Attenuation of some Metabolic Deterioration Induced by Diabetes Mellitus using Nepeta cataria Extracts.

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Abstract: The present research is design to evaluate the pharmacological effects of successive as well as 70% ethanolic extracts of Nepeta cataria on some biochemical parameters in Streptozotocin diabetic rats compared to the currently used Gliclazide drug. The investigated parameters included, glucose, insulin, carbohydrate hydrolyzing enzymes; α–amylase, α–glucosidase, β–galactosidase, liver steatosis markers; total cholesterol, HDL-cholesterol, LDL–cholesterol, triglycerides, total lipid, liver function enzymes; alanin aminotransferase(ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total protein; antioxidant activity of extracts using nitric oxide (NO). In addition, histopathological investigations were performed. The results obtained revealed anti-glycemic, antioxidant, antiplatelet effects of chloroform, petroleum ether as well as 70% ethanolic extracts in comparison with Gliclazide as reference antidiabetic drug . Moreover, these extracts have principle role in treatment and normalized liver and pancreas architecture. Hence, it could be concluded that Nepeta cataria extracts may be applied clinically for reducing complications against diabetes mellitus paralleling with the ideal anti-diabetic Gliclazide drug.

Key words: Diabetes mellitus, Nepeta cataria, oxidative stress, liver function enzymes, carbohydrate hydrolyzing enzymes, lipid profile.

1. Introduction:

Diabetes mellitus (DM) is a serious health problem being the third greatest cause of death all over the world, and if not treated, it is responsible for many complications affecting various organs in the body. Diabetes mellitus is a disease results from abnormality of carbohydrate metabolism and characterized by absolute (type I) or relative (type II) deficiencies in insulin secretion or receptor insensitivity to endogenous insulin, resulting in hyperglycemia. Hyperglycemia that is initiating from unregulated glucose level is widely recognized as the causal link between diabetes and diabetic complications. It was found that, hyperglycemia cause tissue damage by mechanisms involving repeated changes in cellular metabolism. One of the key metabolic pathways as being major contributors to hyperglycemia induced cell damage, is the non enzymatic reaction between excess glucose and several proteins (as hemoglobin and albumin) to form advanced glycosylated end product (AGE). Production of AGE interferes with cell integrity by modifying protein function or by inducing receptors mediated production of reactive oxygen species (ROS) (Thornalley, 2002).

Hyperglycemia-evoked oxidative stress plays a crucial role in the development of diabetic complications, including nephropathy, neuropathy, retinopathy and hepatopathy, which are considered to result from both augmented reactive oxygen species generation and decreased antioxidant defenses (Tepe et al., 2007).

Hypoglycemic plants are still prevalent in developing countries, where they have been used to treat diabetes for many centuries. More than 1200 species of plants have been used empirically for their alleged hypoglycemic activity. This fact is attributed to the high cost and the lack of availability of current therapies for the majority of patients in developing countries. Nevertheless, many medicinal plants claimed effective by folk medicine require scientific investigation to ascertain their effectiveness, toxicity and then provide alternative drugs and therapeutic strategies (Marles ; Farnsworth, 1994).

Nepeta cataria L (family , Lamiaceae; order Lamiales) , comprises about 400 species, most of which found in the Eastern Mediterranean, Southern Asia and China, is commonly known as Catnip or Catmint because of its irresistible action on cats. Due to lemony mint flavor it finds the ways in the herbal teas as well as in cooking. Medicinally, the plants are used in gastrointestinal and respiratory hyperactive disorders such as, colic, diarrhea, cough, asthma and bronchitis (Miceli et al., 2005). A limited number of studies exists on its biological activities include antibacterial (Kalpoutzakis et al., 2001), antifungal (Nostro et al., 2001) and analgesic (Aydin et al., 1998). Various compounds have been identified by different groups of workers in the essential oil of Nepeta cataria. The main constituents so far identified, include β-caryophyllene, caryophyllene oxide, 1,8 -cineol, citronellol, geraniol, eugenol, nerol (Mortua –Semmanni and Saeedi, 2004; Schultz et al., 2004; Sajjadi, 2005). Also, urosolic acid, β-sitosterol, campesterol, α-amyrin, β-amyrin, and sitosterol β-glucopyranoside have been reported previously (Miceli et al., 2005). In addition the plant also contained neptalactones and alkaloids, such as actinidine and iridomyecine (Kalpoutzakis et al., 2001).

So, the present study is design to demonstrate the hypoglycemic efficiency of petroleum ether, chloroform as well as 70% ethyl alcohol extracts of Nepeta cataria [compared with antidiabetic Gliclazide (diamicon) reference drug] in Streptozotocin induced diabetes mellitus in rats through measuring glucose, insulin, carbohydrate hydrolyzing enzymes, nitric oxide, liver function enzymes, total protein and lipid profile. Moreover histological examination of liver and pancreas was performed.

2. Materials and Methods:

Chemicals:

All chemicals in the present study were of analytical grade, product of Sigma, Merek and Aldrich. All kits were the products of Biosystems (Spain), Sigma Chemical Company (USA), Biodiagnostic (Egypt).

Plant materials:

Seeds of catnip (Nepeta cataria L.) was obtained from company of Jelitto stadensamen, Schwarmstedt, Germany). The seeds of the plant were cultivated in the experimental farm of the Cultivation and Production of Aromatic Plants Department of the National Research Center, Giza, during two successive seasons of 2006 and 2007.

The seeds of catnip were sown in nursery on 15th of October in the two seasons. Two months later after sowing, the seedlings were transplanted in 8 cm pots in medium of 1:1:1(by volume) loam, sand and peat moss. The seedlings were planted in the field on 15th of March in hills 25 cm apart on rows 60 cm in-between. The flowering aerial parts of Nepeta cataria were collected from the plants during two successive seasons of 2006 and 2007 and raised from seeds obtained from company of Jelitto stadensamen, Schwarmstedt, Germany).
Preparation of extracts and fractions:
The powdered air-dried aerial parts of Nepeta cataria (720 g) were extracted with petroleum ether (60-80 %) and chloroform in succession, to afford 35 (4.8%) and 28 g (3.88%) respectively. In addition, 400g of the same dried parts were extracted with 70% ethanol to yield 26 g (6.5%) of total ethanolic extract.

Determination of hypoglycemic activity of Nepeta cataria extracts:

Animals:
Male Wister albino rats (120-150g.) were obtained from animal house of National Research Centre, Dokki, Giza, Egypt. Rats were fed on a standard diet and free access to tap water. They were kept for one week to be acclimatized to the environmental conditions.

Doses:
All plant extracts were orally administrated with a dose of 50 mg/kg body weight for 30 consecutive days according to Miceli et al. (2005); Rabbani et al. (2007).

Experimental design:
98 male albino rats were selected for this study and divided to fourteen groups (seven rats in each group) as follows:
- Group 1: normal healthy control rats.
- Groups 2-4: normal healthy rats orally administrated different Nepeta cataria extracts (30 mg/kg body weight daily for 30 days, each rat received 7.5 mg /0.5 ml bidistilled water).
- Groups 5-7: considered as diabetic groups; where type 1 diabetes was induced by Streptozotocin, each rat was injected intraperitoneally with a single dose of Streptozotocin (65mg/Kg)
- Groups 8-10: Diabetic animals treated with petroleum ether extract
- Groups 11-12: Diabetic animals treated with Glicalized (diamicron) preliabetic extract (as previously group).
- Group 13: Diabetic +ve control group sacrificed after 2 days of STZ injection
- Group 14: Diabetic +ve control group sacrificed after 10 days of STZ injection
- Group 15: Diabetic +ve control group sacrificed after 40 days of STZ injection and considered as recovery group.

Preparation of samples:
Serum sample: each animal was weighted, blood collected by puncture the sub-tongual vein in clean and dry test tube, left 10 minutes to clot and centrifuged at 3000 rpm for serum separation. The separated serum was stored at -80°C for further determinations of lipid profile, liver function tests, carbohydrate metabolizing enzymes and serum total protein.

Tissue sample: liver tissue was weighted and homogenized in ice cold 0.9 N NaCl, centrifuged at 3000 rpm for 10 min, separated the supernatant and stored at -80°C for further estimation. For determination of NO in liver tissue ,the extraction method was carried out using 1g tissue and homogenized in 10 ml 10% trichloroacetic acid (to give 10% homogenate 1%w/v ).Then the sample was centrifuged at 3,000 rpm for 10 min and the supernatant was separated.

Blood biochemical analysis:
i-Determination of blood glucose:-
Glucose was determined in serum by colorimetric assay according to Trinder (1969).

ii- Human insulin enzyme immunoassay :
Insulin was determined by quantitative test kit according to the method of Sacks (1994).

iii- Determination of total cholesterol and cholesterol - HDL in rat serum:
Total cholesterol and HDL-cholesterol were determined by the method of Stein (1986).

iv- Determination of triglyceride:
Triglyceride was measured in rat serum by the method of Wahlenfed (1974).

v- Determination of total lipid:
Total lipid was measured in rat serum by the method of Zollner and Kirsch (1962).

vi- Determination of alkaline phosphatase enzyme activity :
Alkaline phosphatase enzyme activity was measured in rat serum by the method of Belfield and Goldberg (1971).

vii- Determination of nitric oxide (NO ) :
NO was measured in liver tissue homogenates according to Moshage et al. (1995).

viii- Determination of carbohydrate hydrolyzing enzymes :
- α- Amylase enzyme activity was performed in liver tissue homogenates according to the method of Caraway (1959).
- Glucosidase and β-galactosidase enzyme activities were performed in liver tissue homogenates by the method of Sanchez andHardisson (1979).

Histopathology :
Liver and pancreas specimens were fixed in 10% formalin, processed to paraffin blocks, sectioned (4 µm thick) and stained with Hematoxyline and Eosin .They were examined using light microscope (Gomori, 1941).

Statistical analysis:
Data were analyzed by comparing values for different treatment groups with the values for individual controls. Results are expressed as mean ± S.D. The significant differences among values were analyzed using analysis of variance (one-way Anova)
coupled with post-Hoc, least significance difference (LSD). Anova at p ≤ 0.05 using Co-stat computer program.

3. Results:
The present results demonstrate the biochemical effects, mechanism(s) of the hypoglycemic actions of *Nepeta cataria* extracts and their possible hepatoprotective roles against liver disorders induced by reactive oxygen species associated with diabetic complications in diabetic rats. The investigated parameters included blood glucose, insulin, total protein content, ALT, AST, ALP, total lipid, HDL-cholesterol, triglycerides, and total protein content as well as hepatic nitric oxide, α- amyalase, α-glucosidase and β-galactosidase enzyme activities.

Blood glucose and insulin levels in serum of control, normal- treated, diabetic and diabetic – treated groups are demonstrated in Table (1) and Fig (1). It is shown that, there is significant change between control and different normal –treated groups either in blood glucose or in insulin levels except normal –treated with 70% ethanolic extract which exhibits significant reduction in blood glucose levels amounted 94.50±16.05 mg/dl at P≤ 0.05 with percentage of reduction -13.69%. Concerning diabetic groups, significant increase is noticed in blood glucose levels which is concomitant with significant reduction in insulin levels at day 2, 10 and 40 post STZ injection recorded 373.00±2.94, 363.00±2.94 and 364.25±3.77 mg/dl with percentage increase +240.64, +231.51 and +232.62% for glucose respectively and 0.59±0.01, 0.34±0.05 and 0.35±0.01 μIU/ml with percentage of reduction reached to -88.97, -93.64 and -93.45% for insulin respectively. Significant amelioration is noticed in blood glucose and insulin levels in all diabetic – treated groups recorded the most pronounced effect for 70% ethanolic extract (+5.94% for glucose and -14.21% for insulin ) followed by petroleum ether extract (+17.12 and -22.8%, respectively) then Gliclazide as a reference drug (+20.55 and -27.66%, respectively) and finally chloroform extract (+21.46 and -28.41%, respectively).

Lipid profile, total cholesterol, HDL- cholesterol, LDL-cholesterol, triglycerides and total lipid in control, normal- treated, diabetic and diabetic – treated groups are demonstrated in Table (2) and Fig (2). It can be easily noticed that, there is no significant change in total cholesterol, HDL- cholesterol, LDL-cholesterol and total lipid between different normal – treated groups as compared to untreated control one. While triglycerides show significant reduction in 70% ethanolic extract treated – normal group reached to 93.32±22.07 mg/dl with percentage of reduction amounted -44.62 %. at day 2, 10 and 40 post STZ injection respectively. The significant increase in triglycerides content still recorded significant reduction post petroleum ether, chloroform and total ethanolic extracts. On the contrary significant inhibition is noticed in all normal –treated groups recorded 2.94±0.40, 2.74±0.25 and 2.78±0.26 umole/mg protein/min with percentage decrease amounted -17.99, -13.27 and -19.17% for chloroform, 70% ethanol and petroleum ether extracts respectively as compared to the normal control group. With regard to diabetic condition, significant increase in all enzyme activities is noticed at day 2,10 and 40 post STZ injection reached to 2.73±0.24, 3.23±0.23, 3.37±0.11 μmole/mg protein/min for AST, ALT and ALP, respectively. Significant improvement is noticed in LDL- cholesterol level post treatment of diabetic rats with different extracts where, insignificant change is observed as compared to each other and significant increase as compared to normal control group amounted 107.89±14.17, 118.02±11.50 and 111.25±2.15 mg /dl with percentage of elevation +28.73, +40.82 and +32.74% for petroleum ether, chloroform and 70% ethanol extracts, respectively. Controversy Gliclazide -treated diabetic group exhibits insignificant change as compared to normal control and significant decrease as compared to other extracts. Remarkable, significant enhancement is noticed in total protein level post different types of treatments, where insignificant change is recorded as compared to normal control except for petroleum ether extract-treated diabetic rats, significant increase is noticed amounted 125.74±10.83 mg/dl (+13.40%) as compared to normal control group. Total lipid reveals an enhanced significant mean value of 373.17±41.65, 1414.58±195.92 , 1325±99.71 and 327.50±33.04 for petroleum ether, chloroform, 70% ethanol extracts and Gliclazide drug as compared to normal control980.62±30.71) with percent +40.03, +44.25, +35.12 and +26.20%, respectively.

Table (3) and Fig (3) demonstrate the level of liver function enzyme activities AST, ALT, ALP and total protein in serum of control, normal- treated, diabetic and diabetic- treated groups. It is obvious that insignificant change is recorded in AST level in serum of normal control treated either with chloroform or 70% ethanolic extracts. On the contrary significant inhibition is noticed in AST activity in normal rats treated with petroleum ether extract amounted 1.97±0.25 u mole /mg protein /min (-12.83 %). With respect to ALT and total protein, insignificant change is observed in their levels in different normal - treated groups as compared to untreated control one. Concerning ALP, significant inhibition is noticed in all normal –treated groups recorded 3.23±0.23, 3.37±0.11 μmole/mg protein/min (-12.83 %). For reference drug (+20.55 and -27.66% respectively) then Gliclazide as a reference drug (+20.55 and -27.66%, respectively). While , ALT recorded 1.97±0.09, 2.44±0.17 and 2.48±0.08 μmole/ mg protein /min with percentage increase +23.13, +5.55% and +5.00% respectively. ALP shows a value of 4.55±0.36, 5.55±0.60 and 5.61±0.13 μmole/mg protein/min with percentage of elevation +33.04, +63.72 and +65.40% respectively. Total protein content shows insignificant change at day 2,10 and 40 post STZ injection, while marked significant reduction at day 40 amounted 84.3 ±1.74 mg / ml (-22.12%). The curative effect of petroleum ether, chloroform, 70% ethanol extracts and Gliclazide drug on diabetic rats can easily be noticed through the normalization of all enzymes tested returned more or less to the level of normal control, where an insignificant change is observed. While total protein content still recorded significant reduction post petroleum ether, chloroform and total ethanolic extracts treatments (although, it shows normalized level with Gliclazide drug) amounted 83.25±5.37, 89.25±4.92 and 83.50±5.06 mg/ml with percentage decrease -23.09, -17.55 and -22.86% respectively.

NO level in hepatic tissue of control, normal- treated, diabetic and diabetic- treated groups is manipulated in Table (4) and Fig (4). It can be easily noticed that NO level is insignificantly affected post various extracts- treated normal rats as compared to untreated control one. In response to diabetic state, NO shows significant increase of a value 62.96±2.32, 72.55±1.87 and 72.55 ±2.00 μg/g tissue with percentage increase +42.86,+64.62 and +64.62 %at day 2, 10 and 40 post STZ injection respectively. The level of NO is significantly improved as a result of different treatments, shows the best result for chloroform petroleum ether extracts, where insignificant change is recorded either as compared to normal control or diabetic- Gliclazide treated groups. In spite of, significant elevation in NO level is
noticed in diabetic –chloroform extract treated group amounted 51.30 ±2.69µg/g tissue with percent +16.41% compared to control. In addition, carbohydrate hydrolyzing enzymes, α-amylose, β-galactosidase and α-glucosidase show insignificant change in different normal - treated groups as compared to the untreated control one. While α-glucosidase shows significant increase as a result of treatment with both 70% ethanolic (0.342±0.01 µmole/mg protein/min) and petroleum ether extracts (0.370±0.06 µmole/mg protein/min) with percentage increase amounted +25.74 and +36.03 %, respectively. On the other hand, total protein content reveals a significant reduction with a value of 39.50±8.22, 36.25±10.30 and 41.25±8.54 mg/g tissue in response to treatment of normal rats with petroleum ether, chloroform and 70% ethanol extracts with percent reduction -38.27, -45.28 and -43.65% respectively. While, β-galactosidase enzyme activity recorded 0.052±0.02, 0.050±0.01 and 0.054±0.01 µmole/mg protein/min with percent +62.72 and +25.93 % respectively. Moreover, α-glucosidase shows a value of 0.192±0.01, 0.187±0.01 and 0.190±.01 µmole/mg protein/min with percent -21.0, -17.5 and -17.5 %, respectively. It can be deduced that carbohydrate metabolizing enzymes are strongly affected with diabetic condition show significant inhibition at day 2, 10 and 40 post STZ injection reached to 20.07 ±1.40, 17.97 ±0.68 and 18.32 ±0.69 µmole/ mg protein/min for α-amylose with percent of reduction -38.27 , - 45.28 and -43.65% respectively. While, β-galactosidase enzyme activity recorded 0.052±0.02, 0.050±0.01 and 0.054±.01 µmole/mg protein/min with percent +62.72 and +25.93 % respectively. The curative effect of the different extracts can be also seen through improvement in β- galactosidase enzyme activity which is returned to its normal value as compared to both normal control and reference drug. In addition, α-glucosidase activity shows insignificant change post chlorofrom treatment, while it recorded significant increase post petroleum ether, 70% ethanol extracts and Gliclazide drug (0.335±0.02, 0.357±0.02 and 0.372±0.03 µmole/mg protein/ min respectively) with percentage increase +23.16, +31.25 and +36.76 % respectively as compared to normal control rats (40 days). Liver weight /body weight ratio shows significant increase in all diabetic –treated groups as compared to normal untreated one. Normal liver weight /body weight ratio is recorded significant increase amounted 8.50 ±0.37 g with percentage reached to +23.14 %. While, it shows insignificant change in other treatments. It is clearly noticed from the present study that diabetic condition is always associated with a significant reduction in body weight. This result is ascertainment through the degradable , remarkable significant reduction in body weight at day 2,10 and 40 post STZ injection amounted 142.25±4.34, 112.75 ±8.77 and 98.50±5.68 g with percentage of reduction -5.79, - 28.86 and - 45.58% respectively. On the contrary, liver weight and liver weight /body weight ratio shows insignificant change amounted 8.50 ±0.37 g with percentage +26.96, +53.49 and +22.06 % for liver weight and 0.0525±0.005, 0.085±0.013 and 0.088±0.005 for liver weight / body weight ratio with percentage increase +31.25, +112.50 and +118.75 % respectively. Treatment of diabetic rats with different extracts clearly produces improvement in body weight, liver weight and their ratios, while chloroform and 70% ethanolic extract–treated rats shows significant increase in body weight amounted 123.50±6.02 g with percent +25.38% as compared to untreated diabetic group at day 40 (recovery group), although, with respect to normal control rats at day 40, significant reduction is recorded (-31.77%). Nearly the same results are achieved for 70% ethanolic extract treated– diabetic rats. In addition, diabetic rats –treated with petroleum ether extract and Gliclazide drug show significant increase in body weight amounted 131.00±4.24 and 153.25 ± 2.36 g with percent +32.99 and +55.58 % respectively as compared to diabetic - untreated one ,while as compared to normal control, significant reduction is noticed (-27.62 and -15.33 %, respectively). Concerning liver weight, insignificant change is observed in chloroform extract treated-diabetic rats and Gliclazide drug, while significant reduction is recorded in petroleum ether and total ethanol extracts amounted 5.00±0.47 and 5.26±0.50 g with percent reduction -27.56 and -23.83% respectively as compared to normal control rats (40 days). Liver weight /body weight ratio exhibits insignificant change in all diabetic –treated groups except chloroform extract which shows significant increase as compared to normal control group at day 40 (0.05±0.02) with percentage of increase +37.5%. In addition, significant reduction is observed in liver weight and liver weight / body weight ratio in all diabetic –treated groups as compared to diabetic untreated one (40 days).

Histological studies on pancreas and liver:

Normal architecture in liver and pancreas of treated –normal groups at the cellular level as compared to the control untreated one. Gradual cellular changes include imperfection, reduction in β-cells numbers, degeneration and atrophic changes are appears in pancreas of streptozotocin treated rats at different durations (Figs 11-13). On the other hand, sections of diabetic liver at day 2, 10 and 40 show degeneration of hepatocytes, necrosis and congestion of central vein (Figs 22-24). Successive as well as total ethanolic extracts of Nepeta cataria and Gliclazide drug appear to regulate diabetes at the cellular level resulting in , restoration of normal architecture of pancreatic islets (Figs 14-17) and hepatocytes (Figs 25-28) in the diabetic- treated groups. These suggested a possible regeneration or repair of the cells in diabetic –treated rats. 
Table (1): Evaluation of successive and 70% ethanol extracts of *Nepeta cataria* on blood glucose and insulin levels in control, normal- treated, diabetic and diabetic-treated groups.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
<th>Glucose</th>
<th>Insulin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>109.5±6.65 de</td>
<td>5.35±0.62 a</td>
</tr>
<tr>
<td>Normal treated chloroform extract</td>
<td></td>
<td>105.25±7.36 de</td>
<td>5.21±1.07 ab</td>
</tr>
<tr>
<td>Normal treated 70% ethanol extract</td>
<td></td>
<td>94.50±16.03 f</td>
<td>5.07±1.47 ab</td>
</tr>
<tr>
<td>Normal treated petroleum ether extract</td>
<td></td>
<td>113.75±1.25 d</td>
<td>5.03±1.66 abc</td>
</tr>
<tr>
<td>Diabetes after 2 days</td>
<td></td>
<td>373.00±2.94 a</td>
<td>0.59±0.01 e</td>
</tr>
<tr>
<td>Diabetes after 10 days</td>
<td></td>
<td>363.00±2.94 b</td>
<td>0.34±0.05 e</td>
</tr>
<tr>
<td>Diabetes after 40 days</td>
<td></td>
<td>364.25±3.77 ab</td>
<td>0.35±0.01 e</td>
</tr>
<tr>
<td>Diabetes treated chloroform extract</td>
<td></td>
<td>133.00±5.35 c</td>
<td>3.83±0.59 d</td>
</tr>
<tr>
<td>Diabetes treated 70% ethanol extract</td>
<td></td>
<td>103.00±2.44 ef</td>
<td>4.59±0.59abcd</td>
</tr>
<tr>
<td>Diabetes treated petroleum ether extract</td>
<td></td>
<td>128.25±2.87 c</td>
<td>4.13±0.57 bcde</td>
</tr>
<tr>
<td>Diabetes treated gliclazide (Ref.Drug)</td>
<td></td>
<td>132.00±2.44 ef</td>
<td>3.87±0.05cde</td>
</tr>
</tbody>
</table>

Blood glucose is expressed in mg/dl while insulin level is expressed in uIU/ml.

Data are mean ±SD of 7 rats in each group.

Statistical analysis is carried out using one way analysis of variance (ANOVA) using Co-Stat computer program.

Unshared superscript letters between treatments are significance values at P<0.001.

Table (2): Evaluation of successive as well as 70% ethanol extracts of *Nepeta cataria* on lipid profile in serum of control, normal- treated, diabetic and diabetic-treated groups.

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
<th>T- cholesterol</th>
<th>HDL- Cho</th>
<th>LDL- Cho</th>
<th>Tri glyceride</th>
<th>Total lipid</th>
</tr>
</thead>
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<tr>
<td>Normal control</td>
<td></td>
<td>131.34±6.26 e</td>
<td>67.04±3.00 cd</td>
<td>83.81±2.27 d</td>
<td>110.88±7.36 de</td>
<td>980.62±30.71 d</td>
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<tr>
<td>Normal treated chloroform extract</td>
<td></td>
<td>126.72±6.16 e</td>
<td>65.45±3.89 cd</td>
<td>81.99±5.27 d</td>
<td>103.61±7.55 ef</td>
<td>1033.50±23.70 d</td>
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<tr>
<td>Normal treated 70% ethanol extract</td>
<td></td>
<td>128.79±7.54 e</td>
<td>69.00±4.54 c</td>
<td>80.01±7.29 d</td>
<td>93.32±22.07 f</td>
<td>931.94±145.94 d</td>
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<tr>
<td>Normal treated petroleum ether extract</td>
<td></td>
<td>125.50±6.40 e</td>
<td>65.00±4.16 cd</td>
<td>83.30±6.08 d</td>
<td>114.06±11.09 ed</td>
<td>1049.00±11.04 d</td>
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<td>Diabetes after 2 days</td>
<td></td>
<td>202.50±4.51 b</td>
<td>81.62±3.35 b</td>
<td>163.22±4.57 b</td>
<td>211.75±8.22 b</td>
<td>1867.19±202.10 a</td>
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<td>Diabetes after 10 days</td>
<td></td>
<td>239.91±10.89 a</td>
<td>90.75±1.89 a</td>
<td>210.72±9.96 a</td>
<td>307.77±8.60 a</td>
<td>1805.55±103.93 a</td>
</tr>
<tr>
<td>Diabetes after 40 days</td>
<td></td>
<td>239.75±4.11 a</td>
<td>90.75±0.95 a</td>
<td>210.75±4.98 a</td>
<td>308.75±1.25 a</td>
<td>1808.75±8.54 a</td>
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<tr>
<td>Diabetes treated chloroform extract</td>
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<td>158.15±8.20 c</td>
<td>64.37±3.53 cd</td>
<td>118.02±11.30 c</td>
<td>121.16±4.65 cd</td>
<td>1414.58±193.92 b</td>
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<tr>
<td>Diabetes treated 70% ethanol extract</td>
<td></td>
<td>150.81±6.46 cd</td>
<td>61.92±6.46 d</td>
<td>111.25±2.15 c</td>
<td>112.72±5.57 ede</td>
<td>1325.00±99.71 bc</td>
</tr>
<tr>
<td>Diabetes treated petroleum ether extract</td>
<td></td>
<td>145.99±10.97 d</td>
<td>63.25±3.76 ed</td>
<td>107.89±14.17 c</td>
<td>125.74±10.83 c</td>
<td>1373.17±41.65 bc</td>
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<tr>
<td>Diabetes treated Gliclazide (Ref.Drug)</td>
<td></td>
<td>72.75±0.96 f</td>
<td>61.25±0.95 c</td>
<td>81.90±1.68 d</td>
<td>124.50±4.20 cd</td>
<td>1237.50±33.04 c</td>
</tr>
</tbody>
</table>

Lipid profile (total cholesterol,HDL-cholesterol,LDL-cholesterol,Triglycerides and Total Lipid) are expressed in mg/dL.

Data are mean ±SD of 7 rats in each group.

Statistical analysis is carried out using one way analysis of variance (ANOVA) using Co-Stat computer program.

Unshared superscript letters between treatments are significance values at P<0.001.
Table (3) Evaluation of successive and 70% ethanol extracts of Nepeta cataria on liver function enzyme activities in serum of control, normal-treated, diabetic and diabetic-treated groups

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<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
<th>AST</th>
<th>ALT</th>
<th>ALP</th>
<th>Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>2.26 ±0.02 c</td>
<td>1.60± 0.09 cd</td>
<td>3.39± 0.13 c</td>
<td>108.25± 5.67 ab</td>
</tr>
<tr>
<td>Normal treated chloroform extract</td>
<td></td>
<td>2.48 ±0.08 bc</td>
<td>1.72± 0.14 c</td>
<td>2.94± 0.40 d</td>
<td>112± 1.41 a</td>
</tr>
<tr>
<td>Normal treated 70% ethanol extract</td>
<td></td>
<td>2.50 ±0.07 bc</td>
<td>1.65± 0.03 cd</td>
<td>2.74± 0.25 de</td>
<td>109± 6.21 ab</td>
</tr>
<tr>
<td>Normal treated petroleum ether extract</td>
<td></td>
<td>1.97± 0.25 d</td>
<td>1.64± 0.19 cd</td>
<td>2.78± 0.26 de</td>
<td>102± 7.70 b</td>
</tr>
<tr>
<td>Diabetes after 2 days</td>
<td></td>
<td>2.73± 0.24 b</td>
<td>1.97± 0.09 b</td>
<td>4.51± 0.36 b</td>
<td>104.5± 8.42 ab</td>
</tr>
<tr>
<td>Diabetes after 10 days</td>
<td></td>
<td>3.23± 0.23 a</td>
<td>2.44± 0.17 a</td>
<td>5.55± 0.60 a</td>
<td>108.75± 6.29 ab</td>
</tr>
<tr>
<td>Diabetes after 40 days</td>
<td></td>
<td>3.37± 0.11 a</td>
<td>2.48± 0.08 a</td>
<td>5.61± 0.13 a</td>
<td>84.3± 1.74 c</td>
</tr>
<tr>
<td>Diabetes treated chloroform extract</td>
<td></td>
<td>2.48± 0.36 bc</td>
<td>1.72± 0.04 c</td>
<td>2.41± 0.08 ef</td>
<td>89.25± 4.92 c</td>
</tr>
<tr>
<td>Diabetes treated 70% ethanol extract</td>
<td></td>
<td>2.40± 0.27 c</td>
<td>1.66± 0.04 cd</td>
<td>2.52± 0.31 df</td>
<td>83± 5.06 e</td>
</tr>
<tr>
<td>Diabetes treated petroleum ether extract</td>
<td></td>
<td>2.41± 0.13 c</td>
<td>1.59± 0.11 cd</td>
<td>2.18± 0.17 f</td>
<td>83.25± 5.37 c</td>
</tr>
<tr>
<td>Diabetes treated Gliclazide (Ref.Drug)</td>
<td></td>
<td>2.53± 0.03 bc</td>
<td>1.55± 0.04 d</td>
<td>2.19± 0.08f</td>
<td>101.25± 5.05 b</td>
</tr>
<tr>
<td>LSD 5%</td>
<td></td>
<td>0.28</td>
<td>0.16</td>
<td>0.42</td>
<td>8.11</td>
</tr>
</tbody>
</table>

AST, ALT and ALP are expressed in µmole/mg protein/min
Total protein is expressed in mg /ml
Data are mean ±SD of 7 rats in each group.
Statistical analysis is carried out using one way analysis of variance (ANOVA) using Co-Stat computer program.
Unshared superscript letters between treatments are significance values at P<0.001.

Table (4) Evaluation of successive as well as 70% ethanol extracts of Nepeta cataria on nitric oxide level in liver of control, normal-treated, diabetic and diabetic-treated groups

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
<th>NO</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>44.07± 1.37 d</td>
</tr>
<tr>
<td>Normal treated chloroform extract</td>
<td></td>
<td>43.32± 0.70 d</td>
</tr>
<tr>
<td>Normal treated 70% ethanol extract</td>
<td></td>
<td>44.03± 0.96 d</td>
</tr>
<tr>
<td>Normal treated petroleum ether extract</td>
<td></td>
<td>43.45± 1.25 d</td>
</tr>
<tr>
<td>Diabetes after 2 days</td>
<td></td>
<td>62.96± 2.32 b</td>
</tr>
<tr>
<td>Diabetes after 10 days</td>
<td></td>
<td>72.55± 1.87 a</td>
</tr>
<tr>
<td>Diabetes after 40 days</td>
<td></td>
<td>72.55± 2.00 a</td>
</tr>
<tr>
<td>Diabetes treated chloroform extract</td>
<td></td>
<td>43.92± 1.47 d</td>
</tr>
<tr>
<td>Diabetes treated 70% ethanol extract</td>
<td></td>
<td>43.12± 0.83 d</td>
</tr>
<tr>
<td>Diabetes treated petroleum ether extract</td>
<td></td>
<td>44.75± 1.70 d</td>
</tr>
<tr>
<td>Diabetes treated Gliclazide (Ref.Drug)</td>
<td></td>
<td>44.75± 1.70 d</td>
</tr>
<tr>
<td>LSD 5%</td>
<td></td>
<td>2.4</td>
</tr>
</tbody>
</table>

Nitric oxide (NO) is expressed in µg/g tissue
Data are mean ±SD of 7 rats in each group.
Statistical analysis is carried out using one way analysis of variance (ANOVA) using Co-Stat computer program.
Unshared superscript letters between treatments are significance values at P<0.001.

Table (5) Evaluation of successive and 70% ethanol extracts of Nepeta cataria on carbohydrate hydrolyzing enzymes in liver of control, normal-treated, diabetic and diabetic-treated groups

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Parameters</th>
<th>α-amylase</th>
<th>β-glactosidase</th>
<th>α-glucosidase</th>
<th>Total protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td></td>
<td>32.51 ±±4.99 de</td>
<td>0.167± 0.02 bc</td>
<td>0.272± 0.02 c</td>
<td>50.00±10.80 abc</td>
</tr>
<tr>
<td>Normal treated chloroform extract</td>
<td></td>
<td>32.36± 4.06 e</td>
<td>0.167± 0.03 bc</td>
<td>0.305± 0.01 bc</td>
<td>36.25± 10.30 d</td>
</tr>
<tr>
<td>Normal treated 70% ethanol extract</td>
<td></td>
<td>38.08± 5.43 bc</td>
<td>0.142± 0.03 c</td>
<td>0.342± 0.04 ab</td>
<td>41.25± 8.54 bcd</td>
</tr>
<tr>
<td>Normal treated petroleum ether extract</td>
<td></td>
<td>35.83± 2.20 bde</td>
<td>0.167± 0.01 bc</td>
<td>0.370± 0.06 a</td>
<td>39.50± 8.22 cde</td>
</tr>
<tr>
<td>Diabetes after 2 days</td>
<td></td>
<td>20.07± 1.44 f</td>
<td>0.052± 0.02 d</td>
<td>0.192± 0.01 d</td>
<td>55.00± 9.13 a</td>
</tr>
<tr>
<td>Diabetes after 10 days</td>
<td></td>
<td>17.79± 0.68 f</td>
<td>0.037± 0.01 d</td>
<td>0.187± 0.01 d</td>
<td>48.75± 11.08 abc</td>
</tr>
<tr>
<td>Diabetes after 40 days</td>
<td></td>
<td>18.32± 0.69 f</td>
<td>0.050± 0.01 d</td>
<td>0.190± 0.01 d</td>
<td>56.30± 2.38 a</td>
</tr>
<tr>
<td>Diabetes treated chloroform extract</td>
<td></td>
<td>40.94± 2.29 b</td>
<td>0.197± 0.02 a</td>
<td>0.282± 0.04 c</td>
<td>51.25± 8.54 abc</td>
</tr>
<tr>
<td>Diabetes treated 70% ethanol extract</td>
<td></td>
<td>34.12± 1.18 cde</td>
<td>0.150± 0.02 c</td>
<td>0.357± 0.02 a</td>
<td>52.50± 6.45 ab</td>
</tr>
<tr>
<td>Diabetes treated petroleum ether extract</td>
<td></td>
<td>52.90± 6.22 a</td>
<td>0.165± 0.01 bc</td>
<td>0.335± 0.02 ab</td>
<td>35.00± 8.16 d</td>
</tr>
<tr>
<td>Diabetes treated Gliclazide (Ref.Drug)</td>
<td></td>
<td>37.60± 2.06 bcd</td>
<td>0.180± 0.01 ab</td>
<td>0.372± 0.03 a</td>
<td>53.92± 1.78 a</td>
</tr>
<tr>
<td>LSD 5%</td>
<td></td>
<td>5.13</td>
<td>0.029</td>
<td>0.044</td>
<td>11.95</td>
</tr>
</tbody>
</table>

Enzymes (α-amylase, β-glactosidase and α-glucosidase) are expressed in µmole/mg protein /min
Total protein is expressed in mg /g tissue
Data are mean ±SD of 7 rats in each group.
Statistical analysis is carried out using one way analysis of variance (ANOVA) using Co-Stat computer program.
Unshared superscript letters between treatments are significance values at P<0.001.

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Table (6): Evaluation of successive and 70% ethanol extracts of *Nepeta cataria* on body weight, liver weight and liver weight/body weight ratio in control, normal-treated, diabetic and diabetic-treated groups

<table>
<thead>
<tr>
<th>Treatments</th>
<th>Body weight</th>
<th>Liver weight</th>
<th>Liver weight/body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control zero time</td>
<td>151.50 ± 1.29 b</td>
<td>5.925 ± 0.25 def</td>
<td>0.04 ± 0 c</td>
</tr>
<tr>
<td>Normal control after 2 days</td>
<td>151.00 ± 5.47 b</td>
<td>6.0375 ± 0.149 def</td>
<td>0.04 ± 0 c</td>
</tr>
<tr>
<td>Normal control after 10-days</td>
<td>158.50 ± 3.10 b</td>
<td>6.225 ± 0.22 def</td>
<td>0.04 ± 0 c</td>
</tr>
<tr>
<td>Normal control after 40-days</td>
<td>181.00 ± 2.58 a</td>
<td>6.9025 ± 0.297 cd</td>
<td>0.04 ± 0.008 c</td>
</tr>
<tr>
<td>Normal treated chloroform extract</td>
<td>186.500 ± 10.96 a</td>
<td>7.81 ± 0.64 bc</td>
<td>0.04 ± 0 c</td>
</tr>
<tr>
<td>Normal treated 70% ethanol extract</td>
<td>187.75 ± 3.5 a</td>
<td>8.50 ± 0.37 ab</td>
<td>0.0475 ± 0.005 bc</td>
</tr>
<tr>
<td>Normal treated petroleum ether extract</td>
<td>184.00 ± 4.69 a</td>
<td>7.7925 ± 0.80bc</td>
<td>0.04 ± 0 c</td>
</tr>
<tr>
<td>Diabetes after 2 days</td>
<td>142.25 ± 4.34 c</td>
<td>7.665 ± 0.87 bc</td>
<td>0.0525 ± 0.005 b</td>
</tr>
<tr>
<td>Diabetes after 10-days</td>
<td>112.75 ± 8.77 e</td>
<td>9.555 ± 1.86 a</td>
<td>0.085 ± 0.0129 a</td>
</tr>
<tr>
<td>Diabetes after 40-days</td>
<td>98.50 ± 5.68 f</td>
<td>8.425 ± 0.419 ab</td>
<td>0.0875 ± 0.005 a</td>
</tr>
<tr>
<td>Diabetes treated chloroform extract</td>
<td>123.50 ± 6.02 d</td>
<td>7.0075 ± 2.248 cd</td>
<td>0.055 ± 0.017 b</td>
</tr>
<tr>
<td>Diabetes treated 70% ethanol extract</td>
<td>123.75 ± 3.68 d</td>
<td>5.2575 ± 0.50 ef</td>
<td>0.0425 ± 0.005 c</td>
</tr>
<tr>
<td>Diabetes treated petroleum ether extract</td>
<td>131.000 ± 4.24 d</td>
<td>5.00 ± 0.467 f</td>
<td>0.0425 ± 0.005 c</td>
</tr>
<tr>
<td>Diabetes treated Gliclazide (Ref.Drug)</td>
<td>153.25 ± 2.36 b</td>
<td>6.30 ± 0.29 de</td>
<td>0.04 ± 0 c</td>
</tr>
</tbody>
</table>

Body weight, liver weight and liver weight/body weight ratio are expressed in g
Data are mean ±SD of 7 rats in each group.
Statistical analysis is carried out using one way analysis of variance (ANOVA) using Co-Stat computer program.
Unshared superscript letters between treatments are significance values at P<0.001.

![Graph showing glucose and insulin levels](image)

Fig. (1): % change of *Nepeta cataria* extracts on blood glucose and insulin levels in normal control and various treated groups.
Fig. (2): % change of *Nepeta cataria* extracts on lipid profile in serum of normal control and various treated groups.

Fig. (3): % change of *Nepeta cataria* extracts on AST, ALT, ALP and total protein in normal control and various treated groups.
Fig. (4): % change of *Nepeta cataria* extracts on nitric oxide (NO) level in liver of normal control and various treated groups.

Fig. (5): % change of *Nepeta cataria* extracts on α-amylase, β-galactosidase and α-glucosidase in normal control and various treated groups.
Fig. (6): % change *Nepeta cataria* extracts on body weight, liver weight and liver weight/body weight ratio in control, different normal-treated, diabetic and diabetic-treated groups.

Fig 7: Photomicrograph in the islet of Langerhans of normal control rats showing normal cellular elements (Hx. E stain X200)

Fig 8: Photomicrograph in the islet of Langerhans of normal-treated petroleum ether showing normal different cellular elements with a typical morphology and without any lymphoid infiltration (Hx. E stain X200)

Fig 9: Photomicrograph in the islet of Langerhans of normal -treated chloroform extract showing normal different cellular elements (Hx. E stain X200)

Fig10: Photomicrograph in the Islet of Langerhans of normal treated - 70%ethyl alcohol showing normal different cellular elements with a typical morphology and without any lymphoid infiltration (Hx. E stain X200).
Fig 11: Photomicrograph of section in the islet of Langerhans after 2 days of STZ injection showing degenerative changes and decrease in number of β-cells (Hx. E stain X200).

Fig 12a: Photomicrograph in the islet of Langerhans at day 10 post STZ injection showing degenerative cells (Hx. E stain X200).

Fig 12b: Photomicrograph in the islet of Langerhans after 10 days of STZ injection showing degenerative changes and decrease in islet size and β-cells number (Hx. E stain X200).

Fig 13a: Photomicrograph in the islet of Langerhans of diabetic rats (after 40 days of STZ injection) showing imperfections with lymphoid infiltration, atrophic changes and only small regions with preserved structure. (Hx. E stain X200).

Fig 13b: Photomicrograph in the islet of Langerhans after 40 days of STZ injection showing degenerative cells decrease in β-cells number (Hx. E stain X200).

Fig 14: Photomicrograph in the islet of Langerhans of chlorophorm extract-treated diabetic rats showing less vaculation and more healthy beta cells (Hx. E stain X200).

Fig 15: Photomicrograph in the islet of Langerhans of petroleum ether treated diabetic rats showing no observed cellular changes (Hx. E stain X200).
Fig 16: Photomicrograph in the islet of langerhans of diabetic rats treated with 70% ethyl alcohol extract showing no observed cellular changes (Hx. E stain X200).

Fig 17: Photomicrograph section in the islet of langerhans in diabetic rats treated with Gliclazed antidiabetic drug, showing less cellular vaculation and more healthy beta cells (Hx. E stain X200).

Fig 18: A photomicrograph of control rat liver section showing normal hepatic cells, sinusoidal space and central vein. (HX & E x200).

Fig 19: A photomicrograph of normal rat liver section treated with petroleum ether showing normal hepatic cells, sinusoidal space and central vein. (HX & E x200).

Fig 20: A photomicrograph section of normal rat liver section treated with chloroform showing normal hepatic cells, sinusoidal space and central vein. (HX & E x200).

Fig 21a: A photomicrograph section of normal rat liver section treated with total extract showing normal hepatic cells, sinusoidal space and central vein. (HX & E x200).

Fig 21b: A photomicrograph of normal rat liver section treated with 70% ethanol extract, showing normal hepatic cells, sinusoidal space and central vein. (HX & E x200).

Fig 22a: A photomicrograph of rat liver section after 2 days of STZ injection, exhibited hepatocyte degeneration and necrosis (HX & E x200).

Fig 22b: Photomicrograph of rat liver section after 2 days of STZ injection showing degeneration of hepatocytes (Hx. E stain X200).

Fig 23a: Photomicrograph of rat liver section after 10 days of STZ injection showing degeneration of hepatocytes and congestion of central vein (Hx. E stain X 200).
Fig 23 b: A photomicrograph section of rat liver section after 10 days of STZ injection (H & E X 200) exhibited hepatocyte degeneration, necrosis and congestion of central vein.

Fig 24: A photomicrograph section of rat liver after 40 days of STZ injection exhibited severe hepatocyte degeneration, necrosis and congestion of central vein (H & E X 200).

Fig 25: A photomicrograph section of diabetic rat liver treated with petroleum ether, showing moderate degeneration of hepatic cells and un-congested central vein. (H & E X 200).

Fig 26: A photomicrograph section of diabetic rat liver treated with chloroform extract, showing mild hepatocyte degeneration, less congested and dilated central vein and blood sinusoids (H & E X 200).

Fig 27 a: A photomicrograph of diabetic rat liver section treated with 70% ethanol extract, showing moderate degeneration of hepatic cells. (H & E X 200)

Fig 27b: A photomicrograph of diabetic rat liver section treated with 70% ethanol extract, showing, almost normal cell architecture with dilated blood sinusoids (H & E X 200).

Fig 28: A photomicrograph of diabetic rat liver section treated with Gliclazide drug, showing near normal hepatic cells, sinusoidal space and central vein (H & E X 200).

4. Discussion:
Diabetes mellitus (DM) is a chronic disease caused by inherited or acquired deficiency in insulin secretion (IDDM) or by decreased responsiveness of the organs to secreted insulin (Non IDDM), resulting in increased blood glucose level. This, in turn, can damage many of the body's systems, including blood vessels, nerves and causes oxidative tissue damage (Matsui et al., 2007).

Reactive oxygen species (ROS), superoxide anion, hydrogen peroxide and hydroxyl, nitric oxide and peroxynitrite radicals, play an important role in oxidative stress related to the pathogenesis of various important diseases as diabetes. In healthy individuals, the production of free radicals is balanced by the antioxidative defense system, while defense system is greatly affected during diseases (Shyur et al., 2005).

The potential role of the medicinal plants as hypoglycemic agents has been reviewed by several authors, supported by the ethnobotanical surveys and traditional medicines of different cultures (Yeh et al., 2003; Biesalski, 2004; Li et al., 2004).

The current study has demonstrated insignificant change between normal-control and different normal-treated groups (G2-G4) either in blood glucose or insulin levels except for normal rats treated with 70% ethanol extract that, recorded significant decrease in blood glucose level (13.69%). Injection of rats with STZ (G5-G7) induced significant elevation in fasting blood glucose with concomitant reduction in insulin levels at day 2, 10 and 40 post STZ treatment as compared to control untreated group (G1) (from 109.5± 6.65 mg/dl to 373.00±2.94, 363.00±2.94 and 364.25±3.77 mg/dl,
respectively for glucose and 5.35 µU/ml to 0.59 ±0.01, 0.34±0.05 and 0.35±0.01 µU/ml for insulin, respectively). These histological examinations at the cellular level, reveal atrophy, necrosis and degenerative changes in both hepatocytes and β-cells of pancreas, indicating establishment of diabetic state (Figs 11-13 ; 22-24 pancreas and liver respectively). Holemans et al. (1997) demonstrated that, Streptozotocin induced beta cells destruction by necrosis; it is an antibiotic and anticancer agent which is widely used for inducing diabetes (Type 1 IDDM) in several animal. It interferes with cellular oxidative mechanisms (Bagri et al., 2008). It selectively induces degenerative alterations and necrosis of pancreatic β-cells resulting in insulin deficiency and impairment in glucose oxidation (DeCarvalho et al., 2005). Ikehukuro et al. (2002) have reported that, the use of lower dose of Streptozotocin produced an incomplete destruction of pancreatic β-cells even though rats became hyperglycemic. In accordance to the present study, Mitra et al. (1996) earlier reported that, the diabetic liver showed degeneration and congestion two hours after injection of STZ, hyperglycemia is observed with a concomitant drop in blood insulin level. The changes in blood glucose and insulin concentrations reflect abnormalities in beta cell functions. The fluctuation in the blood sugar might also be attributed to the sensitivity of STZ that varies with species, strain, sex and nutritional state and there are batch differences in activity (Mir et al., 2008). In a good agreement with the present results, Akbarzadeh et al. (2007) confirmed the destruction of islet cells in pancreatic biopsies of diabetic rats due to the effect of Streptozotocin and added that 60 mg /kg dose of STZ ensured induction of diabetes in rats and hyperglycemia, hypoinsulinemia, polyphagia, polyuria and polydipsia were seen in adult rats within 3 days of STZ treatment and the amounts of these relevant factors were almost stable, which indicates irreversible destruction of langerhans islets cells. Previous studies have reported that, Streptozotocin enters the beta cells via a glucose transporter and causes alkalylation of DNA. DNA damage induces activation of poly ADP ribosylation, a process that is more important for the diabetogeneity of Streptozotocin than DNA damage itself. Poly ADP- ribosylation leads to depletion of cellular NAD and ATP. Enhanced ATP dephosphorylation after Streptozotocin treatment supplies a substrate for xanthine oxidase resulting in the formation of super oxide radicals. Consequently, hydrogen peroxide and hydroxyl radicals are generated. Furthermore Streptozotocin liberates toxic amounts of nitric oxide that inhibits aconitase activity and participates in DNA damage (Fiordaliso et al., 2000). With respect to lipid state, degradation of liver glycogen and gluconeogenesis are increased while glucose utilization is inhibited. Glucose -6-phosphatase increases in the liver, facilitating glucose release into the blood. The opposing enzyme which phosphorlyates gluconeogensis are increased while glucose utilization is inhibited. The authors added that inhibition of disaccharide enzymes sucrase and maltase seems to be one of the factors which explain the hypoglycemic action of many antidiabetic plants, which have an antihyperglycemic action to increase th e insulin sensitivity of target tissues and the antihyperglycemic effect to increase the insulin sensitivity of target tissues and the antihyperglycemic effect to increase the insulin sensitivity of target tissues and the antihyperglycemic effect to increase the insulin sensitivity of target tissues and the antihyperglycemic effect to increase the insulin sensitivity of target tissues and the antihyperglycemic effect to increase the insulin sensitivity of target tissues and the antihyperglycemic effect to increase the insulin sensitivity of target tissues and the antihyperglycemic effect to increase the insulin sensitivity of target tissues and the antihyperglycemic effect to increase the insulin sensitivity of target tissues and the antihyperglycemic effect 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deficiency results in failure to activate the enzyme, thereby causing hyper-triglyceridemia (Shirwaikar et al., 2004). On the other hand, in insulin deficiency, the plasma free fatty acids concentration is elevated as a result of increased free fatty acids outflow from fat depots, where the balance of the free fatty acids esterification, triglycerides lipolysis is displaced in favors of lipolysis (Shirwaikar et al., 2004). Also elevated cortisol promotes the liberation of free fatty acids from adipose tissue into blood stream by inducing and maintaining the synthesis of the hormone sensitive lipase, thus increasing free fatty acids level which contribute to cardio-vascular risk (Lundberg, 2005).

The elevation in cardioprotective HDL-C means increase of cholesterol efflux from the tissues, the first step in reverse cholesterol transport from the peripheral tissues to the liver. The antioxidant and antiatherogenic activities of HDL-C are enhanced when its circulating level is increased. LDL-C particles become smaller and dense which enhances fibrinogen, thus leading to a diabetic complication (Kalousova et al., 2002). In addition, Mir et al. (2008) reported hypercholesterolemia, hypertriglyceridemia associated with DM and explained these increments at the basis of Streptozotocin induced diabetes. There is excess of fatty acids in the serum, which promotes conversion of excess fatty acids into phospholipids and cholesterol in liver. These two substances along with excess triglycerides formed at the same time in liver may be discharged into blood in the form of lipoproteins.

In parallel results, Mir et al. (2008) found high concentration of total lipid in serum of diabetic rabbits and attributed this elevation mainly to increase mobilization of free fatty acids from the peripheral fat depots, since insulin inhibits the hormone sensitive lipase.

Significant amelioration in lipid indices was observed after treatment of diabetic rats with the test extracts (petroleum ether, chloroform and 70% ethanol) with percentage of amelioration amounting to 71.51, 62.13, 67.75 %, respectively for total cholesterol as compared to 127.15% in Gliclazide-treated diabetic group). HDL-cholesterol recorded enhanced level of 41.02, 39.35, 43.00 %, respectively as compared to 44.00% for Gliclazide. The improved level of LDL-cholesterol recorded to 122.73, 110.64, 118.72 %, respectively (153.74 %for Gliclazide). Triglycerides showed normalized level of 165.05, 169.18, 176.77% respectively and 166.17 % for Gliclazide. Total lipid recorded percent of improvement amounted to 40.19, 44.42, 49.33%, respectively (58.25% for Gliclazide). In concomitant with the present results, El-Hilaly and Lyoussi (2002); Brinker et al. (2007) found relative high percentage of essential oil with nerol, geraniol and citral as well as ursoic acid, polyphenols (flavonoids, phenolic acid ) and steroids in Nepeta cataria (Labiatae) which may related to hypolipidemic and cholesterol –lowering effect. The mechanism(s) of hypolipidemic effect of many medicinal plants such as Ajuga Iva (L). Schreber L. (Labiatae). was mediated through insulin –enhancing lipolytic activity by inhibition of hormone –sensing lipase (Al-Shamaony et al., 1994) or lipogenic enzymes (Pari ;Venkates, 2004) , and / or activation of lipoprotein lipase (Ahmed et al., 2001). In addition, the hypolidemic lowering effect may be related to several active constituents extracted such as diglycerides, eddyzones, eddyestones, iridoides, phenylcarboxylic acids, steroid compounds which is considered as anti-inflammatory agents (Brinker et al., 2007) , thus the mechanism(s) of action of such family (Labiatae) as antihyperlipidemia may involve insulin – like effect (Kushbukhtova et al., 2001).

However Sethi et al. (2004) found that, leaves of Ocimum sanctum (Labiatae) significantly reduce lipid profile in serum and tissue in normal and diabetic rats through inhibition of oxygen free radical incorporated in pathogenesis of diabetes and enhancement of cellular enzymatic (SOD) and non enzymatic antioxidants (GSH).

The present results demonstrate, insignificant change in AST level in serum of normal treated rats either with chloroform or total ethanol extracts, while significant inhibition was noticed with petroleum ether extract. Serum total protein content and ALT showed insignificant change in different normal concentrations of liver and pancreas cells and hence normalization of liver enzymes (El Hilaly and Lyoussi , 2002; Li et al., 2004; Zheng et al. 2007; Gilani et al., 2009).

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Significant decrease was observed in serum total protein content in different-diabetic treated groups as compared to both normal control and Gliclazide-treated diabetic group. In contrast, Otuski and Williams (1982); Sethi et al. (2004) demonstrated enhanced level of serum proteins post treatment of diabetic rats with aqueous extract of Ocimum sanctum (Labiatae) and attributed this effect to insulin-like factors contained in the extract, since insulin is reported to increase protein synthesis. In addition, the total thiols in Labiatae family play a vital role in the structure, activity and transport function of proteins, membranes and enzymes.

With respect to oxidative stress marker NO, insignificant change was observed in normal control treated group as compared to untreated one, while a significant increase is noticed in various diabetic groups. It was reported that NO over production has been linked to a variety of clinical inflammatory diseases (Kim et al., 2002). Experimental studies suggested that antioxidants, free radical scavengers and metal chelators exist that may be responsible for increased liver injury (Ma et al., 1995). The direct toxicity of NO is enhanced by reacting with superoxide radical to give powerful secondary toxic oxidizing species, such as peroxynitrite (ONOO) which is capable of oxidizing cellular structure and causes lipid peroxidation (Sayed Ahmed et al., 2001), a process leading to membrane damage and considered the proximal cause of cell death. Lipids peroxidation can damage protein, lipid, carbohydrates and nucleic acids. Also, it has been found that lipid peroxidation is one of the risk factor of protein degradation. The present results indicate, significant elevation in NO in liver of diabetic rats. This increment may be due to oxidative stress which is considered as one of the necessary causative factors that link diabetes with the pathogenic complications of several tissues (Anwar and Meki, 2003).

Significant improvement in NO level post various treatments with percent amounted 146.15, 176.92, 169.23% for chloroform, petroleum ether and 70% ethanol extracts respectively and 169.23% for Gliclazide. In concomitant with the present research, Sethi et al. (2004); Vats et al. (2004) found that treatment of diabetic rats either with aqueous or ethanolic extracts of Ocimum sanctum (OS -Labiatae) significantly increased activity of two antioxidant enzymes in liver, namely, superoxide dismutase (SOD) and catalase. The protective effect of the plant extracts can be brought about directly by scavenging free radicals or indirectly by elevating glutathione levels (GSH). GSH protects the cell against oxidative stress by reacting with peroxides and hydroperoxides. SOD detoxifies superoxide radicals and converts them to H₂O₂ which is further converted to H₂O by catalase. Thus, the antioxidant and antiproliferative activity of OS is partly due to its antioxidant and antiproliferative activity and partly due to scavenging free radicals. Furthermore, it has been proposed that these factors, both antioxidant and antiproliferative, play an important role in the antihyperglycemic activity of OS. In normal control treatment, there was a decrease in amylase level in pancreas, parotid glands and liver of diabetic rat, significant inhibition was demonstrated that is in vivo.

Significant improvement in all carbohydrate hydrolyzing enzymes post chloroform, petroleum ether as well as 70% ethanol extracts amounted 69.58, 106.37, 48.60%, respectively for amylase (as compared to 59.30 % for Gliclazide); 33.82, 53.31, 61.39 % respectively for α-glucosidase comparing to 66.91% in Gliclazide-treated diabetic group and 88.02, 68.88, 59.88% respectively for β-galactosidase (77.84% for Gliclazide). The enhanced levels of carbohydrate hydrolyzing enzymes α-amylase, α-glucosidase and β-galactosidase may be related to the phenolic compounds inhibited the disaccharide enzyme activities as mucosal sucrase and maltase. The inhibition of glycogenic activity of brush border enzymes by polyphenolic compounds seems to be one of the factors which explain the discussed hypoglycemic action of Nepeta cataria (Jurgonski et al., 2008). The improvement in the level of carbohydrate metabolizing enzymes can be also explained at the basis of Nepeta cataria extracts contained fluctuated level of flavonoids. Flavonoids, like other antioxidants may prevent the progressive decline of pancreatic β cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes (Bhandari et al., 2008).
The present results demonstrate, insignificant change in normal -treated body weight, liver weight (except for liver weight treated with 70% ethanol extract which recorded significant increase as compared to normal control at day 40) and liver weight/ body weight ratio post treatment with chloroform ,petroleum ether and 70% ethanol extracts. Gradual significant reduction is noticed in body weight with concomitant increase in liver weight and liver weight / body weight ratio at day 2, 10 and 40 post STZ injection .In concomitant with the present results , several authors (Vats et al., 2004; Akbarzadeh et al., 2007;Mir et al., 2008) reported that diabetes state is usually accompanied by weight loss and increase in liver weight were seen in adult rats within three days of Streptozotocin induction. The literature regarding the effect of diabetes on liver weight is contradictory as some workers have shown an increase in hepatic weight in animals (Chen ; Ianuzzo, 1982;Sadique et al., 1987) as well as human (Van Lancker , 1976) while others have reported no change (Gupta et al., 1999). Exact reasons of hepatic hypertrophy are not known , however fat deposition has been proposed to be the cause. The pattern of increase in liver weight/body weight ratio was manifested by the reduction in body weight and increase in liver weight of diabetic rats (Vats et al., 2004).

Significant amelioration in body weight, liver weight and liver weight/body weight ratio post treatment of diabetic rats with chloroform ,petroleum ether and 70% ethanol extracts as well as Gliclazide as reference drug amounted 13.81, 13.95 , 17.96, 30.25%, respectively for body weight 20.54, 49.62, 45.89 , 30.79% respectively for liver weight and 81.25, 112.5 , 118.75% respectively for liver weight /body weight ratio .The enhancement in body weight may be attributed to anabolic action of ecidyones and ecdysterones found in Lbiatae family (EL Hilaly et al., 2004) and Sembulingam et al.(2005) reported that ethanol extract of Ocimum sanctum treated rats showed higher and significant gain in body weight in comparison to diabetic controls but was lower than in the normal controls. In addition, it prevents increase in organ weight due to the protective action of urosolic acid concerned with free radical inhibition . Based on this findings, increase in organ weight due to the protective action of urosolic acid but was lower than in the normal controls. In addition, it prevents increase in liver weight due to the protective action of urosolic acid but was lower than in the normal controls. In addition, it prevents increase in liver weight due to the protective action of urosolic acid but was lower than in the normal controls. In addition, it prevents increase in liver weight due to the protective action of urosolic acid but was lower than in the normal controls. In addition, it prevents increase in liver weight due to the protective action of urosolic acid but was lower than in the normal controls. In addition, it prevents increase in liver weight due to the protective action of urosolic acid but was lower than in the normal controls. In addition, it prevents increase in liver weight due to the protective action of urosolic acid but was lower than in the normal controls. 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