute to various cardiovascular complications. The hypertriglyceridemia observed in fat-fed/STZ rats may be due to increased absorption and formation of triglycerides in the form of chylomicrons following exogenous consumption of diet rich in fat or through increased endogenous production of TG-enriched hepatic very low density lipoprotein (VLDL) and decreased TG uptake in peripheral tissues<sup>[29]</sup>.

In the present study, serum leptin levels were significantly increased in HFD fed diabetic rats. An increase in leptin levels after feeding of high fat diet to diabetic rats has been reported<sup>[30]</sup>. Zhao *et al.* have reported that the HFD feeding rats; develop insulin resistance<sup>[31]</sup>. At the same time, low-dose STZ has been known to induce a mild impairment of insulin secretion which is similar to the feature of the later stage of type-2 diabetes characterized by a progressive decline in insulin action (insulin resistance), followed by hyperinsulinemia<sup>[24]</sup>.

Serum glucose concentrations were elevated in the diabetic rats maintained on the HFD relative to the normal control group, these concentrations were significantly decreased by water soluble fraction of *G. sylvestre* extract treated group. Serum apolipoprotein B levels were increased in the diabetic rats maintained on HFD as compared to the normal control group as apolipoprotein B is associated with low density lipoprotein, intermediate-density lipoprotein (IDL), very low-density lipoprotein (VLDL) and chylomicrons<sup>[32]</sup>.

The treatment with water soluble fraction of *G. sylvestre* extract for 3 weeks suppressed the increase in caspase-3 levels, Na<sup>+</sup>/K<sup>+</sup> ATPase activity,

organ's weight (liver, heart, kidney and pancreas) and weights of perirenal, mesentric and epididymal fat in HFD fed diabetic rats. DNA electrophoresis demonstrates the presence of small DNA fragments in the form of a DNA ladder in HFD fed group, while in the *G. sylvestre* ethanol extract treatment group, DNA laddering was preserved.

In the present study, antioxidant enzymes levels were significantly increased by water soluble fraction of *G. sylvestre* extract treated group as compared to the HFD fed diabetic group. This may be due to the high levels of fat increase fat-mediated oxidative stress and decrease antioxidative enzyme activity<sup>[33]</sup>, as well as hyperglycemia was found to increase the production of free radicals that is associated with increased production of reactive oxygen species (ROS), resulting in tissue damage that is assessed by the measurement of lipid peroxides<sup>[34]</sup>.

The results of the present study confirm that water soluble fraction of *G. sylvestre* ethanol extract effectively alleviates the deleterious effects produced by HFD in diabetic rats. The possible mechanism of water soluble fraction of *Gymnema sylvestre* ethanol extract for antiobesity activity is due to the reduction of serum leptin, insulin, hyperlipidemia and oxidative stress in HFD fed diabetic rats. Further, *G. sylvestre* ethanol extract offers cardiac protection by decreasing cardiac caspase-3 levels, Na<sup>+</sup>/K<sup>+</sup> ATPase activity, DNA laddering, oxidative stress, and maintaining normal architecture of myocardium. The present study supports the potential of *G. sylvestre* in diaobesity disorder.

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