Chapter VI

RESULTS
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RESULTS OF HIGH FAT DIET-INDUCED OBESITY IN NORMAL HEALTHY WISTAR RATS
Obesity and its associated conditions such as insulin resistance, type 2 diabetes, dyslipidemia, and hepatic steatosis termed as the metabolic syndrome, represent major challenges for basic science and clinical research. It is obvious that appropriate animals models are crucial for studies on the pathogenesis and therapy of this complex metabolic disorder (Buettner et al., 2007).

The continued growth in the numbers of obese individuals in developed countries indicates an important role of environmental factors. The consumption of a high energy density (high fat diet) is to be one of the main factors. A high fat diet has been reported to adversely affect the health of humans and animal species (Ghosh et al., 2001). Chronic consumption of a HFD induces obesity, insulin resistance, dyslipidemia, and type 2 diabetes (Shin et al., 2008).

It has been observed that the disorders achieved by high-fat feeding resemble the human metabolic syndrome as was characterized by the increased body weight (obesity), mild hyperglycemia, hypertriglyceridemia, hypercholesterolemia and compensatory hyperinsulinemia together with reduced glucose disappearance rate, and this also may extend to the cardiovascular complications (Woods et al., 2003; Srinivasan et al., 2005). A model of high fat diet (HFD) - induced obesity in rats is well controlled and shares many features with human obesity. A rodent model of obesity based on the intake of HFD is advantageous in studying obesity-related cardiovascular abnormalities (Carroll et al., 2006).

Therefore, it was thought worthwhile to investigate the antiobesity activity of standardized ethanolic extract of Gymnema sylvestre on high fat diet-induced obesity in Wistar rats.
RESULTS OF PROTOCOL I

In order to evaluate the effects of *G. sylvestre* on high fat diet – induced obesity in Wistar rats, serial investigations were carried out in all the groups of rats for the estimations of serum leptin, insulin, lipids, visceral fat pad weights, antioxidant enzymes (GSH, GPx, GR, GST, SOD and catalase) levels, in heart and liver homogenate followed by histopathology of heart and liver tissues.

A. ANTHROPOMETRIC PARAMETERS

A. Body Mass Index

The mean body mass index was significantly (p< 0.01) increased in high fat diet-fed group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). While body mass index was significantly (p<0.01) decreased in water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) but not in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the body mass index in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Figure 5).

B. Body Weight Gain

Body weight was significantly (p<0.01) gained in group II (i.e. 126.25±13.90) as compared to the group I (75.13±5.02). Body weight was less significantly gained in group III (i.e. 95±5.74) and group V (i.e. 100±3.53), but non significantly in group IV (i.e. 111.25±8.78) as compared to the group II. There were no significant (p>0.05) changes in the body weight gain in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Figure 6).

C. Food Intake

There was significant (p<0.05) increase in food intake in group II as compared to the group I. While there was no significant changes in food intake in group III,
group IV and group V as compared to the group II. There were no significant (p>0.05) changes in the daily food intake in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Figure 7).

### D. Water Intake

There was significant (p<0.05) increase in water intake in group II as compared to the group I. While there was no significant changes in water intake in group III, group IV and group V as compared to the group II. There were no significant (p>0.05) changes in the water intake in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Figure 8).

### II. HEMODYNAMIC PARAMETERS USING CODA NIBP INSTRUMENT (USA) BY TAIL CUFF METHOD

#### A. Effect on systolic BP (mm Hg)

The mean systolic BP was significantly (p<0.01) increased in high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III), water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment decreased the increase in systolic BP as compared to the HFD control group (i.e. group II) significantly (p<0.05). There were no significant (p>0.05) changes in the systolic BP in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 5).

#### B. Effect on diastolic BP (mm Hg)

The mean diastolic BP was increased significantly (p<0.01) in high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). There was significant (p<0.01) decrease in diastolic BP with water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III), water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment
decreased the increase in diastolic BP as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the diastolic BP in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 5).

**C. Effect on mean arterial BP (mm Hg)**

The mean arterial BP was increased significantly (p<0.01) in high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). There was significant (p<0.01) decrease in mean arterial BP with water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III), water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment decreased the increase in mean arterial BP as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean arterial BP in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 5).

**D. Effect on heart rate (beats/minute)**

A significant (p<0.01) increase in the mean heart rate was observed in high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). There was significant (p<0.01) decrease in mean heart rate with water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III), water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment decreased the increase in mean heart rate as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean heart rate in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 5).
III. EFFECT ON LIPID PROFILE LEVELS

A. Effect on serum TC levels (mg/dl)

The mean serum TC levels, in the rats fed on normal diet alone (i.e. normal healthy control rats, group I) were stable throughout the experimental period. Conversely, in the high fat diet treated group (i.e. HFD control, group II), there was a significant (p<0.01) increase in the serum TC levels as compared to the group I rats. High fat diet fed rats when treated with water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) significantly (p<0.01) but water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV), treatment for 21 days less significantly (p<0.05) decrease in the serum TC level as compared with the HFD control (i.e. group II). There were no significant (p>0.05) changes in the mean serum TC levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 6).

B. Effect on serum TG levels (mg/dl)

The mean serum TG levels were significantly (p<0.01) increased in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) significantly (p<0.01) reduced the increased TG levels in serum as compared to HFD group (i.e. group II). There was less significant (p>0.05) decrease in the serum TG levels in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD group (i.e. group II). There were no significant (p>0.05) changes in the mean serum TGs levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 6).

C. Effect on serum HDL-C levels (mg/dl)

The mean serum HDL-C was significantly (p<0.01) decreased in high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I), water soluble fraction of *Gymnema sylvestre* ethanolic
extract (120 mg/kg/p.o., i.e. group III), water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days, significantly (p<0.01) elevated the reduced HDL-C levels in serum as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean serum HDL-C levels in G. sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 6).

D. Effect on serum LDL-C levels (mg/dl)
The mean serum LDL-C levels were significantly (p<0.01) increased in high fat diet treated group (i.e. HFD control, group II) as compared to the levels in normal healthy control rats (i.e. group I). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) significantly (p<0.01) reduced the increased LDL-C levels in serum as compared to HFD group (i.e. group II). There was less significant (p<0.05) decrease in the serum LDL-C levels in water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD group (i.e. group II). There were no significant (p>0.05) changes in the mean serum LDL-C levels in G. sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 6).

E. Effect on serum VLDL-C levels (mg/dl)
The mean serum VLDL-C levels were significantly (p<0.01) increased in high fat diet treated group (i.e. HFD control, group II) as compared to the levels in normal healthy control rats (i.e. group I). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) significantly (p<0.01) reduced the increased VLDL-C levels in serum as compared to HFD group (i.e. group II). There was no significant (p>0.05) change in the serum VLDL-C levels in water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD group (i.e. group II). There were no significant (p>0.05) changes in the mean
serum VLDL-C levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 6).

F. Atherogenic Indexes

i) TC/HDL-C

The mean TC/HDL-C was significantly (p<0.01) increased in the in high fat diet treated group (i.e. HFD control, group II) as compared to the levels in normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) significantly (p<0.01) reduced the increased TC/HDL-C levels as compared to HFD group (i.e. group II). There was less significant (p<0.05) decrease in the TC/HDL-C levels in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD group (i.e. group II). There were no significant (p>0.05) changes in the mean TC/HDL-C in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 6).

ii) LDL-C/HDL-C

The mean LDL-C/HDL-C was significantly (p<0.01) increased in the high fat diet treated group (i.e. HFD control, group II) as compared to the levels in normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) significantly (p<0.01) reduced the increased LDL-C/HDL-C levels as compared to HFD group (i.e. group II). There was less significant (p<0.05) decrease in the LDL-C/HDL-C levels in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD group (i.e. group II). There were no significant (p>0.05) changes in the mean LDL-C/HDL-C in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 6).
G. Effect on serum Apolipoprotein A1 (Apo A1) levels (mg/ml)
The mean serum Apo A1 levels were significantly decreased (p<0.01) in the high fat diet treated group (i.e. HFD control, group II) as compared to the levels in normal healthy control rats (i.e. group I). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days significantly (p<0.01) increased the serum Apo A1 levels as compared to the group II. While there was no significant change in the Apo A1 levels in water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean serum Apo A1 levels in G. sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 7).

H. Effect on serum Apolipoprotein B (Apo B) levels (mg/ml)
The mean serum Apo B levels were significantly (p<0.01) increased in the high fat diet treated group (i.e. HFD control, group II) as compared to the levels in normal healthy control rats (i.e. group I). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V), significantly (p<0.01), but water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) treatment for 21 days less significantly (p<0.05) decreased the serum Apo B levels as compared to the group II. There were no significant (p>0.05) changes in the mean serum Apo B levels in G. sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 7).

B. Effect on ratio of Apolipoprotein A1/Apolipoprotein B (Apo A1/Apo B)
The mean ratio of apo A1/apo B, significantly decreased in high fat diet treated group (i.e. HFD control, group II) as compared to the levels in normal healthy control rats (i.e. group I). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days significantly (p<0.01) increased the apo A1/apo B as compared to the group II. While there was no significant change in the apo A1/apo
C. Effect on Hepatic Cholesterol levels (mg/dl)

The mean hepatic cholesterol levels, in the rats fed on normal diet alone (i.e. normal healthy control rats, group I) were stable throughout the experimental period. Conversely, in the high fat diet treated group (i.e. HFD control, group II), there was a significant (p<0.01) increase in the hepatic cholesterol levels as compared to the group I rats. High fat diet fed rats when treated with water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) significantly (p<0.01) but water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV), treatment for 21 days less significantly (p<0.05) decrease the hepatic cholesterol levels as compared with the HFD control (i.e. group II). There were no significant (p>0.05) changes in the mean hepatic cholesterol levels in G. sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 7).

IV. EFFECT ON VISCERAL FAT PAD WEIGHTS (g)

a. Mesenteric fat (g)

The mean mesenteric fat weight was significantly (p<0.01) increased by 1.9-folds in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days significantly (p<0.01) decreased by 1.8-folds the increase in the mesenteric fat weight as compared to the HFD control group (i.e. group II). While there was no significant change in the mesenteric fat weight in water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD control group (i.e. group II).
There was no significant (p>0.05) change in the mean mesenteric fat weight in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 13).

b. Perirenal fat (g)
The mean perirenal fat weight was significantly (p<0.01) increased by 2-folds in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days significantly (p<0.01) decreased by 1.9-folds the increase in the perirenal fat weight as compared to the HFD control group (i.e. group II). While there was no significant change in the perirenal fat weight in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD control group (i.e. group II). There was no significant (p>0.05) change in the mean perirenal fat weight in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 13).

c. Epididymal fat (g)
The mean epididymal fat weight was significantly (p<0.01) increased by 2.5-folds in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) by 1.7-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days significantly (p<0.01) decreased by 1.9-folds the increase in the epididymal fat weight as compared to the HFD control group (i.e. group II). While there was no significant change in the epididymal fat weight in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD control group (i.e. group II). There was no significant (p>0.05) change in the mean epididymal fat weight in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 13).
V. Effect on Organ’s weight (g)

a. Heart (g)
There was significantly (p<0.01) increase by 1.3-folds, in the mean heart weight in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). While there was less significant (p<0.05) decrease in the mean heart weight by water insoluble fraction of *Gymnema sylvestre* ethanolic extract treatment (80 mg/kg/p.o., i.e. group IV) and rimonabant (10 mg/kg/p.o., i.e. group V) but water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) treatment for 21 days significantly (p<0.01) decrease the mean heart weight as compared to the HFD control group (i.e. group II). There was no significant (p>0.05) change in the mean heart weight in *G. sylvestre* (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 12).

b. Liver (g)
There was significantly (p<0.01) increase by 1.4-folds, in the mean liver weight in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) by 1.6-folds and rimonabant (10 mg/kg/p.o., i.e. group V) by 1.25-folds treatment for 21 days significantly (p<0.01) decrease the mean liver weight as compared to the HFD control group (i.e. group II) but water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV), less significantly (p<0.05) decreased the increase in the mean liver weight as compared to the HFD control group (i.e. group II). There was no significant (p>0.05) change in the mean liver weight in *G. sylvestre* (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 12).

c. Kidneys (Right + Left) (g)
There was significantly (p<0.01) increase by 1.6-folds, in the mean kidneys weight in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days significantly (p<0.01) decrease by 1.5-folds in the
mean kidneys weight as compared to the HFD control group (i.e. group II) but water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV), less significantly (p<0.05) decreased the increase in the mean kidneys weight as compared to the HFD control group (i.e. group II). There was no significant (p>0.05) change in the mean kidney weight in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 12).

VI. EFFECT ON SERUM BIOCHEMICAL PARAMETERS

i. Effect on serum LDH levels (IU/L)

The mean serum LDH levels was significantly (p<0.01) increased in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days significantly (p<0.01) but water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV), less significantly (p<0.05) decreased the increase in the serum LDH levels as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean serum LDH levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 7).

ii. Effect on serum Leptin levels (pg/ml)

The mean serum leptin levels were significantly (p<0.05) increased in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days significantly (p<0.05) decreased the serum leptin levels as compared to the group II. While there was no significant change in the leptin levels in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean serum leptin levels in *G. sylvestre* (200
mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 7).

iii. Effect on serum Insulin levels (ng/ml)

The mean serum insulin levels were significantly \( p<0.01 \) increased in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) significantly \( p<0.01 \), but water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) treatment for 21 days less significantly \( p<0.05 \) decreased the serum insulin levels as compared to the group II. There were no significant \( p>0.05 \) changes in the mean serum insulin levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 7).

iv. Effect on serum Glucose levels (mg/ml)

The mean serum glucose levels were significantly \( p<0.01 \) increased in the high fat diet treated group (i.e. HFD control, group II) as compared to the normal healthy control rats (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) significantly \( p<0.01 \), but water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) treatment for 21 days less significantly \( p<0.05 \) decreased the serum glucose levels as compared to the group II. There were no significant \( p>0.05 \) changes in the mean serum glucose levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 7).

VII. EFFECT ON MYOCARDIAL APOPTOSIS

A. Effect on Caspase-3 activity (units/mg protein/hour)

The mean Caspase-3 levels were significantly \( p<0.01 \) increased by 3.4 folds in high fat diet treated group (i.e. HFD control, group II) as compared to the caspase-3 levels in hearts of the normal healthy control rats (i.e. group I). Water insoluble
fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the caspase-3 levels but water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) decrease in the caspase-3 levels in the cardiac tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean caspase-3 levels in *G. sylvestre* (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 8).

**B. Effect on Na\(^+\)-K\(^+\) ATPase activity (Pj/min/mg tissue)**

The mean Na\(^+\)-K\(^+\) ATPase levels were significantly (p<0.01) decreased by 1.7-folds in hearts of high fat diet treated group (i.e. HFD control, group II) as compared to the hearts of the normal healthy control group (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) increase by 1.35-folds in the decreased Na\(^+\)-K\(^+\) ATPase levels in the cardiac tissue as compared to the HFD control group (i.e. group II) hearts. There was no significant (p>0.05) change in the cardiac Na\(^+\)-K\(^+\) ATPase levels in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) treated rats as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean Na\(^+\)-K\(^+\) ATPase levels in *G. sylvestre* (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 8).

**C. DNA gel electrophoresis**

Electrophoresis of DNA extracted from the left ventricle region of the heart of high fat diet treated rats (i.e. group II) showed DNA laddering indicating apoptotic inter-nucleosomal DNA fragmentation. Ladders were not detected in normal healthy control group (i.e. group I), water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treated group, where genomic DNA band was preserved. Little DNA laddering was shown in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80
mg/kg/p.o., i.e. group IV) treated group as compared to HFD control group (i.e. group II). There were no significant (p>0.05) changes in the DNA laddering in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Figure 9).

VIII. Effect on oxidative stress parameters

1. Lipid peroxidation

The mean TBARS (nmoles MDA/ mg protein) levels were significantly (p<0.01) increased by 3.25-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the TBARS levels in hearts of the normal healthy control rats (i.e. group I). Water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the TBARS levels but water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.4-folds and rimonabant (10 mg/kg/p.o., i.e. group V) 1.5-folds treatment for 21 days showed significant (p<0.01) decrease in the TBARS levels in the cardiac tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean cardiac TBARS levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 8).

The mean TBARS (nmoles MDA/ mg protein) levels were significantly (p<0.01) increased by 1.9-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the TBARS levels in liver of the normal healthy control rats (i.e. group I). Water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the TBARS levels but water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.4-folds and rimonabant (10 mg/kg/p.o., i.e. group V) 1.6-folds treatment for 21 days showed significant (p<0.01) decrease in the TBARS levels in the hepatic tissue as compared to the HFD control group (i.e. group II) rats. There were no
significant (p>0.05) changes in the mean hepatic TBARS levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 9).

2. **Superoxide dismutase levels (IU/mg protein)**

The mean superoxide dismutase (SOD) levels significantly (p<0.01) decreased in high fat diet treated group (i.e. HFD control, group II) as compared to the SOD levels in hearts of the normal healthy control rats (i.e. group I). Water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the SOD levels but water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed less significant (p<0.05) increase in the SOD levels in the cardiac tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean cardiac SOD levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 8).

The mean superoxide dismutase (SOD) levels significantly (p<0.01) decreased in high fat diet treated group (i.e. HFD control, group II) as compared to the SOD levels in liver of the normal healthy control rats (i.e. group I) (i.e. from 1.93±0.01 to 1.25±0.013). Water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the SOD levels but water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) less significantly (p<0.05) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) increase in the SOD levels in the hepatic tissue as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean hepatic SOD levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 9).
3. Catalase (nmol H₂O₂ -consumed/min/mg protein)

The mean catalase levels significantly (p<0.01) decreased by 2.5-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the catalase levels in hearts of the normal healthy control rats (i.e. group I). Water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the catalase levels but water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.5-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) 1.5-folds increase in the catalase levels in the cardiac tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean cardiac catalase levels in G. sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 8).

The mean catalase levels significantly (p<0.01) decreased by 1.9-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the catalase levels in liver of the normal healthy control rats (i.e. group I). Water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed less significant (p<0.05) increase in the catalase levels but water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.8-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) 2.1-folds increase in the catalase levels in the hepatic tissue as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean hepatic catalase levels in G. sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 9).

4. Reduced glutathione (µmol of phosphorus liberated/min/mg protein)

The mean glutathione levels were significantly (p<0.01) decreased by 2.7-folds in high fat diet treated group (i.e. HFD control, group II) as compared to
the glutathione levels in hearts of the normal healthy control rats (i.e. group I). Water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the glutathione levels but water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) 2.9-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) 3.0-folds increase in the glutathione levels in the cardiac tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean cardiac glutathione levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 10).

The mean glutathione levels were significantly (p<0.01) decreased by 3-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the glutathione levels in liver of the normal healthy control rats (i.e. group I). Water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the glutathione levels but water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) 2.5-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) 3.0-folds increase in the glutathione levels in the hepatic tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean hepatic glutathione levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 11).

5. Glutathione peroxidase (nmol NADPH oxidized/min/mg of protein)

The mean glutathione peroxidase (GPx) levels were significantly (p<0.01) decreased by 1.3-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the GPx levels in hearts of the normal healthy control rats (i.e. group I). Water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the
GPx levels but water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.2-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) 1.3-folds increase in the GPx levels in the cardiac tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean cardiac glutathione peroxidase (GPx) levels in Gymnema sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 10).

The mean glutathione peroxidase (GPx) levels were significantly (p<0.01) decreased by 1.5-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the GPx levels in liver of the normal healthy control rats (i.e. group I). Water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the GPx levels but water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.2-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed less significant (p<0.05) 1.3-folds increase in the GPx levels in the hepatic tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean hepatic glutathione peroxidase (GPx) levels in Gymnema sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Table 11).

6. Glutathione reductase (nmol NADPH oxidized/min/mg of protein)

There was significantly (p<0.05) decrease in mean glutathione reductase (GR) levels by 1.5-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the GR levels in hearts of the normal healthy control rats (i.e. group I). Water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the GR levels but water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.7-folds and rimonabant (10 mg/kg/p.o., i.e. group
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V) treatment for 21 days showed significant (p<0.01) 1.8-folds increase in the GR levels in the cardiac tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean cardiac glutathione reductase (GR) levels in \textit{G. sylvestre} (200 mg/kg/p.o.) \textit{per se} treated rats (i.e. group VI) as compared to the group I rats (Table 10).

There was significantly (p<0.05) decrease in mean glutathione reductase (GR) levels by 1.6-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the GR levels in liver of the normal healthy control rats (i.e. group I). Water insoluble fraction of \textit{Gymnema sylvestre} ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the GR levels but water soluble fraction of \textit{Gymnema sylvestre} ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.7-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) 1.8-folds increase in the GR levels in the hepatic tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean hepatic glutathione reductase (GR) levels in \textit{G. sylvestre} (200 mg/kg/p.o.) \textit{per se} treated rats (i.e. group VI) as compared to the group I rats (Table 11).

7. Glutathione-S-transferase (nmol CDNB conjugate formed / min / mg protein)

There was significantly (p<0.01) decrease in mean glutathione-S-transferase (GST) levels by 2-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the GST levels in hearts of the normal healthy control rats (i.e. group I). Water insoluble fraction of \textit{Gymnema sylvestre} ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed no significant (p>0.05) change in the GST levels but water soluble fraction of \textit{Gymnema sylvestre} ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.4-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) 1.6-folds increase in the GST levels in the cardiac tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean
cardiac glutathione-S-transferase (GST) levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 10).

There was significantly (p<0.01) decrease in mean glutathione-S-transferase (GST) levels by 1.85-folds in high fat diet treated group (i.e. HFD control, group II) as compared to the GST in liver of the normal healthy control rats (i.e. group I). Water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) showed less significant (p<0.05) change in the GST levels but water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) 1.4-folds and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) 1.6-folds increase in the GR levels in the hepatic tissue as compared to the HFD control group (i.e. group II) rats. There were no significant (p>0.05) changes in the mean hepatic glutathione-S-transferase (GST) levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 11).

IX. Effect on hepatic Na⁺-K⁺ ATPase activity (P/ min/mg tissue)

The mean Na⁺-K⁺ ATPase levels were significantly (p<0.01) decreased by 1.85-folds in livers of high fat diet treated group (i.e. HFD control, group II) as compared to the livers of the normal healthy control group (i.e. group I). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group III) and rimonabant (10 mg/kg/p.o., i.e. group V) treatment for 21 days showed significant (p<0.01) increase by 1.3-folds in the decreased Na⁺-K⁺ ATPase levels in the hepatic tissue as compared to the HFD control group (i.e. group II) livers. There was no significant (p>0.05) change in the hepatic Na⁺-K⁺ ATPase levels in water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o., i.e. group IV) treated rats as compared to the HFD control group (i.e. group II). There were no significant (p>0.05) changes in the mean hepatic Na⁺-K⁺ ATPase levels in *G. sylvestre* (200 mg/kg/p.o.) *per se* treated rats (i.e. group VI) as compared to the group I rats (Table 9).
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X. EFFECT ON HISTOPATHOLOGICAL STUDIES

Histopathology of heart tissue
The section of heart tissue of normal healthy control rats (i.e. vehicle control group) showed no fatty changes with normal architecture as well as normal morphology of myocardium. The heart section of HFD control group (i.e. high fat diet group), showed deposition of fat globules in myocardial cells. Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o.) (i.e. group III) and rimonabant (10 mg/kg/p.o.) (i.e. group V) treatment showed normal fat deposition in myocardial cells while water insoluble fraction of Gymnema sylvestre ethanolic extract (80 mg/kg/p.o.) (i.e. group IV) treatment showed microvascular fat deposition in myocardial cells. There were no significant (p>0.05) pathological changes in the cardiac tissue of G. sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Figure 10).

Histopathology of liver tissue
Histopathology of rat’s liver tissue of high fat diet group showed marked fatty changes within liver cells as compared to normal healthy control rat’s liver tissue which showed normal fat deposition in hepatocytes i.e. within normal limit. Water soluble fraction of G. sylvestre extract (120 mg/kg/p.o.) and rimonabant (10 mg/kg/p.o.) groups showed normal morphology of liver tissue while water insoluble fraction of G. sylvestre extract (80 mg/kg/p.o.) showed ballooning degeneration but no fatty changes as compared to the HFD fed group. There were no significant (p>0.05) pathological changes in the hepatic tissue of G. sylvestre (200 mg/kg/p.o.) per se treated rats (i.e. group VI) as compared to the group I rats (Figure 11).
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Figure 5: Effect of ethanolic *Gymnema sylvestre* extract on body mass index in HFD-induced obesity in rats

Figure 6: Effect of ethanolic *Gymnema sylvestre* extract on body weight gain in HFD-induced obesity in rats

**P< 0.01 as compared to the Control group; ns$ - non significant as compared to the Control group; #P< 0.05, ##P< 0.01, ns - non significant, as compared to the HFD group
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Figure 7: Effect of ethanolic *Gymnema sylvestre* extract on food intake in HFD-induced obesity in rats

Figure 8: Effect of ethanolic *Gymnema sylvestre* extract on water intake in HFD-induced obesity in rats

*P* < 0.05 as compared to the Control group; ns$ - non significant as compared to the Control group; ns - non significant, as compared to the HFD group
### Table 4: Effect of ethanolic Gymnema sylvestre extract on heart rate, systolic, Diastolic, Mean blood pressure in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart Rate (BPM)</th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP (mm Hg)</th>
<th>Mean BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td>421.83±15.77</td>
<td>127.83±6.86</td>
<td>96±4.65</td>
<td>106.16±5.00</td>
</tr>
<tr>
<td>II (HFD 20 g/d/rat/p.o. i.e. HFD control)</td>
<td>539.20±37.68**</td>
<td>165.16±10.58**</td>
<td>119±4.56**</td>
<td>135.33±6.09**</td>
</tr>
<tr>
<td>III HFD (20 g/d/rat/p.o.) + Water soluble fraction of ethanolic Gymnema sylvestre extract 120 mg/kg/p.o.)</td>
<td>396.66±14.14**a</td>
<td>134±2.03a</td>
<td>96.16±2.58**a</td>
<td>108.5±2.12**a</td>
</tr>
<tr>
<td>IV HFD (20 g/d/rat/p.o.) + Water insoluble fraction of ethanolic Gymnema sylvestre extract (80 mg/kg/p.o.)</td>
<td>381.33±16.37**a</td>
<td>133±1.69**a</td>
<td>95.83±3.51**a</td>
<td>106.66±2.67**a</td>
</tr>
<tr>
<td>V HFD (20 g/d/rat/p.o.) + Rimonabant (10 mg/kg/p.o.)</td>
<td>452±8.09**b</td>
<td>118.25±4.26**b</td>
<td>87.5±3.39**b</td>
<td>97.5±3.56**b</td>
</tr>
<tr>
<td>VI Ethanolic Gymnema sylvestre extract (200 mg/kg/p.o. i.e. perse group)</td>
<td>406.66±14.14**a</td>
<td>128±2.03**a</td>
<td>97.16±2.38**a</td>
<td>109.5±2.12**a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10); **P<0.01 as compared to the Group I; ##P<0.01, †P<0.05 as compared to the Group II; ns$- non significant compared to the Group I; a, b- non significant as compared to the Group III (ANOVA followed by Dunnett’s test)
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Table 5: Effect of ethanolic Gymnema sylvestre extract on Serum Total cholesterol (TC), Triglycerides (TGs), High density lipoprotein (HDL), Low density lipoprotein (LDL), Very low density lipoprotein (VLDL) and atherogenic index in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>High density lipoprotein (mg/dl)</th>
<th>Low density lipoprotein (mg/dl)</th>
<th>Very low density lipoprotein (mg/dl)</th>
<th>Atherogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td>101.69±2.00</td>
<td>64.97±2.19</td>
<td>32.26±1.01</td>
<td>56.64±0.31</td>
<td>13.23±0.44</td>
<td>3.25±0.09</td>
</tr>
<tr>
<td>II (HFD 20 g/d/rat/p.o. i.e. HFD control)</td>
<td>167.06±4.74**</td>
<td>156±11.63**</td>
<td>25.28±0.94**</td>
<td>110.61±5.87**</td>
<td>31.2±3.32**</td>
<td>6.02±0.52**</td>
</tr>
<tr>
<td>III HFD (20 g/rat/p.o.)+ Water soluble fraction of ethanolic Gymnema sylvestre extract (120 mg/kg/p.o.)</td>
<td>119.29±2.71**</td>
<td>107.59±2.42**</td>
<td>39.36±0.64**</td>
<td>58.04±2.93**</td>
<td>21.5±0.48**</td>
<td>3.02±0.08**</td>
</tr>
<tr>
<td>IV HFD (20 g/rat/p.o.) + Water insoluble fraction of ethanolic Gymnema sylvestre extract (80 mg/kg/p.o.)</td>
<td>138.98±2.46**</td>
<td>157.25±4.34**</td>
<td>30.56±0.91**</td>
<td>76.96±2.92**</td>
<td>31.45±0.56**</td>
<td>4.55±0.12**</td>
</tr>
<tr>
<td>V HFD (20 g/rat/p.o.) + Rimonabant (10 mg/kg/p.o.)</td>
<td>115.08 ± 3.30**</td>
<td>103.62 ± 3.43**</td>
<td>44.14±0.79**</td>
<td>50.22±3.89**</td>
<td>20.72±0.68**</td>
<td>2.60±0.08**</td>
</tr>
<tr>
<td>VI Ethanolic Gymnema sylvestre extract (200 mg/kg/p.o. i.c. per se group)</td>
<td>102.29±2.71 ns$^b$</td>
<td>68.59±2.42 ns$^b$</td>
<td>33.86±0.64 ns$^b$</td>
<td>57.46±0.31 ns$^b$</td>
<td>13.58±0.44 ns$^b$</td>
<td>3.52±0.19 ns$^b$</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10); **P<0.01, *P<0.05 as compared to the Group I; #P<0.01, $P<0.05 as compared to the Group II; ns= non significant as compared to the Group II; ns$= non significant as compared to the Group I; a, b- non significant as compared to the Group III (ANOVA followed by Dunnett’s test)

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### Table 6: Effect of ethanolic *Gymnema sylvestre* extract on serum leptin, insulin, glucose, lactate dehydrogenase (LDH) and apolipoprotein-A1 & B, and serum in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum leptin (pg/ml)</th>
<th>Serum insulin (ng/ml)</th>
<th>Serum glucose (mg/dl)</th>
<th>Serum LDH (IU/L)</th>
<th>Serum apolipoprotein-A1 (mg/dl)</th>
<th>Serum apolipoprotein-B (mg/dl)</th>
<th>A1/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td>159.37±6.26</td>
<td>0.116±0.02</td>
<td>94.14±2.16</td>
<td>23.48±0.64</td>
<td>1.33±0.08</td>
<td>4.38±0.17</td>
<td>0.303±0.11</td>
</tr>
<tr>
<td>II (HFD 20 g/d/rat/p.o. i.e. HFD control)</td>
<td>242.96±9.32*</td>
<td>1.330±0.35**</td>
<td>211.21±4.65**</td>
<td>208.82±12.46**</td>
<td>0.20±0.02**</td>
<td>21.8±2.04**</td>
<td>0.009±0.01**</td>
</tr>
<tr>
<td>III HFD (20 g/d/rat/p.o.)* Water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (120 mg/kg/p.o.)</td>
<td>158.85±5.05*</td>
<td>0.228±0.09**</td>
<td>112.66±1.24**</td>
<td>60.76±5.31**</td>
<td>1.16±0.08**</td>
<td>4.53±0.12**</td>
<td>0.256±0.06**</td>
</tr>
<tr>
<td>IV HFD (20 g/d/rat/p.o.) + Water insoluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (80 mg/kg/p.o.)</td>
<td>223.35±38.18**</td>
<td>0.240±0.02**</td>
<td>146.26±1.85**</td>
<td>113±5.88**</td>
<td>0.43±0.08**</td>
<td>8.56±0.28**</td>
<td>0.050±0.02**</td>
</tr>
<tr>
<td>V HFD (20 g/d/rat/p.o.) + Rimonabant (10 mg/kg/p.o.)</td>
<td>189.99±11.12**</td>
<td>0.171±0.05**</td>
<td>96.25±2.21**</td>
<td>61.44±4.48**</td>
<td>1.23±0.12**</td>
<td>4.31±0.24**</td>
<td>0.285±0.08**</td>
</tr>
<tr>
<td>VI Ethanolic <em>Gymnema sylvestre</em> extract (200 mg/kg/p.o. i.e. perse group)</td>
<td>159.69±5.05 ns</td>
<td>0.124±0.02 ns</td>
<td>96.17±2.19 ns</td>
<td>24.84±0.84 ns</td>
<td>1.34±0.09 ns</td>
<td>4.43±0.25 ns</td>
<td>0.302±0.13 ns</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10); *P<0.01, †P<0.05 as compared to the Group I; **P<0.01, ††P<0.05 as compared to the Group II; ns= non significant as compared to the Group II; ns$^*$= non significant as compared to the Group I; a, b- non significant as compared to the Group III (ANOVA followed by Dunnett’s test)
Table 7: Effect of ethanolic *Gymnema sylvestre* extract on lipid peroxides (TBARS), catalase (CAT), superoxide dismutase (SOD), Caspase-3 and Sodium potassium ATPase levels in HEART tissue of Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmol MDA/mg protein)</th>
<th>SOD (IU/mg protein)</th>
<th>CAT (nmol H₂O₂ - consumed/min/mg protein)</th>
<th>Caspase-3 (nmol/hr/mg protein)</th>
<th>Na-K ATPase activity (µmol of Pi liberated/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td>0.200±0.003</td>
<td>1.77±0.01</td>
<td>55.75±1.88</td>
<td>62.34±9.28</td>
<td>0.725±0.04</td>
</tr>
<tr>
<td>II (HFD 20 g/d/rat/p.o. i.e. HFD control)</td>
<td>0.729±0.01**</td>
<td>1.23±0.33**</td>
<td>24.99±1.28**</td>
<td>209.30±8.51**</td>
<td>0.426±0.01**</td>
</tr>
<tr>
<td>III HFD (20 g/d/rat/p.o.)+ Water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (120 mg/kg/p.o.)</td>
<td>0.291±0.01ms</td>
<td>1.37±0.03s</td>
<td>37.48±1.76ms</td>
<td>113.05±3.40ms</td>
<td>0.593±0.02s</td>
</tr>
<tr>
<td>IV HFD (20 g/d/rat/p.o.)+ Water insoluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (80 mg/kg/p.o.)</td>
<td>0.489±0.01sa</td>
<td>1.24±0.01m</td>
<td>27.58±1.63m</td>
<td>186.12±1.43m</td>
<td>0.416±0.01ms</td>
</tr>
<tr>
<td>V HFD (20 g/d/rat/p.o.) + Rimonabant (10 mg/kg/p.o.)</td>
<td>0.247±0.013sb</td>
<td>1.39±0.02sb</td>
<td>36.01±0.37sb</td>
<td>104.75±3.24sb</td>
<td>0.589±0.003b</td>
</tr>
<tr>
<td>VI Ethanolic <em>Gymnema sylvestre</em> extract (200 mg/kg/p.o. i.e. perse group)</td>
<td>0.205±0.01ms</td>
<td>1.78±0.02ms</td>
<td>57.59±1.96ms</td>
<td>63.45±9.48ms</td>
<td>0.729±0.05ms</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10); **P<0.01 as compared to the Group I; *@P<0.05 as compared to the Group II; ns= non significant as compared to the Group II; ns$= non significant as compared to the Group I; a, b= non significant as compared to the Group III (ANOVA followed by Dunnett's test)
Table 8: Effect of ethanolic Gymnema sylvestre extract on lipid peroxides (TBARS), catalase (CAT), superoxide dismutase (SOD) and Sodium potassium ATPase levels in LIVER tissue of Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmol MDA/ mg protein)</th>
<th>SOD (IU/mg protein)</th>
<th>CAT (nmol H2O2 – consumed/min/ mg protein)</th>
<th>Na-K ATPase activity (μmol of Pi liberated/ min/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td>0.315±0.01</td>
<td>1.93±0.01</td>
<td>35.63±1.45</td>
<td>0.852±0.01</td>
</tr>
<tr>
<td>II (HFD 20 g/rat/p.o. i.e. HFD control)</td>
<td>0.704±0.022**</td>
<td>1.25±0.013**</td>
<td>18.03±0.64**</td>
<td>0.462±0.01**</td>
</tr>
<tr>
<td>III HFD (20 g/rat/p.o.) + Water soluble fraction of ethanolic Gymnema sylvestre extract (120 mg/kg/p.o.)</td>
<td>0.482±0.03##</td>
<td>1.37±0.03#</td>
<td>32.07±2.04##</td>
<td>0.600±0.01##</td>
</tr>
<tr>
<td>IV HFD (20 g/rat/p.o.) + Water insoluble fraction of ethanolic Gymnema sylvestre extract (80 mg/kg/p.o.)</td>
<td>0.672±0.01##</td>
<td>1.24±0.01##</td>
<td>28.95±0.85##a</td>
<td>0.427±0.01##</td>
</tr>
<tr>
<td>V HFD (20 g/rat/p.o.) + Rimonabant (10 mg/kg/p.o.)</td>
<td>0.439 ± 0.01##b</td>
<td>1.43 ± 0.04##b</td>
<td>38.11 ± 0.39##b</td>
<td>0.614 ± 0.003##b</td>
</tr>
<tr>
<td>VI Ethanolic Gymnema sylvestre extract (200 mg/kg/p.o. i.e. perse group)</td>
<td>0.318±0.02##S</td>
<td>1.92±0.01##S</td>
<td>35.47±1.45##S</td>
<td>0.848±0.01##S</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10); ** P<0.01 as compared to the Group I; ##P<0.01, #P<0.05 as compared to the Group II; m# non significant as compared to the Group II, mS non significant as compared to the Group I; a, b- non significant as compared to the Group III (ANOVA followed by Dunnett’s test)
### Table 9: Effect of ethanolic *Gymnema sylvestre* extract on reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in HEART tissue of Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (μmol of phosphorus liberated/min/mg protein)</th>
<th>Glutathione peroxidase (nmol NADPH oxidized/min/mg of protein)</th>
<th>Glutathione reductase (nmol NADPH oxidized/min/mg of protein)</th>
<th>Glutathione S transferase (nmol CDNB conjugate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td>26.75±0.97</td>
<td>184.81±7.54</td>
<td>29.90±0.43</td>
<td>492.31±9.01</td>
</tr>
<tr>
<td>II (HFD 20 g/d/rat/p.o., i.e. HFD control)</td>
<td>9.54±0.47**</td>
<td>142.66±3.14*</td>
<td>18.95±0.25*</td>
<td>246.43±2.06**</td>
</tr>
<tr>
<td>III (HFD 20 g/d/rat/p.o.)+ Water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (120 mg/kg/p.o.)</td>
<td>27.17±1.24#</td>
<td>171.11±8.09§</td>
<td>33.58±1.40##</td>
<td>359.67±16.37###</td>
</tr>
<tr>
<td>IV (HFD 20 g/d/rat/p.o.)+ Water insoluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (80 mg/kg/p.o.)</td>
<td>11.92±0.84##</td>
<td>120.91±4.32##</td>
<td>23.85±0.69##</td>
<td>212.68±9.49##</td>
</tr>
<tr>
<td>V (HFD 20 g/d/rat/p.o.) + Rimonabant (10 mg/kg/p.o.)</td>
<td>29.01±0.18#</td>
<td>188.66±4.55#</td>
<td>35.67±1.61##</td>
<td>400.87±5.92##</td>
</tr>
<tr>
<td>VI (Ethanolic <em>Gymnema sylvestre</em> extract (200 mg/kg/p.o., i.e. per se group)</td>
<td>27.56±0.98ns$</td>
<td>183.96±6.95ns$</td>
<td>29.78±0.39ns$</td>
<td>491.13±8.93ns$</td>
</tr>
</tbody>
</table>

All values were expressed as Mean±SEM, (n=10); **P<0.01, *P<0.05 as compared to the Group I; #P<0.01, §P<0.05 as compared to the Group II; ns$= non significant as compared to the Group II; ns$= non significant as compared to the Group I; b- non significant as compared to the Group III (ANOVA followed by Dunnett’s test)
Table 10: Effect of ethanolic *Gymnema sylvestre* extract on reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in LIVER tissue of Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (μmol of phosphorus liberated /min /mg protein)</th>
<th>Glutathione peroxidase (nmol/NADPH oxidized/min/mg of protein)</th>
<th>Glutathione reductase (nmol/NADPH oxidized/min/mg of protein)</th>
<th>Glutathione S transferase (nmol/CDNB conjugate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>(Normal healthy Control)</td>
<td>28.46±0.21</td>
<td>197.97±8.64</td>
<td>36.15±0.85</td>
</tr>
<tr>
<td>II</td>
<td>(HFD 20 g/d/rat/p.o. i.e. HFD control)</td>
<td>8.79±0.13**</td>
<td>136.19±3.43**</td>
<td>21.50±0.34**</td>
</tr>
<tr>
<td>III</td>
<td>HFD (20 g/d/rat/p.o.)+ Water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (120 mg/kg/p.o.)</td>
<td>25.94±1.28**</td>
<td>166.82±6.32**</td>
<td>38.76±1.56**</td>
</tr>
<tr>
<td>IV</td>
<td>HFD (20 g/d/rat/p.o.) + Water insoluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (80 mg/kg/p.o.)</td>
<td>13.26±0.25**</td>
<td>127.81±5.49**</td>
<td>27.39±0.52**</td>
</tr>
<tr>
<td>V</td>
<td>HFD (20 g/d/rat/p.o.) + Rimonabant (10 mg/kg/p.o.)</td>
<td>30.14±0.13**</td>
<td>176.03±8.25**</td>
<td>42.47±0.61**</td>
</tr>
<tr>
<td>VI</td>
<td>Ethanolic <em>Gymnema sylvestre</em> extract (200 mg/kg/p.o. i.e. perse group)</td>
<td>28.08±0.20**</td>
<td>196.79±8.48**</td>
<td>36.04±0.58**</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10); **P<0.01 as compared to the Group I; ***P<0.01, ^P<0.05 as compared to the Group II; ns= non significant as compared to the Group II; nS= non significant as compared to the Group I; a, b- non significant as compared to the Group III (ANOVA followed by Dunnett’s test)
## Table 11: Effect of ethanolic *Gymnema sylvestre* extract on various organs weight in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (gm)</th>
<th>Heart (gm)</th>
<th>Kidney (Rt + Lt) (gm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td>6.95 ± 0.09</td>
<td>0.737 ± 0.01</td>
<td>1.30 ± 0.006</td>
</tr>
<tr>
<td>II (HFD 20 g/d/rat/p.o. i.e. HFD control)</td>
<td>9.81 ± 0.26**</td>
<td>0.958 ± 0.02**</td>
<td>2.06 ± 0.04**</td>
</tr>
<tr>
<td>III HFD (20 g/d/rat/p.o.) + Water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (120 mg/kg/p.o.)</td>
<td>6.29 ± 0.28**</td>
<td>0.755 ± 0.01**</td>
<td>1.36 ± 0.01**</td>
</tr>
<tr>
<td>IV HFD (20 g/d/rat/p.o.) + Water insoluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (80 mg/kg/p.o.)</td>
<td>8.16 ± 0.24**</td>
<td>0.869 ± 0.009**</td>
<td>1.52 ± 0.02**</td>
</tr>
<tr>
<td>V HFD (20 g/d/rat/p.o.) + Rimonabant (10 mg/kg/p.o.)</td>
<td>7.78 ± 0.32**</td>
<td>0.864 ± 0.03**</td>
<td>1.37 ± 0.02**</td>
</tr>
<tr>
<td>VI Ethanoligic <em>Gymnema sylvestre</em> extract (200 mg/kg/p.o. i.e. perse group)</td>
<td>6.91 ±0.08ns</td>
<td>0.735 ±0.02ns</td>
<td>1.31 ±0.01ns</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10); **P<0.01 as compared to the Group I; ***P<0.01, #P<0.05 as compared to the Group II; ns= non significant as compared to the Group II; nsS= non significant as compared to the Group I; a, b- non significant as compared to the Group III (ANOVA followed by Dunnett’s test)
### Table 12: Effect of ethanolic Gymnema sylvestre extract on visceral fat pad weight in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mesenteric Fat (gm)</th>
<th>Perirenal Fat (gm)</th>
<th>Epididymal Fat (gm)</th>
<th>Hepatic Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I (Normal healthy Control)</td>
<td>0.399±0.014</td>
<td>0.399±0.005</td>
<td>0.440±0.006</td>
<td>65.93±4.39</td>
</tr>
<tr>
<td>II (HFD 20 g/d/rat/p.o. i.e. HFD control)</td>
<td>0.795±0.022**</td>
<td>0.803±0.03**</td>
<td>1.069±0.07**</td>
<td>156.03±3.19**</td>
</tr>
<tr>
<td>III HFD (20 g/d/rat/p.o.) + Water soluble</td>
<td>0.441±0.04**</td>
<td>0.414±0.02**</td>
<td>0.631±0.02**</td>
<td>75.28±4.63**</td>
</tr>
<tr>
<td>fraction of ethanolic Gymnema sylvestre</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>extract (120 mg/kg/p.o.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV HFD (20 g/d/rat/p.o.) + Water insoluble</td>
<td>0.695±0.03**</td>
<td>0.643±0.03**</td>
<td>0.888±0.06**</td>
<td>118.09±4.86**</td>
</tr>
<tr>
<td>fraction of ethanolic Gymnema sylvestre</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>extract (80 mg/kg/p.o.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>V HFD (20 g/d/rat/p.o.) + Rimonabant (10</td>
<td>0.403±0.03**</td>
<td>0.413±0.01**</td>
<td>0.557±0.03**</td>
<td>94.09±2.98**</td>
</tr>
<tr>
<td>mg/kg/p.o.)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VI Ethanolic Gymnema sylvestre extract</td>
<td>0.402±0.04 ns$^b$</td>
<td>0.402±0.02 ns$^b$</td>
<td>0.438±0.02 ns$^b$</td>
<td>67.28±4.63 ns$^b$</td>
</tr>
<tr>
<td>(200 mg/kg/p.o. i.e. perse group)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10); ** P< 0.01 as compared to the Group I; ***P< 0.01, ##P<0.05 as compared to the Group II; ns= non significant as compared to the Group II; ns$^s$= non significant as compared to the Group I; a, b- non significant as compared to the Group III (ANOVA followed by Dunnett’s test)
Figure 9: Effect of ethanolic *Gymnema sylvestre* extract on DNA fragmentation detected by agarose gel electrophoresis in HFD-induced obesity in Wistar rats. Lane M = Marker, V1 = normal control group, V2 = HFD control group, V3 = water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o.) treated group, V4 = water insoluble fraction of *Gymnema sylvestre* ethanolic extract (80 mg/kg/p.o.) treated group, V5 = rimonabant (10 mg/kg/p.o.) treated group, V6 = *Gymnema sylvestre* ethanolic extract (200 mg/kg/p.o. i.e. perse group)
Chapter VI

Results of High fat diet-induced Obesity in Wistar rats

Histopathological Studies (Haematoxylin-Eosin stained)
Photomicrographs of Cardiac Tissues (I-VI)

Group I: Normal healthy control group (i.e. vehicle control group) showed no fatty changes with normal architecture.

Group II: HFD control group (i.e. high fat diet group), showed deposition of fat globules in myocardial cells.

Group III: Water soluble fraction of G. sylvestre extract (120 mg/kg/p.o.) showed normal fat deposition in myocardial cells.
Chapter VI

Results of High fat diet-induced Obesity in Wistar rats

Group IV: Water insoluble fraction of *G. sylvestre* extract (80 mg/kg/p.o.) showed microvasicular fat deposition in myocardial cells

Group V: Rimonabant (10 mg/kg/p.o.) showed normal fat deposition in myocardial cells

Group VI: Ethanolic *G. sylvestre* extract (200 mg/kg/p.o.) showed myocardium with normal architecture

Figure 10: Photomicrographs of heart tissues of HFD-induced obesity in rats
Chapter VI
Results of High fat diet-induced Obesity in Wistar rats

Histopathological Studies (Haematoxylin-Eosin stained)
Photomicrographs of Liver Tissues (I-VI)

Group I: Normal healthy control group (i.e. vehicle control group) showed no fat globules deposition

Group II: HFD control group (i.e. high fat diet group), showed marked fatty changes within liver cells

Group III: Water soluble fraction of *G. sylvestre* extract (120 mg/kg/p.o.) showed normal fat deposition i.e. within normal limit
Chapter VI

Results of High fat diet-induced Obesity in Wistar rats

Group IV: Water insoluble fraction of *G. sylvestre* extract (80 mg/kg/p.o.)
Showed ballooning degeneration and congestion in the liver cells

Group V: Rimonabant (10 mg/kg/p.o.)
showed ballooning degeneration but no fatty changes

Group VI: Ethanolic *G. sylvestre* extract (200 mg/kg/p.o.)
showed normal fat deposition i.e. within normal limit

Figure 11: Photomicrographs of liver tissues of HFD-induced obesity in rats
Chapter VI

RESULTS OF STREPTOZOTOCIN INDUCED DIABETES IN WISTAR RATS
Protocol II: STZ – induced diabetes in Wistar rats

Diabetes is a serious condition associated with overweight and obesity (Geiss et al., 2006). There are two types of diabetes. Type 1 diabetes is "insulin-dependent diabetes." Type 2 diabetes is "insulin-resistant diabetes". Type 2 diabetes is strongly associated with obesity and cardiovascular risk. According to data from the National Health and Nutrition Examination Survey (2005-2006), the crude prevalence of diagnosed diabetes in persons aged 20 years and older rose from 5.1 percent in 1988-1994 to 7.7 percent in 2005-2006. A recent report released by the Centers for Disease Control and Prevention (CDC) reported that the rate of new diabetes diagnoses has nearly doubled over the last decade. The average-age-adjusted incidence of diabetes rose from 4.8 new cases per 1,000 persons (between 1995 and 1997) to 9.1 new cases per 1,000 persons (between 2005 and 2007). Streptozotocin (STZ) is a well documented diabetogen to induce diabetes mellitus in experimental models (Nakhaee et al., 2009). Low dose of STZ (45 mg/kg, i.v. single dose in citrate buffer, pH 4.5) was used to induce the diabetes in the present study. Low-doses of STZ (25, 35, 45 and 55 mg/kg) have been known to induce a mild impairment of insulin secretion which is similar to the feature of the later stage of type 2 diabetes (Srinivasan et al., 2005).

Diabetes was induced by streptozotocin. STZ was injected to Wistar rats in a dose of 45 mg/kg, in citrate buffer pH 4.5. Those rats having fasting blood glucose levels \( \geq 200 \, \text{mg/dl} \) after 3 days (72 hrs) were selected for the study. Hyperphagia and polydepsia was observed in rats. However, no mortality was observed in these rats in a dose of STZ (45 mg/kg).

The present study was planned to evaluate the antidiabetic (antihyperglycemic) effect of water soluble fraction of ethanolic Gymnema sylvestre extract in low dose STZ (45 mg/kg) -induced diabetes in Wistar rats. Since water soluble fraction of ethanolic Gymnema sylvestre extract exhibited significant obesity lowering effect on HFD-induced obesity in Wistar rats, as compared to the water insoluble fraction of ethanolic Gymnema
RESULTS OF PROTOCOL II

I. ANTHROPOMETRIC PARAMETERS

A. Food Intake

There was significant (p<0.01) increase in food intake in group VIII (i.e. STZ treated rats) as compared to the group VII (i.e. normal healthy control rats). While there was no significant change in food intake in group IX (i.e. water soluble fraction of Gymnema sylvestre ethanolic extract 120 mg/kg/p.o. treatment group) and group X (i.e. pioglitazone 20 mg/kg/p.o. treatment group) as compared to the group VIII (Figure 12).

B. Water Intake

There was significant (p<0.01) increase in water intake in group VIII as compared to the group VII. While there was significant (p<0.01) decrease in water intake in group IX and group X as compared to the group VIII (Figure 13).

II. HEMODYNAMIC PARAMETERS USING CODA NIBP INSTRUMENT (USA) BY TAIL CUFF METHOD

A. Effect on systolic BP (mm Hg)

The mean systolic BP (158.45±2.29) was less significantly (p<0.05) increased in STZ treated diabetic group (i.e. group VIII) as compared to systolic BP (127.83±6.86) of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX), and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment significantly (p<0.01) decreased the increase in systolic BP as compared to the group VIII (Table 15).
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B. Effect on diastolic BP (mm Hg)

The mean diastolic BP was increased less significantly (p<0.05) in STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). There was significant (p<0.01) decrease in diastolic BP with water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX), and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment as compared to the group VIII (Table 15).

C. Effect on mean BP (mm Hg)

The mean BP was less significantly (p<0.05) in increased STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). There was significant (p<0.01) decrease in mean BP with water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX), and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment decreased the increase in mean BP as compared to the group VIII (Table 15).

D. Effect on heart rate (beats/minute)

A significant (p<0.01) increase in the mean heart rate was observed in STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). There was significant (p<0.01) decrease in mean heart rate with water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX), and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment decreased the increase in mean heart rate as compared to the group VIII (Table 15).

III. EFFECT ON LIPID PROFILE LEVELS

A. Effect on serum TC levels (mg/dl)

The mean serum TC levels, in the rats fed on normal diet alone (i.e. normal healthy control rats, group VII) were stable throughout the experimental period. Conversely, in the STZ treated diabetic group (i.e. group VIII), there was a significant (p<0.01) increase by 3.18-folds in the serum TC levels as compared to the group VII rats. STZ treated diabetic rats when treated with water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) by 2.9-folds and pioglitazone (20
mg/kg/p.o., i.e. group X) significantly ($p<0.01$) decrease by 3.2-folds in the serum TC level as compared with the group VIII (Table 16).

**B. Effect on serum TG levels (mg/dl)**

The mean serum TG levels were significantly ($p<0.01$) increased by 4.7-folds in the STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) by 3.1-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) significantly ($p<0.01$) reduced the increased TG levels by 3-folds in serum as compared to pathogenic group (i.e. group VIII) (Table 16).

**C. Effect on serum HDL-C levels (mg/dl)**

The mean serum HDL-C was significantly ($p<0.01$) decreased by 1.3-folds in STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) by 1.7-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days, significantly ($p<0.01$) elevated the reduced HDL-C levels by 2-folds in serum as compared to the group VIII (Table 16).

**D. Effect on serum LDL-C levels (mg/dl)**

The mean serum LDL-C levels were significantly ($p<0.01$) increased by 7.5-folds in STZ treated diabetic group (i.e. group VIII) as compared to the levels in normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) by 4.1-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) significantly ($p<0.01$) reduced the increased LDL-C levels by 4.15-folds in serum as compared to the group VIII (Table 16).

**E. Effect on serum VLDL-C levels (mg/dl)**

The mean serum VLDL-C levels were significantly ($p<0.01$) increased by 4.8-folds in STZ treated diabetic group (i.e. group VIII) as compared to the levels in normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) by 3.1-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) significantly ($p<0.01$) reduced the increased VLDL-C levels by 3-folds in serum as compared to the group VIII (Table 16).
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F. Atherogenic Indexes

i) TC/HDL-C
The mean TC/HDL-C was significantly (p<0.01) increased by in the STZ treated diabetic group (i.e. group VIII) as compared to the levels in normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) significantly (p<0.01) reduced the increased TC/HDL-C levels as compared to the group VIII (Table 16).

ii) LDL-C/HDL-C
The mean LDL-C/HDL-C was significantly (p<0.01) increased in the STZ treated diabetic group (i.e. group VIII) as compared to the levels in normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) significantly (p<0.01) reduced the increased LDL-C/HDL-C levels as compared to the group VIII (Table 16).

G. Effect on serum Apolipoprotein B (Apo B) levels (mg/ml)
The mean serum Apo B levels were significantly (p<0.01) increased by 3-folds in the STZ treated diabetic group (i.e. group VIII) as compared to the levels in normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) by 2.8-folds and pioglitazone (20 mg/kg/p.o., i.e. group X), treatment for 21 days significantly (p<0.01) decreased by 2.9-folds the serum Apo B levels as compared to the group VIII (Table 17).

H. Effect on Hepatic Cholesterol levels (mg/dl)
The mean hepatic cholesterol levels, in the rats fed on normal diet alone (i.e. normal healthy control rats, group VII) were stable throughout the experimental period. Conversely, in the STZ treated diabetic group (i.e. group VIII), there was a significant (p<0.01) increase by 3.6-folds in the hepatic cholesterol levels as compared to the group VII rats. STZ treated diabetic rats when treated with water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) and pioglitazone
(20 mg/kg/p.o., i.e. group X) for 21 days less significantly (p<0.05) decreased (2.3-folds) the hepatic cholesterol levels as compared with the group VIII (Table 19).

IV. EFFECT ON VISCERAL FAT PAD WEIGHT (g)

a. Mesenteric fat (g)

The mean mesenteric fat weight was significantly (p<0.05) decreased in the STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). While there was significantly (p<0.05) increased in the mesenteric fat weight by water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) as compared to the group VIII (Table 18).

b. Perirenal fat (g)

There was no significant changes in the mean perirenal fat weight in the STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days, produced non significant changes in the perirenal fat weight as compared to the group VIII (Table 18).

c. Epididymal fat (g)

The mean epididymal fat weight was significantly (p<0.01) increased by 1.9-folds in the STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). While water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days decreased non significantly (p>0.05) the increase in the epididymal fat weight as compared to the group VIII (Table 18).

V. EFFECT ON ORGAN’S WEIGHT (g)

a. Heart (g)

There was significantly (p<0.05) decrease in the mean heart weight in the STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). While there was no significant (p>0.05) change in the mean heart weight by water
soluble fraction of *Gymnema sylvestre* ethanolic extract treatment (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days as compared to the pathogenic control group (i.e. group VIII) (Table 19).

b. Liver (g)

There was significantly (p<0.05) decrease in the mean liver weight in the STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). While there was no significant (p>0.05) change in the mean liver weight by water soluble fraction of *Gymnema sylvestre* ethanolic extract treatment (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days as compared to the group VIII (Table 19).

c. Kidneys (Right + Left) (g)

There was no significant change in the mean kidneys weight in the STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). There was no significant (p>0.05) change in the mean kidneys weight produced by water soluble fraction of *Gymnema sylvestre* ethanolic extract treatment (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days as compared to the group VIII (Table 19).

d. Pancreas (g)

The mean pancreas weight was significant (p<0.05) decrease in STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). there was significant (p<0.05) increase in the mean pancreas weight by water soluble fraction of *Gymnema sylvestre* ethanolic extract treatment (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days as compared to the group VIII (Table 19).

VI. EFFECT ON SERUM BIOCHEMICAL PARAMETERS

(a) Effect on serum LDH levels (IU/L)

The mean serum LDH levels was significantly (p<0.01) increased by 11.6-folds in the STZ treated diabetic group (i.e. group VIII) as compared to the normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract
VII. EFFECT ON MYOCARDIAL APOPTOSIS

A. Effect on Caspase-3 activity (units/mg/protein/hour)

The mean Caspase-3 levels were significantly (p<0.01) increased by 3.9-folds in STZ treated diabetic group (i.e. group VII) as compared to the caspase-3 levels in hearts of
the normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) decrease (2.3-folds) in the caspase-3 levels in the cardiac tissue as compared to the group VIII rats (Table 18).

**B. Effect on Na\(^+\)-K\(^+\) ATPase activity (P./min/mg tissue)**

The mean Na\(^+\)-K\(^+\) ATPase levels were significantly (p<0.01) decreased by 1.25-folds in hearts of STZ treated diabetic group (i.e. group VIII) as compared to the hearts of the normal healthy control group (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) (1.58-folds) and pioglitazone (20 mg/kg/p.o., i.e. group X) (1.64-folds) treatment for 21 days showed significant (p<0.01) increase in the decreased Na\(^+\)-K\(^+\) ATPase levels in the cardiac tissue as compared to the group VIII (Table 20).

**C. DNA gel electrophoresis**

Electrophoresis of DNA extracted from the left ventricle region of the heart of STZ treated rats (i.e. group VIII) showed DNA laddering, indicating apoptotic inter-nucleosomal DNA fragmentation. Ladders were not detected in normal control group (i.e. group VII), while water soluble fraction of *G. sylvestre* extract treated group (i.e. group IX) and pioglitazone treated group (i.e. group X), little DNA laddering was observed as compared to the group VIII (Figure 14).

**VIII. EFFECT ON OXIDATIVE STRESS PARAMETERS**

**A. Lipid peroxidation**

The mean TBARS (nmol MDA/ mg protein) levels were significantly (p<0.01) increased by 4.2-folds in STZ treated diabetic group (i.e. group VIII) as compared to the TBARS levels in hearts of the normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) by 2.3-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) decrease in the TBARS levels by 2.9-folds in the cardiac tissue as compared to the group VIII (Table 20).
The mean TBARS (nmol MDA/ mg protein) levels were significantly (p<0.01) increased by 2.7-folds in STZ treated diabetic group (i.e. group VIII) as compared to the TBARS levels in liver of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) (1.85-folds) and pioglitazone (20 mg/kg/p.o., i.e. group X) (2.35-folds) treatment for 21 days showed significant (p<0.01) decrease in the TBARS levels in the hepatic tissue as compared to the group VIII (Table 21).

B. Superoxide dismutase levels (IU/mg protein)

The mean superoxide dismutase (SOD) levels significantly (p<0.01) decreased in STZ treated diabetic group (i.e. group VIII) as compared to the SOD levels in hearts of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed less significant (p<0.05) increase in the SOD levels in the cardiac tissue as compared to the group VIII (Table 20).

The mean superoxide dismutase (SOD) levels significantly (p<0.01) decreased by 1.7-folds in STZ treated diabetic group (i.e. group VIII) as compared to the SOD levels in liver of the normal healthy control rats (i.e. group VII) (i.e. from 1.935±0.01 to 1.150±0.05). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) increase in the SOD levels in the hepatic tissue as compared to the group VIII (Table 21).

C. Catalase (nmol H₂O₂-consumed/min/mg protein)

The mean catalase levels significantly (p<0.01) decreased by 5-folds in STZ treated diabetic group (i.e. group VIII) as compared to the catalase levels in hearts of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) (1.75-folds) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed...
significant (p<0.01) increase by 2-folds in the catalase levels in the cardiac tissue as compared to the group VIII (Table 20).

The mean catalase levels significantly (p<0.01) decreased by 2.3-folds in STZ treated diabetic group (i.e. group VIII) as compared to the catalase levels in liver of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) (1.5-folds) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) 1.7-folds increase in the catalase levels in the hepatic tissue as compared to the group VIII (Table 21).

D. Reduced glutathione (µmol of phosphorus liberated/min/mg protein)

The mean glutathione levels were significantly (p<0.01) decreased by 2-folds in STZ treated diabetic group (i.e. group VIII) as compared to the glutathione levels in hearts of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) (1.9-folds) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) 2.1-folds increase in the glutathione levels in the cardiac tissue as compared to the group VIII (Table 22).

The mean glutathione levels were significantly (p<0.01) decreased by 2.1-folds in STZ treated diabetic group (i.e. group VIII) as compared to the glutathione levels in liver of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) (2-folds) and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) 2.1-folds increase in the glutathione levels in the hepatic tissue as compared to the group VIII (Table 23).
E. Glutathione peroxidase (nmol NADPH oxidized/min/mg of protein)

The mean glutathione peroxidase (GPx) levels were significantly (p<0.01) decreased by 1.5-folds in STZ treated diabetic group (i.e. group VIII) as compared to the glutathione peroxidase levels in hearts of the normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) 1.51-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) 1.8-folds increase in the GPx levels in the cardiac tissue as compared to the group VIII (Table 22).

The mean glutathione peroxidase (GPx) levels were significantly (p<0.01) decreased by 1.45-folds in STZ treated diabetic group (i.e. group VIII) as compared to the glutathione peroxidase levels in liver of the normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) 1.51-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed less significant (p<0.05) 1.6-folds increase in the GPx levels in the hepatic tissue as compared to the group VIII (Table 23).

F. Glutathione reductase (nmol NADPH oxidized/min/mg of protein)

There was significantly (p<0.01) decrease in mean glutathione reductase (GR) levels by 2-folds in STZ treated diabetic group (i.e. group VIII) as compared to the glutathione levels in hearts of the normal healthy control rats (i.e. group VII). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group IX) 1.8-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) 2.25-folds increase in the GR levels in the cardiac tissue as compared to the group VIII (Table 22).
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Results of STZ - induced diabetes in Wistar rats

There was significantly (p<0.01) decrease in mean glutathione reductase (GR) levels by 1.8-folds in STZ treated diabetic group (i.e. group VIII) as compared to the glutathione levels in liver of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) 1.7-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) 1.8-folds increase in the GR levels in the hepatic tissue as compared to the group VIII (Table 23).

G. Glutathione-S-transferase (nmol CDNB conjugate formed / min / mg protein)

There was significantly (p<0.01) decrease in mean glutathione-S-transferase (GST) levels by 3.2-folds in STZ treated diabetic group (i.e. group VIII) as compared to the glutathione levels in hearts of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) 2.2-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) 2.4-folds increase in the GR levels in the cardiac tissue as compared to the group VIII (Table 22).

There was significantly (p<0.01) decrease in mean glutathione-S-transferase (GST) levels by 2.7-folds in STZ treated diabetic group (i.e. group VIII) as compared to the glutathione levels in liver of the normal healthy control rats (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) 2.45-folds and pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant (p<0.01) 2.6-folds increase in the GR levels in the hepatic tissue as compared to the group VIII (Table 23).

IX. Effect on hepatic Na\(^+\)-K\(^+\) ATPase activity (P\(_i\)/min/mg tissue)

The mean Na\(^+\)-K\(^+\) ATPase levels were significantly (p<0.01) decreased by 1.45-folds in livers of STZ treated diabetic group (i.e. group VIII) as compared to the livers of the normal healthy control group (i.e. group VII). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group IX) by 1.75-folds and
pioglitazone (20 mg/kg/p.o., i.e. group X) treatment for 21 days showed significant 
(p<0.01) increase by 1.88-folds in the decreased Na\(^+\)-K\(^+\) ATPase levels in the hepatic 
tissue as compared to the group VIII (Table 21).

X. EFFECT ON HISTOPATHOLOGICAL STUDIES

i) Histopathology of heart tissue (Photomicrographs I-IV)

Histopathological sections of rat's heart tissue of STZ treated group showed few 
calcified lesions with fatty particles in the myocardial cells as compared to normal 
healthy control rat's heart tissue which showed normal architecture with regular 
morphology of myocardial cell membrane and well preserved cytoplasm. Water 
soluble fraction of G. sylvstre ethanolic extract (120 mg/kg/p.o.) (i.e. group IX) and 
standard drug i.e. pioglitazone (20 mg/kg/p.o., i.e. group X) showed no pathological 
changes with regular architecture of myocardium (Figure 15).

ii) Histopathology of liver tissue (Photomicrographs I-IV)

Histopathological observation of rat's liver tissue of STZ treated group (i.e. group VIII) 
showed few fatty particles in the hepatocytes and no other pathological changes 
observed as compared to normal healthy control rat's liver tissue which showed 
normal fat deposition in hepatocytes i.e. within normal limit. Water soluble fraction of 
G. sylvstre ethanolic extract (120 mg/kg/p.o.) (i.e. group IX) showed microvesicular 
fat accumulation in the hepatocytes while standard drug i.e. pioglitazone group (20 
mg/kg/p.o., i.e. group X) showed normal morphology of liver tissues as compared to 
the STZ treated group (Figure 16).
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Figure 12: Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on food intake in STZ-induced diabetes in Wistar rats

Figure 13: Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on water intake in STZ-induced diabetes in Wistar rats

**P<0.01 as compared to the Control group; **P<0.01, ns# - non significant as compared to the STZ treated group
Table 14. Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on blood pressure and heart rate in STZ-induced diabetes in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart Rate (BPM)</th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP (mm Hg)</th>
<th>Mean BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VII (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>421.83±15.77</td>
<td>127.83±6.86</td>
<td>96±4.65</td>
<td>106.16±5.00</td>
</tr>
<tr>
<td>Group VIII (Diabetic pathogenic group i.e. STZ 45 mg/kg, i.v. single dose+1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>641.05±7.32**</td>
<td>158.45±2.29**</td>
<td>136.9±1.71**</td>
<td>145.85±1.69**</td>
</tr>
<tr>
<td>Group IX (STZ 45 mg/kg, i.v. single dose+ water soluble fraction of ethanolic Gymnema sylvestre extract 120 mg/kg/p.o. for 21 days)</td>
<td>494.64±12.48##</td>
<td>110.14±2.03##</td>
<td>91.78±2.00##</td>
<td>100±1.91##</td>
</tr>
<tr>
<td>Group X (STZ 45 mg/kg, i.v. single dose+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>600.5±22.89#a</td>
<td>110.62±3.41##a</td>
<td>92±3.04##a</td>
<td>99±3.32##a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P< 0.01 as compared to the Group VII; ##P< 0.01, #P<0.05 as compared to the Group VIII; a – non significant as compared to the Group IX (ANOVA followed by Dunnett’s test)
### Chapter VI

Results of STZ – induced diabetes in Wistar rats

Table 15. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on lipid profile levels in STZ-induced diabetes in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>Atherogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VII (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>101.69±2.00</td>
<td>32.26±1.01</td>
<td>64.97±2.19</td>
<td>30.83±5.63</td>
<td>12.99±0.43</td>
<td>3.25±0.09</td>
</tr>
<tr>
<td>Group VIII (Diabetic pathogenic group i.e. STZ 45 mg/kg, i.v. single dose+ 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>317.24±12.39**</td>
<td>24.48±0.92**</td>
<td>305.41±16.31**</td>
<td>231.67±14.45**</td>
<td>61.08±3.26**</td>
<td>13.03±0.61*</td>
</tr>
<tr>
<td>Group IX (STZ 45 mg/kg, i.v. single dose+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract 120 mg/kg/p.o. for 21 days)</td>
<td>110.35±7.19##</td>
<td>41.74±1.62##</td>
<td>99.18±5.35##</td>
<td>56.64±0.31##</td>
<td>19.83±1.07##</td>
<td>2.68±0.25##</td>
</tr>
<tr>
<td>Group X (STZ 45 mg/kg, i.v. single dose+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>101.31±5.46###a</td>
<td>50.07±2.81###a</td>
<td>102.02±5.11###a</td>
<td>48.77±8.23###a</td>
<td>20.40±1.02###a</td>
<td>2.04±0.11###a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P<0.01 as compared to the Group VII; ###P<0.01 as compared to the Group VIII; a – non significant as compared to the Group IX (ANOVA followed by Dunnett’s test)
Table 16. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on serum leptin, insulin, apolipoprotein-B, LDH, glucose and glycated hemoglobin in STZ-induced diabetes in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum insulin (ng/ml)</th>
<th>Serum apolipoprotein-B (mg/dl)</th>
<th>Serum LDH (IU/L)</th>
<th>Serum glucose (mg/dl)</th>
<th>Glycated hemoglobin (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VII (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>0.116±0.02</td>
<td>4.65±0.22</td>
<td>23.48±0.64</td>
<td>94.14±2.16</td>
<td>7.94±0.11</td>
</tr>
<tr>
<td>Group VIII (Diabetic pathogenic group i.e. STZ 45 mg/kg, i.v. single dose+ 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>0.110±0.01&lt;sup&gt;ns&lt;/sup&gt;</td>
<td>14.26±1.57&lt;sup&gt;**&lt;/sup&gt;</td>
<td>275.14±21.99&lt;sup&gt;**&lt;/sup&gt;</td>
<td>354.16±16.67&lt;sup&gt;**&lt;/sup&gt;</td>
<td>14.61±0.73&lt;sup&gt;**&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group IX (STZ 45 mg/kg, i.v. single dose+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract 120 mg/kg/p.o. for 21 days)</td>
<td>0.163±0.01&lt;sup&gt;###&lt;/sup&gt;</td>
<td>5.31±0.37&lt;sup&gt;###&lt;/sup&gt;</td>
<td>97.89±9.05&lt;sup&gt;##&lt;/sup&gt;</td>
<td>94.29±4.14&lt;sup&gt;##&lt;/sup&gt;</td>
<td>8.91±0.28&lt;sup&gt;##&lt;/sup&gt;</td>
</tr>
<tr>
<td>Group X (STZ 45 mg/kg, i.v. single dose+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>0.157±0.01&lt;sup&gt;###&lt;/sup&gt;</td>
<td>5.10±0.41&lt;sup&gt;###&lt;/sup&gt;</td>
<td>101.83±11.61&lt;sup&gt;##&lt;/sup&gt;</td>
<td>97.51±4.42&lt;sup&gt;###&lt;/sup&gt;a</td>
<td>10.13±0.40&lt;sup&gt;##&lt;/sup&gt;a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean±SEM, (n=10 rats/group). **P< 0.01, ns-non significant as compared to the Group VII; ###P< 0.01 as compared to the Group VIII, a – non significant as compared to the Group IX (ANOVA followed by Dunnett’s test)
### Table 17. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on visceral fat pad weights and caspase-3 activity in STZ-induced diabetes in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mesenteric fat (g)</th>
<th>Perirenal fat (g)</th>
<th>Epididymal fat (g)</th>
<th>Caspase -3 activity (nmole/hr/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VII (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>0.399±0.014</td>
<td>0.799±0.005</td>
<td>0.440±0.006</td>
<td>62.34 ± 9.28</td>
</tr>
<tr>
<td>Group VIII (Diabetic pathogenic group i.e. STZ 45 mg/kg, i.v. single dose+ 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>0.279±0.007*</td>
<td>0.850±0.01ns</td>
<td>0.704±0.007**</td>
<td>240.46±12.32**</td>
</tr>
<tr>
<td>Group IX (STZ 45 mg/kg, i.v. single dose+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract 120 mg/kg/p.o. for 21 days)</td>
<td>0.336±0.008#</td>
<td>0.917±0.01nsf</td>
<td>0.696±0.02nsf</td>
<td>105.62±2.37###</td>
</tr>
<tr>
<td>Group X (STZ 45 mg/kg, i.v. single dose+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>0.324±0.005*a</td>
<td>0.893±0.008ns#a</td>
<td>0.617±0.005 ns#a</td>
<td>104.25±4.97###</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P< 0.01, *P<0.05, ns-non significant as compared to the Group VII; #P< 0.01, #P<0.05 as compared to the Group VIII; ns#, non significant as compared to the Group VIII; a – non significant as compared to the Group IX (ANOVA followed by Dunnett’s test)
### Table 18. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on organs weight and hepatic cholesterol in STZ-induced diabetes in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (gm)</th>
<th>Heart (gm)</th>
<th>Kidney (Rt + Lt) (gm)</th>
<th>Pancreas (gm)</th>
<th>Hepatic Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VII (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>6.95 ± 0.09</td>
<td>0.737 ± 0.01</td>
<td>1.30 ± 0.006</td>
<td>0.348±0.04</td>
<td>65.93±4.39</td>
</tr>
<tr>
<td>Group VII (Diabetic pathogenic group i.e. STZ 45 mg/kg, i.v. single dose+ 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg, p.o.) daily for 28 days)</td>
<td>6.17±0.37*</td>
<td>0.614±0.03ns</td>
<td>1.34±0.03ns</td>
<td>0.306±0.01*</td>
<td>237.28±5.70**</td>
</tr>
<tr>
<td>Group IX (STZ 45 mg/kg, i.v. single dose+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract 120 mg/kg/p.o. for 21 days)</td>
<td>7.55±0.18##</td>
<td>0.712±0.01ns</td>
<td>1.61±0.03ns</td>
<td>0.419±0.009</td>
<td>111.02±6.44##</td>
</tr>
<tr>
<td>Group X (STZ 45 mg/kg, i.v. single dose+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>6.97±0.16##a</td>
<td>0.664±0.01ns</td>
<td>1.47±0.01ns</td>
<td>0.381±0.01##a</td>
<td>105.98±3.93##a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). *P< 0.05, **P< 0.01 as compared to the Group VII; ns- non significant as compared to the Group VII; ##P< 0.01, #P<0.05 as compared to the Group VIII; ns## non significant as compared to the Group VIII; a – non significant as compared to the Group IX (ANOVA followed by Dunnett’s test)
Table 19. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on lipid peroxides (TBARS), catalase (CAT), superoxide dismutase (SOD) and Sodium potassium ATPase levels in Heart tissue of STZ-induced diabetes in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmol MDA/mg protein)</th>
<th>SOD (IU/mg protein)</th>
<th>CAT (nmol H$_2$O$_2$ - consumed/min/mg protein)</th>
<th>Na-K ATPase activity (µmol of Pi liberated/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VII (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>0.200±0.003</td>
<td>1.77±0.01</td>
<td>55.75±1.88</td>
<td>0.725±0.04</td>
</tr>
<tr>
<td>Group VIII (Diabetic pathogenic group i.e. STZ 45 mg/kg, i.v. single dose+ 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg, p.o.) daily for 28 days)</td>
<td>0.822±0.02**</td>
<td>1.08±0.009**</td>
<td>11.67±0.26**</td>
<td>0.565±0.01**</td>
</tr>
<tr>
<td>Group IX (STZ 45 mg/kg, i.v. single dose+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract 120 mg/kg/p.o. for 21 days)</td>
<td>0.366±0.01##</td>
<td>1.21±0.008#</td>
<td>20.75±0.70##</td>
<td>0.859±0.006##</td>
</tr>
<tr>
<td>Group X (STZ 45 mg/kg, i.v. single dose+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>0.289±0.02##a</td>
<td>1.23±0.008##a</td>
<td>23.36±1.02##a</td>
<td>0.925±0.008##a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P< 0.01 as compared to the Group VII, ##P< 0.01, #P<0.05 as compared to the Group VIII; a – non significant as compared to the Group IX (ANOVA followed by Dunnett’s test)
Table 20. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on lipid peroxides (TBARS), catalase (CAT), superoxide dismutase (SOD) and Sodium potassium ATPase levels in liver tissue of STZ-induced diabetes in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmol MDA/ mg protein)</th>
<th>SOD (IU/mg protein)</th>
<th>CAT (nmol H₂O₂ – consumed/min/ mg protein)</th>
<th>Na-K ATPase activity (μmol of Pi liberated/min/ mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VII (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>0.315±0.01</td>
<td>1.935±0.01</td>
<td>35.63±1.45</td>
<td>0.852±0.01</td>
</tr>
<tr>
<td>Group VIII (Diabetic pathogenic group i.e. STZ 45 mg/kg, i.v. single dose+ 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>0.868±0.03**</td>
<td>1.15±0.005**</td>
<td>15.78±0.32**</td>
<td>0.576±0.01**</td>
</tr>
<tr>
<td>Group IX (STZ 45 mg/kg, i.v. single dose+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract 120 mg/kg/p.o. for 21 days)</td>
<td>0.471±0.02##</td>
<td>1.26±0.03#</td>
<td>23.95±0.82##</td>
<td>0.993±0.01##</td>
</tr>
<tr>
<td>Group X (STZ 45 mg/kg, i.v. single dose+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>0.378±0.02##a</td>
<td>1.34±0.01#a</td>
<td>28.11±0.73##a</td>
<td>1.06±0.03##a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P<0.01 as compared to the Group VII, ##P<0.01, #P<0.05 as compared to the Group VIII; a – non significant as compared to the Group IX (ANOVA followed by Dunnett’s test)
Table 21. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in Heart tissue of STZ-induced diabetes in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (μmol of phosphorus liberated/min/mg protein)</th>
<th>Glutathione peroxidase (nmol/NADPH oxidized/min/mg of protein)</th>
<th>Glutathione reductase (nmol/NADPH oxidized/min/mg of protein)</th>
<th>Glutathione S transferase (nmolCDNB conjugate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VII (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>26.75±0.97</td>
<td>184.81±7.54</td>
<td>29.90±0.43</td>
<td>492.31±9.01</td>
</tr>
<tr>
<td>Group VIII (Diabetic pathogenic group i.e. STZ 45 mg/kg, i.v. single dose+ 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>13.08±0.35**</td>
<td>120.20±6.25**</td>
<td>14.99±0.58**</td>
<td>156.48±5.68**</td>
</tr>
<tr>
<td>Group IX (STZ 45 mg/kg, i.v. single dose+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract (20 mg/kg/p.o. for 21 days))</td>
<td>25.61±0.10***</td>
<td>191.22±10.97***</td>
<td>27.38±0.52***</td>
<td>344.31±3.47***</td>
</tr>
<tr>
<td>Group X (STZ 45 mg/kg, i.v. single dose+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>27.22±0.13***</td>
<td>216.90±9.07***</td>
<td>34.69±1.06***</td>
<td>375.74±5.67***</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P< 0.01 as compared to the Group I; ***P< 0.01 as compared to the Group II; a – non significant as compared to the Group IX (ANOVA followed by Dunnett’s test)
Table 22. Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in liver tissue of STZ-induced diabetes in Wistar rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (μmol of phosphorus liberated /min /mg protein)</th>
<th>Glutathione peroxidase (nmolNADPH oxidized/min/mg of protein)</th>
<th>Glutathione reductase (nmolNADPH oxidized/min/mg of protein)</th>
<th>Glutathione S transferase (nmolCDNB conjugate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group VII (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>28.46±0.21</td>
<td>197.97±8.64</td>
<td>36.15±0.85</td>
<td>508.35±8.87</td>
</tr>
<tr>
<td>Group VIII (Diabetic pathogenic group i.e. STZ 45 mg/kg, i.v. single dose+ 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/ p.o.) daily for 28 days)</td>
<td>13.38±0.29**</td>
<td>133.32±7.15**</td>
<td>19.11±0.67**</td>
<td>188.93±3.89**</td>
</tr>
<tr>
<td>Group IX (STZ 45 mg/kg, i.v. single dose+ water soluble fraction of ethanolic Gymnema sylvestre extract 120 mg/kg/p.o. for 21 days)</td>
<td>26.64±1.06##</td>
<td>204.15±3.24##</td>
<td>35.06±1.11##</td>
<td>465.73±9.61##</td>
</tr>
<tr>
<td>Group X (STZ 45 mg/kg, i.v. single dose+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>27.99±1.00###a</td>
<td>220.38±4.29###a</td>
<td>37.72±1.90###a</td>
<td>491.12±15.72###a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P< 0.01 as compared to the Group VII; ###P< 0.01 as compared to the Group VIII; a – non significant as compared to the Group IX (ANOVA followed by Dunnett’s test)
Figure 14. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on DNA fragmentation detected by agarose gel electrophoresis in STZ-induced diabetes in Wistar rats. Lane M = Marker, S1 = normal control group, S2 = Diabetic control group (STZ 45 mg/kg i.v.), S3 = water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o.) treated group, S4 = pioglitazone (20 mg/kg/p.o.) treated group.
Histopathological Studies (Haematoxylin-Eosin stained)

Photomicrographs of Cardiac Tissues (VII-X)

Group VII: Normal healthy control group (i.e. vehicle control group) showed no pathological changes with normal architecture.

Group VIII: Diabetic control group (i.e. STZ treated group), showed few calcified lesions with fatty particles in myocardial cells.

Group IX: Water soluble fraction of G. sylvestre extract (120 mg/kg/p.o.) showed no pathological changes in myocardial cells.

Group X: Pioglitazone (20 mg/kg/p.o.) showed no pathological changes in myocardial cells.

Figure 15: Photomicrographs of heart tissues of STZ-induced diabetes in Wistar rats
Chapter VI

Results of STZ – induced diabetes in Wistar rats

Histopathological Studies (Haematoxylin-Eosin stained)

Photomicrographs of Liver Tissues (VII-X)

Group VII: Normal healthy control group (i.e. vehicle control group) showed no pathological changes.

Group VIII: Diabetic control group (i.e. STZ treated group), showed hypertrophy of hepatocytes with dense cytoplasm and fatty changes.

Group IX: Water soluble fraction of *G. sylvestre* extract (120 mg/kg/p.o.) showed no pathological changes i.e. within normal limit.

Group X: Pioglitazone (20 mg/kg/p.o.) showed no changes in the hepatocytes.

Figure 16: Photomicrographs of liver tissues of STZ-induced diabetes in Wistar rats
Chapter VI

RESULTS OF HIGH FAT DIET-INDUCED OBESITY IN DIABETIC WISTAR RATS
PROTOCOL III: High fat diet-induced Obesity in diabetic rats

Obesity is associated with diabetes mellitus, hypertension, dyslipidemia and cardiovascular disease (Marinou et al., 2010). 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 (Nisoli et al., 2000). 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. The increase in the prevalence of type 2 diabetes is closely linked to the upsurge in obesity. About 90% of type 2 diabetes is attributable to excess weight.

In Model II i.e. high fat diet-induced obesity in streptozotocin-induced diabetes in rats, we had developed Model II i.e. high-fat diet-fed and STZ-injected rats as a model for study of antiobesity activity in diabetic rats. The present study was planned to evaluate the antiobesity effect of water soluble fraction of ethanolic Gymnema sylvestre extract in HFD-induced obesity in diabetic rats. Since, water insoluble fraction of ethanolic Gymnema sylvestre extract showed non significant (P>0.05) decrease in body mass index, body weight gain, serum leptin, lipid levels, visceral fat pad weights, cardiac caspase-3 levels and lipid peroxide levels hence, we used water soluble fraction of ethanolic Gymnema sylvestre extract in this Model II, which showed significant effect on obesity biomarkers viz. body mass index, body weight gain, food and water intake, hemodynamic parameters, serum leptin, insulin, glucose, lipids, apolipoproteins, visceral fat pad and organs weights, cardiac caspase-3, antioxidant enzymes levels and histopathological changes. We used Pioglitazone as a standard drug in this Model (i.e. Model II). Ding et al. (2005) have reported that treatment with pioglitazone improves insulin sensitivity in low-dose STZ and high sucrose-fat diet induced obese rats.

Streptozotocin (STZ) is a well documented diabetogen to induce diabetes mellitus (NIDDM) in experimental models (Nakhaee et al., 2009). 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 (Steinberger and Daniels, 2003). 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 is supported by the findings of Ding et al. (2005) 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. Similarly, Srinivasan et al. (2005) 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. Further, Zhang et al. (2008) 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.

Therefore, it was thought worthwhile to investigate the antiobesity activity of standardized water soluble fraction of ethanolic extract of Gymnema sylvestre on high fat diet-induced obesity in diabetic Wistar rats.

RESULTS OF PROTOCOL III

In order to evaluate the effects water soluble fraction of G. sylvestre ethanolic extract on high fat diet – induced obesity in diabetic rats, serial investigations were carried out in all the groups of rats for the estimations of serum leptin, insulin, glucose, lipids, visceral fat pad weights, organs weight, antioxidant enzymes (GSH, GPx, GR, GST, SOD and catalase) levels in heart and liver homogenate followed by histopathology of heart and liver tissues.
I. ANTHROPOMETRIC PARAMETERS

A. Body Mass Index

The mean body mass index was significantly (p<0.01) increased in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). While body mass index was decreased significantly (p<0.01) in water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) as compared to the Toxic control group (i.e. group XII) (Figure 17).

B. Body Weight Gain

Body weight gain was significantly (p<0.01) decreased in group XII (i.e. 40.66±11.61) as compared to the group XI (75.13±5.02). There was no significant (p>0.01) changes in body weight gain in group XIII (i.e. 59.33±8.44) and group XIV (i.e. 55±13.81), as compared to the group XII (Figure 18).

C. Food Intake

There was no significant (p>0.05) change in food intake in group XII as compared to the group XI. While there was significant (p<0.01) increase in food intake in group XIII and group XIV as compared to the group XII (Figure 19).

D. Water Intake

There was significant (p<0.01) increase in water intake in group XII as compared to the group XI. While there was significant (p<0.01) decrease in water intake in group XIII and group XIV as compared to the group II (Figure 20).

II. HEMODYNAMIC PARAMETERS USING CODA NIBP INSTRUMENT (USA) BY TAIL CUFF METHOD

A. Effect on systolic BP (mm Hg)

The mean systolic BP (161±1.43) was less significantly (p<0.05) increased in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to systolic BP (127.83±6.86) the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII), and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment significantly (p<0.01)
decreased the increase in systolic BP as compared to the Toxic control group (i.e. group XII) (Table 25).

B. Effect on diastolic BP (mm Hg)
The mean diastolic BP was increased less significantly (p<0.05) in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). There was significant (p<0.01) decrease in diastolic BP with water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII), and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment as compared to the Toxic control group (i.e. group XII) (Table 25).

C. Effect on mean BP (mm Hg)
The mean BP was less significantly (p<0.05) in increased HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). There was significant (p<0.01) decrease in mean BP with water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII), and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment decreased the increase in mean BP as compared to the Toxic control group (i.e. group XII) (Table 25).

D. Effect on heart rate (beats/minute)
A significant (p<0.05) increase in the mean heart rate was observed in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). There was significant (p<0.01) decrease in mean heart rate with water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII), and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment decreased the increase in mean heart rate as compared to the Toxic control group (i.e. group XII) (Table 25).

III. EFFECT ON LIPID PROFILE LEVELS
A. Effect on serum TC levels (mg/dl)
The mean serum TC levels, in the rats fed on normal diet alone (i.e. normal healthy control rats, group XI) were stable throughout the experimental period. Conversely, in the HFD fed diabetic group (i.e. Toxic control, group XII), there was a significant (p<0.01) increase by 3.7-folds in the serum TC levels as compared to the group XI rats. HFD fed diabetic rats when treated with water soluble fraction of
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Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 2.8-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) significantly (p<0.01) decrease by 3.1-folds in the serum TC level as compared with the Toxic control (i.e. group XII) (Table 26).

B. Effect on serum TG levels (mg/dl)

The mean serum TG levels were significantly (p<0.01) increased by 5.4-folds in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 3-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) significantly (p<0.01) reduced the increased TG levels by 3.3-folds in serum as compared to Toxic group (i.e. group XII) as compared to the Toxic group (i.e. group XII) (Table 26).

C. Effect on serum HDL-C levels (mg/dl)

The mean serum HDL-C was significantly (p<0.01) decreased by 1.15-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 1.7-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days, significantly (p<0.01) elevated the reduced HDL-C levels by 1.6-folds in serum as compared to the Toxic control group (i.e. group XII) (Table 26).

D. Effect on serum LDL-C levels (mg/dl)

The mean serum LDL-C levels were significantly (p<0.01) increased by 5-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the levels in normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 4.5-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) significantly (p<0.01) reduced the increased LDL-C levels by 4.8-folds in serum as compared to Toxic group (i.e. group XII) (Table 26).

E. Effect on serum VLDL-C levels (mg/dl)

The mean serum VLDL-C levels were significantly (p<0.01) increased by 5-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the levels in normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema
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*Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 3-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) significantly (p<0.01) reduced the increased VLDL-C levels by 3.2-folds in serum as compared to Toxic group (i.e. group XII) (Table 26).

**F. Atherogenic Indexes**

1) **TC/HDL-C**

The mean TC/HDL-C was significantly (p<0.01) increased by in the in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the levels in normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) significantly (p<0.01) reduced the increased TC/HDL-C levels as compared to Toxic group (i.e. group XII) (Table 26).

2) **LDL-C/HDL-C**

The mean LDL-C/HDL-C was significantly (p<0.01) increased in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the levels in normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) significantly (p<0.01) reduced the increased LDL-C/HDL-C levels as compared to Toxic group (i.e. group XII) (Table 26).

**G. Effect on serum Apolipoprotein B (Apo B) levels (mg/ml)**

The mean serum Apo B levels were significantly (p<0.01) increased by 7.8-folds in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the levels in normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 5-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV), treatment for 21 days significantly (p<0.01) decreased by 6-folds the serum Apo B levels as compared to the group XII (Table 27).

**H. Effect on Hepatic Cholesterol levels (mg/dl)**

The mean hepatic cholesterol levels, in the rats fed on normal diet alone (i.e. normal healthy control rats, group XI) were stable throughout the experimental period. Conversely, in the HFD fed diabetic group (i.e. Toxic control, group XII), there was a significant (p<0.01) increase by 5.8-folds in the hepatic cholesterol
levels as compared to the group XI rats. HFD fed diabetic rats when treated with water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) for 21 days less significantly (p<0.05) decreased (2.6-folds) the hepatic cholesterol levels as compared with the Toxic control (i.e. group XII) (Table 29).

**IV. EFFECT ON VISCERAL FAT PAD WEIGHT (g)**

a. **Mesenteric fat (g)**

The mean mesenteric fat weight was significantly (p<0.05) decreased in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). While there was no significant change in the mesenteric fat weight in water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) as compared to the Toxic control group (i.e. group XII) (Table 28).

b. **Perirenal fat (g)**

The mean perirenal fat weight was significantly (p<0.01) increased by 1.2-folds in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days significantly (p<0.01) decreased by 1.2-folds the increase in the perirenal fat weight as compared to the Toxic control group (i.e. group XII) (Table 28).

c. **Epididymal fat (g)**

The mean epididymal fat weight was significantly (p<0.01) increased by 1.9-folds in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days less significantly (p<0.05) decreased the increase in the epididymal fat weight as compared to the Toxic control group (i.e. group XII) (Table 28).
V. EFFECT ON ORGAN'S WEIGHT (g)

a. Heart (g)
There was significantly (p<0.01) increase by 1.2-folds, in the mean heart weight in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). While there was no significant (p>0.05) change in the mean heart weight by water soluble fraction of Gymnema sylvestre ethanolic extract treatment (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days as compared to the Toxic control group (i.e. group XII) (Table 29).

b. Liver (g)
There was significantly (p<0.01) increase by 1.2-folds, in the mean liver weight in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). While there was no significant (p>0.05) change in the mean liver weight by water soluble fraction of Gymnema sylvestre ethanolic extract treatment (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days as compared to the Toxic control group (i.e. group XII) (Table 29).

c. Kidneys (Right + Left) (g)
There was significantly (p<0.01) increase by 1.3-folds, in the mean kidneys weight in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). While there was no significant (p>0.05) change in the mean kidneys weight by water soluble fraction of Gymnema sylvestre ethanolic extract treatment (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days as compared to the Toxic control group (i.e. group XII) (Table 29).

d. Pancreas (g)
The mean pancreas weight was no significant (p>0.05) change in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). there was no significant (p>0.05) change in the mean pancreas weight by water soluble fraction of Gymnema sylvestre ethanolic extract treatment (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV)
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treatment for 21 days as compared to the Toxic control group (i.e. group XII) (Table 29).

VI. EFFECT ON SERUM BIOCHEMICAL PARAMETERS

(a) Effect on serum LDH levels (IU/L)

The mean serum LDH levels were significantly (p<0.01) increased by 12-folds in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 3.5-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days significantly (p<0.01) decreased the increase in the serum LDH levels by 2.6-folds as compared to the Toxic control group (i.e. group XII) (Table 27).

(b) Effect on serum Leptin levels (pg/ml)

The mean serum leptin levels were significantly (p<0.01) increased by 3.2-folds in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 3.3-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days significantly (p<0.01) decreased by 3.5-folds in the serum leptin levels as compared to the group XII (Table 27).

(c) Effect on serum Insulin levels (ng/ml)

The mean serum insulin levels were significantly (p<0.05) increased by 1.25-folds in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) (1.2-folds) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) (1.1-folds) treatment for 21 days less significantly (p<0.05) increased the serum insulin levels as compared to the group XII (Table 27).

(d) Effect on serum Glucose levels (mg/ml)

The mean serum glucose levels were significantly (p<0.01) increased by 4.9-folds in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema
sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 4.8-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days significantly (p<0.01) decreased (4.7-folds) the serum glucose levels as compared to the group XII (Table 27).

(e) **Effect on Glycosylated hemoglobin (in %)**

The mean glycosylated hemoglobin levels were significantly (p<0.01) increased by 1.5-folds in the HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days, significantly (p<0.01) decreased (1.4-folds) the glycosylated hemoglobin levels as compared to the group XII (Table 27).

**VII. EFFECT ON MYOCARDIAL APOPTOSIS**

A. **Effect on Caspase-3 activity (units/mg/protein/hour)**

The mean Caspase-3 levels were significantly (p<0.01) increased by 4.5-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the caspase-3 levels in hearts of the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 2.3-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) decrease by 2.5-folds in the caspase-3 levels in the cardiac tissue as compared to the Toxic control group (i.e. group XII) rats (Table 28).

B. **Effect on Na⁺-K⁺ ATPase activity (P₁/min/mg tissue)**

The mean Na⁺-K⁺ ATPase levels were significantly (p<0.05) decreased by 1.1-folds in hearts of HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the hearts of the normal healthy control group (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) increase by 1.5-folds in the decreased Na⁺-K⁺ ATPase.
levels in the cardiac tissue as compared to the Toxic control group (i.e. group XII) hearts (Table 30).

C. DNA gel electrophoresis

Electrophoresis of DNA extracted from the left ventricle region of the heart of HFD fed diabetic rats (i.e. group XII) showed DNA laddering indicating apoptotic inter-nucleosomal DNA fragmentation. Ladders were not detected in normal control group (i.e. group XI), water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treated group, where genomic DNA band was preserved (Figure 21).

VIII. EFFECT ON OXIDATIVE STRESS PARAMETERS

A. Lipid peroxidation

The mean TBARS (nmol MDA/ mg protein) levels were significantly (p<0.01) increased by 5.7-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the TBARS levels in hearts of the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 2-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) decrease in the TBARS levels by 2.5-folds in the cardiac tissue as compared to the Toxic control group (i.e. group XII) rats (Table 30).

The mean TBARS (nmol MDA/ mg protein) levels were significantly (p<0.01) increased by 3.25-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the TBARS levels in liver of the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 1.6-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) 2.2-folds treatment for 21 days showed significant (p<0.01) decrease in the TBARS levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) rats (Table 31).
B. Superoxide dismutase levels (IU/mg protein)

The mean superoxide dismutase (SOD) levels significantly (p<0.01) decreased in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the SOD levels in hearts of the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) increase in the SOD levels in the cardiac tissue as compared to the Toxic control group (i.e. group XII) rats (Table 30).

The mean superoxide dismutase (SOD) levels significantly (p<0.01) decreased by 1.7-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the SOD levels in liver of the normal healthy control rats (i.e. group XI) (i.e. from 1.935±0.01 to 1.165±0.016). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) increase in the SOD levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) (Table 31).

C. Catalase (nmol H₂O₂ consumed/min/mg protein)

The mean catalase levels significantly (p<0.01) decreased by 5-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the catalase levels in hearts of the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) increase by 2.5-folds in the catalase levels in the cardiac tissue as compared to the Toxic control group (i.e. group XII) rats (Table 30).

The mean catalase levels significantly (p<0.01) decreased by 2.4-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the catalase levels in liver of the normal healthy control rats (i.e. group XI). Water soluble
fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 2-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 2.4-folds increase in the catalase levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) (Table 31).

D. Reduced glutathione (μmol of phosphorus liberated/min/mg protein)
The mean glutathione levels were significantly (p<0.01) decreased by 1.7-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione levels in hearts of the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 1.6-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 1.9-folds increase in the glutathione levels in the cardiac tissue as compared to the Toxic control group (i.e. group XII) rats (Table 32).

The mean glutathione levels were significantly (p<0.01) decreased by 2.35-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione levels in liver of the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 2-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 2.3-folds increase in the glutathione levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) rats (Table 33).

E. Glutathione peroxidase (nmol NADPH oxidized/min/mg of protein)
The mean glutathione peroxidase (GPx) levels were significantly (p<0.01) decreased by 1.5-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione peroxidase levels in hearts of the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema
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Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 1.4-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 1.8-folds increase in the GPx levels in the cardiac tissue as compared to the Toxic control group (i.e. group XII) rats (Table 32).

The mean glutathione peroxidase (GPx) levels were significantly (p<0.01) decreased by 1.5-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione peroxidase levels in liver of the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 1.4-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed less significant (p<0.05) 1.7-folds increase in the GPx levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) rats (Table 33).

F. Glutathione reductase (nmol NADPH oxidized/min/mg of protein)

There was significantly (p<0.01) decrease in mean glutathione reductase (GR) levels by 1.7-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione levels in hearts of the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 1.8-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 2.1-folds increase in the GR levels in the cardiac tissue as compared to the Toxic control group (i.e. group XII) rats (Table 32).

There was significantly (p<0.01) decrease in mean glutathione reductase (GR) levels by 2-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione levels in liver of the normal healthy control rats (i.e. group XI). Water soluble fraction of Gymnema sylvestre ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 1.8-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 2-folds
increase in the GR levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) rats (Table 33).

**G. Glutathione-S-transferase (nmol CDNB conjugate formed / min / mg protein)**

There was significantly (p<0.01) decrease in mean glutathione-S-transferase (GST) levels by 3.1-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione levels in hearts of the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 2.2-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 2.4-folds increase in the GR levels in the cardiac tissue as compared to the Toxic control group (i.e. group XII) rats (Table 32).

There was significantly (p<0.01) decrease in mean glutathione-S-transferase (GST) levels by 2.7-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione levels in liver of the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 2.5-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 2.7-folds increase in the GR levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) rats (Table 33).

**IX. Effect on hepatic Na\(^+\)-K\(^+\) ATPase activity (P\(_i\)/min/mg tissue)**

The mean Na\(^+\)-K\(^+\) ATPase levels were significantly (p<0.01) decreased by 1.5-folds in livers of HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the livers of the normal healthy control group (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 1.4-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) increase by 1.55-folds in the decreased Na\(^+\)-K\(^+\) ATPase levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) (Table 31).
increase in the GR levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) rats (Table 33).

G. Glutathione-S-transferase (nmol CDNB conjugate formed / min / mg protein)

There was significantly (p<0.01) decrease in mean glutathione-S-transferase (GST) levels by 3.1-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione levels in hearts of the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 2.2-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 2.4-folds increase in the GR levels in the cardiac tissue as compared to the Toxic control group (i.e. group XII) rats (Table 32).

There was significantly (p<0.01) decrease in mean glutathione-S-transferase (GST) levels by 2.7-folds in HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the glutathione levels in liver of the normal healthy control rats (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) 2.5-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) 2.7-folds increase in the GR levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) rats (Table 33).

IX. Effect on hepatic Na\(^+\)-K\(^+\) ATPase activity (P\(_i\)/min/mg tissue)

The mean Na\(^+\)-K\(^+\) ATPase levels were significantly (p<0.01) decreased by 1.5-folds in livers of HFD fed diabetic group (i.e. Toxic control, group XII) as compared to the livers of the normal healthy control group (i.e. group XI). Water soluble fraction of *Gymnema sylvestre* ethanolic extract (120 mg/kg/p.o., i.e. group XIII) by 1.4-folds and pioglitazone (20 mg/kg/p.o., i.e. group XIV) treatment for 21 days showed significant (p<0.01) increase by 1.55-folds in the decreased Na\(^+\)-K\(^+\) ATPase levels in the hepatic tissue as compared to the Toxic control group (i.e. group XII) (Table 31).
X. EFFECT ON HISTOPATHOLOGICAL STUDIES

i) Histopathology of heart tissue

Histopathological sections of rat's heart tissue of STZ+HFD treated group showed dense focal fatty infiltration in the myocardial cells as compared to normal healthy control rat's heart tissue which showed normal architecture with regular morphology of myocardial cell membrane and well preserved cytoplasm. Water soluble fraction of G. sylvstre ethanolic extract (120 mg/kg/p.o.) (i.e. group XIII) and standard drug i.e. pioglitazone groups (20 mg/kg/p.o., i.e. group XIV) showed normal morphology and no other pathological changes within the myocardium (Figure 22).

ii) Histopathology of liver tissue

Histopathological observation of rat's liver tissue of STZ+HFD treated group (i.e. group XII) showed few fatty particles in the hepatocytes and no other pathological changes observed as compared to normal healthy control rat's liver tissue which showed normal fat deposition in hepatocytes i.e. within normal limit. Water soluble fraction of G. sylvstre ethanolic extract group (120 mg/kg/p.o.) (i.e. group XIII) showed microvesicular fat accumulation in the hepatocytes while standard drug i.e. pioglitazone group (20 mg/kg/p.o., i.e. group XIV) showed normal morphology of liver tissues as compared to the STZ+HFD treated group (Figure 23).
Chapter VI

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Figure 17: Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on body mass index in HFD-induced obesity in diabetic rats

Figure 18: Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on body weight gain in HFD-induced obesity in diabetic rats

**P< 0.01 as compared to the Control group; ###P< 0.01 as compared to the HFD group; ns# - non significant as compared to the STZ+HFD group
Figure 19: Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on food intake in HFD-induced obesity in diabetic rats

Figure 20: Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on water intake in HFD-induced obesity in diabetic rats

*P< 0.05, as compared to the Control group; ns - non significant as compared to the Control group; **P< 0.01, #P< 0.05, as compared to the STZ+HFD group
Table 23. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on blood pressure and heart rate in HFD-induced obesity in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart Rate (BPM)</th>
<th>Systolic BP (mmHg)</th>
<th>Diastolic BP (mm Hg)</th>
<th>Mean BP (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group XI (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/ p.o.) daily for 28 days)</td>
<td>421.83±15.77</td>
<td>127.83±6.86</td>
<td>96±4.65</td>
<td>106.16±5.00</td>
</tr>
<tr>
<td>Group XII (Toxic control group i.e. STZ 45 mg/kg, i.v. single dose+ HFD for 28 days)</td>
<td>463.66±23.65*</td>
<td>161±1.43*</td>
<td>126.66±1.80*</td>
<td>137.83±0.94*</td>
</tr>
<tr>
<td>Group XIII (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract 120 mg/kg/p.o. for 21 days)</td>
<td>350.33±9.65**</td>
<td>106±1.52**</td>
<td>79.16±2.65**</td>
<td>90.83±1.07**</td>
</tr>
<tr>
<td>Group XIV (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>360.16±19.29#a</td>
<td>107.66±2.57#a</td>
<td>80.16±2.99#a</td>
<td>91.66±2.43#a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). *P < 0.05 as compared to the Group XI; **P < 0.01 as compared to the Group XII; #P < 0.01 as compared to the Group XIII (ANOVA followed by Dunnett’s test)
### Table 24. Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on lipid profile levels in HFD-induced obesity in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dl)</th>
<th>HDL-C (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
<th>LDL-C (mg/dl)</th>
<th>VLDL-C (mg/dl)</th>
<th>Atherogenic index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group XI (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>101.69±2.00</td>
<td>32.26±1.01</td>
<td>64.97±2.19</td>
<td>56.64±0.31</td>
<td>12.99±0.43</td>
<td>3.25±0.09</td>
</tr>
<tr>
<td>Group XII (Toxic control group i.e. STZ 45 mg/kg, i.v. single dose+ HFD for 28 days)</td>
<td>373.22±17.51**</td>
<td>28.54±0.86**</td>
<td>346.57±18.07**</td>
<td>275.37±18.74**</td>
<td>69.31±3.61**</td>
<td>13.06±0.38**</td>
</tr>
<tr>
<td>Group XIII (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ water soluble fraction of ethanolic Gymnema sylvestre extract 120 mg/kg/p.o. for 21 days)</td>
<td>132.08±8.61**a</td>
<td>48.45±1.04**a</td>
<td>113.23±7.30**a</td>
<td>60.99±9.27**a</td>
<td>22.64±1.46**a</td>
<td>2.73±0.19**a</td>
</tr>
<tr>
<td>Group XIV (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>120.9±3.11**a</td>
<td>44.46±1.74**a</td>
<td>105.07±5.73**a</td>
<td>55.41±2.39**a</td>
<td>21.01±1.14**a</td>
<td>2.73±0.09**a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P<0.01 as compared to the Group XI; ***P<0.01 as compared to the Group XII; a- non significant as compared to the Group XIII (ANOVA followed by Dunnett's test)
Table 25. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on serum leptin, insulin, apolipoprotein-B, LDH, glucose, and glycated hemoglobin in HFD-induced obesity in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Serum leptin (pg/ml)</th>
<th>Serum insulin (ng/ml)</th>
<th>Serum apolipoprotein-B (mg/dl)</th>
<th>Serum LDH (IU/L)</th>
<th>Serum glucose (mg/dl)</th>
<th>Glycated hemoglobin (in %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group XI (Normal healthy control rats treated with 1% CMC in normal saline (2 ml/kg/ p.o.) once daily for 28 days)</td>
<td>159.37±6.26</td>
<td>0.116±0.02</td>
<td>4.65±0.22</td>
<td>23.48±0.64</td>
<td>94.14±2.16</td>
<td>7.94±0.11</td>
</tr>
<tr>
<td>Group XII (Toxic control group i.e. STZ 45 mg/kg, i.v. single dose+ HFD for 28 days)</td>
<td>513.5±64.01&quot;&quot;</td>
<td>0.143±0.076*</td>
<td>32.78±2.01&quot;&quot;</td>
<td>285.26±11.49&quot;&quot;</td>
<td>430.62±8.78&quot;&quot;</td>
<td>13.88±0.19&quot;&quot;</td>
</tr>
<tr>
<td>Group XIII (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract 120 mg/kg/p.o. for 21 days)</td>
<td>155.5±8.19&quot;&quot;</td>
<td>0.175±0.02&quot;</td>
<td>6.18±0.26&quot;&quot;</td>
<td>79.89±9.61&quot;##</td>
<td>95.72±3.30&quot;&quot;</td>
<td>8.82±0.18&quot;##</td>
</tr>
<tr>
<td>Group XIV (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>144±16.34&quot;&quot;*##</td>
<td>0.155±0.02&quot;&quot;</td>
<td>5.30±0.39&quot;&quot;##</td>
<td>101.83±11.61&quot;##</td>
<td>97.51±4.42&quot;##</td>
<td>8.73±0.26&quot;##</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). *P<0.05, **P<0.01 as compared to the Group XI; *P<0.05, **P<0.01 as compared to the Group XII; a- non significant as compared to the Group XIII (ANOVA followed by Dunnett's test)
Table 26. Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on visceral fat pad weights and caspase-3 activity in HFD-induced obesity in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mesenteric fat (g)</th>
<th>Perirenal fat (g)</th>
<th>Epididymal fat (g)</th>
<th>Caspase -3 activity (nmole/hr/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group XI (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/ p.o.) daily for 28 days)</td>
<td>0.399±0.014</td>
<td>0.799±0.005</td>
<td>0.440±0.006</td>
<td>62.34 ± 9.28</td>
</tr>
<tr>
<td>Group XII (Toxic control group i.e. STZ 45 mg/kg, i.v. single dose+ HFD for 28 days)</td>
<td>0.325±0.01*</td>
<td>0.951±0.01**</td>
<td>0.865±0.02**</td>
<td>288.80±8.36**</td>
</tr>
<tr>
<td>Group XIII (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ water soluble fraction of ethanolic <em>Gymnema sylvestre</em> extract 120 mg/kg/p.o. for 21 days)</td>
<td>0.296±0.006ns#</td>
<td>0.819±0.006##</td>
<td>0.734±0.01#</td>
<td>124.45±3.77##</td>
</tr>
<tr>
<td>Group XIV (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>0.283±0.008ns*a</td>
<td>0.799±0.006##a</td>
<td>0.718.016#a</td>
<td>115.98±2.30##a</td>
</tr>
</tbody>
</table>

All values were expressed as Mean±SEM, (n=10 rats/group). **P< 0.01, *P<0.05 as compared to the Group I; ##P< 0.01, #P<0.05 as compared to the Group II; ns# non significant as compared to the Group II; a- non significant as compared to the Group XIII (ANOVA followed by Dunnett’s test)
# Chapter VI

## Results of High fat diet-induced Obesity in diabetic rats

### Table 27. Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on organs weight and hepatic cholesterol in HFD-induced obesity in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver (gm)</th>
<th>Heart (gm)</th>
<th>Kidney (Rt + Lt) (gm)</th>
<th>Pancreas (gm)</th>
<th>Hepatic Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group XI (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/ p.o.) daily for 28 days)</td>
<td>6.95 ± 0.09</td>
<td>0.737 ± 0.01</td>
<td>1.30 ± 0.006</td>
<td>0.620±0.04</td>
<td>65.93±4.39</td>
</tr>
<tr>
<td>Group XII (Toxic control group i.e. STZ 45 mg/kg, i.v. single dose+ HFD for 28 days)</td>
<td>8.80±0.48**</td>
<td>0.915±0.03**</td>
<td>1.78±0.08**</td>
<td>0.653±0.07**</td>
<td>306.19±9.63**</td>
</tr>
<tr>
<td>Group XIII (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ water soluble fraction of ethanolic Gymnema sylvestre extract 120 mg/kg/p.o. for 21 days)</td>
<td>8.79±0.39**</td>
<td>0.922±0.03**</td>
<td>1.94±0.06**</td>
<td>0.634±0.07**</td>
<td>118.02±4.82**</td>
</tr>
<tr>
<td>Group XIV (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>7.84±0.50**</td>
<td>0.844±0.03**</td>
<td>1.74±0.07**</td>
<td>0.528±0.04**</td>
<td>105.88±3.22**</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P< 0.01 as compared to the Group I; ns- non significant as compared to the Group I; ###P< 0.01, ##P<0.05 as compared to the Group II; ns# non significant as compared to the Group II; a- non significant as compared to the Group XIII (ANOVA followed by Dunnett’s test)
Table 28. Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on lipid peroxides (TBARS), catalase (CAT), superoxide dismutase (SOD) and Sodium potassium ATPase levels in Heart tissue of HFD-induced obesity in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmol MDA/mg protein)</th>
<th>SOD (IU/mg protein)</th>
<th>CAT (nmol H₂O₂ - consumed/min/mg protein)</th>
<th>Na-K ATPase activity (μmol of Pi liberated/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group XI (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>0.200±0.003</td>
<td>1.77±0.01</td>
<td>55.75±1.88</td>
<td>0.725±0.04</td>
</tr>
<tr>
<td>Group XII (Toxic control group i.e. STZ 45 mg/kg, i.v. single dose+ HFD for 28 days)</td>
<td>1.149±0.02**</td>
<td>1.123±0.015**</td>
<td>10.64±0.34**</td>
<td>0.654±0.03*</td>
</tr>
<tr>
<td>Group XIII (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ water soluble fraction of ethanolic Gymnema sylvestre extract 120 mg/kg/p.o. for 21 days)</td>
<td>0.583±0.02##</td>
<td>1.308±0.011##</td>
<td>26.34±0.35##</td>
<td>0.909±0.02##</td>
</tr>
<tr>
<td>Group XIV (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>0.418±0.02##*</td>
<td>1.368±0.001##*</td>
<td>25.57±1.09##*</td>
<td>0.967±0.02##*</td>
</tr>
</tbody>
</table>

All values were expressed as Mean±SEM, (n=10 rats/group), *P< 0.05, **P< 0.01 as compared to the Group I; ##P< 0.01 as compared to the Group II; a- non significant as compared to the Group XIII (ANOVA followed by Dunnett’s test)
### Table 29. Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on lipid peroxides (TBARS), catalase (CAT), superoxide dismutase (SOD) and Sodium potassium ATPase levels in LIVER tissue of HFD-induced obesity in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>TBARS (nmol MDA/mg protein)</th>
<th>SOD (IU/mg protein)</th>
<th>CAT (nmol H₂O₂ consumed/min/mg protein)</th>
<th>Na-K ATPase activity (μmol of Pi liberated/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group XI (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>0.315±0.01</td>
<td>1.935±0.01</td>
<td>35.63±1.45</td>
<td>0.832±0.01</td>
</tr>
<tr>
<td>Group XII (Toxic control group i.e. STZ 45 mg/kg, i.v. single dose+ HFD for 28 days)</td>
<td>1.016±0.03**</td>
<td>1.165±0.016**</td>
<td>14.98±0.20**</td>
<td>0.560±0.006**</td>
</tr>
<tr>
<td>Group XIII (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ water soluble fraction of ethanolic Gymnema sylvestre extract 120 mg/kg/p.o. for 21 days)</td>
<td>0.666±0.01#*</td>
<td>1.410±0.017##</td>
<td>29.27±0.51##</td>
<td>0.797±0.01##</td>
</tr>
<tr>
<td>Group XIV (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>0.492±0.03##*</td>
<td>1.488±0.007##*</td>
<td>35.42±1.92##*</td>
<td>0.890±0.009##*</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P< 0.01 as compared to the Group I; ##P< 0.01 as compared to the Group II; # - non significant as compared to the Group XIII (ANOVA followed by Dunnett’s test)
### Table 30. Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in Heart tissue of HFD-induced obesity in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (µmol of phosphorus liberated/min/mg protein)</th>
<th>Glutathione peroxidase (nmolNADPH oxidized/min/mg of protein)</th>
<th>Glutathione reductase (nmolNADPH oxidized/min/mg of protein)</th>
<th>Glutathione S transferase (nmolCDNB conjugate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group XI (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/p.o.) daily for 28 days)</td>
<td>26.75±0.97</td>
<td>184.81±7.54</td>
<td>29.90±0.43</td>
<td>492.31±9.01</td>
</tr>
<tr>
<td>Group XII (Toxic control group i.e. STZ 45 mg/kg, i.v. single dose+ HFD for 28 days)</td>
<td>15.86±0.48**</td>
<td>121.28±6.46**</td>
<td>17.39±1.22**</td>
<td>155.80±9.71**</td>
</tr>
<tr>
<td>Group XIII (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ water soluble fraction of ethanolic Gymnema sylvestre extract 120 mg/kg/p.o. for 21 days)</td>
<td>26.26±0.45##</td>
<td>173.47±10.46##</td>
<td>33.70±1.09##</td>
<td>399.79±5.68##</td>
</tr>
<tr>
<td>Group XIV (STZ 45 mg/kg, i.v. single dose+ HFD for 28 days+ pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>29.24±0.85##a</td>
<td>219.04±9.49##</td>
<td>36.76±0.95##</td>
<td>434.65±8.73##</td>
</tr>
</tbody>
</table>

All values were expressed as Mean±SEM, (n=8 rats/group). **P < 0.01 as compared to the Group I; ##P < 0.01 as compared to the Group II; a- non significant as compared to the Group XIII (ANOVA followed by Dunnett’s test)
Table 31. Effect of water soluble fraction of ethanolic Gymnema sylvestre extract on reduced glutathione (GSH), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in LIVER tissue of HFD-induced obesity in diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>GSH (μmol of phosphorus liberated /min /mg protein)</th>
<th>Glutathione peroxidase (nmol NADPH oxidized/min/mg of protein)</th>
<th>Glutathione reductase (nmol NADPH oxidized/min/mg of protein)</th>
<th>Glutathione S transferase (nmol CDNB conjugate formed/min/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group XI (Normal healthy control rats treated with 1% Carboxy Methyl Cellulose in normal saline (2 ml/kg/ p.o.) daily for 28 days)</td>
<td>28.46±0.21</td>
<td>197.97±8.64</td>
<td>36.15±0.85</td>
<td>508.35±8.87</td>
</tr>
<tr>
<td>Group XII (Toxic control group i.e. STZ 45 mg/kg, i.v. single dose + HFD for 28 days)</td>
<td>12.24±0.27**</td>
<td>131.74±5.57**</td>
<td>18.66±0.39**</td>
<td>185.60±3.97**</td>
</tr>
<tr>
<td>Group XIII (STZ 45 mg/kg, i.v. single dose + HFD for 28 days + water soluble fraction of ethanolic Gymnema sylvestre extract 120 mg/kg/p.o. for 21 days)</td>
<td>24.29±0.30##</td>
<td>194.99±7.31##</td>
<td>32.58±0.90##</td>
<td>413.68±8.49##</td>
</tr>
<tr>
<td>Group XIV (STZ 45 mg/kg, i.v. single dose + HFD for 28 days + pioglitazone 20 mg/kg/p.o. for 21 days)</td>
<td>27.29±0.30##</td>
<td>224.74±6.95##</td>
<td>36.40±1.03##</td>
<td>464.47±8.76##</td>
</tr>
</tbody>
</table>

All values were expressed as Mean± SEM, (n=10 rats/group). **P < 0.01 as compared to the Group I; ##P < 0.01 as compared to the Group II; a- non significant as compared to the Group XIII (ANOVA followed by Dunnett’s test)
Figure 21: Effect of water soluble fraction of ethanolic *Gymnema sylvestre* extract on DNA fragmentation 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* ethanolic extract (120 mg/kg/p.o.) treated group, L4 = Pioglitazone (20 mg/kg/p.o.) treated group.
Chapter VI

Results of High fat diet-induced Obesity in diabetic rats

Histopathological Studies (Haematoxylin-Eosin stained)

Photomicrographs of Cardiac Tissues (XI-XIV)

Group XI: Normal healthy control group (i.e. vehicle control group) showed no pathological changes with normal architecture.

Group XII: Toxic control group (i.e. STZ+HFD treated group), showed dense focal fatty infiltration in myocardial cells.

Group XIII: Water soluble fraction of *G. sylvestre* extract (120 mg/kg/p.o.) showed no pathological changes with normal architecture of myocardium.

Group XIV: Pioglitazone (20 mg/kg/p.o.) showed no pathological changes, pathological changes with regular morphology of myocardium.

Figure 22: Photomicrographs of heart tissues of HFD-induced obesity in diabetic rats
Chapter VI

Results of High fat diet-induced Obesity in diabetic rats

Histopathological Studies (Haematoxylin-Eosin stained)

Photomicrographs of Liver Tissues (XI-XIV)

Group XI: Normal healthy control group (i.e. vehicle control group) showed no pathological changes

Group XII: Toxic control group (i.e. STZ+HFD treated group), showed few fatty particles in the hepatocytes and no other pathological changes

Group XIII: Water soluble fraction of G. sylvestre extract (120 mg/kg/p.o.) showed some microvesicular fat accumulation in hepatocytes

Group XIV: Pioglitazone (20 mg/kg/p.o.) showed no changes in the hepatocytes

Figure 23: Photomicrographs of liver tissues of HFD-induced obesity in diabetic rats