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 Glicalized 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), aspatrate 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, antilipidemic 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 Glicalized drug. [Journal of American Science 2010;6(8):436-455]. (ISSN: 1545-1003).

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).

Nepata cataria L. (family, Limiaceae; 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 bronchosis (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 , elemol, nerol (Mortuza –Semmani and Saeedi, 2004; Schultz *et al.*, 2004; Sajjadi, 2005). Also, urosolic acid, β–sisoterol, 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 (diamicron) 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, Merck 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 staudensamen, 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 staudensamen , 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 (50 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 body weight dissolved in 0.01M citrate buffer immediately before use, each rat received 9.75 mg /0.5ml citrate buffer) (Vats *et al..*, 2004). After injection, animals had free access to food and water and were given 5% glucose solution to drink overnight to counter hypoglycemic shock (Bhandari *et al*., 2005). After 2 days of STZ injection fasting blood samples were obtained and fasting blood sugar was determined (>300 mg/dl). Hyperglycemic rats were used for the experiment and classified as follows:

Group5: Diabetic +ve control group sacrificed after 2 days of STZ injection

Group 6: Diabetic +ve control group sacrificed after 10 days of STZ injection

Group 7: Diabetic +ve control group sacrificed after 40 days of STZ injection and considered as recovery group.

Group 8: Diabetic animals treated with petroleum ether extract 50mg/kg body weight for 30 days, each rat received 7.5 mg /0.5 ml bidistilled water)
Group 9: Diabetic animals treated with chloroform extract (as previously group).
Group 10: Diabetic animals treated with 70% ethanol extract (as previously group).
Group11: Diabetic animals treated with Glicalized (diamicron) 10 mg/kg body weight (each rat received 1.5 mg/0.5 bidstilled water) and considered as reference drug.
Group 12-14: healthy control groups sacrificed after 2, 10 and 40 days and considered as normal control groups for diabetic groups at the same times.

Sample preparations:

Serum sample: each animal was weighed, 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^{o}C for further determinations of lipid profile, liver function tests, carbohydrate metabolizing enzymes and serum total protein.

Tissue sample: liver tissue was weighed and homogenized in ice cold 0.9 N NaCl, centrifuged at 3000 rpm for 10 min, separated the supernatant and stored at -80^{o}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).

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v- Determination of cholesterol- LDL:
Cholesterol- LDL was calculated according to Friedewald *et al*. (1972).

vi- Determination of triglyceride:

Triglyceride was measured in rat serum by the method of Wahelfed (1974).

vii- Determination of total lipid:

Total lipid was measured in rat serum by the method of Zollner and Kirsch (1962).

viii- Determination of alkaline phosphatase enzyme activity:

Alakaline phosphatase enzyme activity was measured in rat serum by the method of Belfield and Goldberg (1971).

x- Determination of aspartate and alanine aminotransferases (AST and ALT) enzyme activities:

AST and ALT were measured in rat serum by the method of Reitman and Frankel (1957).

xi- Determination of total protein:

Total protein was assayed in rat serum according to the method of Bradford (1976).

xii- Determination of nitric oxide (NO):

NO was measured in liver tissue homogentes according to Moshage *et al.* (1995).

xiii- 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 and Hardisson (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 $p \leq 0.05$ 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 cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides, total lipid, ALT, AST, ALP enzyme activities and total protein content as well as hepatic nitric oxide , α-amaylase, 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 obvious that, there is no 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 Glicalized as a reference drug (+20.55and -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 is shown 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 -15.84% as compared to untreated control group. With regard to diabetic groups, significant elevation in lipid profile is observed 2 days post STZ injection as compared to control group , recorded 202.50±4.5, 81.62 ±3.35, 163.22±4.57, 211.75 ±8.22 and 1687.19 ±202.10 mg/dl with percent of elevation +54.17, +21.75 , +94.75, +90.97 and +72.05% for total cholesterol, HDL-cholesterol, LDL– cholesterol, triglycerides and total lipid, respectively. On the other hand, nearly simultaneously elevated levels in lipid profile is recorded 10 and 40 days post STZ injection with percentage of increase +82.66 , +35.37 ,+151.46 ,+177.57 and +84.12% at 10 day and+82.54,+35.37, +151.46, +178.45 and +84.45% at 40 day respectively. All diabetic – treated groups (G8- G11) show significant enhancement in lipid profile level as compared to the normal control group, since in petroleum ether extract –treated diabetic group, total cholesterol recorded 145.99± 10.97mg/dl with percentage increase amounted +11.15%.While in both 70% ethanolic and chloroform extracts , total cholesterol reached to 150.81±6.46 and 158.15±8.20 mg /dl, respectively with percent +14.82 and +20.41%, respectively. With respect to Gliclazide as reference drug, total cholesterol amounted 72.75±0.96 mg/dl with percent of reduction - 44.61% as compared to control group. Concerning HDL –cholesterol nearly similar results is obtained for different extracts-treated diabetic groups and Gliclazide as reference drug, where insignificant change is observed either compared to each other or to control group. In addition, 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 triglycerides 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 1373.17±41.65, 1414.58±195.92 , 1325±99.71 and 1237.50±33.04 for petroleum ether , chloroform , 70% ethanol extracts and Gliclazide drug as compared to normal control(980.62±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 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 with percentage increase +20.80, +42.92 and +49.12% 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, +52.50 and +55.00% respectively. ALP shows a value of 4.51±0.36, 5.55±0.60 and 5.61±0.13 µmole/mg protein/min with percentage of elevation +33.04, +63.72 and +65.49% respectively. Total protein content shows insignificant change at day 2 and 10 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 ethanol 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 pronounced effect for 70% ethanol and 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%.

The level of carbohydrate hydrolyzing enzymes, α-amylase, β-galactosidase and α-glucosidase in liver tissue homogenates of the different studied groups is recorded in Table (5) and Fig (5). Careful inspection of the data would reveal that, α - amylase and β-galactosidase 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±0.04 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 -21.0, -27.5 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, 17.79±0.68 and 18.32±0.69 µmole/ mg protein/min for α-amylase with percent of reduction -38.27, - 45.28 and -43.65% respectively. While, β-galactosidase enzyme activity recorded 0.052±0.02, 0.037±0.01 and 0.050±0.01 µmol/mg protein / min with percent -68.86, -77.84 and -70.06%, respectively. In addition, α-glucosidase shows a value of 0.192±0.01, 0.187±0.01 and 0.190± 0.01 µmole/mg protein /min with percent -29.41, -31.25 and -30.15 % respectively. On the contrary, total protein content shows insignificant change at the different durations post STZ injection.

Treatments of the diabetic rats with the different extracts of *Nepeta cataria* produce obvious enhancement in all carbohydrate hydrolyzing enzymes tested. This can be easily seen through normalization of α-amylase level to show insignificant change as a result of diabetic rats treatments with 70% ethanol extract and as compared to either normal or Glicalized-treated diabetic groups. While α-amylase shows significant increase post treatments with petroleum ether (52.90±6.22 µmole /mg protein/min) and chloroform extracts (40.94±2.2 µmole/mg protein/min) with percentage increase +62.72 and +25.93% respectively. The curative effect of the different extracts can be also seen through improvement in β- galactosidase enzyme activity that is returned to its normal value as compared to both normal control and reference drug. In addition, α-glucosidase activity shows insignificant change post

chloroform 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. Concerning total protein content, insignificant change is observed in all diabetic –treated groups except in petroleum ether extract, significant reduction is obtained amounted 35.00 ±8.16 mg /g tissue with reduction percent -30% as compared to normal control.

Table (6) and Fig (6) illustrate body weight, liver weight and liver weight / body weight ratio in control and different treated groups. It is obvious that body weight of normal control rats recorded significant increase (181.00 ±2.58 gm) with concomitant increase in liver weight (6.90±0.297) at day 40 post experiment as compared to body weight and liver weight at zero time with percentage increase amounted +19.47 and +16.50 % respectively. While, body weight and liver weight show insignificant change at other durations (2 and 10 days). Moreover, normal control liver weight/body weight ratio shows insignificant change at different durations as compared to zero time. On the other hand, body weight and liver weight /body weight ratio of normal rats treated - chloroform, 70% ethanol and petroleum ether extracts show insignificant change either as compared to normal control at 40 day or as compared to each other. Normal liver weight of rats treated with 70% ethanolic extract exhibits 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 ascertained 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 manipulated significant increase of 7.67 ± 0.87, 9.56±1.86 and 8.25±0.42 g with percent +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 extract- treated diabetic 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 percentage -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 (0.055±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 un-treated one. Gradual cellular changes include imperfection, reduction in β-cells numbers, degeneration and atrophic changes are appears in pancreas of Streptozotocin - treated groups 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

Parameters \ Treatments	Glucose	Insulin
Normal control	109.5 ±6.65 de	5.35± 0.62 a
Normal treated chloroform extract	105.25± 7.36 de	5.21 ±1.07 ab
Normal treated 70% ethanol extract	94.50 ±16.05 f	5.07 ±1.47 ab
Normaltreated petroleum ether extract	113.75 ±1.25 d	5.03 ±1.66 abc
Diabetes after 2 days	373.00± 2.94 a	0.59± 0.01 e
Diabetes after 10 days	363.00± 2.94 b	0.34± 0.05 e
Diabetes after 40 days	364.25± 3.77 ab	0.35 ±0.01 e
Diabetes treated chloroform extract	133.00± 5.35 c	3.83 ±0.59 d
Diabetes treated 70% ethanol extract	103.00± 2.44 ef	4.59± 0.59 abcd
Diabetes treated petroleum ether extract	128.25± 2.87 c	4.13± 0.57 bcd
Diabetes treated gliclazide (Ref.Drug)	132.00± 2.44 c	3.87± 0.05cd
LSD 5%	9.08	1.19

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.

Parameters \ Treatments	T- cholesterol	HDL- Cho	LDL- Cho	Tri glycride	Total lipid
Normal control	131.34± 6.26 e	67.04±3.00 cd	83.81± 2.27 d	110.88±7.36 de	980.62± 30.71 d
Normal treated chloroform extract	126.72± 6.16 e	65.45 ±3.89 cd	81.99± 5.27 d	103.61± 7.55 ef	1033.50± 23.70 d
Normal treated 70% ethanol extract	128.79± 7.54 e	69.00 ±4.54 c	80.01± 7.29 d	93.32 ±22.07 f	931.94± 145.94 d
Normal treated petroleum ether extract	125.50± 6.40 e	65.00 ±4.16 cd	83.30± 6.08 d	114.06 ±11.09 cde	1049.00± 11.04 d
Diabetes after 2 days	202.50± 4.51 b	81.62± 3.35 b	163.22± 4.57 b	211.75± 8.22 b	1687.19 ±202.10 a
Diabetes after 10 days	239.91 ±10.89 a	90.75 ±1.89 a	210.72 ±9.96 a	307.77± 8.60 a	1805.55 ±103.93 a
Diabetes after 40 days	239.75 ±4.11 a	90.75±0.95 a	210.75± 4.98 a	308.75 ±1.25 a	1808.75± 8.54 a
Diabetes treated chloroform extract	158.15± 8.20 c	64.37±4.53 cd	118.02± 11.50 c	121.16± 4.65 cd	1414.58 ±195.92 b
Diabetes treated 70% ethanol extract	150.81± 6.46 cd	61.92 ±6.46 d	111.25± 2.15 c	112.72± 5.57 cde	1325.00 ±99.71 bc
Diabetes treated petroleum ether extract	145.99± 10.97 d	63.25 ±7.36 cd	107.89± 14.17 c	125.74±10.83 c	1373.17± 41.65 bc
Diabetes treated gliclazide (Ref.Drug)	72.75 ±0.96 f	61.25± 0.95c	81.90± 1.68 d	124.50± 4.20 cd	1237.50± 33.04 c
LSD 5%	10.27	6.06	10.7	14.07	153.84

Lipid profile (total chloesterol,HDL-cholesterol,LDL-cholesterol,Triglycrides 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

Parameters \ Treatments	AST	ALT	ALP	Total Protein
Normal control	2.26 ±0.02 c	1.60± 0.09 cd	3.39± 0.13 c	108.25± 5.67 ab
Normal treated chloroform extract	2.48 ±0.08 bc	1.72± 0.14 c	2.94± 0.40 d	112± 1.41 a
Normal treated 70% ethanol extract	2.50 ±0.07 bc	1.65 ±0.03 cd	2.74± 0.25 de	109± 6.21 ab
Normal treated petroleum ether extract	1.97± 0.25 d	1.64± 0.19 cd	2.78± 0.26 de	102± 7.70 b
Diabetes after 2 days	2.73± 0.24 b	1.97 ±0.09 b	4.51± 0.36 b	104.5± 8.42 ab
Diabetes after 10 days	3.23 ±0.23 a	2.44 ±0.17 a	5.55 ±0.60 a	108.75 ± 6.29 ab
Diabetes after 40 days	3.37 ±0.11 a	2.48 ±0.08 a	5.61 ±0.13 a	84.3± 1.74 c
Diabetes treated chloroform extract	2.48 ±0.36 bc	1.72± 0.04 c	2.41 ±0.08 ef	89.25± 4.92 c
Diabetes treated 70% ethanol extract	2.40± 0.27 c	1.66± 0.04 cd	2.52 ±0.31 def	83.5± 5.06 c
Diabetes treated petroleum ether extract	2.41± 0.13 c	1.59±0.11 cd	2.18± 0.17 f	83.25± 5.37 c
Diabetes treated gliclazide (Ref.Drug)	2.53 ±0.03 bc	1.55±0.04 d	2.19 ±0.08f	101.25 ± 5.05 b
LSD 5%	0.28	0.16	0.42	8.11

AST, ALT and ALP are expressed in µ mole/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

Parameters \ Treatments	NO
Normal control	44.07 ±1.37 d
Normal treated chloroform extract	43.32 ±0.70 d
Normal treated 70% ethanol extract	44.03± 0.96 d
Normal treated petroleum ether extract	43.45 ±1.25 d
Diabetes after 2 days	62.96 ±2.32 b
Diabetes after 10 days	72.55 ±1.87 a
Diabetes after 40 days	72.55 ±2.00 a
Diabetes treated chloroform extract	51.30± 2.69 c
Diabetes treated 70% ethanol extract	43.92± 1.47 d
Diabetes treated petroleum ether extract	43.12 ±0.83 d
Diabetes treated gliclazide (Ref.Drug)	44.75 ±1.70 d
LSD 5%	2.4

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.

Parameters \ Treatments	α- amylase	β- glactosidase	α- glucosidase	Total protein
Normal control	32.51 ±4.49 de	0.167± 0.02 bc	0.272± 0.02 c	50.00±10.80 abc
Normal treated chloroform extract	32.36± 4.06 e	0.167± 0.03 bc	0.305± 0.01 bc	36.25± 10.30 d
Normal treated 70% ethanol extract	38.08± 5.43 bc	0.142± 0.03 c	0.342± 0.04 ab	41.25± 8.54 bcd
Normal treated petroleum ether extract	35.83 ±2.20 bcde	0.167± 0.01 bc	0.370± 0.06 a	39.50 ±822 cd
Diabetes after 2 days	20.07± 1.44 f	0.052± 0.02 d	0.192± 0.01 d	55.00 ±9.13 a
Diabetes after 10 days	17.79 ±0.68 f	0.037± 0.01 d	0.187± 0.01 d	48.75± 11.08 abc
Diabetes after 40 days	18.32 ±0.69 f	0.050± 0.01 d	0.190± 0.01 d	56.50± 2.38 a
Diabetes treated chloroform extract	40.94± 2.29 b	0.197± 0.02 a	0.282± 0.04 c	51.25±8.54 abc
Diabetes treated 70% ethanol extract	34.12 ±4.18 cde	0.150± 0.02 c	0.357± 0.02 a	52.50 ±6.45 ab
Diabetes treated petroleum ether extract	52.90 ±6.22 a	0.165± 0.01 bc	0.335± 0.02 ab	35.00± 8.16 d
Diabetes treated diamicron (Ref drug)	37.60 ±2.06 bcd	0.180± 0.01 ab	0.372± 0.03 a	53.92± 1.78 a
LSD 5%	5.13	0.029	0.044	11.95

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.

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

Parameters \ Treatments	Body weigh	Liver weight	Liver weight/ body weight
Normal control zero time	151.50 ± 1.29 b	5.925 ± 0.25 def	0.04± 0 c
Normal control after 2 days	151.00 ± 5.47 b	6.0375± 0.149 def	0.04 ± 0 c
Normal control after10-days	158.50 ± 3.10 b	6.225 ± 0.22 def	0.04 ± 0 c
Normal control after 40-days	181.00± 2.58 a	6.9025± 0.297 cd	0.04 ± 0.008 c
Normal treated chloroform extract	186.5.00± 10.96 a	7.81± 0.64 bc	0.04 ± 0 c
Normal treated 70% ethanol extract	187.75 ± 3.5 a	8.50± 0.37 ab	0.0475± 0.005 bc
Normal treated petroleum ether extract	184.00± 4.69 a	7.7925± 0.80bc	0.04 ± 0 c
Diabetes after 2 days	142.25± 4.34 c	7.665± 0.87 bc	0.0525± 0.005 b
Diabetes after 10-days	112.75± 8.77 e	9.555 ± 1.86 a	0.085 ± 0.0129 a
Diabetes after 40-days	98.50± 5.68 f	8.425± 0.419 ab	0.0875± 0.005 a
Diabetes treated chloroform extract	123.50± 6.02 d	7.0075± 2.248 cd	0.055± 0.017 b
Diabetes treated 70% ethanol extract	123.75 ± 3.68 d	5.2575± 0.50 ef	0.0425± 0.005 c
Diabetes treated petroleum ether extract	131.00± 4.24 d	5.00± 0.467 f	0.0425± 0.005 c
Diabetes treated gliclazide (Ref.Drug)	153.25± 2.36 b	6.30± 0.29 de	0.04± 0 c
LSD 5%	7.66	1.29	0.009

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.

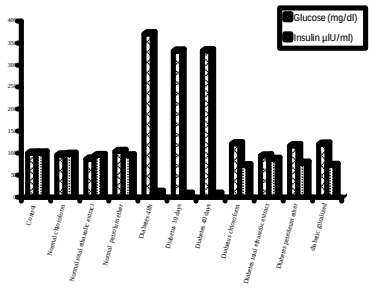

Fig. (1): % change of *Nepeta cataria* extracts on blood glucose and insulin levels in normal control and various treated groups.

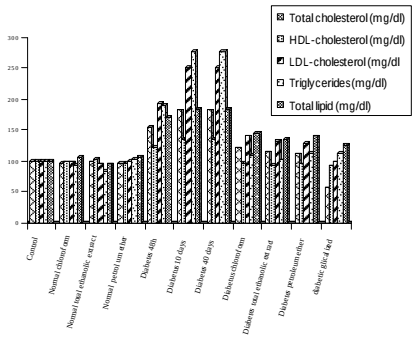

Fig. (2): % change of *Nepeta cataria* extracts on lipid profile in serum of normal control and various treated groups.

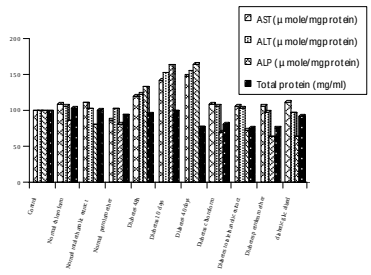

Fig. (3): % change of *Nepeta cataria* extracts on AST,ALT , ALP and total protein in normal control and various treated groups.

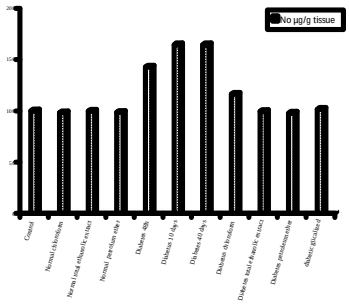

Fig. (4): % change of *Nepeta cataria* extracts on nitric oxide (NO) level in liver of normal control and various treated groups.

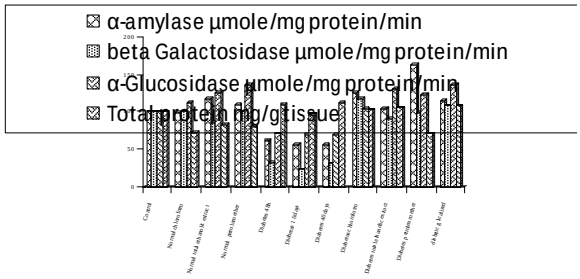

Fig. (5): % change of *Nepeta cataria* extracts on α-amylase , β-galactosidase and α-glucosidase in normal control and various treated groups.

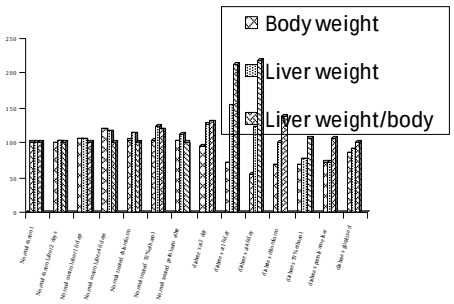

Fig. (6): % change *Nepeta cataria* extracts on body weight, liver weight and liver weight/body weight ratio in control , different normal- treated, diabetic and diabetic- treated groups.

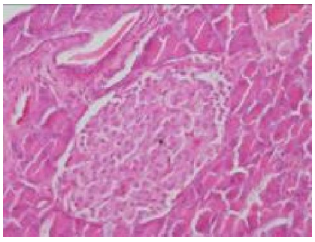

Fig 7: Photomicrograph in the islet of langerhans of normal control rats showing normal cellular elements (Hx. E stain X200)

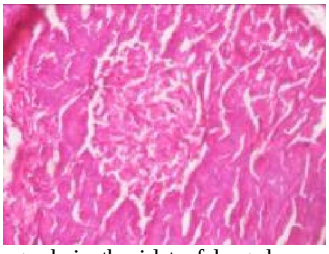

Fig 8: Photomicrograph in the islet of langerhans of normal- treated petroleum ether showing normal different cellular elements with a typical morphology and without any lymphoid infiltration (Hx. E stain X200)

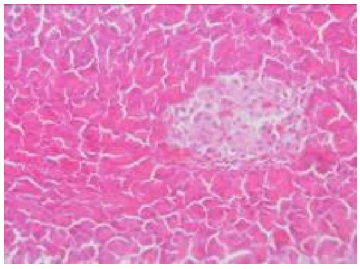

Fig 9: Photomicrograph in the islet of langerhans of normal - treated chloroform extract showing normal different cellular elements (Hx. E stain X200)

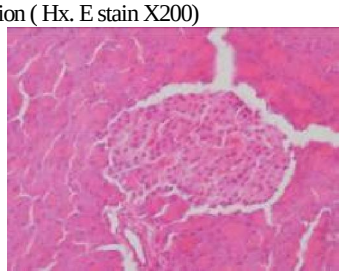

Fig10: Photomicrograph in the Islet of langerhans of normal treated -70%ethyl alcohol showing normal different cellular elements with a typical morphology and without any lymphoid infiltration (Hx. E stain X200).

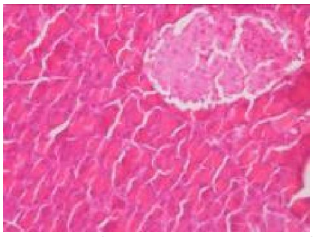

Fig 11: Photomicrograph of section in the islet of langerhans after 2days of s STZ injection showing degenerative changes and decrease in number of β- cells (Hx. E stain X200)

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

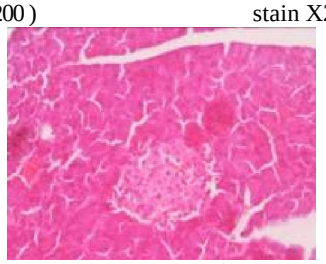

Fig 12b:Photomicrograph in the islet of langerhans after 10 days of STZ injection showing degenerative changes and decrease in islet size and β-cells number (Hx. E stain X200).

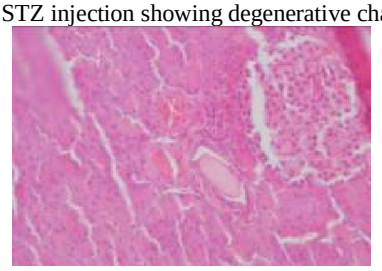

Fig 13a: Photomicrograph in the islet of langerhans of diabetic rats (after 40 days of STZ injection) showing imperfections with lymphoid infiltration , atrophic changes and only small regions with preserved structure..(Hx. E stain X 200).

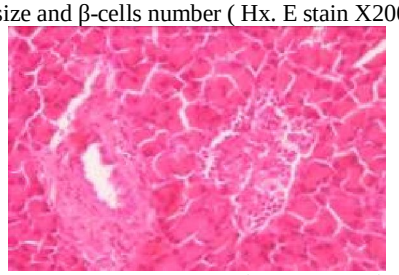

Fig13b: Photomicrograph in the islet of langerhans after 40 days of STZ injection showing degenerative cells decrease in β-cells number (Hx. E stain X200).

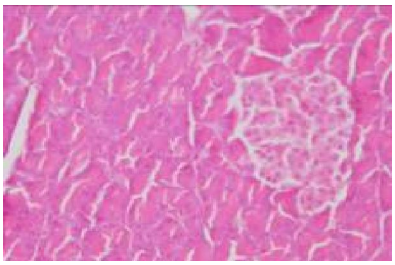

Fig 14: Photomicrograph in the islet of langerhans of cholorophorm extract- treated diabetic rats showing less vaculation and more healthy beta cells (Hx. E stain X200)

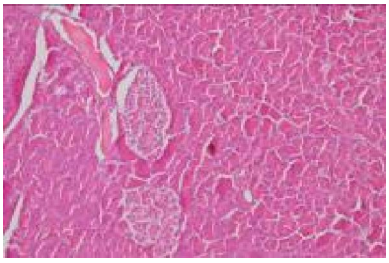

Fig 15: Photomicrograph in the islet of langerhans of petroleum ether treated diabetic rats showing no observed cellular changes (Hx. E stain X 200)

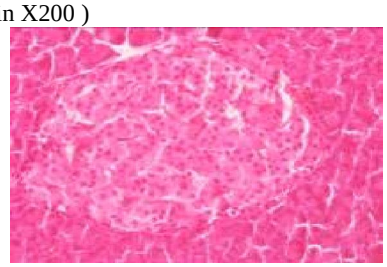

Fig 16: Photomicrograph in the islet of langerhans of diabetic rats treated with 70% ethyl alcohol extract showing no observed cellular changes (Hx. E stain X200)

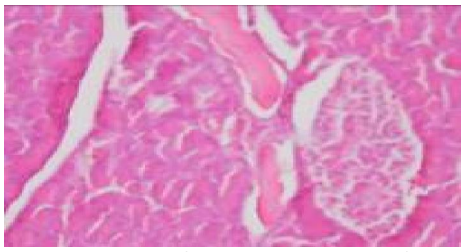

Fig 17: Photomicrograph of section in the islet of langerhans in diabetic rats treated with Glicalized antidiabetic drug , showing less cellular vaculation and more healthy beta cells (Hx. E stain X200)

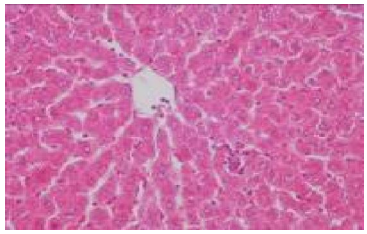

Fig 18: A photomicrograph of control rat liver section showing normal hepatic cells, sinusoidal space and central vein. (HX & E x200).

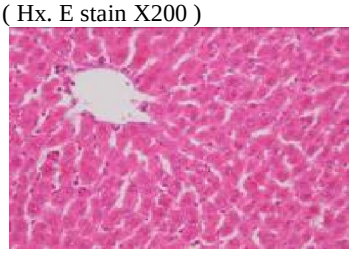

Fig 19: A photomicrograph of normal rat liver section treated with petroleum ether showing normal hepatic cells , sinusoidal space and central vein . (HX & E x200**).**

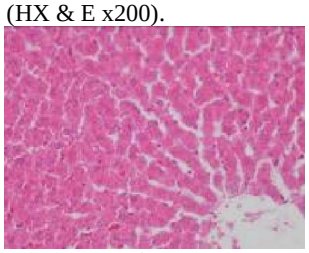

Fig 20: A photomicrograph of normal rat liver section treated with chloroform showing normal hepatic cells , sinusoidal space and central vein . (HX & E x200).

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

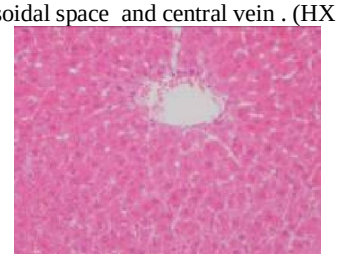

Fig 21 b: A photomicrograph of normal rat liver section treated with 70% ethanol extract, showing normal hepatic cells , sinusoidal space and central vein . (HX & E x200).

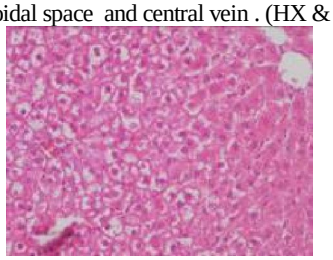

Fig 22 a : A photomicrograph of rat liver section after 2 days of STZ injection , exhibited hepatocyte degeneration and necrosis (HX & E x200).

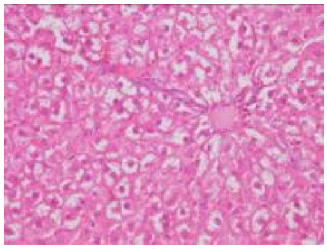

Fig 22b : Photomicrograph of rat liver section after 2 days of STZ injection showing degeneration of hepatocytes (Hx. E stain X200).

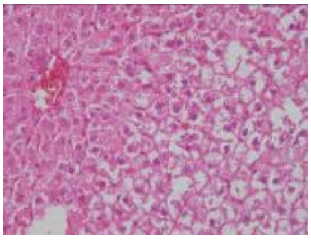

Fig23 a : Photomicrograph of rat liver section after 10 days of STZ injection showing degeneration of hepatocytes and congestion of central vein (Hx. E stain X 200) .

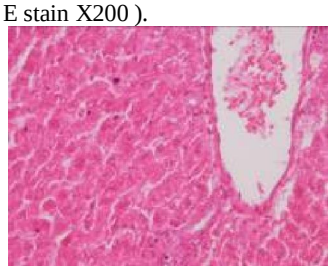

Fig 23 b : A photomicrograph of rat liver t section after 10 days of STZ injection (Hx & E X 200) exhibited hepatocyte degeneration, necrosis and congestion of central vein.

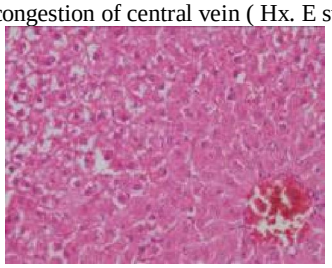

Fig 24: A photomicrograph of rat liver section after 40 days of STZ injection exhibited severe hepatocyte degeneration, necrosis and congestion of central vein (Hx & E X 200)

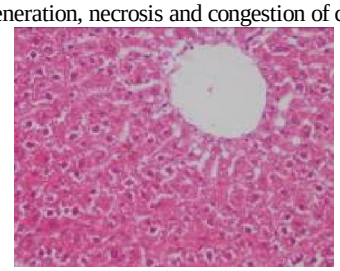

Fig 25: A photomicrograph of diabetic rat liver section treated with petroleum ether, showing moderate degeneration of hepatic cells and un – congested central vein . (Hx & E X 200).

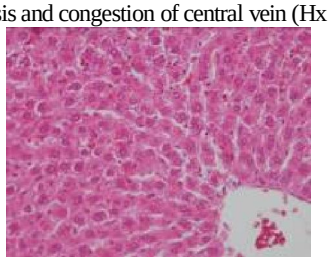

Fig 26:Aphotomicrograph of diabetic rat liver section treated with chloroform extract, showing mild hepatocyte degeneration less congested and dilated central vein and blood sinusoids (Hx & E X 200).

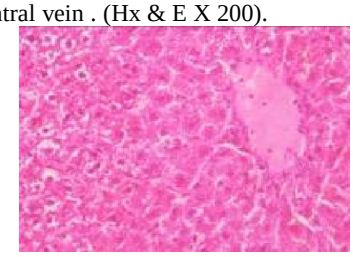

Fig27 a: A photomicrograph of diabetic rat liver section treated with 70% ethanol extract, showing moderate degeneration of hepatic cells. (Hx & E X 200)

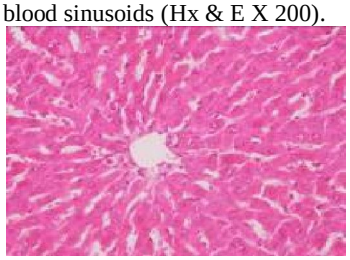

Fig 27b: A photomicrograph of diabetic rat liver section treated with 70% ethanol extract, showing, almost normal cell architecture with dilated blood sinusoids (Hx & E X 200).

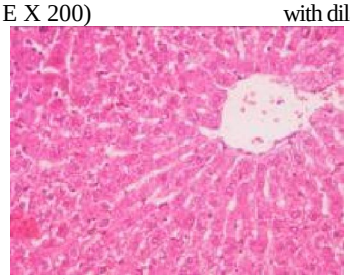

Fig 28 : Aphotomicrograph of diabetic rat liver section treated with Gliclazide drug, showing near normal hepatic cells, ,sinusoidal space and central vein ((Hx& E X200) .

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) (from109.5±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 µIU/ml to 0.59 ±0.01, 0.34±0.05 and 0.35±0.01 µIU/ml for insulin, respectively).

The present 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 a variety of animals. It interferes with cellular metabolic 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). Ikebukuro *et al* . (2002) have reported that, the use of lower dose of Streptozotocin produced an incomplete destruction of pancreatic beta cells even though rats became permanently diabetic. In accordance to the present study, Mitra *et al.* (1996) earlier reported that, diabetic liver showed degeneration and congestation 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. Fluctuation in the blood sugar might also be attributed to sensitivity to 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 biopsy 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 alkylation of DNA. DNA damage induces activation of poly ADP ribosylation, a process that is more important for the diabetogenecity 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. Consequantly, 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).

In diabetes state, degradation of liver glycogen and gluconeogensis 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 glucose, i.e hexokinase, is unaffected by insulin while glucokinase is decreased in diabetes. As a result, the liver continues to produce glucose even with severe hyperglycemia. Under these circumstances the normal liver would shut off and deposit glycogen (Shelia and James, 1993).

The present results reveal, significant amelioration in blood glucose and insulin levels post treatment of diabetic rats with the 70% ethanol, petroleum ether and chloroform extracts of *Nepeta cataria* with percent of improvement amounted 239.68, 215.29 and 211.19 % for glucose and 79.25, 70.65 and 65.05% for insulin, respectively. The current investigation also showed that, treatment of diabetic rats with Gliclazide (reference drug) modulated the alterations in blood glucose and insulin within its normal levels (212.10 and 65.79%, respectively). In

addition, the histological examination showed improvement in hepatocytes and pancreas β- cells (Figs 14-17;25-28). In line with the present study, Vats *et al.* (2004) found that the 70% ethanol extract of *Ocimum sanctum* (Labiatae) leaves significantly improve β–cells function and enhances insulin secretion leading to lowering blood glucose level. These authors added that the antihyperglycemic effect of *Ocimum sanctum* is at least partially dependent upon insulin release from the pancreas and significantly increased the activity of three key enzymes involved in carbohydrate metabolism, namely phosphofructokinase, glucokinase and hexokinase (PFK, GK, HK) towards normal levels. In addition ,it increases glycogen in muscle and liver by stimulating glycogen synthase, suggesting that, the antihyperglycemic action is the result of increased glucose utilization at the level of skeletal muscle as well as liver. 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 plant extracts. Immune-stimulation also might be one of the mechanisms contributing towards the protective actions of *Ocimum sanctum* (Sembulingam *et al.,* 2005). In addition, Zheng *et al.* (2007) studied the hypoglycemic effect of the aqueous ethanol extract of *Prunella vulgaris L.* (Labiatae) in STZ diabetic mice and attributed the hypoglycemic effect to increase the insulin sensitivity of target tissues and the antihyperglycemic effect of insulin is enhanced and prolonged, which could result from increased tissue metabolism or from suppressed levels of non-esterified fatty acids (NEFA). Excess plasma NEFA can inhibit insulin – stimulated glucose utilization in muscle and promote hepatic production of glucose .Whereas, reduction of plasma NEFA concentration improves glucose utilization and enhances the suppression of hepatic glucose production by insulin. Stimulation of glucose uptake by peripheral tissues and inhibition of endogenous glucose production may be involved in hypoglycemic mechanisms of Labiatae family. Some constituents in the *Prunella vulgaris L.* have been identified such as phenolic acids (rosmarinic, caffeic), triterpenoids (methyl oleanolate, methyl ursolate, methyl maslinate), flavonoides (quercetin, campherol, rutin), tannis and polysaccharide . The antihyperglycemic activity of *Prunella vulgaris L.* (Labiatae) may be due to any one or more of the constituents in the extracts. Based on this findings , the hypoglycemic action of the 70% ethanol, petroleum and chloroform extracts of *Nepeta cateria* may be insulin –mediated by mechanism(s) in common with Gliclazide. Another explanation for the hypoglycemic action of *Nepeta cataria* extracts is depend on the presence of antioxidants such as flavonoides which may prevent the progressive impairment of pancreatic beta-cell function due to oxidative stress and may thus reduce the occurrence of type 2 diabetes (Li *et al.,* 2004; Bhandari *et al.,* 2008). *Nepeta cataria* was found to possess relatively high percentage of essential oil (nerol, geraniol and citral as well as ursolic acid) and polyphenols (falvonoids, phenolic acid) and the antihyperglycemic, antisplasmodic and myorelaxant effects may be related to these constituents (Gilani *et al.,* 2009).

With respect to lipid profile, the current results demonstrate, insignificant change in total cholesterol, HDL-cholesterol, LDL-cholesterol and total lipid in different normal- treated groups, with significant reduction in triglycerides in normal – 70% ethanolic extract treated group. Meanwhile, significant elevation in lipid profile was noticed at day 2, 10 and 40 post STZ injections. In addition, to abnormal glucose metabolism, DM often involves abnormal lipid metabolism which is considered as additional metabolic disorder, in diabetic complications. The same results were achieved by Sethi *et al.* (2004) who found significant elevation in lipid profile in serum of diabetic rats. In a good agreement with the present data, some authors revealed that hyperglycemia produced marked increased level of serum triglycerides, total –cholesterol and LDL- cholesterol (LDL-C), while in contrast to the present data HDL-cholesterol (HDL-C) showed reduced concentration in diabetic rats (Abou –Seif and Youssef ,2004 ; Jurgonski *et al.,* 2008). Levinthal and Tavill (1999) reported that, hepatic fat accumulation is a well – recognized complication of DM. The most common clinical presentation in DM is hepatomegaly. This hyperlipidemia associated with DM may be attributed to insulin deficiency (Morel and Chisolm, 1989) and elevated cortisol level, which have an important role in the process of fat accumulation (Hristova and Aloe, 2006). Under normal circumstances insulin activates lipoprotein lipase which hydrolyzes triglycerides. Insulin 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 estrification , 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 afflux 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 small and dense which undergo oxidative modification, 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 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 reached 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 a*l. (2007) found relative high percentage of essential oil with nerol, geraniol and citrol as well as ursolic acid, polyphenols (flavonoids , phenolic acid) and steroids in *Nepeta cataria* (Lbiatae) 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.* (Liabiatae) was mediated through insulin –enhancing lipolytic activity by inhibition of hormone –sensitive 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 hypolidimic lowering effect may be related to several active constituents extracted such as diglycerides , ecdysones , ecdysterones , 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 (Khushbaktova *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 –treated groups. Considering ALP, significant inhibition was noticed in all normal treated groups. With respect to different diabetic- groups, high serum levels of these enzymes at day 2, 10 and 40 post STZ treatment was recorded which are associated with inflammation and /or injury to liver cells , a condition known as hepatocellular liver injury and apopotosis (Fiordaliso *et al.*,2000). Histological examination of diabetic rat liver showed congestion, destruction of cells by necrosis and hepatocytes degeneration. In parallel with the present work, Hickman *et al.* (2008) revealed significant increased activities of serum enzymes relative to their normal levels. Supporting our findings, it has been found that hyperglycemia resulted in hepatolysis reflected by histopathological investigation and increased blood serum aminotransferases as one of the consequences of diabetic complication. The increment of such serum markers may be due to the leakage of these enzymes from the liver cytosol into the blood stream as a result of hepatomegaly (fatty liver) (Muhammad *et al.*, 2008).

The present results reveal also, insignificant change in serum total protein content at day 2, 10 post STZ injection and significant reduction at day 40. In concomitant with the present results , Otsuki ; Williams (1982) found significant reduction in serum total protein concentrations in diabetic rats and this may be due to reduction in the three major phases in protein secretion, intracellular transport and discharge. Also, Alderson *et al.* (2004) demonstrated a significant increase of total protein excretion, albuminuria, glucosuria and urinary urea levels indicating impaired renal function. The reduction in serum total protein content in the present results may be related to reduction in albumin which is the most abundant blood plasma protein (70%) produced in liver. Non –enzymatic glycation of albumin was found the

potential to alter its biological structure and function (Mendez *et al.*, 2005). It is mainly due to the formation of a Schiff base between amino – group of lysine (and sometimes arginine) residues and excess glucose molecules in blood to form glycoalbumin. Elevated glycoalbumin was observed in diabetes mellitus accompanied by decrease in albumin and this is confirmed with early studies suggested that the level of glycosylated albumin may indeed be a sensitive indicator of moderate hyperglycemia and of early glucose intolerance (Miwa *et al.*, 2005). Hypoalbuminemia is one of the factors responsible for the onset of ascites related to liver fibrosis (Horie *et al*., 1998).

Significant improvement in liver function enzyme markers was noticed in treatment of diabetic rats with petroleum ether , chloroform as well as 70% ethanol extracts with percent of amelioration amounted to 42.48, 39.38, 42.92%, respectively for AST as compared to 37.17% for Gliclazide .While ALT recorded 55.63, 47.5, 55.63 %, respectively (58.13 % for reference drug). ALP showed ameliorated level amounted to 94.39, 101.18, 91.15% respectively as well as 100.88% for Gliclazide drug. It was shown that administration of successive extracts and 70% ethanol one to diabetic rats reflect an improvement of cellular damage as determined in the current research by histopathological examination of liver and pancreas (Figs 25-28;14-17) and as shown by normalization of altered liver enzymes in response to diabetic complications. Our results are consistent with previous studies that administration of some antioxidants (as zinc , selenium , vitamin C and E) to diabetic rats , normalized the elevated activities of liver function enzymes AST, ALT, ALP induced in response to diabetes mellitus (Abdel Mageed, 2005). The mechanism of hepatoprotective ability of extracts may be attributed to numerous bioactive compounds such as terpenoids, flavonoids , sterols , essential oil, alkaloids and polysaccharides. Most of them (especially falvonoids, triterpenoids such as ursolic acid) showed a mechanism to improve the function 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).

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, Otsuki; 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 NO 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 glycation. 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 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 peroxiodes and hydroperoxides . SOD detoxifies superoxide radicals and converts them to H_2O_2 which is further converted to H_2O by catalase. Thus, the antihyperglycemic activity of OS supplemented with its adaptogenic and antioxidant activity will be an ideal multi-prolonged treatment for managing diabetes as it will target the stress, catabolism and glycemic effects associated with disease. Moreover, Sembulingam *et al*. (2005) ascertained the reduction in the NO level by the component ursolic acid separated from OS. Essential oil in many species in the Labiatae family are composed of mono –and – sesquiterpenes in addition to phenolic compounds and favonoids such as cinnamic acid, caffeic acid, sinapic acid, ferulic acid

and rosmarinic acid have also been reported as antioxidants , free radical scavengers and metal chelators (Manosroi *et al*., 2006). In this context, Tepe *et al*. (2007) proved that , *Nepeta flavida(Nepeta species)* essential oils have various biological effects , including antioxidant activity due to the presence of 1,8-cineole, phenolic compounds especially terpenoids and phenolic acids. Furthermore, Souri *et al*. (2008) found that, different antioxidant and radical scavenging activity of several array of medicinal plants may partly be due to wide variety of antioxidant constituents such as phenolics, ascorbate and carotenoids .

Concerning carbohydrate hydrolyzing enzymes, ά-amylase, ά-glucosidase , β-galactosidase as well as liver total protein content, insignificant change was observed in ά- amylase, β-galactosidase post different normal -treated groups as compared to untreated one . While significant increase in ά-glucosidase post treatment of normal rats with petroleum ether and total ethanol extracts (70%). Liver total protein content exhibited significant reduction in all normal –treated groups. Significant inhibition of all carbohydrate hydrolyzing enzymes was noticed in diabetic rats at the different durations post STZ treatment. However, total protein content showed insignificant change. Messer and Dean (1975) reported that liver and serum amylases are immunological identity and both are very similar to parotid gland and their differences from pancreatic amylase strengthens previous suggestion that liver is the main source of serum amylase and, further, eliminates the possibility of the pancreas being a source . In the same context, Terada ; Nakanuma (1995) found pancreatic enzymes in bile ducts and hepatocytes due to common cell lineage. The results of present study are in agreement with the previous reports that indicated a decreased in pancreatic amylase activity in diabetic rats (Otsuki and Williams, 1982). Moreover, the pancreatic content of ribonuclease is also significantly reduced in diabetic acini's. The fall in amylase content either in pancreas or in liver is may be due to increased secretion or intracellular degradation *in vivo* and a decreased rate of synthesis. In addition, the reduction in amylase content is paralleled by a change in specific messenger RNA content suggesting that, insulin regulates the synthesis of amylase at the level of transcription (Otsuki and Williams, 1982). Kim *et al*. (1990) has reported that STZ induced diabetes resulted in reductions in glandular contents of DNA, RNA and amylase protein . However, the changes in amylase protein and its mRNA levels did not exactly parallel each other during diabetogenesis or subsequent insulin treatment. One reason for this discrepancy might be related to fluctuations in glands contents of amylase protein due to variations in the secretary activity of the glands in diabetic rats. The possibilities exists that the lower level of parotid amylase was related to an elevated rate of secretion due to increased mastication associated with hyperphagia in diabetic rats (Anderson, 1983). However, it is unlikely that the level of a secretary protein (amylase) mRNA will be reduced in glands with an increased secretary activity. The increase in secretary activity is likely to affect the glandular levels of all secretary proteins equally. Furthermore, Roy *et al*. (2005) found a decrease in amylase level in liver, parotid glands and pancreas during STZ induced diabetes and this is due to a decrease in the gene expression of amylase RNA.

With regard to α-glucosidase and β-glactosidase in liver of diabetic rat, significant inhibition was demonstrated that is parallel with results of Otsuki and Williams (1982) who noticed reduced maximal amounts of digestive enzymes released from acini of diabetic rats and explained this inhibition at the basis of, reduced secretary capacity, alterations in nutritional or other hormones states, a decrease of secretagogues or a combination of these factors.

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 glycolytic 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 antioxidants may prevent the progressive impairment of pancreatic beta 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 a*l., 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, 112.5 , 118.75% respectively for liver weight /body weight ratio .The enhancement in body weight may be attributed to anabolic action of ecdysones and ecdysterones found in Lbiatae family (EL Hilaly and Lyoussi, 2002). In concomitant with the present results , Vats *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, the ameliorative effect of *Nepeta cataria* may be due to various investigated phytochemicals compounds(as polyphenols, urosolic acid, essential oils, terpenoids , flavonids and phenolic acids) that can ameliorate physiological response to stress (Gilani *et al*.,2009).

In conclusion, the present study demonstrate the anti-glycemic, antioxidant, antilipidemic effects of chloroform, petroleum ether as well as 70% ethanol extracts in comparison with Gliclazide as reference antidiabetic drug. The present data reveal that ,these extracts have significant beneficial glycemic control, scavenging free radical, normalized liver function , inhibited lipid synthesis associated with diabetic complication, as well as they have principle role in treatment and amelioration liver damage at the cellular level. Thus, the safely promising therapeutic dose used in the current study, can be effective in treatment and enhanced liver tissue from the damage induced by diabetes and may candidate as natural antidiabetic drugs.

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