5.0 RESULTS

5.1 PLANT MATERIAL

5.1.1 Pharmacognostic studies

(a) Macroscopic study

Samples of Lagerstroemia speciosa L. (L. speciosa) leaves collected from Valsad showed following morphological characters.

Leaves 10-20 by 3.8-7.5 cm, oblong-lanceolate or elliptic, subacute, glabrous and finely reticulate on both surfaces, pale beneath, base acute or rounded, main nerves 10-13 pairs, prominent curving upwards, petioles 6-10 mm long, stout.

(b) Microscopic study

Free hand transverse sections (T.S) of fresh samples of L. speciosa leaf was taken and studied for their histological characters.

T.S of Leaf (Fig. 5).

T.S of leaf shows midrib and lamina portion. Midrib from upperside it is domshaped. It is broadly convex with two narrow laminar extension on either side. The later being dorsiventral showing two layer palisade tissure. Below upper epidermis the rest of the tissue occupied by spongy parenchyma travels by obliquely cut vascular bundle and Ca Oxalate cluster crystal. The midrib shows bicolateral vascular bundle in the centre, the bigger one being ‘U’ shaped occupies the major area of the section while other one which is resting on its upper side forms lid like structure. Both the merysteles are encyed by a sclerenchymatus sheath further in the centre paranchymatus tissue of triangular shape is found. Colenchyma is present as 5-6 layers tissue below upper epidermis and above the lower epidermis. Rest of the space occupied by spongy parenchyma embedded places with clusters of Ca Oxalate.
5.1.2 Physico-chemical evaluations (Table 16a, 16b and 16c)

Phyto-chemical evaluation was done for presence of various phytoconstituents. Preliminary chemical tests indicated presence of saponins, carbohydrates, steroids, triterpenoids, tannins, phenolics, coumarins, quinone glycosides.

Table 16a: Physicochemical parameters

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Quality Parameters</th>
<th>% w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ash Value</td>
<td>8.55%</td>
</tr>
<tr>
<td></td>
<td>Total Ash Value</td>
<td>8.55%</td>
</tr>
<tr>
<td></td>
<td>Acid Insoluble Ash</td>
<td>1.2%</td>
</tr>
<tr>
<td></td>
<td>Water Soluble Ash</td>
<td>6.55%</td>
</tr>
<tr>
<td>2</td>
<td>Extractive Value</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Water Soluble Extractive</td>
<td>16.72%</td>
</tr>
<tr>
<td></td>
<td>Alcohol Soluble Extractive</td>
<td>10.08%</td>
</tr>
<tr>
<td></td>
<td>Petroleum Ether Extractive</td>
<td>3.3%</td>
</tr>
<tr>
<td>3</td>
<td>Moisture Content</td>
<td>1%</td>
</tr>
<tr>
<td>4</td>
<td>Foreign matter</td>
<td>1%</td>
</tr>
</tbody>
</table>
Results

Table 16b: Phytochemical Screening

<table>
<thead>
<tr>
<th>Phytoconstituents</th>
<th>Tests</th>
<th>Positive (+ve)</th>
<th>Negative (-ve)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloid</td>
<td>Dragonroff’s Reagent Test</td>
<td></td>
<td>-ve</td>
</tr>
<tr>
<td>Flavanoid</td>
<td>Shinoda Test</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluorescence Test</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>Froth Test</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Haemolytic Zone Test</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>Molisch’s Test</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fehling Test</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Steroids and triterpenoids</td>
<td>Liberman Burchard Test</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Salkowski Reaction</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>Test with Gelatin</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Test with Lead Acetate</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Phenolics</td>
<td>With FeCl₃</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With Folin Ciocalteu Reagent</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Coumarins</td>
<td>With Ammonia</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>With Hydroxylamine HCl</td>
<td>+ve</td>
<td></td>
</tr>
<tr>
<td>Quinone Glycoside</td>
<td>Borntrager’s Test</td>
<td>-ve</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Modified Borntrager’s Test</td>
<td>+ve</td>
<td></td>
</tr>
</tbody>
</table>

Yield of extract and fractions of L. speciosa

The yield of the 50% methanolic extract (MEL) was found to be 5.13% and the yield of the petroleum ether fraction of 50% methanolic extract (PFML) was found to be 3.33% and yield of the solvent ether insoluble fraction i.e. tannin fraction (TFML) was found to be 10%.
Estimation of corosolic acid content in PFML

(A)

(B)
Results
Results

(E)

(F)
Results

(G)

(H)
Results

Figure 6. HPTLC Chromatogram of (A) Corosolic acid standard (0.184 ng) (B) Corosolic acid standard (368.00 ng) (C) Corosolic acid standard (552.00 ng) (D) Corosolic acid standard (736.00 ng) (E) Corosolic acid standard (920.00 ng) (F) Corosolic acid standard (1.104 µg) (G) Corosolic acid in the sample (PFML) (H) TLC of PFML and standard (I) Overlay Spectra

Table 17: Calculation of Corosolic acid concentration in PFML

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Sample</th>
<th>Amount</th>
<th>Area</th>
<th>Rf</th>
<th>X(calc)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Standard 1</td>
<td>184.00 ng</td>
<td>329.03</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Standard 2</td>
<td>368.00 ng</td>
<td>737.05</td>
<td>0.72</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Standard 3</td>
<td>552.00 ng</td>
<td>910.4</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Standard 4</td>
<td>736.00 ng</td>
<td>1058.93</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Standard 5</td>
<td>920.00 ng</td>
<td>1229.73</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Standard 6</td>
<td>1.104 µg</td>
<td>1687.15</td>
<td>0.71</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>PFML Extract</td>
<td>X</td>
<td>536.28</td>
<td>0.75</td>
<td>295.29 ng</td>
</tr>
</tbody>
</table>

$r^2=0.959$

X calculated from $Y=mx+C$

Upon performing HPTLC analysis of the extract and fraction of *L. speciosa* following percentage weight by weight quantity of corosolic acid was found 0.25% in petroleum ether fraction of 50% methanolic extract.

5.1.3 HPTLC method validation

The composition of the mobile phase for TLC was optimized by testing different solvent mixtures of varying polarity. The best results were obtained Hexane: Ethyl formate (6:3) the selected mobile phase produced highly symmetrical peaks showing good resolution. The compound with an $R_f$ value of $0.71 \pm 0.04$ was identified as corosolic acid. The
specificity of the method was ascertained by analyzing standards and samples. The spot for corosolic acid in the sample was confirmed by comparing the Rf value. Spectral studies revealed that the peaks obtained from both standard corosolic acid and test samples were identical, because they had similar pattern. The precision of the method was studied by applying six replicates of the three different concentration of the standard. The results in Table 19 showed that the % RSD for intra- and Inter - day analysis was found to be in the range 0.91 – 1.94, that is less than 2%. The accuracy of the method was determined from recovery studies. A known but varying amount of standards of corosolic acid was added to the pre-analyzed sample and analyzed according to the procedure. The results reported in Table 20. The average recovery percentage value was found to be 96.50 ± 0.602%. Linearity was evaluated by determining six standard working concentrations containing 184 – 1104 ng/ml. of corosolic acid. Peak area and concentration were subjected to least square linear regression analysis to calculate the calibration equation and correlation coefficient. Linearity was found over the concentration range 0.920 –5.520 ng / spot with a correlation coefficient of 0.959 ± 0.0010. The linearity of the calibration curve and adherence to the system to Beer’s law was validated by a high value correlation coefficient. In order to estimate the limit of detection (LOD) and limit of quantification (LOQ), blank, mixed solvent methanol was spotted six times. LOD and LOQ were determined based on the standard deviation of the response of the blank and slope estimated from the calibration curve of the corosolic acid. The LOD and LOQ were found to be 17.45 and 52.89 ng/spot respectively for corosolic acid.

Table 18: Summary of Method Validation parameters

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Linear range (ng/spot)</th>
<th>Correlation coefficient (R2)</th>
<th>Linear regression</th>
<th>LOD (ng/spot)</th>
<th>LOQ (ng/spot)</th>
<th>Repeatability (RSD, n=6)</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corosolic acid</td>
<td>184-1104 ng/spot</td>
<td>0.959</td>
<td>y = 1.307x + 150.33</td>
<td>17.4551</td>
<td>52.89423</td>
<td>0.71456</td>
<td>Specific</td>
</tr>
</tbody>
</table>

Table 19: Intra- and Inter-day precision of HPTLC methods

<table>
<thead>
<tr>
<th>HPTLC Method</th>
<th>Corosolic acid (ng)</th>
<th>Intra-day</th>
<th></th>
<th>Inter-day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Area</td>
<td>%RSD</td>
<td>Area</td>
<td>%RSD</td>
</tr>
<tr>
<td></td>
<td>184</td>
<td>329.29 ± 3.0</td>
<td>0.91</td>
<td>323.79 ± 5.13</td>
</tr>
<tr>
<td></td>
<td>552</td>
<td>923.43 ± 12.93</td>
<td>1.40</td>
<td>891.23 ± 15.90</td>
</tr>
<tr>
<td></td>
<td>920</td>
<td>1243.5 ± 24.17</td>
<td>1.94</td>
<td>1195.9 ± 20.83</td>
</tr>
</tbody>
</table>

Table 20: Recovery Study

<table>
<thead>
<tr>
<th>HPTLC Method</th>
<th>Amount of Corosolic Acid Added (ng)</th>
<th>Area</th>
<th>Area</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>184</td>
<td>368</td>
<td>359</td>
<td>97.55</td>
</tr>
<tr>
<td></td>
<td>552</td>
<td>936</td>
<td>910</td>
<td>96.46</td>
</tr>
<tr>
<td></td>
<td>920</td>
<td>1204</td>
<td>1254</td>
<td>95.47</td>
</tr>
</tbody>
</table>
5.2 Effect of 50% methanolic extract of *L. speciosa* leaves (MEL) on HFD/STZ-induced diabetic rats:

5.2.1(a) Effect of MEL on serum glucose levels (after 3 week):

HFD control rats showed significant increase in serum glucose levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significantly higher fasting serum glucose level as compared to HFD control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (100 mg/kg, p.o.) did not produce significant decrease in fasting serum glucose level when compared with HFD/STZ-induced diabetic control group. Treatment of HFD/STZ-induced diabetic rats with MEL (300, 500 mg/kg, p.o.) produced significantly lower fasting serum glucose level as compared to HFD/STZ-induced diabetic control group. This effect was comparable to pioglitazone (20 mg/kg, p.o.) and glipizide (10 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Normal rats treated with MEL (500mg/kg, p.o.) did not show any significant change when compared with normal control rats (fig. 7).

![Figure 7](image-url)

Figure 7. Effect of MEL on fasting serum glucose level in HFD/STZ-induced diabetic rats (after 3 week). Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL100=HFD+STZ rats treated with 100 mg/kg MEL, MEL300=HFD+STZ rats treated with 300 mg/kg MEL, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.2.1(b) Effect of MEL on serum glucose levels (after 8 week):

HFD control rats showed significant increase in serum glucose levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significantly higher fed (Fig. 8A) and fasting (Fig. 8B) serum glucose level as compared to HFD control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (100mg/kg, p.o.) did not produce any significant change in fed serum glucose while significant decrease in fasting serum glucose level was found when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (300, 500 mg/kg, p.o.) produced significant decrease in fed and fasting serum glucose level when compared with HFD/STZ-induced diabetic control group. This effect was comparable to pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not produce any significant change in fed and fasting serum glucose when compared with HFD/STZ-induced diabetic control rats. Normal rats treated...
Results

with MEL (500mg/kg, p.o.) did not show any significant change when compared with normal control rats.

During OGTT, HFD/STZ-induced diabetic rats showed significantly higher serum glucose levels as compared to HFD control rats. Dose dependent treatment of HFD/STZ-induced diabetic group with MEL (100, 300 and 500 mg/kg, p.o.) as well as pioglitazone (20 mg/kg, p.o.) produced significant decrease in serum glucose level when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum glucose when compared with HFD/STZ-induced diabetic control rats (Fig. 8C).

The AUC_{glucose} was found to be significantly higher in HFD/STZ-induced diabetic rats as compared to HFD control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (100mg/kg, p.o.) did not produce any significant change AUC_{glucose} when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (300 and 500 mg/kg, p.o.) showed significant decrease in AUC_{glucose} when compared with HFD/STZ-induced diabetic control rats. In our study, treatment of HFD/STZ-induced diabetic rats with pioglitazone (20mg/kg, p.o.) and glipizide (10 mg/kg, p.o.) group did not show any change in AUC_{glucose} when compared with HFD/STZ-induced diabetic control rats (Fig. 8D).

(8A)

![Fed serum glucose (mg/dl)](image)

(8B)

![Fasting serum glucose (mg/dl)](image)

(8C)
Figure 8. Effect of MEL on serum glucose level in HFD/STZ-induced diabetic rats (after 8 week). (8A) Effect on fed serum glucose level; (8B) Effect on fasting serum glucose level; (8C) Effect on oral glucose tolerance test; (8D) Effect on AUC<sub>glucose</sub>. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL100=HFD+STZ rats treated with 100 mg/kg MEL, MEL300=HFD+STZ rats treated with 300 mg/kg MEL, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); †significantly different from control (p<0.05).

5.2.2(a) Effect of MEL on serum insulin levels (after 3 week):

HFD control rats showed significant increase in serum insulin levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significantly lower fasting serum insulin level as compared to HFD control rats. Treatment with MEL (100, 300, 500 mg/kg, p.o.) to HFD/STZ-induced diabetic rats did not show significant decrease fasting serum insulin level when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Normal rats treated with MEL (500mg/kg, p.o.) did not show any significant change when compared with normal control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) produced significantly higher serum insulin level as compared to HFD/STZ-induced diabetic control rats (Fig. 9).
Results

Figure 9. Effect of MEL on fasting serum insulin level in HFD/STZ-induced diabetic rats (after 3 week). Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL100=HFD+STZ rats treated with 100 mg/kg MEL, MEL300=HFD+STZ rats treated with 300 mg/kg MEL, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05);

5.2.2(b)Effect of MEL on serum insulin levels (after 8 week):

HFD control rats showed significant increase in serum insulin levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significantly higher fed (Fig. 10A) and fasting (Fig. 10B) serum insulin level as compared to HFD control rats. Treatment with MEL (300, 500 mg/kg, p.o.) to HFD/STZ-induced diabetic rats produced significantly lower fed and fasting serum insulin level as compared to HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Normal rats treated with MEL (500mg/kg, p.o.) did not show any significant change when compared with normal control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum insulin level when compared with HFD/STZ-induced diabetic control rats

During OGTT, HFD rats showed significant increase in AUC_{insulin} when compared with non diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (300, 500 mg/kg, p.o.) and pioglitazone (20 mg/kg, p.o.) produced significantly lower AUC_{insulin} as compared to HFD/STZ-induced diabetic control group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in AUC_{insulin} level when compared with HFD/STZ-induced diabetic control rats (Fig. 10C).

Animals fed on high fat diet showed significantly lower insulin sensitivity and higher T1/2 as compared to animals fed on normal diet. HFD/STZ-induced diabetic rats showed significant decrease in insulin sensitivity and increase in T1/2 when compared with HFD control group. Treatment with pioglitazone (20 mg/kg, p.o.), in HFD/STZ-induced diabetic rats produced significant decrease in serum insulin and T1/2 when compared with HFD/STZ-induced diabetic control group. Treatment with glipizide (10 mg/kg, p.o.) in HFD/STZ-induced diabetic rats did not produce significant decrease in serum insulin and T1/2 when compared with HFD/STZ-induced diabetic control group. Administration of MEL (500 mg/kg, p.o.), in HFD/STZ-induced diabetic rats significantly increased insulin sensitivity by decreasing serum insulin level and increased glucose disposal rate by decreasing the T1/2 when compared with HFD/STZ-induced diabetic control group (Fig. 10D).
Results

(10A) Fed serum insulin (µU/ml)

(10B) Fasting serum insulin (µU/ml)

(10C) AUCinsulin (µU/ml/min×10^3)

(10D) KITT and T1/2
Figure 10. Effect of MEL on serum insulin level in HFD/STZ-induced diabetic rats (after 8 week). (10A) Effect on fed serum insulin level; (10B) Effect on fasting serum insulin level; (10C) Effect on AUC insulin; (10D) Effect on Kit and T1/2. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL100=HFD+STZ rats treated with 100 mg/kg MEL, MEL300=HFD+STZ rats treated with 300 mg/kg MEL, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.2.3 Effect of MEL on activities of hepatic enzymes:

Diabetic animals showed abnormal activities of the hepatic enzymes hexokinase and phosphoenolpyruvate carboxykinase (PEPCK). HFD control rats showed significant decrease activities of hepatic hexokinase (Fig. 11A) and increased activities of hepatic PEPCK (Fig. 11B) when compared with normal control rats. HFD/STZ-induced diabetic rats showed significant decrease activities of hepatic hexokinase and increased activities of hepatic PEPCK when compared with HFD control rats. Administration of MEL (500mg/kg, p.o.) to HFD/STZ-induced diabetic rats significantly increased the activity of hexokinase and significantly decreased the activity of PEPCK when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with pioglitazone (20mg/kg, p.o.) resulted in significantly increase hexokinase activity and significantly decreases PEPCK activity as compared to HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in hexokinase activity and PEPCK activity when compared with HFD/STZ-induced diabetic control rats.
Figure 11. Effect of MEL on activities of hepatic enzymes in HFD/STZ-induced diabetic rats. (11A) Effect on liver hexokinase level; (11B) Effect on liver PEPCK level. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.2.4 Effect of MEL on cardinal signs:

HFD control rats showed significant increase in body weight, food intake and water intake when compared with normal control rats. HFD/STZ-induced diabetic rats showed significant decrease in body weight (Fig.12A) and increase in food (Fig.12B) and water (Fig.12C) intake when compared with HFD control rats. MEL (500mg/kg, p.o.) and pioglitazone (20 mg/kg, p.o.) produced significant decrease in food intake and water intake and significant increase in body weight of HFD/STZ-induced diabetic rats when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change cardinal signs when compared with HFD/STZ-induced diabetic control rats.
Figure 12. Effect of MEL on cardinal signs in HFD/STZ-induced diabetic rats. (12A) Effect on body weight; (12B) Effect on food intake; (12C) Effect on water intake. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ = high fat diet fed/STZ-induced diabetic control, MEL500 = HFD+STZ rats treated with 500 mg/kg MEL, GS = HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS = HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.) *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.2.5 Effect of MEL on lipid profile:

HFD control rats showed significant increase in serum lipid levels when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant increase in serum cholesterol (Fig. 13A), triglycerides (Fig. 13B), VLDL (Fig. 13D), LDL (Fig. 13E), atherogenic index (Fig. 13F), decrease in HDL levels (Fig. 13C) when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (500 mg/kg, p.o.) produced significant decrease in serum cholesterol, triglycerides, and VLDL, LDL and atherogenic index while significant increase in serum HDL levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change serum lipid levels when compared with HFD/STZ-induced diabetic control rats.
Results

(13B)

Serum triglyceride (mg/dl)

0.00 100.00 200.00 300.00

CON CONT HFD HFD+STZ MEL500 GS PS

(13C)

Serum HDL (mg/dl)

0 20 40 60 80

CON CONT HFD HFD+STZ MEL500 GS PS

(13D)

Serum VLDL (mg/dl)

0.00 20.00 40.00 60.00

CON CONT HFD HFD+STZ MEL500 GS PS
5.2.6 Effect of MEL on serum Lactate dehydrogenase, Creatine kinase and Collagen levels in heart:

HFD control rats exhibited significantly higher serum LDH levels (Fig. 14A), serum CK levels (Fig. 14B) and collagen levels in heart (Fig. 14C) as compared to normal control rats. HFD/STZ-induced diabetic rats produced significant increase in serum LDH levels, CK levels and collagen levels in heart when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with MEL (500 mg/kg, p.o.) produced significant decrease in elevated serum LDH levels, CK levels, and collagen levels in heart when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in cardiac parameters when compared with HFD/STZ-induced diabetic control rats.
Figure 14. Effect of MEL on serum lactate dehydrogenase, creatine kinase and collagen levels in heart in HFD/STZ-induced diabetic rats. (14A) Effect on lactate dehydrogenase; (14B) Effect on creatine kinase; (14C) Effect on collagen levels. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).
5.2.7 Effect of MEL on force of contraction, heart rate and blood pressure:

The force of contraction of the HFD control rat’s heart was found to be significantly higher as compared to normal control rats. Further, HFD/STZ-induced diabetic rats produced significant increase in the force of contraction when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (500 mg/kg, p.o.) produced significant decrease in force of contraction when compared with HFD control group. This effect was comparable with the effect of pioglitazone (20 mg/kg, p.o.) but not to glipizide (10 mg/kg, p.o.) in HFD/STZ-induced diabetic group (Fig. 15A).

The heart rate was found to be significantly lower in HFD control rat as compared to normal control rats. HFD/STZ-induced diabetic rats showed significant decrease in heart rate when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (500 mg/kg, p.o.) produced significantly higher heart rate as compared to HFD/STZ-induced diabetic control group. This effect was comparable with the effect of pioglitazone (20 mg/kg, p.o.) but not to glipizide (10 mg/kg, p.o.) in HFD/STZ-induced diabetic group (Fig. 15B).

The blood pressure of the HFD control rat was found to be significantly higher as compared to normal control rats. HFD/STZ-induced diabetic rats showed significant increase in blood pressure when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (500 mg/kg, p.o.) produced significant decrease in blood pressure when compared with HFD/STZ-induced diabetic control rats. This effect was comparable with the effect of pioglitazone (20 mg/kg, p.o.) but not to glipizide (10 mg/kg, p.o.) in HFD/STZ-induced diabetic group (Fig. 15C).
Figure 15. Effect of MEL on force of contraction, heart rate and blood pressure in HFD/STZ-induced diabetic rats. (15A) Effect on force of contraction; (15B) Effect on heart rate; (15C) Effect on blood pressure. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.2.8 Effect of MEL on cardiac hypertrophy index and left ventricular hypertrophy index:

Diabetic rats exhibited increase heart weight and LV weight. However, the ratio of heart weight to body weight which is measure of cardiac hypertrophy index was significantly high in HFD control rats as compared to normal control rats and ratio of LV weight to heart weight which is measure of LV hypertrophy index was significantly high in HFD control rats as compared to normal control rats. HFD/STZ-induced diabetic rats showed significant increase in cardiac hypertrophy index and LV hypertrophy index when compared with HFD control group. Treatment of HFD/STZ diabetic rats with MEL (500 mg/kg, p.o.) produced significant decrease in cardiac hypertrophy index (Fig. 16A) and LV hypertrophy index (Fig. 16B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable with the effect of pioglitazone (20 mg/kg, p.o.) but not to glipizide (10 mg/kg, p.o.) in HFD/STZ-induced diabetic group.
Results

Figure 16. Effect of MEL on cardiac hypertrophy index and left ventricular hypertrophy index in HFD/STZ-induced diabetic rats. (16A) Effect on cardiac hypertrophy index; (16B) Effect on left ventricular hypertrophy index. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.2.9 Effect of MEL on serum GOT and GPT:

HFD control rats exhibited significantly higher SGOT and SGPT levels as compared to normal control rats. HFD/STZ-induced diabetic rats produced significant increase in SGOT and SGPT levels when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with MEL (500 mg/kg, p.o.) produced significant decrease in elevated SGOT (Fig. 17A) and SGPT levels (Fig. 17B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum GOT and GPT levels when compared with HFD/STZ-induced diabetic control rats.
Figure 17. Effect of MEL on SGOT and SGPT in HFD/STZ-induced diabetic rats. (17A) Effect on SGOT; (17B) Effect on SGPT. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.2.10 Effect of MEL on serum urea, creatinine, albumin, total protein:

HFD control rats exhibited significant increase in serum urea, creatinine while significant decrease in total protein and serum albumin levels when compared with normal control rats. HFD/STZ-induced diabetic showed significant increase in serum urea, creatinine while significant decrease in total protein and serum albumin levels when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with MEL (500 mg/kg, p.o.) produced significant decrease in urea, creatinine while significant increase in total protein and serum albumin levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable with the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum urea (Fig. 18A), creatinine (Fig. 18B), albumin (Fig. 18C) and total protein (Fig. 18D) when compared with HFD/STZ-induced diabetic control rats.
Figure 18. Effect of MEL on serum urea, creatinine, albumin, total protein in HFD/STZ-induced diabetic rats. (18A) Effect on serum urea; (18B) Effect on serum creatinine; (18C) Effect on serum albumin; (18D) Effect on serum total protein. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS=HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS=HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05). 

5.2.11 Effect of MEL on serum Na, K, levels:
HFD control rats exhibited significantly higher sodium and potassium levels as compared to normal control rats. HFD/STZ-induced diabetic produced significant increase in serum sodium and potassium levels when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (500 mg/kg, p.o.) produced significant decrease in elevated serum sodium and potassium levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable with the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum Na (Fig. 19A) and K levels (Fig. 19B) when compared with HFD/STZ-induced diabetic control rats.

**Figure 19.** Effect of MEL on serum sodium and potassium level in HFD/STZ-induced diabetic rats. (19A) Effect on serum sodium; (19B) Effect on Serum potassium. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = High fat diet fed rats, HFD+STZ=High fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.2.12 Effect of MEL on urine creatinine, albumin, total protein & creatinine clearance:

HFD control rats exhibited significant decrease in urine creatinine, creatinine clearance and increase in urine albumin and total protein levels when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant decrease in urine creatinine, creatinine clearance and increase in urine albumin and total protein levels when
compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with MEL (500 mg/kg, p.o.) produced significant increase in urine creatinine, creatinine clearance and decrease in urine albumin levels and total protein levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable with the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in urine creatinine (Fig. 20A), creatinine clearance (Fig. 20D), albumin (Fig. 20B) and total protein levels (Fig. 20C) when compared with HFD/STZ-induced diabetic control rats.
Figure 20. Effect of MEL on urine creatinine, albumin, total protein in HFD/STZ-induced diabetic rats. (20A) Effect on urine creatinine; (20B) Effect on urine albumin; (20C) Effect on urine total protein; (20D) Effect on creatinine clearance. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from control (p<0.05).

5.2.13 Effect of MEL on urine Na, K, levels:

HFD control rats exhibited significant decrease in urine sodium and potassium levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significantly lower urine sodium and potassium levels as compared to HFD control rats. Treatment of HFD/STZ diabetic rats with MEL (500 mg/kg, p.o.) produced significant increase in urine sodium (Fig. 21A) and potassium levels (Fig. 21B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable with the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in urine Na and K levels when compared with HFD/STZ-induced diabetic control rats.
Results

Figure 21. Effect of MEL on urine sodium and potassium level in HFD/STZ-induced diabetic rats. (21A) Effect on urine sodium; (21B) Effect on urine potassium. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.2.14 Effect of MEL on kidney weight to body weight:

HFD control rats exhibited significantly higher kidney to body weight ratio as compared to normal control rats. HFD/STZ-induced diabetic produced significant increase in kidney to body weight ratio when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with MEL (500 mg/kg, p.o.) produced significant decrease in kidney to body weight ratio when compared with HFD/STZ-induced diabetic control group. This effect was comparable with the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in kidney weight to body weight ratio when compared with HFD/STZ-induced diabetic control rats (Fig. 22).

Figure 22. Effect of MEL on kidney weight to body weight ratio in HFD/STZ-induced diabetic rats. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, MEL500=HFD+STZ rats treated with 500 mg/kg MEL, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).
5.3 Effect of Petroleum ether fraction of 50% methanolic extract of *L. speciosa* leaves (PFML) on HFD/STZ-induced diabetic rats:

5.3.1(a) Effect of PFML on serum glucose levels (after 3 week):

HFD control rats showed significant increase in serum glucose levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significantly higher fasting serum glucose level as compared to HFD control rats. Treatment of HFD/STZ-induced diabetic rats with PFML (100, 300, 500 mg/kg, p.o.) produced significant decrease in fasting serum glucose level when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg/day) and glipizide (10 mg/kg, p.o.) in HFD/STZ-induced diabetic group (Fig. 23).

![Figure 23](image)

**Figure 23.** Effect of PFML on fasting serum glucose level in HFD/STZ-induced diabetic rats. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control PFML100=HFD+STZ rats treated with 100 mg/kg PFML, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, PFML500=HFD+STZ rats treated with 500 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); #significantly different from control (p<0.05).

5.3.1(b) Effect of PFML on serum glucose levels (after 8 week):

HFD control rats showed significant increase in serum glucose levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significant increase in fed (Fig. 24A) and fasting (Fig. 24B) serum glucose level when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with PFML (100, 300, 500 mg/kg, p.o.) produced significantly lower fed and fasting serum glucose level as compared to HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg/day) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in fed and fasting serum glucose when compared with HFD/STZ-induced diabetic control rats.

During OGTT, HFD/STZ-induced diabetic rats showed significant increase in glucose levels when compared with HFD control rats. Dose dependent treatment of HFD/STZ-induced diabetic group with PFML (100, 300 and 500 mg/kg, p.o.) as well as pioglitazone (20 mg/kg, p.o.) produced significant decrease in serum glucose level when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum glucose when compared with HFD/STZ-induced diabetic control rats (Fig. 24C).

The AUCglucose was found to be significantly higher in HFD/STZ-induced diabetic rats as compared to HFD control rats. Treatment of HFD/STZ-induced diabetic rats with
PFML (100, 300 and 500 mg/kg, p.o.) showed significant decrease in $AUC_{\text{glucose}}$ when compared with HFD/STZ-induced diabetic control rats. In our study, treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) and pioglitazone (20mg/kg, p.o.) did not show any change in $AUC_{\text{glucose}}$ when compared with HFD/STZ-induced diabetic control rats (Fig. 24D).
Figure 24. Effect of PFML on serum glucose level in HFD/STZ-induced diabetic rats. (24A) Effect on fed serum glucose level; (24B) Effect on fasting serum glucose level; (24C) Effect on oral glucose tolerance test; (24D) Effect on AUC\(_{\text{glucose}}\). Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, PFML100=HFD+STZ rats treated with 100 mg/kg PFML, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, PFML500=HFD+STZ rats treated with 500 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.3.2(a) Effect of PFML on insulin levels (after 3 week):

HFD control rats showed significant increase in serum insulin levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significantly lower fasting serum insulin level as compared to HFD control rats. Treatment with PFML (100, 300, 500 mg/kg, p.o.) to HFD/STZ-induced diabetic rats did not produce significant change in fasting serum insulin level when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) produced significant increase in fasting serum insulin levels when compared with HFD/STZ-induced diabetic control rats (Fig. 25).

Figure 25. Effect of PFML on fasting serum insulin level in HFD/STZ-induced diabetic rats. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = High fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control PFML100=HFD+STZ rats treated with 100 mg/kg PFML, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, PFML500=HFD+STZ rats treated with 500 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.3.2(b) Effect of PFML on insulin levels (after 8 week):
HFD control rats showed significant increase in serum insulin levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significantly higher fed (Fig. 26A) and fasting (Fig. 26B) serum insulin level as compared to HFD control rats. Treatment with PFML (100, 300, 500 mg/kg, p.o.) to HFD/STZ-induced diabetic rats produced significantly lower fed and fasting serum insulin level as compared to HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum insulin levels when compared with HFD/STZ-induced diabetic control rats.

During OGTT, HFD rats showed significant increase in AUC$_{\text{insulin}}$ when compared with non diabetic control rats. Treatments of HFD/STZ-induced diabetic rats with PFML (100, 300, 500 mg/kg, p.o.) and pioglitazone (20 mg/kg, p.o.) produced significant decrease in AUC$_{\text{insulin}}$ when compared with HFD/STZ-induced diabetic control group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not produce any significant change in AUC$_{\text{insulin}}$ when compared with HFD/STZ-induced diabetic control rats (Fig. 26C).

Animals fed on high fat diet showed significantly lower insulin sensitivity and higher T1/2 as compared to animals fed on normal diet. HFD/STZ-induced diabetic rats showed significant decrease in insulin sensitivity and increase in T1/2 when compared with HFD/STZ-induced diabetic control group. Treatment with pioglitazone (20 mg/kg/day), in HFD/STZ-induced diabetic rats increase in insulin sensitivity and T1/2 when compared with HFD/STZ-induced diabetic control group. Administration of PFML (300 mg/kg, p.o.), in HFD/STZ-induced diabetic rats significantly increased insulin sensitivity by decreasing serum insulin level and increased glucose disposal rate by decreasing the T1/2 when compared with HFD/STZ-induced diabetic control group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in insulin sensitivity and glucose disposal rate when compared with HFD/STZ-induced diabetic control rats (Fig. 26D).
Figure 26. Effect of PFML on serum insulin level in HFD/STZ-induced diabetic rats. (26A) Effect on fed serum insulin level; (26B) Effect on fasting serum insulin level; (26C) Effect on AUC_{insulin}; (26D) Effect on Kitt and T_{1/2}. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ = high fat diet fed/STZ-induced diabetic control, PFML100 = HFD+STZ rats treated with 100 mg/kg PFML, PFML300 = HFD+STZ rats treated with 300 mg/kg PFML, PFML500 = HFD+STZ rats treated with 500 mg/kg PFML, GS = HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS = HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); *significantly different from control (p<0.05).

5.3.3 Effect of PFML on activities of hepatic enzymes:
Results

Diabetic animals showed abnormal activities of the hepatic enzymes hexokinase and PEPCK. HFD control rats showed significant decrease activities of hepatic hexokinase and increased activities of hepatic PEPCK when compared with normal control rats. HFD/STZ-induced diabetic rats showed significant decrease activities of hepatic hexokinase (Fig. 27A) and increased activities of hepatic PEPCK (Fig. 27B) when compared with HFD control rats. Administration of PFML (300mg/kg, p.o.) to HFD/STZ-induced diabetic rats significantly increased the activity of hexokinase and significantly decreased the activity of PEPCK when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with pioglitazone (20mg/kg, p.o.) resulted in significantly increase hexokinase activity and significantly decreases PEPCK activity when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in hexokinase and PEPCK activity when compared with HFD/STZ-induced diabetic control rats.

![Graph 27A](image)

![Graph 27B](image)

**Figure 27.** Effect of PFML on activities of hepatic enzymes in HFD/STZ-induced diabetic rats. (27A) Effect on liver hexokinase level; (27B) Effect on liver PEPCK level. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.) *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ. (p<0.05); #significantly different from control (p<0.05).

5.3.4 Effect of PFML on cardinal signs HFD/STZ-induced diabetic rats:
HFD control rats showed significant increase in body weight, food intake and water intake when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant decrease in body weight (Fig. 28A) and increase in food (Fig. 28B) and water (Fig. 28C) intake when compared with HFD control rats. PFML (300mg/kg, p.o.) and pioglitazone (20mg/kg, p.o.) produced significant decrease in food intake and water intake and significant increase in body weight in HFD/STZ-induced diabetic rats when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in cardinal signs when compared with HFD/STZ-induced diabetic control rats.

Figure 28. Effect of PFML on cardinal signs in HFD/STZ-induced diabetic rats. (28A) Effect on body weight; (28B) Effect on food intake; (28C) Effect on water intake. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ = high fat diet...
fed/STZ-induced diabetic control, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.) *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ. (p<0.05); *significantly different from control (p<0.05).

5.3.5 Effect of PFML on lipid profile:

HFD control rats showed significant increase in serum lipid levels when compared with normal control rats. HFD/STZ-induced diabetic rats produced significantly higher serum cholesterol, triglycerides, VLDL, LDL, atherogenic index while significantly lower HDL levels as compared to HFD control rats. Treatment of HFD/STZ-induced diabetic rats with PFML (300 mg/kg, p.o.) produced significant decrease in serum cholesterol (Fig. 29A), triglycerides (Fig. 29B), and VLDL (Fig. 29D), LDL (Fig. 29E) and atherogenic index (Fig. 29F) while significant increase in serum HDL levels (Fig. 29C) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum lipid levels when compared with HFD/STZ-induced diabetic control rats.
Results

(29D)

(29E)

(29F)
Figure 29. Effect of PFML on lipid profile in HFD/STZ-induced diabetic rats. (29A) Effect on cholesterol level; (29B) Effect on triglyceride level; (29C) Effect on HDL level; (29D) Effect on VLDL level; (29E) Effect on LDL level; (29F) Effect on atherogenic index. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.) *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.3.6 Effect of PFML on serum Lactate dehydrogenase, Creatine kinase and Collagen levels in heart:

HFD control rats exhibited significantly higher serum LDH levels, serum CK levels and collagen levels in heart as compared to normal control rats. HFD/STZ-induced diabetic rats produced significant increase in serum LDH levels (Fig. 30A), CK levels (Fig. 30B) and collagen levels in heart (Fig. 30C) when compared with HFD control rats Treatment of HFD/STZ diabetic rats with PFML (300 mg/kg, p.o.) produced significant decrease in elevated serum LDH levels, CK levels, and collagen levels in heart when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum Lactate dehydrogenase, Creatine kinase and Collagen levels in heart when compared with HFD/STZ-induced diabetic control rats.

(30A)

(30B)
Results

Figure 30. Effect of PFML on serum lactate dehydrogenase, creatine kinase and collagen levels in heart of HFD/STZ-induced diabetic rats. (30A) Effect on lactate dehydrogenase; (30B) Effect on creatine kinase; (30C) Effect on collagen levels. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ = high fat diet fed/STZ-induced diabetic control, PFML300 = HFD+STZ rats treated with 300 mg/kg PFML, GS = HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS = HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.3.7 Effect of PFML on force of contraction, heart rate and blood pressure:

The force of contraction of the HFD control rat’s heart was found to be significantly higher as compared to normal control rats. Further, HFD/STZ-induced diabetic rats produced significant increase in the force of contraction when compared with HFD control rats. Treatment of HFD control rats with PFML (300 mg/kg, p.o.) produced significant decrease in force of contraction (Fig. 31A) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in force of contraction when compared with HFD/STZ-induced diabetic control rats.

The heart rate was found to be significantly decreased in HFD control rat when compared with normal control rats. HFD/STZ-induced diabetic rats showed significantly lower heart rate as compared to HFD control rats. Treatment of HFD/STZ-induced diabetic rats with PFML (300 mg/kg, p.o.) produced significantly higher heart rate (Fig. 31B) as compared to HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of
HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in heart rate when compared with HFD/STZ-induced diabetic control rats.

The blood pressure of the HFD control rat was found to be significantly increased when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant increase in blood pressure (Fig. 31C) when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with PFML (300 mg/kg, p.o.) produced significant decrease in blood pressure when compared with HFD/STZ-induced diabetic control rats. This effect was comparable to the pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in blood pressure when compared with HFD/STZ-induced diabetic control rats.

(31A)

(31B)

(31C)
Results

**Figure 31.** Effect of PFML on force of contraction, heart rate and blood pressure in HFD/STZ-induced diabetic rats. (31A) Effect on force of contraction; (31B) Effect on heart rate; (31C) Effect on blood pressure. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

### 5.3.8 Effect of PFML on cardiac hypertrophy index and left ventricular hypertrophy index:

Diabetic rats exhibited increased heart weight and LV weight. However, the ratio of heart weight to body weight which is measure of cardiac hypertrophy index was significantly high in HFD control rats as compared to normal control rats and ratio of LV weight to heart weight which is measure of LV hypertrophy index was significantly high in HFD control rats as compared to normal control rats. HFD/STZ-induced diabetic rats showed significant increase in cardiac hypertrophy index and LV hypertrophy index when compared with HFD control group. Treatment of HFD/STZ diabetic rats with PFML (300 mg/kg, p.o.) produced significant decrease in cardiac hypertrophy index (Fig. 32A) and LV hypertrophy index (Fig. 32B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in cardiac hypertrophy index and LV hypertrophy index when compared with HFD/STZ-induced diabetic control rats.
Results

Figure 32. Effect of PFML on cardiac hypertrophy index and left ventricular hypertrophy index in HFD/STZ-induced diabetic rats. (32A) Effect on cardiac hypertrophy index; (32B) Effect on left ventricular hypertrophy index. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-

induced diabetic control, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.3.9 Effect of PFML on serum GOT and GPT:

HFD control rats exhibited significantly higher SGOT and SGPT levels as compared to normal control rats. HFD/STZ-induced diabetic rats produced significant increase in SGOT and SGPT levels when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with PFML (300 mg/kg, p.o.) produced significant decrease in elevated SGOT (Fig. 33A) and SGPT levels (Fig. 33B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum GOT and GPT when compared with HFD/STZ-induced diabetic control rats

(33A)
Figure 33. Effect of PFML on SGOT and SGPT in HFD/STZ-induced diabetic rats. (33A) Effect on SGOT; (33B) Effect on SGPT. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.3.10 Effect of PFML on serum urea, creatinine, albumin, total protein:

HFD control rats exhibited significant increase in serum urea, creatinine while decrease in total protein and serum albumin levels when compared with normal control rats. HFD/STZ-induced diabetic showed significant increase in serum urea (Fig. 34A), creatinine (Fig. 34B) while decrease in total protein (Fig. 34D) and serum albumin levels (Fig. 34C) when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with PFML (300 mg/kg, p.o.) produced significant decrease in urea, creatinine while significant increase in total protein and serum albumin levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum urea, creatinine, albumin and total protein when compared with HFD/STZ-induced diabetic control rats.
Results

(34B)

(34C)

(34D)
Figure 34. Effect of PFML on serum urea, creatinine, albumin, total protein in HFD/STZ-induced diabetic rats. (34A) Effect on serum urea; (34B) Effect on serum creatinine. (34C) Effect on serum albumin. (34D) Effect on serum total protein. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.3.11 Effect of PFML on serum Na, K, levels:

HFD control rats exhibited significant increase in serum sodium and potassium levels when compared with normal control rats. HFD/STZ-induced diabetic produced significant increase in serum sodium (Fig. 35A) and potassium levels (Fig. 35B) when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with PFML (300 mg/kg, p.o.) produced significant decrease in elevated serum sodium and potassium levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum Na and K levels when compared with HFD/STZ-induced diabetic control rats.
Figure 35. Effect of PFML on serum sodium and potassium level in HFD/STZ-induced diabetic rats. (35A) Effect on serum sodium; (35B) Effect on serum potassium. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ = high fat diet fed/STZ-induced diabetic control, PFML300 = HFD+STZ rats treated with 300 mg/kg PFML, GS = HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS = HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.3.12 Effect of PFML on urine creatinine, albumin, total protein and creatinine clearance:

HFD control rats exhibited significant decrease in urine creatinine, creatinine clearance and increase in urine albumin and total protein levels when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant decrease in urine creatinine (Fig. 36A), creatinine clearance (Fig. 36D) and increase in urine albumin (Fig. 36B) and total protein levels (Fig. 36C) when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with PFML (300 mg/kg, p.o.) produced significant increase in urine creatinine, creatinine clearance and decrease in urine albumin levels and total protein levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in urine creatinine, creatinine clearance, albumin and total protein when compared with HFD/STZ-induced diabetic control rats.

(36A)

(36B)
Results

Figure 36. Effect of PFML on urine creatinine, albumin, total protein in HFD/STZ-induced diabetic rats. (36A) Effect on urine creatinine; (36B) Effect on urine albumin. (36C) Effect on urine total protein (36D) Effect on creatinine clearance. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ= high fat diet fed/STZ-induced diabetic control, PFML300= HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.3.13 Effect of PFML on urine Na, K, levels:
HFD control rats exhibited significant decrease in urine sodium and potassium levels when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant decrease in urine sodium and potassium levels when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with PFML (300 mg/kg, p.o.) produced significant increase in urine sodium (Fig. 37A) and potassium levels (Fig. 37B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in urine Na and K levels when compared with HFD/STZ-induced diabetic control rats. 

**Figure 37.** Effect of PFML on urine sodium and potassium level in HFD/STZ-induced diabetic rats. (37A) Effect on urine sodium; (37B) Effect on urine potassium. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.) *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ. (p<0.05); #significantly different from control (p<0.05).

### 5.3.14 Effect of PFML on kidney weight to body weight:

HFD control rats exhibited significant increase in kidney to body weight ratio when compared with normal control rats. HFD/STZ-induced diabetic produced significant increase in kidney to body weight ratio when compared with HFD control rats. Treatment of PFML (300 mg/kg, p.o.) to HFD/STZ diabetic rats produced significant decrease in kidney to body weight ratio when compared with HFD/STZ-induced diabetic control group. This effect was
comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in kidney weight to body weight ratio when compared with HFD/STZ-induced diabetic control rats (Fig. 38).

Figure 38. Effect of PFML on kidney weight to body weight ratio in HFD/STZ-induced diabetic rats. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, PFML300=HFD+STZ rats treated with 300 mg/kg PFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.4 Effect of Tannin fraction of 50% methanolic extract of L. speciosa leaves (TFML) on HFD/STZ-induced diabetic rats:

5.4.1(a) Effect of TFML on serum glucose levels (after 3 week):

HFD control rats showed significant increase in serum glucose levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significant increase in fasting serum glucose level when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (100, 300 mg/kg, p.o.) did not produce any significant change in fasting serum glucose level while TFML 500 mg/kg produced significant decrease in fasting serum glucose level when compared with HFD/STZ-induced diabetic control group. This effect (reduction in glucose level) was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) produced significant decrease in serum fasting glucose levels when compared with HFD/STZ-induced diabetic control rats (Fig. 39).
Figure 39. Effect of TFML on fasting serum glucose level in HFD/STZ-induced diabetic rats. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ = high fat diet fed/STZ-induced diabetic control, TFML100 = HFD+STZ rats treated with 100 mg/kg TFML, TFML300 = HFD+STZ rats treated with 300 mg/kg TFML, TFML500 = HFD+STZ rats treated with 500 mg/kg TFML, GS = HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS = HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.4.1(b) Effect of TFML on serum glucose levels (after 8 week):

HFD control rats showed significant increase in serum glucose levels when compared with normal control rats. HFD/STZ-induced diabetic rats showed significant increase in fed (Fig. 40A) and fasting (Fig. 40B) serum glucose level when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (100, 300 mg/kg, p.o.) did not produce any significant change in fed and fasting serum glucose level while TFML (500 mg/kg, p.o.) produced significant decrease fed and fasting serum glucose level when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum fed and fasting glucose levels when compared with HFD/STZ-induced diabetic control rats.

During OGTT, HFD/STZ-induced diabetic rats showed significant increase in glucose levels when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (100, 300 mg/kg, p.o.) did not produce any significant change in serum glucose levels while TFML (500 mg/kg, p.o.) as well as pioglitazone (20 mg/kg, p.o.) produced significant decrease serum glucose level when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum glucose levels when compared with HFD/STZ-induced diabetic control rats (Fig. 40C).

The AUC_{glucose} was found to be significantly higher in HFD/STZ-induced diabetic rats as compared to HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (100, 300 and 500 mg/kg, p.o.), glipizide (10 mg/kg, p.o.) and pioglitazone (20 mg/kg, p.o.) did not show any significant change in AUC_{glucose} when compared with HFD/STZ-induced diabetic control rats (Fig. 40D).
Results

(40A)

(40B)

(40C)
Results

Figure 40. Effect of TFML on serum glucose level in HFD/STZ-induced diabetic rats. (40A) Effect on fed serum glucose level; (40B) Effect on fasting serum glucose level; (40C) Effect on oral glucose tolerance test; (40D) Effect on $AUC_{glucose}$. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML100=HFD+STZ rats treated with 100 mg/kg TFML, TFML300=HFD+STZ rats treated with 300 mg/kg TFML, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.4.2(a) Effect of TFML on serum insulin levels (after 3 week):

HFD/STZ-induced diabetic rats showed significant decrease in fasting serum insulin level when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (100, 300, 500 mg/kg, p.o.) as well as pioglitazone (20 mg/kg, p.o.) did not show any significant change in fasting serum insulin levels when compared with HFD/STZ-induced diabetic control group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) produced significant increase in serum insulin levels when compared with HFD/STZ-induced diabetic control rats (Fig. 41).

Figure 41. Effect of TFML on fasting serum insulin level in HFD/STZ-induced diabetic rats. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML100=HFD+STZ rats treated with 100 mg/kg TFML, TFML300=HFD+STZ rats treated with 300 mg/kg TFML, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).
5.4.2(b) Effect of TFML on serum insulin levels (after 8 week):

HFD/STZ-induced diabetic rats showed significant increase in fed (Fig. 42A) and fasting (Fig. 42B) serum insulin level when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (100, 300 mg/kg, p.o.) did not produce any significant change in serum insulin levels while TFML (500 mg/kg, p.o.) as well as pioglitazone (20 mg/kg, p.o.) produced significant decrease fed and fasting serum insulin when compared with HFD/STZ-induced diabetic control group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not produce any significant change in serum insulin levels when compared with HFD/STZ-induced diabetic control rats.

During OGTT, HFD rats showed significant increase in AUC$_{\text{insulin}}$ when compared with non diabetic control rats. HFD/STZ-induced diabetic rats showed significant increase in AUC$_{\text{insulin}}$ when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (100, 300 mg/kg, p.o.) did not produce any significant change in AUC$_{\text{insulin}}$ while TFML (500 mg/kg, p.o.) and pioglitazone (20 mg/kg, p.o.) produced significant decrease in AUC$_{\text{insulin}}$ when compared with HFD/STZ-induced diabetic control group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum insulin levels when compared with HFD/STZ-induced diabetic control rats (Fig. 42C).

Animals fed on high fat diet showed significantly lower insulin sensitivity and higher T1/2 as compared to animals fed on normal diet. HFD/STZ-induced diabetic rats showed significant decrease in insulin sensitivity and increase in T1/2 when compared with HFD/STZ-induced diabetic control group. Treatment of HFD/STZ-induced diabetic rats with pioglitazone (20 mg/kg, p.o.) decrease serum insulin level and T1/2 when compared with HFD/STZ-induced diabetic control group. Administration of TFML (500 mg/kg, p.o.), in HFD/STZ-induced diabetic rats significantly increased insulin sensitivity by decreasing serum insulin level and increased glucose disposal rate by decreasing the T1/2 when compared with HFD/STZ-induced diabetic control group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in insulin sensitivity and glucose disposal rate when compared with HFD/STZ-induced diabetic control rats (Fig. 42D).
Figure 42. Effect of TFML on serum insulin level in HFD/STZ-induced diabetic rats. (42A) Effect on fed serum insulin level; (42B) Effect on fasting serum insulin level; (42C) Effect on AUC \(_{\text{insulin}}\); (42D) Effect on Kitt and T\(_{1/2}\). Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML100=HFD+STZ rats treated with 100 mg/kg TFML, TFML300=HFD+STZ rats treated with 300 mg/kg TFML, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.4.3 Effect of TFML on activities of hepatic enzymes:

Diabetic animals showed abnormal activities of the hepatic enzymes hexokinase and PEPCK. HFD control rats showed significant decrease activities of hepatic hexokinase and
increased activities of hepatic PEPCK when compared with normal control rats. HFD/STZ-induced diabetic rats showed significant decrease activities of hepatic hexokinase (Fig. 43A) and increased activities of hepatic PEPCK (Fig. 43B) when compared with HFD control rats. Administration of TFML (500mg/kg, p.o.) to HFD/STZ-induced diabetic rats significantly increased the activity of hexokinase and significantly decreased the activity of PEPCK when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with pioglitazone (20mg/kg, p.o.) resulted in significantly higher hexokinase activity and significantly lower PEPCK activity as compared to HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in activities of hepatic enzymes when compared with HFD/STZ-induced diabetic control rats.

\[\text{Figure 43. Effect of TFML on activities of hepatic enzymes in HFD/STZ-induced diabetic rats. (43A) Effect on liver hexokinase level; (43B) Effect on liver PEPCK level. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.), PS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).}\]

5.4.4 Effect of TFML on cardinal signs:

HFD control rats showed significant increase in body weight, food intake and water intake when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant decrease in body weight (Fig. 44A) and increase in food (Fig. 44B) and water (Fig. 44C) intake when compared with HFD control rats. TFML (500mg/kg, p.o.) and
pioglitazone (20 mg/kg, p.o.) produced significant decrease in food intake and water intake and significant increase in body weight in HFD/STZ-induced diabetic rats when compared with HFD/STZ-induced diabetic control rats. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in cardinal signs when compared with HFD/STZ-induced diabetic control rats.

(44A)

Figure 44. Effect of TFML on cardinal signs in HFD/STZ-induced diabetic rats. (44A) Effect on body weight; (44B) Effect on food intake; (44C) Effect on water intake. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20
mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05);
#significantly different from control (p<0.05).

5.4.5 Effect of TFML on lipid profile:

HFD control rats showed significant increase in serum lipid levels when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant increase in serum cholesterol (Fig. 45A), triglycerides (Fig. 45B), VLDL (Fig. 45D), LDL (Fig. 45E), atherogenic index (Fig. 45F) while decrease in HDL levels (Fig. 45C) when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (500 mg/kg, p.o.) produced significant decrease in serum cholesterol, triglycerides, VLDL, LDL and atherogenic index while significant increase in serum HDL levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum lipid levels when compared with HFD/STZ-induced diabetic control rats.

(45A)

(45B)
Results

(45C)

Serum HDL (mg/dl)

0 40 80

CON HFD HFD-STZ TFML500 GS PS

(45D)

Serum VLDL (mg/dl)

0 20 40 60

CON HFD HFD-STZ TFML500 GS PS

(45E)

Serum LDL (mg/dl)

0 40 80 120 160

CON HFD HFD-STZ TFML500 GS PS

(45F)
5.4.6 **Effect of TFML on serum Lactate dehydrogenase, Creatine kinase and Collagen levels in heart:**

HFD control rats exhibited significantly higher serum LDH levels, serum CK levels and collagen levels in heart as compared to normal control rats. HFD/STZ-induced diabetic rats produced significant increase in serum LDH levels (Fig. 46A), CK levels (Fig. 46B) and collagen levels in heart (Fig. 46C) when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with TFML (500 mg/kg, p.o.) produced significant decrease in elevated serum LDH levels, CK levels, and collagen levels in heart when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in cardiac parameters when compared with HFD/STZ-induced diabetic control rats.
Figure 46. Effect of TFML on serum lactate dehydrogenase, creatine kinase and collagen levels in heart of HFD/STZ-induced diabetic rats. (46A) Effect on lactate dehydrogenase; (46B) Effect on creatine kinase; (46C) Effect on collagen levels. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.4.7 Effect of TFML on force of contraction, heart rate and blood pressure:

The force of contraction of the HFD control rat’s heart was found to be significantly higher as compared to normal control rats. Further, HFD/STZ-induced diabetic rats produced significant increase in the force of contraction when compare with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (500 mg/kg, p.o.) produced significant decrease in force of contraction when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in force of contraction when compared with HFD/STZ-induced diabetic control rats (Fig. 47A).

The heart rate was found to be significantly lower in HFD control rat as compared to normal control rats. HFD/STZ-induced diabetic rats showed significantly lower heart rate as compared to HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (500 mg/kg, p.o.) produced significant increase in heart rate when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in heart rate when compared with HFD/STZ-induced diabetic control rats (Fig. 47B).
The blood pressure of the HFD control rat was found to be significantly higher as compared to normal control rats. HFD/STZ-induced diabetic rats produced significant increase in blood pressure when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (500 mg/kg, p.o.) produced significant decrease in blood pressure when compared with HFD/STZ-induced diabetic control rats. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in blood pressure when compared with HFD/STZ-induced diabetic control rats (Fig. 47C).

**Figure 47.** Effect of TFML on force of contraction, heart rate and blood pressure in HFD/STZ-induced diabetic rats. (47A) Effect on force of contraction; (47B) Effect on heart rate; (47C) Effect on blood pressure. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high.
Results

fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); †significantly different from control (p<0.05).

5.4.8 Effect of TFML on cardiac hypertrophy index and left ventricular hypertrophy index:

Diabetic rats exhibited increased heart weight and LV weight. However, the ratio of heart weight to body weight which is measure of cardiac hypertrophy index was significantly high in HFD control rats as compared to normal control rats and ratio of LV weight to heart weight which is measure of LV hypertrophy index was significantly high in HFD control rats as compared to normal control rats. HFD/STZ-induced diabetic rats showed significant increase in cardiac hypertrophy index and LV hypertrophy index when compared with HFD control group. Treatment of HFD/STZ diabetic rats with TFML (500 mg/kg, p.o.) produced significant decrease in cardiac hypertrophy index (Fig. 48A) and LV hypertrophy index (Fig. 48B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in cardiac hypertrophy index and left ventricular hypertrophy index when compared with HFD/STZ-induced diabetic control rats.

(48A)

(48B)
Figure 48. Effect of TFML on cardiac hypertrophy index and left ventricular hypertrophy index in HFD/STZ-induced diabetic rats. (48A) Effect on cardiac hypertrophy index; (48B). Effect on left ventricular hypertrophy index. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.4.9 Effect of TFML on serum GOT and GPT:

HFD control rats exhibited significantly higher SGOT and SGPT levels as compared to normal control rats. HFD/STZ-induced diabetic rats produced significant increase in SGOT and SGPT levels when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with TFML (500 mg/kg, p.o.) produced significant decrease in elevated SGOT (Fig. 49A) and SGPT levels (Fig. 49B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum GOT and GPT when compared with HFD/STZ-induced diabetic control rats.
Figure 49. Effect of TFML on SGOT and SGPT in HFD/STZ-induced diabetic rats. (49A) Effect on SGOT; (49B) Effect on SGPT. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ = high fat diet fed/STZ-induced diabetic control, TFML500 = HFD+STZ rats treated with 500 mg/kg TFML, GS = HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS = HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.4.10 Effect of TFML on serum urea, creatinine, albumin, total protein:

HFD control rats exhibited significant increase in serum urea, creatinine while decrease in total protein and serum albumin levels when compared with normal control rats. HFD/STZ-induced diabetic showed significant increase in serum urea (Fig. 50A), creatinine (Fig. 50B) while decrease in total protein levels (Fig. 50D) and serum albumin levels (Fig. 50C) when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with TFML (500 mg/kg, p.o.) produced significant decrease in urea, creatinine while significant increase in total protein and serum albumin levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum urea, creatinine, albumin, total protein when compared with HFD/STZ-induced diabetic control rats.
Figure 50. Effect of TFML on serum urea, creatinine, albumin, total protein in HFD/STZ-induced diabetic rats. (50A) Effect on serum urea; (50B) Effect on serum creatinine. (50C) Effect on serum albumin. (50D) Effect on serum total protein. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); # significantly different from control (p<0.05).

5.4.11 Effect of TFML on serum Na, K, levels:
HFD control rats exhibited significant increase in serum sodium and potassium levels when compared with normal control rats. HFD/STZ-induced diabetic produced significant increase in serum sodium and potassium levels when compared with HFD control rats. Treatment of HFD/STZ-induced diabetic rats with TFML (500 mg/kg, p.o.) produced significant decrease in elevated serum sodium (Fig. 51A) and potassium levels (Fig. 51B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in serum Na and K levels when compared with HFD/STZ-induced diabetic control rats.

![Figure 51](image)

Figure 51. Effect of TFML on serum sodium and potassium level in HFD/STZ-induced diabetic rats. (51A) Effect on serum sodium; (51B) Effect on Serum potassium. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); # significantly different from control (p<0.05).

5.4.12 Effect of TFML on urine creatinine, albumin, total protein & creatinine clearance:

HFD control rats exhibited significant decrease in urine creatinine, creatinine clearance and increase in urine albumin and total protein levels when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant decrease in urine creatinine (Fig. 52A), creatinine clearance (Fig. 52D) and increase in urine albumin (Fig. 52B) and total protein levels (Fig. 52C) when compared with HFD control rats. Treatment of HFD/STZ-
induced diabetic rats with TFML (500 mg/kg, p.o.) produced significant increase in urine creatinine, creatinine clearance and decrease in urine albumin and total protein levels when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in urine creatinine, albumin and total protein when compared with HFD/STZ-induced diabetic control rats.

(52A)

(52B)

(52C)

(52D)
5.4.13 Effect of TFML on urine Na, K, levels:

HFD control rats exhibited significant decrease in urine sodium and potassium levels when compared with normal control rats. HFD/STZ-induced diabetic rats produced significant decrease in urine sodium and potassium levels when compared with HFD control rats. Treatment of HFD/STZ diabetic rats with TFML (500 mg/kg, p.o.) produced significant increase in urine sodium (Fig. 53A) and potassium levels (Fig. 53B) when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) in HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in urine Na and K levels when compared with HFD/STZ-induced diabetic control rats.
Figure 53. Effect of TFML on urine sodium and potassium level in HFD/STZ-induced diabetic rats (53A) Effect on urine sodium; (53B) Effect on urine potassium. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.4.14 Effect of TFML on kidney weight to body weight:

HFD control rats exhibited significant increase in kidney to body weight ratio when compared with normal control rats. HFD/STZ-induced diabetic produced significant increase in kidney to body weight ratio when compared with HFD control rats. Treatment of TFML (500 mg/kg, p.o.) to HFD/STZ diabetic rats produced significant decrease in kidney to body weight ratio when compared with HFD/STZ-induced diabetic control group. This effect was comparable to the effect of pioglitazone (20 mg/kg, p.o.) on HFD/STZ-induced diabetic group. Treatment of HFD/STZ-induced diabetic rats with glipizide (10 mg/kg, p.o.) did not show any significant change in kidney weight to body weight ratio when compared with HFD/STZ-induced diabetic control rats (Fig. 54).

Figure 54. Effect of TFML on kidney weight to body weight ratio in HFD/STZ-induced diabetic rats. Each bar represents mean ± SEM of 7 animals. CON = non diabetic control, CONT = non diabetic treated, HFD = high fat diet fed rats, HFD+STZ=high fat diet fed/STZ-induced diabetic control, TFML500=HFD+STZ rats treated with 500 mg/kg TFML, GS= HFD+STZ rats treated with glipizide standard (10 mg/kg, p.o.), PS= HFD+STZ rats treated with pioglitazone standard (20 mg/kg, p.o.). *Significantly different from HFD (p<0.05); **significantly different from HFD+STZ (p<0.05); #significantly different from control (p<0.05).

5.5 Effect of MEL on streptozotocin-induced type 1 diabetic rats:
5.5.1 Effect of MEL on serum glucose levels:

STZ-induced diabetic rats showed significant increase in fed and fasting serum glucose level when compared with non diabetic control rats. Treatment with MEL (100, 300, 500mg/kg, p.o.) in STZ-induced type 1 diabetic rats significantly lower fed (Fig. 55A) and fasting (Fig. 55B) serum glucose level as compared to diabetic control group. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 55A).

The AUCglucose was found to be significantly higher in diabetic rats as compared to non diabetic control rats. Treatment with MEL 500 mg/kg p.o., significantly reduced AUCglucose while, 100 and 300 mg/kg, p.o. dose failed to produce this effect in STZ-induced type 1 diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 55C).
5.5.2 Effect of MEL on fed and fasting serum insulin levels:

STZ-induced type 1 diabetic rats showed significant decrease in serum insulin level when compared with non diabetic control rats. Treatment with MEL (100, 300, 500mg/kg, p.o.) did not produce significant effect on fed (Fig. 56A) and fasting (Fig. 56B) serum insulin level in diabetic rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.

During OGTT, STZ-induced diabetic rats showed significant decrease in AUCinsulin when compared with non diabetic control rats. Treatment of STZ-induced type 1 diabetic rats with MEL (100, 300, 500mg/kg, p.o.) did not produce significant effect on AUCinsulin in diabetic rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 56C).
Results

Figure 56. Effect of MEL on fed and fasting serum insulin level in streptozotocin-diabetic rats. (56A) Effect of MEL on Fed serum insulin; (56B) Effect of MEL on Fasting serum insulin; (56C) Effect of MEL on AUCinsulin. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non-diabetic treated, DIC = diabetic control, MEL100 = diabetic treated with MEL (100mg/kg, p.o.), MEL300 = diabetic treated with MEL (300mg/kg, p.o.), MEL500 = diabetic treated with MEL (500mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05)

5.5.3 Effect of MEL on cardinal signs:

Injection of STZ in rats produced significant decrease in body weight (Fig.59A) and increase food (Fig.57B) and water (Fig.57C) intake when compared with non diabetic control rats. MEL (500 mg/kg, p.o.) produced significant increase in STZ-induced type 1 diabetic rat’s body weight while significant decrease in food intake and water intake. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.
Figure 57. Effect of MEL on cardinal signs of streptozotocin - diabetic rats. (57A) Effect on body weight; (57B) Effect on food intake; (57C) Effect on water intake. Each bar represents mean ± SEM of 6 animals. CON= non diabetic control, CONT = non-diabetic treated, DIC = diabetic control, MEL500 = diabetic treated with MEL (500mg/kg, p.o.).*Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)

5.5.4 Effect of MEL on activities of hepatic enzymes:
STZ-induced type 1 diabetic rats exhibited significantly lower hexokinase and higher PEPCK levels as compared to non diabetic control rats. Chronic treatment with MEL (500 mg/kg, p.o.) and insulin produced significant increase in hexokinase and decrease in PEPCK levels of STZ-induced type 1 diabetic rats when compared with diabetic control rats (Fig. 58A and 58B).

**Figure 58.** Effect of MEL on liver hexokinase and PEPCK in streptozotocin-diabetic rats. (58A) Effect on liver hexokinase; (58B) Effect on liver PEPCK. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, MEL300= diabetic treated with MEL (500 mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)

5.5.5 Effect of MEL on lipid profile:

STZ in rats produced a significant increase in cholesterol, triglycerides, VLDL and LDL while decrease in HDL levels when compared with non diabetic control rats. Treatment of rats with MEL (500 mg/kg, p.o.) caused a significant decrease in cholesterol (Fig. 59A), triglycerides (Fig. 59B), VLDL (Fig. 59D) and LDL (Fig. 59E) while increase in HDL (Fig. 59C) levels of STZ-induced type 1 diabetic rats when compare with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.
Figure 59. Effect of MEL on lipid profile in streptozotocin-diabetic rats. (59A) Effect on serum cholesterol; (59B) Effect on serum triglycerides; (59C) Effect on serum HDL. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, MEL500 = diabetic treated with MEL (500mg/kg, p.o.). *Significantly different from non diabetic control (p<0.05); **significantly different from diabetic control. (p<0.05)
5.5.6 Effect of MEL on serum GOT and GPT:

STZ-induced type 1 diabetic rats exhibited significantly higher SGOT and SGPT levels when compared with non diabetic control rats. Chronic treatment with MEL (500 mg/kg, p.o.) produced significant decrease in SGOT and SGPT levels of STZ-induced type 1 diabetic rats when compared with diabetic control rats (Fig. 60A and 60B). This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.
5.5.7 Effect of MEL on serum urea:

STZ-induced type 1 diabetic rats exhibited significantly higher serum urea levels when compared with non-diabetic control rats. MEL (500 mg/kg, p.o.) treatment decreased the elevated serum urea levels of STZ-induced type 1 diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 61).

5.5.8 Effect of MEL on serum creatinine:

STZ-induced type 1 diabetic rats showed significant increase in serum creatinine (Fig. 62) level when compared with non-diabetic control rats. Treatment with MEL (500 mg/kg, p.o.) produced significant decrease in serum creatinine in STZ-induced type 1 diabetic rats when compared with diabetic control. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.
Results

5.6 Effect of PFML on streptozotocin-induced type 1 diabetic rats:

5.6.1 Effect of PFML on serum glucose levels:

STZ-induced diabetic rats showed significant increase in fed and fasting serum glucose level when compared with non diabetic control rats. Treatment with PFML (100, 300, 500mg/kg, p.o.) in STZ-induced type 1 diabetic rats significantly decreased fed (Fig. 63A) and fasting (Fig. 63B) serum glucose level when compared with diabetic control group.

The AUCglucose was found to be significantly high in diabetic rats as compared to non diabetic rats. PFML (100, 300, 500mg/kg, p.o.) treatment produced significant decrease in AUCglucose in STZ-induced type 1 diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 63C).
Results

Figure 63. Effect of PFML on serum glucose level in streptozotocin-diabetic rats. (63A) Effect on fed serum glucose level; (63B) Effect on fasting serum glucose level; (63C) Effect on AUCglucose. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, PFML 100 = diabetic treated with PFML (100mg/kg, p.o.), PFML 300 = diabetic treated with PFML (300mg/kg, p.o.), PFML 500 = diabetic treated with PFML (500mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)

5.6.2 Effect of PFML on fed and fasting serum insulin levels:

STZ-induced type 1 diabetic rats showed significant decrease in serum insulin level when compared with non diabetic control rats. Treatment with PFML (100, 300, 500mg/kg, p.o.) did not produce significant effect on fed (Fig. 64A) and fasting (Fig. 64B) serum insulin level in diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.

During OGTT, STZ-induced diabetic rats showed significant decrease in AUCinsulin when compared with non diabetic control rats. Treatment of these rats with PFML (100, 300, 500mg/kg, p.o.) did not produce significant effect on AUCinsulin in diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 64C).
Figure 64. Effect of PFML on fed and fasting serum insulin level in streptozotocin-diabetic rats. (64A) Effect of MEL on Fed serum insulin; (64B) Effect of MEL on Fasting serum insulin; (64C) Effect of MEL on AUCinsulin. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, PFML 100 = diabetic treated with PFML (100mg/kg, p.o.), PFML 300 = diabetic treated with PFML (300mg/kg, p.o.), PFML 500 = diabetic treated with PFML (500mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)

5.6.3 Effect of PFML on cardinal signs:

Injection of STZ in rats produced significant decrease in body weight (Fig.65A) and increase in food (Fig.65B) and water (Fig.65C) intake when compared with non diabetic
Results

PFML (300 mg/kg, p.o.) produced significant increase in STZ-induced diabetic rat’s body weight while significant decrease in food intake and water intake when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.

Figure 65. Effect of PFML on cardinal signs of streptozotocin - diabetic rats. (65A) Effect on body weight; (65B) Effect on food intake; (65C) Effect on water intake. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non-diabetic treated, DIC = diabetic control, PFML300 = diabetic treated with PFML (300mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)
5.6.4 Effect of PFML on activities of hepatic enzymes:

STZ-induced type 1 diabetic rats exhibited significantly lower hexokinase and higher PEPCK levels when compared with non diabetic control rats. Chronic treatment of STZ-induced type 1 diabetic group with PFML (300 mg/kg, p.o.) and insulin produced significant increase in hexokinase and decrease in PEPCK levels of diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 66A and 66B).

![Graph showing effect of PFML on liver hexokinase and PEPCK in streptozotocin-diabetic rats.](image)

**Figure 66.** Effect of PFML on liver hexokinase and PEPCK in streptozotocin-diabetic rats. (66A) Effect on liver hexokinase; (66B) Effect on liver PEPCK. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, PFML300 = diabetic treated with PFML (300 mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)

5.6.5 Effect of PFML on lipid profile:

STZ in rats produced a significant increase in cholesterol, triglycerides, VLDL and LDL while decrease in HDL levels when compared with non diabetic control rats. Treatment of STZ-induced type 1 diabetic rats with PFML (300 mg/kg, p.o.) caused a significant decrease in cholesterol (Fig. 67A), triglycerides (Fig. 67B), VLDL (Fig. 67D) and LDL (Fig. 67E) while increase in HDL (Fig. 67C) levels when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.
Figure 67. Effect of PFML on lipid profile in streptozotocin-diabetic rats. (67A) Effect on serum cholesterol; (67B) Effect on serum triglycerides; (67C) Effect on serum HDL. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, PFML300 = diabetic treated with PFML (300mg/kg, p.o.). *Significantly different from non diabetic control (p<0.05); **significantly different from diabetic control. (p<0.05)
Results

Figure 67. Effect of PFML on lipid profile in streptozotocin-diabetic rats. (67D) Effect on serum VLDL; (67E) Effect on serum LDL. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, PFML300 = diabetic treated with PFML (300mg/kg, p.o.). *Significantly different from non diabetic control (p<0.05); **Significantly different from diabetic control. (p<0.05)

5.6.6 Effect of PFML on serum GOT and GPT:

STZ-induced type 1 diabetic rats exhibited significantly higher SGOT and SGPT levels as compared to non diabetic control rats. Chronic treatment of STZ-induced type 1 diabetic group with PFML (300 mg/kg, p.o.) produced significant decrease in SGOT and SGPT levels when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 68A and 68B).
Results

5.6.7 Effect of PFML on serum urea:

STZ-induced type 1 diabetic rats exhibited significantly higher serum urea levels as compared to non diabetic control rats. PFML (300 mg/kg, p.o.) treatment decreased the elevated serum urea levels of STZ-induced type 1 diabetic group when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 69).

Figure 69. Effect of PFML on serum urea in streptozotocin-diabetic rats. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, PFML300 = diabetic treated with PFML (300 mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)
Results

diabetic treated with PFML (300 mg/kg, p.o.). *Significantly different from non diabetic control (p<0.05);
**Significantly different from diabetic control. (p<0.05)

5.6.8 Effect of PFML on serum creatinine:

STZ-induced type 1 diabetic rats showed significant increase in serum creatinine (Fig. 70) level when compared with non diabetic control rats. Treatment of STZ-induced type 1 diabetic group with PFML (300 mg/kg, p.o.) produced significant decrease in serum creatinine when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.

Figure 70. Effect of PFML on serum creatinine in streptozotocin-diabetic rats. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, PFML300 = diabetic treated with PFML (300 mg/kg, p.o.). *Significantly different from non diabetic control (p<0.05); **significantly different from diabetic control. (p<0.05)

5.7 Effect of TFML on streptozotocin-induced type 1 diabetic rats:

5.7.1 Effect of TFML on serum glucose levels:

STZ-induced diabetic rats showed significant increase in fed and fasting serum glucose level when compared with non diabetic control rats. Treatment of STZ-induced type 1 diabetic group with TFML (100, 300 mg/kg, p.o.) did not affect while TFML (500mg/kg, p.o) produced significant decrease in fed (Fig. 71A) and fasting (Fig. 71B) serum glucose level when compared with diabetic control group. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.

The AUCglucose was found to be significantly high in diabetic rats as compared to non diabetic control rats. Treatment with TFML 500 mg/kg p.o., significantly reduced AUCglucose while, 100 and 300 mg/kg, p.o. dose failed to produce this effect in STZ-induced type 1 diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 71C).
Results

Figure 71. Effect of TFML on serum glucose level in streptozotocin-diabetic rats. (71A) Effect on fed serum glucose level; (71B) Effect on fasting serum glucose level; (71C) Effect on AUCglucose. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, TFML100 = diabetic treated with TFML (100mg/kg, p.o.), TFML300 = diabetic treated with TFML (300mg/kg, p.o.), TFML500 = diabetic treated with TFML (500mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)

5.7.2 Effect of TFML on fed and fasting serum insulin levels:
STZ-induced type 1 diabetic rats showed significant decrease in serum insulin level when compared with non-diabetic control rats. Treatment with TFML (100, 300, 500mg/kg, p.o.) did not produce significant effect on fed (Fig. 72A) and fasting (Fig. 72B) serum insulin level in diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.

During OGTT, STZ-induced diabetic rats showed significant decrease in AUC insulin when compared with non-diabetic control rats. Treatment of STZ-induced type 1 diabetic rats with TFML (100, 300, 500mg/kg, p.o.) did not produce significant effect on AUC insulin of diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 72C).
### Results

**Figure 72.** Effect of TFML on fed and fasting serum insulin level in streptozotocin-diabetic rats. (72A) Effect of TFML on Fed serum insulin; (72B) Effect of TFML on Fasting serum insulin; (72C) Effect of TFML on AUCinsulin. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non-diabetic treated, DIC = diabetic control, TFML100 = diabetic treated with TFML (100mg/kg, p.o.), TFML300 = diabetic treated with TFML (300mg/kg, p.o.), TFML500 = diabetic treated with TFML (500mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)

### 5.7.3 Effect of TFML on cardinal signs:

Injection of STZ in rats produced significant decrease in body weight (Fig.73A) and increase food (Fig.73B) and water (Fig.73C) intake when compared with non diabetic control rats. TFML (500 mg/kg, p.o.) produced significant increase in STZ-induced type 1 diabetic rat’s body weight while significant decrease in food intake and water intake when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.
Results

5.7.4 Effect of TFML on activities of hepatic enzymes:

STZ-induced type 1 diabetic rats exhibited significantly lower hexokinase and higher PEPCK levels as compared to non diabetic control rats. Chronic treatment with TFML (500 mg/kg, p.o.) and insulin (5 IU/kg, i.p.) produced significant increase in hexokinase and decrease in PEPCK levels of STZ- induced type 1 diabetic rats when compared with diabetic control rats (Fig. 74A and 74B).
Results

5.7.5 Effect of TFML on lipid profile:

STZ in rats produced a significant increase in cholesterol, triglycerides, VLDL and LDL while decrease in HDL levels when compared with non diabetic control rats. Treatment of STZ-induced type 1 diabetic rats with TFML (500 mg/kg, p.o.) caused a significant decrease in cholesterol (Fig. 74A), triglycerides (Fig. 74B), VLDL (Fig. 74D) and LDL (Fig. 74E) while increase in HDL (Fig. 74C) levels of when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.

Figure 74. Effect of TFML on liver hexokinase and PEPCK in streptozotocin-diabetic rats. (74A) Effect on liver hexokinase; (74B) Effect on liver PEPCK. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, TFML500 = diabetic treated with TFML (500 mg/kg, p.o.). *Significantly different from non diabetic control. (p<0.05); **significantly different from diabetic control. (p<0.05)
Figure 74. Effect of TFML on lipid profile in streptozotocin-diabetic rats. (74A) Effect on serum cholesterol; (74B) Effect on serum triglycerides; (74C) Effect on serum HDL. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, TFML500 = diabetic treated with TFML (500mg/kg, p.o.). *Significantly different from non diabetic control (p<0.05); **significantly different from diabetic control. (p<0.05)
Results

**Figure 74.** Effect of TFML on lipid profile in streptozotocin-diabetic rats. (74D) Effect on serum VLDL; (74E) Effect on serum LDL. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, TFML500= diabetic treated with TFML (500mg/kg, p.o.). *Significantly different from non diabetic control (p<0.05); **Significantly different from diabetic control (p<0.05)

5.7.6 Effect of TFML on serum GOT and GPT:

STZ-induced type 1 diabetic rats exhibited significantly higher SGOT and SGPT levels as compared to non diabetic control rats. Chronic treatment of STZ-induced type 1 diabetic rats with TFML (500 mg/kg, p.o.) produced significant decrease in SGOT and SGPT levels when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 75A and 75B).
Results

5.7.7 Effect of TFML on serum urea:

STZ-induced type 1 diabetic rats exhibited significantly higher serum urea levels as compared to non diabetic control rats. TFML (500 mg/kg, p.o.) treatment decreased the elevated serum urea levels of STZ-induced type 1 diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group (Fig. 76).
Figure 76. Effect of TFML on serum urea in streptozotocin-diabetic rats. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, TFML500 = diabetic treated with TFML (500 mg/kg, p.o.). *Significantly different from non diabetic control (p<0.05); **Significantly different from diabetic control. (p<0.05)

5.7.8 Effect of TFML on serum creatinine:

STZ-induced type 1 diabetic rats showed significant increase in serum creatinine (Fig. 77) level when compared with non diabetic control rats. Treatment with TFML (500 mg/kg, p.o.) produced significant decrease in serum creatinine of STZ-induced type 1 diabetic rats when compared with diabetic control rats. This effect was comparable to the effect of insulin (5 IU/kg, i.p.) on STZ-induced diabetic group.

Figure 77. Effect of TFML on serum creatinine in streptozotocin-diabetic rats. Each bar represents mean ± SEM of 6 animals. CON = non diabetic control, CONT = non diabetic treated, DIC = diabetic control, TFML500 = diabetic treated with TFML (500 mg/kg, p.o.). *Significantly different from non diabetic control (p<0.05); **significantly different from diabetic control. (p<0.05)

5.8 Renal Histopathology

Figure 78. Non-diabetic Control-Normal histology
Figure 79. Non-diabetic control treated with MEL (500mg/kg/day)-Normal histology

Figure 80. HFD fed/STZ-induced diabetic rats treated with PFML (300 mg/kg, p.o)-Near normal histology

Figure 81. HFD fed/STZ-induced diabetic rats treated with Pioglitazone standard (PS)-Near normal histology
Figure 82. HFD fed control rats - Mild Thickening of glomeruli and focal sclerosis

Figure 83. HFD fed /STZ-induced diabetic Control rats - Thickening of glomeruli and focal sclerosis