AIM OF THE WORK

In this study, we had evaluated the possible effect of *panex ginseng* extract on CYP2E1 mRNA gene expression, some oxidative stress markers (MDA) and some antioxidant parameters (GSH and GPx) in STZ-induced diabetic rats. The histopathological studies were carried out parallel to the biochemical studies to correlate changes in liver and pancreas tissues with the chemical parameters.

MATERIALS AND METHODS

Fifty healthy adult male albino rats approximately 3 months old, weighing (90 -150 g) purchased from Animal House Colony of the National Research Centre Dokki, Giza, Egypt, were used in the present study,

The rats were kept in special plastic rodent cages in laboratory under nearly constant conditions for experimental work, were kept in laboratory for at least one week before initiation of the experiments and were maintained on a standard rodent diet and clean water.

The guidelines of the ethical care and treatment of the animals followed the regulations of the ethical committee of Medical Research Institute.

Animals were divided into five groups as follows:

Group I : 10 rats were served as normal controls (control group).

Group II : 10 diabetic rats (diabetes was induced by a single intraperitoneal injection of STZ at a dose of 60 mg/kg body weight for 3 consecutive days)[187] (diabetic group).

Group III : 10 rats were received daily *Panax ginseng* extract 4 % dissolved in distilled water, (administered orally at a dose of 100 mg/kg body weight for 30 days)[188] (ginseng group).

Group IV : 10 rats were pretreated with *Panax ginseng* extract as in group III for 30 days. Then they were injected by STZ as in group II for 3 consecutive days (ginseng-pretreated diabetic group).

Group V : 10 diabetic rats as in group II, then they were treated with *Panax ginseng* extract as in group III for 30 days (ginseng-treated diabetic group).

- *Panax ginseng* extract (methanolic extract) was purchased from Arab Company for Gelatinous and Pharmaceutical Products Alexandria, Egypt.
- Body weights of rats were recorded at the beginning and at the end of the experiment period (33 days).
- Rats in the control group and the diabetic group were orally administrated with distilled water in a volume matched with that given in ginseng group until the end of the experiment (33 days). As well as rats in both groups (control and ginseng) were injected an equivalent volume of citrate buffer pH 4.5 (the solvent of STZ).

Induction of diabetes:

STZ (Sigma Chemicals Co, St. Louis, MO, USA) was dissolved in cold 0.01 M citrate buffer, pH 4.5 and always freshly prepared for immediate use within 5 min. STZ injections were given intraperitoneally and the doses were determined according to the body weight of animals [189].

Samples collection:

At the end of the experiment (33days) all the animals were kept fasting for 12 h, then anesthetized by ether and sacrificed. One part of blood was withdrawn from the heart of each rat in plain tubes and left to clot, then centrifuged for 10 min at 3000 rpm to separate serum, used for determination of serum cholesterol, serum TG levels, serum ALT and AST activities. Other part of blood was collected on potassium ethylene diamine tetraacetic acid (K_3 EDTA) coated tubes, and then centrifuged for 10 min at 3000 rpm to separate plasma, used for determination of FPG levels.

Liver and pancreas tissues were collected from rats, washed with saline and dried with filter paper. Liver tissues were cut into 3 small pieces and used as follow:

- First part of liver was stored at – 80 °C until used for determination of GSH content, protein content, GPx specific activity and MDA levels.
- Second part of the liver was stored at –80 °C until used for determination of CYP2E1 mRNA gene expression by real time polymerase chain reaction (RT-PCR).
- Third part of liver and pancreas tissue were fixed in 10 % formalin for the histopathological examination.

Biochemical Parameters:

1- Determination of fasting plasma glucose levels [190]:

Glucose was determined using standard Kits Spinreact, S.A. Ctra. Santa Coloma, Spain.

Principle:

Glucose oxidase (GOD) catalyses the oxidation of glucose to gluconic acid. The formed H_2O_2 is detected by a chromogenic oxygen acceptor, phenol and 4–aminophenazone (4-AP) in the presence of peroxidase (POD):

$$\beta\text{-D-Glucose} + O_2 + H_2O \xrightarrow{GOD} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{POD} \text{Quinone} + H_2O$$

The intensity of the color formed is proportional to the glucose concentration in the sample.

Reagents:

R	Tris pH 7.4	92 mmol /L
	Phenol	0.3 mmol /L
	GOD	1500 U/L
	POD	1000 U/L
	4-AP	2.6 mmol/L
Glucose standard	Glucose aqueous primary standard 100 mg/dl	

Procedure:

1. Pipette into the tubes:

	Blank	Standard	Sample
R (ml)	1.0	1.0	1.0
Standard (µl)	----	10	----
Sample(µl)	----	----	10

2. The tubes was mixed and incubated for 10 minutes at 37ºC.
3. The absorbance (A) of the samples and standard were read against the blank at wavelength 505 nm. The color is stable for at least 30 minutes.

Calculations:

$$\frac{\text{(A) Sample}}{\text{(A) Standard}} \times 100 \text{ (Standard conc.)} = \text{mg/dL glucose in the sample}$$

2- Determination of serum cholesterol levels [191]:

Cholesterol was determined using standard Kits Spinreact, S.A. Ctra. Santa Coloma, Spain.

Principle:

The cholesterol present in the sample originates a colored complex, according to the following reactions:

$$\text{Cholesterol esters} + H_2O \xrightarrow{\text{CHE}} \text{Cholesterol} + \text{fatty acids}$$

$$\text{Cholesterol} + O_2 \xrightarrow{\text{CHOD}} \text{4-Cholestenone} + H_2O_2$$

$$2H_2O_2 + \text{Phenol} + \text{4-AP} \xrightarrow{\text{POD}} \text{Quinonimine} + 4H_2O$$

The intensity of the color formed is proportional to the cholesterol concentration in the sample.

Reagents:

R	Pipes pH 6.9	90 mmol/L
	Phenol	26 mmol/L
	Cholesterol esterase (CHE)	1000 U/L
	Cholesterol oxidase (CHOD)	300 U/L
	POD	650 U/L
	4-AP	0.4 mmol/L
Cholesterol standard	Cholesterol aqueous primary standard	200 mg/dl

Procedure:

1. Pipette into the tubes.

	Blank	**Standard**	**Sample**
R (ml)	1.0	1.0	1.0
Standard (µL)	----	10	----
Sample (µL)	----	----	10

2. The tubes was mixed and incubated for 5 minutes at 37ºC.
3. The absorbance (A) of the samples and standard were read against the blank at wavelength 505 nm.The color is stable for at least 60 minutes.

Calculations:

$$\frac{\text{(A) Sample}}{\text{(A) Standard}} \times 200 \text{ (Standard conc.)} = \text{mg/dL cholesterol in the sample}$$

3- Determination of serum triglycerides levels [191]:

TG was determined using standard Kits Spinreact, S.A. Ctra. Santa Coloma, Spain.

Principle:

Sample TG incubated with LPL, liberate glycerol and FFA. Glycerol is converted to glycerol-3-phosphate (G3P) and adenosine-5-diphosphate (ADP) by glycerol kinase (GK) and ATP. G3P is then converted by glycerol phosphate dehydrogenase (GPO) to dihydroxyacetone phosphate (DAP) and H_2O_2. In the last reaction, H_2O_2 reacts with 4-AP and p-chlorophenol in presence of POD to give a red colored dye:

$$TG + H_2O \xrightarrow{LPL} Glycerol + FFA$$

$$Glycerol + ATP \xrightarrow{Gk} G3P + ADP$$

$$G3P + O_2 \xrightarrow{GPO} DAP + H_2O_2$$

$$H_2O_2 + 4\text{-}AP + p\text{-}Chlorophenol \xrightarrow{POD} Quinone + H_2O$$

The intensity of the color formed is proportional to the TG concentration in the sample.

Reagents:

R	Good pH 7.5	50 mmol/L
	p-Chlorophenol	2 mmol/L
	LPL	150000 U/L
	GK	500 U/L
	GPO	2500 U/L
	POD	440 U/L
	4-AP	0.1 mmol/L
	ATP	0.1 mmol/L
TG standard	TG primary standard	200 mg/dL

Procedure:

1. Pipette into the tubes.

	Blank	**Standard**	**Sample**
R (ml)	1.0	1.0	1.0
Standard (µL)	----	10	----
Sample (µL)	----	----	10

2. The tubes was mixed and incubated for 5 minutes at 37ºC.
3. The absorbance (A) of the samples and standard were read, against the blank at wavelength 505 nm.The color is stable for at least 30 minutes.

Calculations:

$$\frac{(A)\ Sample}{(A)\ Standard} \times 200\ (Standard\ conc.) = mg/dl\ TG\ in\ the\ sample.$$

4- Estimation of serum aminotransferase (ALT) activity [192]:

ALT was determined using standard Kits Spinreact, S.A. Ctra. Santa Coloma, Spain

Principle:

ALT or GPT catalyses the reversible transfer of an amino group from alanine to α-ketoglutarate forming glutamate and pyruvate.

The pyruvate produced is reduced to lactate and nicotinamide (NAD^+) by lactate dehydrogenase (LDH) according to the following equation:

$$\text{Alanine} + \alpha\text{-Ketoglutarate} \xrightarrow{\text{ALT}} \text{Glutamate} + \text{Pyruvate}$$

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightarrow{\text{LDH}} \text{Lactate} + NAD^+$$

The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic activity of ALT present in serum.

Reagents:

(R1) Buffer	Tris pH 7.8	100 mmol/L
	LDH	1200 U/L
	L-Alanine	500 mmol/L
(R2) Substrate	NADH	0.18 mmol/L
	α-Ketoglutarate	15 mmol/ L

Preparation:

Working reagent (WR) was prepared by mixing 4 volume of (R1) Buffer + 1 volume of (R2) Substrate.

Procedure:

1- 1 ml of WR was mixed with 100µl of serum, and then incubated for 1 minute.

2- The initial absorbance (A) of each sample was read at wavelength 340 nm, start the stopwatch and absorbance at 1-minute intervals then after for 3 minutes was read also.

3- The difference between the initial absorbance and the average absorbance differences was calculated per minute (ΔA/min).

Calculations:

ΔA/ min x 1750 = U/L of ALT

5- Estimation of serum aspartate aminotransferase (AST) activity [192]:

AST was determined using standard Kits Spinreact, S.A. Ctra. Santa Coloma, Spain.

Principle:

AST formerly called GOT catalyses the reversible transfer of an amino group from aspartate to α-ketoglutarate forming glutamate and oxalacetate. The oxalacetate produced is reduced to malate and NAD^+ by malate dehydrogenase (MDH) according to the following equation:

L-Aspartate + α-Ketoglutarate $\xrightarrow{AST}$ Glutamate + Oxalacetate

Oxalacetate + NADH + H^+ $\xrightarrow{MDH}$ Malate + NAD^+

The rate of decrease in concentration of NADH, measured photometrically, is proportional to the catalytic activity of AST present in serum.

Reagents:

(R1) Buffer)	Tris pH 7.8	80 mmol/L
	L-Asprtate	200 mmol/L
(R2) (Substrate)	NADH	0.18 mmol/
	MDH	600 U/L
	α-Ketoglutarate	12 mmol/ L

Preparation:

WR was prepared by mixing 4 volume of (R1) Buffer + 1 volume of (R2) Substrate.

Procedure

1- 1 ml of WR was mixed with 100µl of serum, and then incubated for 1 minute.

2- The initial absorbance (A) of each sample was read at wavelength 340 nm, start the stopwatch and absorbance at 1-minute intervals then after for 3 minutes was read also.

3- The difference between the initial absorbance and the average absorbance differences was calculated per minute (ΔA/min).

Calculations:

ΔA/ min x 1750 = U/L of AST

Preparation of liver homogenates:

First part of liver was homogenized in cold 0.01 M phosphate buffer saline pH 7.2 (10% W/V) in Teflon Potter-Elvehjem homogenizer. 0.4 ml of total homogenate was added to 0.1 ml of 25 % metaphosphoric acid, vortexed, left 10 minutes on ice to allow protein precipitation and then centrifuged at 3200 Xg for 20 minutes at 4 °C. The supernatant immediately used for determination of GSH content. The remaining homogenate was centrifuged at 3000 rpm for 20 minutes at 4 °C and the supernatant was used for assaying the activities of GPx specific activity and protein content. For the MDA, liver homogenates were prepared in a ratio of (10% W/V) of 1.15 % KCL (w/v) by using Teflon Potter-Elvehjem homogenizer in an ice bath 4 °C.

6) Determination of tissue protein content [193].

Principle:

A modification of the method of Lowery et al [193] was used for the determination of total protein in the samples. The color produced is thought to be due to a complex between the alkaline copper –phenol reagent, tyrosine and tryptophan residues of protein sample.

Reagents:

1- Reagent A : 2% Na_2CO_3 in 0.1 N NaOH.

2- Reagent B : 0.5 % $CuSO_4.5H_2O$ in 1%Na-K tartarate.

3- Reagent C : 50 ml of reagent A + 1 ml of reagent B (reagent C was freshly prepared and discarded after 24 hours).

4- Reagent D : Folin reagent was prepared by diluting the stock reagent 1:2 (V/V) with distilled water immediately before use.

Procedure:

- 1ml of each sample (supernatant) was mixed with 5ml of reagent C.
- After incubation for 10 minutes at room temperature, 0.5 ml of reagent D was added.
- The tubes were then mixed and incubated for 30 minutes at room temperature, after which the absorbance was measured at 750 nm.
- No sample was added to the blank.

Calculation:

The protein concentration in each sample was estimated by referring to a standard curve (Figure 10) which was constructed using bovine serum albumin (BSA).

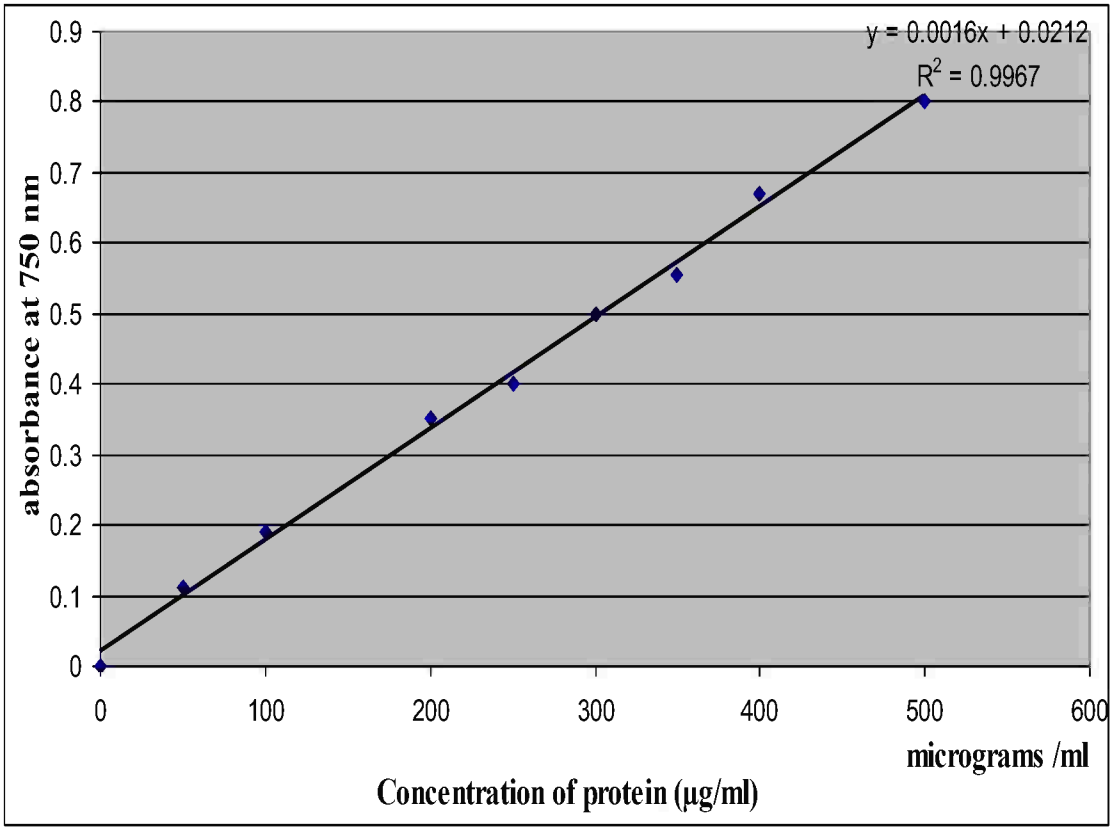

Figure (10): Standard curve of protein content.

7) Determination of tissue glutathione content [194]:

Reduced GSH or total soluble GSH content per gm tissue was assayed by a modification of the spectrophotometric method reported by Sed Lack and Lindsay [194].

Principle:

This method depended on the reductive cleavage of 5,5⁻ - dithiobis-(2- nitrobenzoic acid) {DTNB} by SH group to yield a yellow color with maximum absorbance at 412 nm.

Reagents:

1- 0.1 M sodium phosphate buffer, pH = 8.

2- 0.01 M DTNB dissolved in sodium phosphate buffer, 0.1M, pH = 7.

Procedure:

- The assay mixture contained 0.2 ml of supernatant, 4.7 ml sodium phosphate buffer, 0.1 ml DTNB was added and vortexd immediately.
- Blank contained the same content except the supernatant.
- The absorbance at 412 nm was read within 25 minutes after addition of DTNB.

Calculations:

The GSH concentration in each sample was estimated by referring to a standard curve (Figure 11) which was constructed using standard reduced GSH (1.25 mg/ml).

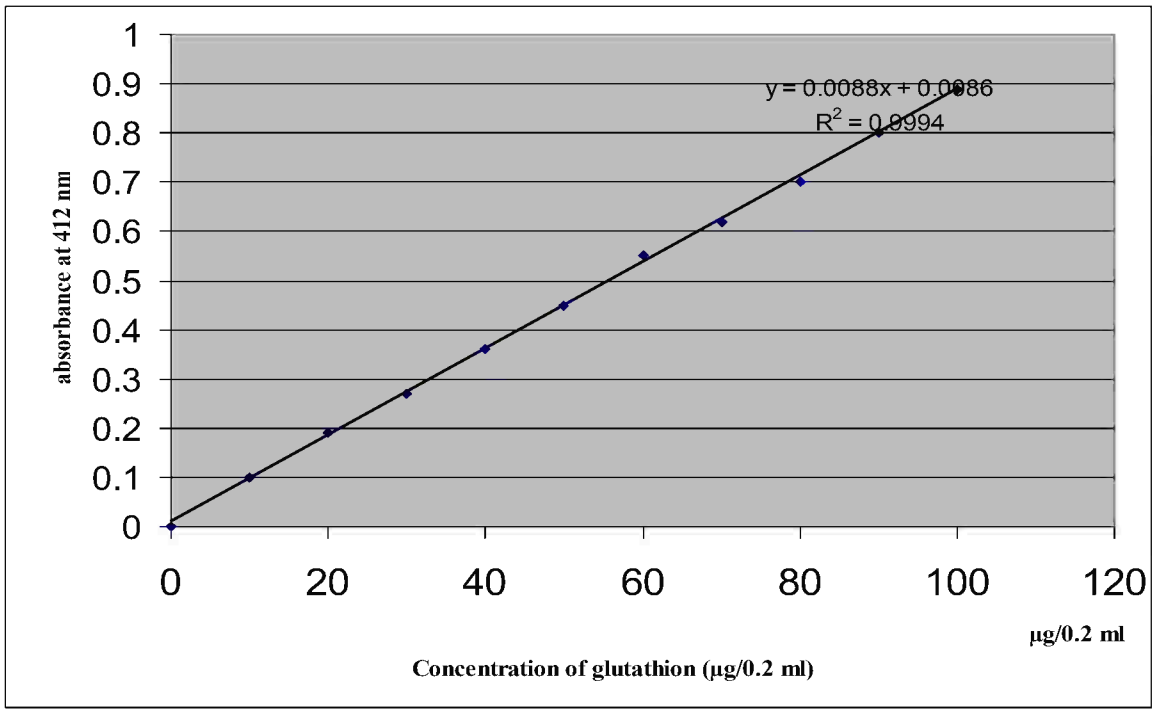

Figure (11): Standard curve GSH.

8) Estimation of malondialdehyde contents in liver tissue [195]:

Principle:

This Assay is based on the reaction between lipid peroxides in the sample with thiobarbituric acid (TBA) at low pH (3.5) forming a red pigment which is extracted by a mixture of n-butanol and pyridine. The optical density (OD) of this pigment, measured at 532 nm, is representative for the concentration of lipid peroxides in the sample.

Reagents:

- Acetic acid 20% in distilled water, pH of this solution was adjusted to 3.5 using 1N NaOH.
- TBA, 0.8 % in distilled water (freshly prepared).
- Sodium dodecyle sulfate (SDS), 8.1 % in distilled water.
- n-Butanol and pyridine (15:1 v/v).

- Tetramethoxy propane(TMP) standard solution:

  a) Stock standard solutions: this solution prepared diluting 25µl of TMP standard solution to 50 ml with absolute ethanol to give 2.02 mM TMP.

  b) Working standard solutions : 25, 50, 75, 100, 150 and 200 µl aliquots were transferred from the stock TMP standard solution to 50 ml volumetric flasks, then completed to the mark with distilled water to give a series of working standard solutions containing concentrations of 1, 2, 3, 4, 6 and 8 nmolTMP/ml, respectively.

Procedure:

1- 0.2 ml of the liver homogenate was mixed with an equal volume of SDS, 1.5 ml acetic acid, 1.5 ml of TBA (freshly prepared) and 0.6 ml of distilled water then the tubes were capped.

2- After heating in a boiling water bath for 60 minutes to allow the reaction of lipid peroxidase with TBA, the tubes were left to cool. To each tube 1 ml of distilled water was added and mixed with its contents. The resulting chromogen was extracted with 5 ml of the n-butanol and pyridine mixture by vigorous shaking.

3- Separation of the organic phase was facilitated by centrifugation at 4000 rpm for 10 minutes and OD was measured at 532 nm against the blank in which the sample was replaced by an equal volume of phosphate buffer (0.2 M, pH 7.4).

Calculations:

A standard curve was prepared under the same experimental conditions relating nmol/ml concentrations of TMP to OD. The MDA concentration in each sample is determined from the standard curve and expressed in nmol/ml (Figure 12).

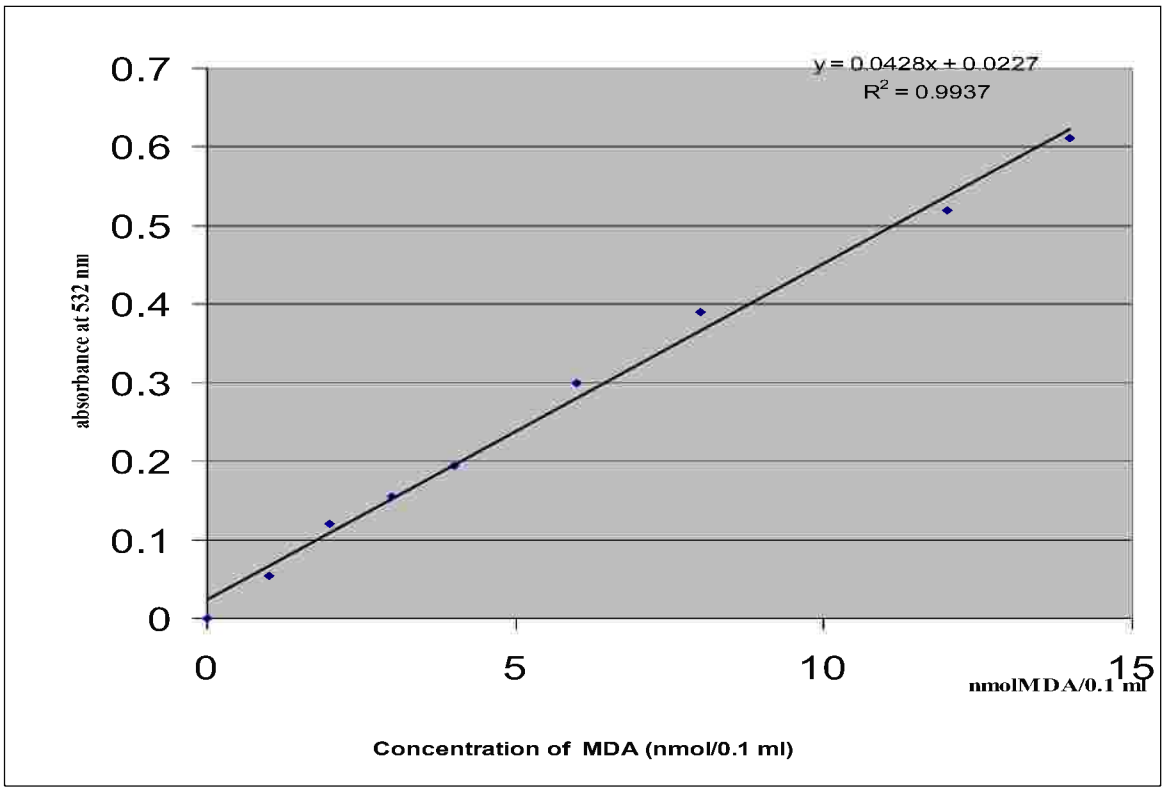

Figure (12): Standard curve of TMP

9) Determination of tissue glutathione peroxidase activity [196]:

Principle:

In these assay cumene hydroperoxide is used as peroxide substrate (ROOH) and GSSG formation is catalyzed by GPx.

$$2GSH + ROOH \xrightarrow{GPx} GSSG + ROH + H_2O$$

Reagents:

1- 50 mM Tris-HCL Buffer pH = 7.6 containing 1 mM EDTA.

2- 1.95 mM GSH dissolved in Tris-HCL buffer pH =7.6

3- Cumene hydroperoxide (prepared by adding 5 µl of cumene hydroperoxide to 10 ml Tris-HCL buffer pH = 7.6).

4- 15 % Trichloro-acetic acid (TCA).

5- 0.4 M Tris-HCL buffer pH = 8.9.

6- 0.01 M DTNB dissolved in methanol.

Procedure:

- To 0.05 ml of the diluted sample, 0.75 ml Tris-HCL buffer pH =7.6, 0.1 ml GSH and 0.1 ml cumene hydroperoxide were added and well mixed. The mixture was then incubated at 37 °C for 5 minutes.
- To another 0.05 ml of diluted supernatant, 0.75 ml Tris-HCL buffer pH =7.6, 0.1 ml of GSH was added and this mixture was incubated at 37 °C for 5 minutes (blank).
- 1 ml of TCA was added to both blank and sample. 0.1 ml of cumene hydroperoxide was added to the blank only and then incubated for 10 minutes at 37 °C followed by centrifugation of both sample and blank at 3000 rpm for 20 minutes at 37 °C. 2 ml of Tris-HCL buffer pH = 8.9, 0.1 ml of DTNB were added to 1 ml of the supernatant of both blank and sample, the OD of the yellow color obtained were measured at 412 nm within 5 minutes.

Calculations:

$$\text{GPx activity (IU/ml)} = \frac{E \quad X \quad 6.2 \text{ X } 10}{13.1 \text{ X } 10 \text{ X } 0.05} \text{ µmol /minute/ml}$$

E = Difference in the absorbance between the blank and sample.

0.05 = sample volume (ml).

6.2 = Molar extinction coefficient for cumene hydroperoxide (mmol $^{-1}$ cm^{-1}).

13.1 = Molar extinction coefficient for DTNB (mmol $^{-1}$ cm^{-1}).

10 = Incubation time (minute)

$$\text{GPx specific activity} = \frac{\text{IU /ml}}{\text{mg protein / ml}} = \text{IU/mg protein}$$

Relative quantification CYP2E1 mRNA gene expression by real time polymerase chain reaction (RT-PCR) [197]:

A – Ribonucleic acid (RNA) extraction:

Reagents:

1- Buffer RLT (lysis buffer) (45 ml)

2- Buffer RW1(Wash buffer) (45 ml)

3- Buffer RPE (Wash buffer) (11 ml)

4 - RNase- free water (10 ml)

5- β –Mercaptoethanol

6- Ethanol (96-100 %)

Things were done before starting:

- β -Mercaptoethanol (β -ME) was added to lysis buffer RLT before using (add 10 µl β-ME per 1 ml lysis buffer RLT), lysis buffer RLT containing β-ME can be stored at room temperature for up to 1 month.
- Buffer RPE is supplied as a concentrate. Before using for the first time, add 4 volumes of ethanol (96-100 %) as indicated on the bottle to obtain a working solution.

Procedure:

1- Weight 30 mg of frozen liver tissue.

2- The liver tissue was disrupted and homogenized the lysate in 600 µl lysis buffer RLT by using glass homogenizer.

4- The lysate was centrifuged for 3 minutes at full speed. Carefully the supernatant was removed by pipetting and transferd it to a new microcentrifuge tube. This supernatant (lysate) was used only in subsequent steps.

Notes:

- Don't use more than 30 mg of liver tissue.
- Transfer the liver tissue to the lysis buffer as quickly as possible.
- The grinded liver tissue should be directly used for RNA purification and should not be stored.
- All grinded material must be thoroughly mixed with the lysis buffer and should not left dry on the walls of the tube (this can cause degradation of RNA). Homogenized the lysate by passing the lysate thoroughly a blunt 20-gauge needle fitted to an RNase-free syringe several times.

5- 1 volume of 70% ethanol was added to the cleared lysate and was mixed immediately by pipetting, don't centrifuge.

6- Transfer up to 700 µl of the sample, including any precipitate that may have formed, to an RNeasy spin column placed in a 2 ml collection tube. Close the lid gently and centrifuged for 15 second (S) at ≥8000 Xg (≥10,000 rpm). Discard the flow-through.

7- Add 700 µl Buffer RW1 to the RNeasy spin column. Close the lid gently and centrifuged for 15 S at ≥8000 Xg (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.

8- Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently and centrifuge for 15 S at ≥8000 Xg (≥10,000 rpm) to wash the spin column membrane. Discard the flow-through.

9- Add 500 µl Buffer RPE to the RNeasy spin column. Close the lid gently and centrifuged for 2 minutes at ≥8000 Xg (≥10,000 rpm) to wash the spin column membrane.

10- Place the RNeasy spin column in a new 2 ml collection tube and discard the old collection tube with the flow-through. Close the lid gently and centrifuge at full speed for 1 minute.

11- Place the RNeasy spin column in a new 1.5 ml collection tube. Add 30–50 µl RNase-free water directly to the spin column membrane. Close the lid gently and centrifuge for 1 minute at ≥8000 Xg (≥10,000 rpm) to elute the RNA.

12- Discard the purification column and stored RNA at – 20 ºC until use.

B- Measurement of messenger RNA (mRNA) concentration:

Total RNA purity (A260/A280) and concentration was determined for each sample using a Nano-Spectrophotometer, in Alexandria Regional Centre for Women's Health and Development. mRNA concentrations results were shown in Table(6)

Table (6): mRNA concentration (ng/µl) for all studied groups.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	17.72	26.65	8.84	40.61	43.72
2	16.43	85.42	17.43	84.10	106.54
3	5.41	10.28	29.08	55.21	66.97
4	6.02	87.97	10.15	25.04	17.31
5	6.77	13.90	5.50	30.04	58.93
6	23.98	124.01	15.54	57.23	85.42
7	11.64	76.84	3.03	8.03	33.40
8	6.59	25.40	10.03	13.88	40.70
9	9.88	112.83	27.81	21.65	11.87
10	10.75	119.70	6.60	10.55	31.24

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

C- Complementry DNA (cDNA) preparation (by High Capacity cDNA Reverse transcription Kits of Applied Biosystem Company):

Preparation of reagents:

Prepared the reverse transcription master mix on ice:

10x reverse transcription buffer	2.0 µL
25x dNTP Mix (100mM)	0.8 µL
10x reverse transcription random primers	2.0 µL
MultiScribe TM Reverse transcriptase	1.0 µL
RNase inhibitor	1.0 µL
Nuclease-free H_2O	3.2 µL
Total per reaction	10.0 µL

Procedure:

1- Pipette the previous reverse transcription master mix into each individual tube.

2- Add 10 µL of RNA sample into each tube, pipetting up and down two times to mix.

3- Seal the tubes.

4- Briefly centrifuge the tubes to spin down the contents and eliminate any air bubbles.

5- Place the plate or tubes on ice until you are ready to load thermal cycle.

6- Program the thermal cycler conditions to perform reverse transcription.

	Step 1	**Step 2**	**Step 3**	**Step 4**
Temperature	25 °C	37 °C	85 °C	4 °C
Time	10 min	120 min	5 min	∞

7- Store cDNA reverse transcription tubes prepared for RT-PCR at -15°C to -25 °C.

D- Real time quantification PCR (by universal master mix reagents kit of Applied Biosystem Company)

Reagents:

- TaqMan Gene Expression Assay (20 X) for CYP2E1
- TaqMan Gene Expression Assay (20 X) for GAPDH
- TaqMan 2X Universal PCR Master Mix
- Nuclease-Free Water

Procedures:

1- Loaded the plate for all templates.

TaqMan 2X Universal PCR Master Mix	12.5 µl
TaqMan 2X Gene Expression Assay (20 X) for CYP2E1	1.25 µl
TaqMan 2X Gene Expression Assay (20 X) for GAPDH	1.25 µl
cDNA template	100 ng
Water	Up to 25 µl

2- Placed the plate on Applied Biosystems StepOne instrument. Use the thermal cycling conditions

Step	AmpliTaq Gold® Polymerase Activation	PCR	
		Cycle (40 cycles)	
	HOLD	Denature	Anneal / Extend
Temperature	95.0 ºC	95.0 ºC	60.0 ºC
Time	10 min	15 sec	1 min

Calculations:

Interpretation of results were done using the comparative CT method for relative quantitation according to this arithmetic formulas: $2^{-\Delta\Delta CT}$

Arithmetic formulas: the amount of target, normalized to an endogenous reference and relative to a calibrator, is given by: $2^{-\Delta\Delta CT}$

Where's CT = threshold cycle.

$\Delta CT = CT\ t - CT\ r$

Where's ΔCT= the difference in threshold cycles for target and reference.

$\Delta\Delta CT = \Delta CT$ cases – ΔCT calibrators

A relative quantitation assay was used to analyze changes in gene expression in a given sample relative to another reference sample (such as an untreated control sample). The comparative CT method requires an endogenous control, which was a gene target used to normalize quantitative PCR results. The endogenous control should consistently express in all samples and the expression level should not be altered by any experimental condition. The CYP2E1 mRNA gene expression in a sample was determined by subtracting the CT of the reference gene from the target gene to get the normalized amount of the CYP2E1 mRNA gene expression, then comparing this value to that of the calibrator.

Histopathological study

Haematoxylin and eosin stain [198].

The parts of the liver and pancreas tissue were fixed in formalin buffer, were washed in running tap water for 24 hours and dehydrated in ascending series of ethyl alcohol.The samples were cleared in xylol and immersed in a mixture of xylol and paraffin in the oven at 60 °C. The tissues were transported to pure paraffin wax with melting point 58 °C in the oven, and then mounted in blocks and left at 4 °C. The paraffin blocks were sectioned on the microtome at thickness of 4 mm and mounted on clean glass slides and left in the oven at 40 °C for dryness.The slides were deparafinized in xylol and then immersed in descending series of ethyl alcohol from 100-50 %. The ordinary haematoxylin and eosin stain was used to stain the slides.

Statistical analyses of the data [199]:

- Data were fed to the computer and analyzed using IBM SPSS software package version 20.0[200].
- Quantitative data were described using mean and standard deviation, median, minimum and maximum.
- The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used.
- For normally distributed data, comparison between different groups were analyzed using F-test (ANOVA) and Post Hoc test (Scheffe) for pair wise comparison, paired t-test is to used to analyse two paired data while. Correlations between normally variables were assessed using Pearson coefficient.
- For abnormally distributed data, comparison between two independent populations were done using Mann Whitney test while Kruskal Wallis test was used to compare between different groups. Correlations between abnormally variables were assessed using Spearman coefficient.
- Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

RESULTS

In the present study, the initial, final body weights of rats, the biochemical parameters and the pathological investigation were estimated in five groups: control group (group I), diabetic group (group II), ginseng group (group III), ginseng-pretreated diabetic group (group IV) and ginseng-treated diabetic group (group V).

The results of biochemical parameters and body weight of rats are represented as individual data, range and mean values ± SD for all studied groups of rats Tables (7a,8a,9a,10a,11a,12a,13a,14a,15a,16a and17a).

The statistical analyses were made to compare all parameters` in all studied groups Tables(7b,8b,9b,10b,11b,12b,13b,14b,15b,16b and 17b) and Figures (13,14,15,16,17,18 ,19,20,21,22 and 23).

The correlations studies between all biochemical parameters with each other through the whole study, were done using Spearman coefficient, ($r > 0.3$ at $p \leq 0.05$ was considered significant) Table (18) Figures (25, 26, 27,28,29,30,31,32,33 and 34).

The results of histopathological study are represented in Figures (35,36a,36b,37,38,39,40,41a,41b,42,43 and 44).

(A) Body weight of rats (gram):

The initial body weight of rats in group I ranged from (110.60-130.0 g) with a mean value of (118.64 ± 6.04), from (114.0 - 137.0 g) with a mean value of (125.06 ± 7.11) in group II, from (111.0 – 140.50 g) with a mean value of (128.02 ± 10.54) in group III, from (106.0 – 129.70 g) with a mean value (115.77 ± 7.47) in group IV and from (114.60 – 146.30 g) with a mean value of (130.48 ± 9.64) in group V.

The final body weight of rats in group I ranged from (130.0-152.0 g) with a mean value of (139.90 ± 7.94), from (84.50 – 154.0 g) with a mean value of (116.15 ± 18.06) in group II, from (133.0 – 194.0 g) with a mean value of (167.0 ± 23.84) in group III, from (116.0 -153.0 g) with a mean value (128.75 ± 13.54) in group IV and from (102.0 – 146.0 g) with a mean value of (124.40 ± 13.26) in group V.

The final body weight was significantly increased as compared to its corresponding initial body weight in groups I, III and IV ($P<0.001$, <0.001, 0.015), respectively. While in groups II and V the final body weight of rats was significantly decreased as compared to its corresponding initial weight ($P=0.002$, 0.041), respectively.

As compared to the control: the final body weight was significantly decreased in group II ($P_1=0.044$). On the other hand, it was significantly increased in group III ($P_1 =0.015$).While in groups IV and V it didn't show any significant difference ($P_1 = 0.672$, 0.350), respectively.

As compared to group II: the final body weight was significantly increased in group III ($P_2 <0.001$). While in groups IV and V it didn't show any significant difference ($P_2 =$ 0.561, 0.861), respectively.

The final body weight was significantly decreased in groups IV and V as compared with group III ($P_3<0.001$). While it didn't show any significant difference in group V as compared to group IV ($P_4 =0.985$).

Table (7a): Individual data of initial and final body weight of rats (gram) for all studied groups of rats.

Number of rats	Group I (n= 10)		Group II (n= 10)		Group III (n=10)		Group IV (n=10)		Group V (n = 10)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
1	119.0	130	114	84.5	115.7	139	116.1	116	120.1	113
2	120.0	149	115.8	109	132	173	120.1	139	114.6	102
3	117.0	144	126	121	118	142	113.8	118.5	120.8	107
4	112.0	135	125.3	119	111	133	116	119	146.3	130
5	124.0	140	128	120	123.8	152	122.5	144	133.5	125
6	118.30	146	137	130	140.5	193	108	118	138	128
7	112.50	130	133	154	127	168	129.7	122	137.4	146
8	110.60	132	122.5	110	136.8	187	118.5	153	133.1	130
9	123.0	141	128	105	138.8	189	107	139	128	133
10	130.0	152	121	109	136.6	194	106	119	133	130
Min.	110.60	130.0	114.00	84.50	111.00	133.00	106.00	116.00	114.60	102.0
Max.	130.0	152.0	137.00	154.00	140.50	194.00	129.70	153.00	146.30	146.0
Mean	118.64	139.90	125.06	116.15	128.02	167.0	115.77	128.75	130.48	124.40
SD	6.04	7.94	7.11	18.06	10.54	23.84	7.47	13.54	9.64	13.26
Median	118.65	140.50	125.65	114.50	129.50	170.50	116.05	120.50	133.05	129.0

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (7b): The statistical analyses of initial and final body weight of rats (gram) for all studied groups of rats.

Weight of rat	Group I (n= 10)		Group II (n= 10)		Group III (n=10)		Group IV (n=10)		Group V (n = 10)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
Min.	110.60	130.0	114.00	84.50	111.00	133.00	106.00	116.00	114.60	102.0
Max.	130.0	152.0	137.00	154.00	140.50	194.00	129.70	153.00	146.30	146.0
Mean	118.64	139.90	125.06	116.15	128.02	167.0	115.77	128.75	130.48	124.40
SD	6.04	7.94	7.11	18.06	10.54	23.84	7.47	13.54	9.64	13.26
Median	118.65	140.50	125.65	114.50	129.50	170.50	116.05	120.50	133.05	129.0
P	<0.001*		0.002*		<0.001*		0.015*		0.041*	
p_1				0.044*		0.015*		0.672		0.350
p_2						<0.001*		0.561		0.861
p_3								<0.001*		<0.001*
p_4										0.985

p: p value for Paired t-test for comparing between initial and final body weights of rats.
P_1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P_2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P_3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P_4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

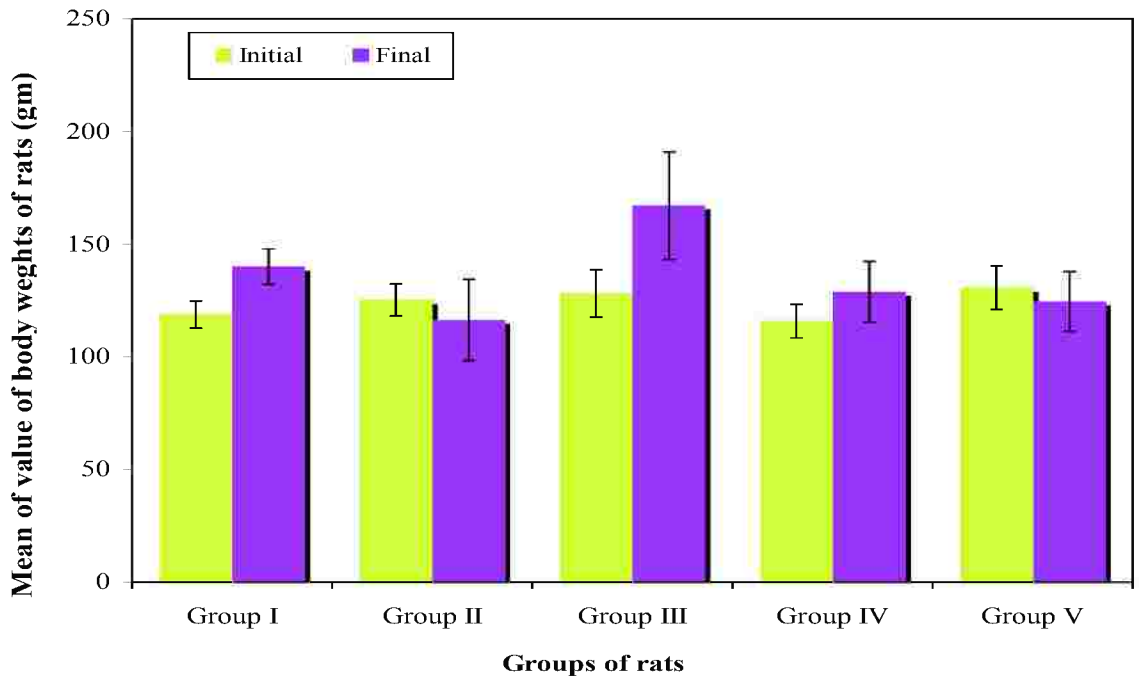

Figure (13): Initial and final body weights (gram) for all studied groups of rats.

(B) Biochemical results:

(1) Fasting plasma glucose levels (mg/dl):

The FPG level in group I ranged from (80.0-99.0 mg/dl) with a mean value of (91.80 ± 5.77), from (321.0 - 482.0 mg/dl) with a mean value of (419.0 ± 50.91) in group II, from (78.0 – 91.0 mg/dl) with a mean value of (83.54 ± 4.65) in group III, from (280.0 -378.0 mg/dl) with a mean value (312.40 ± 26.83) in group IV and from (230.0 – 313.0 mg/dl) with a mean value of (278.90 ± 28.01) in group V.

The FPG level was significantly increased in groups II, IV and V as compared to the control group (P_1 <0.001). While in group III it didn't show any significant difference as compared with the control group (P_1 = 0.981).

The FPG level was significantly decreased in groups III, IV and V as compared to group II (P_2 <0.001). On the other hand, it was significantly increased in groups IV and V as compared to group III (P_3<0.001).

The FPG level in group V didn't show any significant difference when compared to group IV (P_4 = 0.169).

Table (8a): Individual data of FPG levels (mg/dl) for all studied groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	99	482	81	332	309
2	90	395	78	319	260
3	95	355	83.4	280	313
4	88	429	86	303	244
5	97	440	80	301	230
6	91	415	88	307	281
7	98	321	79	305	307
8	80	478	80	294	289
9	92	437	91	378	286
10	88	446	89	305	270
Min. – Max.	80.0 – 99.0	321.0 – 482.0	78.0 – 91.0	280.0 – 378.0	230.0 – 313.0
Mean ± SD	91.80 ± 5.77	419.0 ± 50.91	83.54 ± 4.65	312.40 ± 26.83	278.90 ± 28.01
Median	91.50	433.0	82.20	305.0	283.50

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (8b): The statistical analyses of FPG levels (mg/dl) for all studied groups of rats.

FPG	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	80.0 – 99.0	321.0 – 482.0	78.0 – 91.0	280.0 – 378.0	230.0 – 313.0
Mean ± SD	91.80 ± 5.77	419.0 ± 50.91	83.54 ± 4.65	312.40 ± 26.83	278.90 ± 28.01
Median	91.50	433.0	82.20	305.0	283.50
F(p)	257.424* (<0.001)				
P_1		<0.001*	0.981	<0.001*	<0.001*
P_2			<0.001*	<0.001*	<0.001*
P_3				<0.001*	<0.001*
P_4					0.169

[F]P: P value for F test f (ANOVA) for comparing between the different studied groups.
P_1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P_2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P_3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P_4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

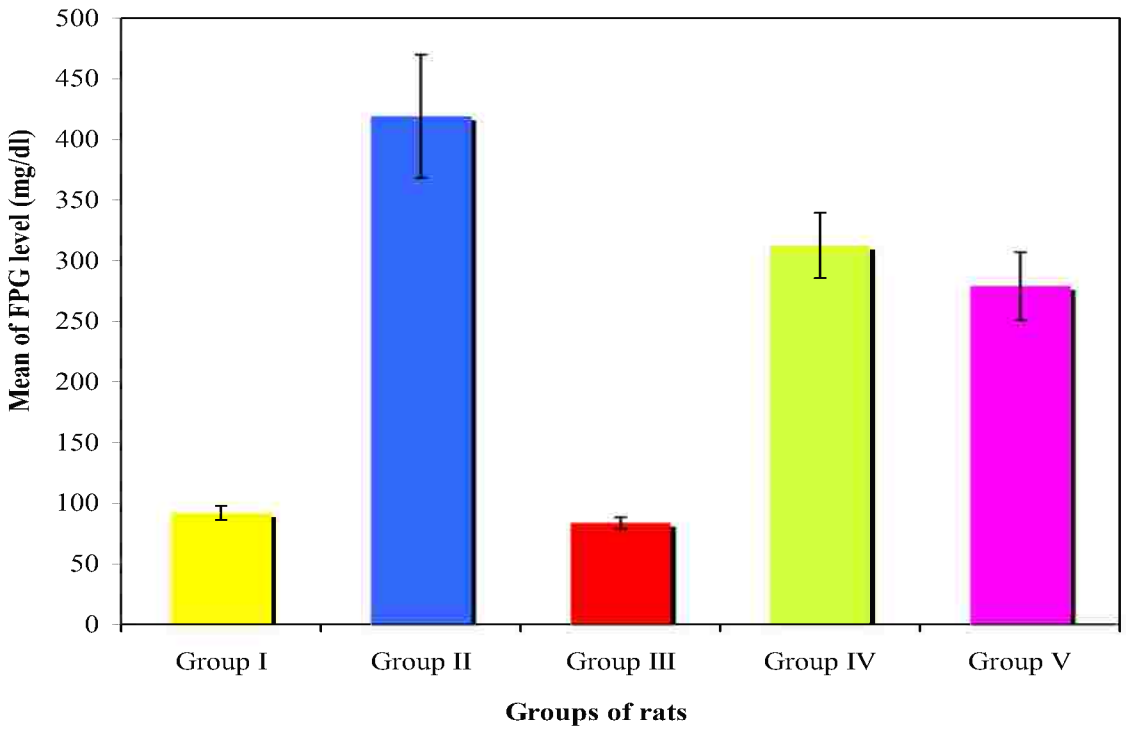

Figure (14): FPG level (mg/dl) for all studied groups of rats.

(2) Serum cholesterol levels (mg/dl):

The cholesterol level in group I ranged from (29.0- 38.0 mg/dl) with a mean value of (34.10 ± 2.95), from (63.0 - 87.0 mg/dl) with a mean value of (76.25 ± 8.55) in group II, from (24.0 – 39.0 mg/dl) with a mean value of (30.60 ± 4.30) in group III, from (42.0 - 56.0 mg/dl) with a mean value (49.30 ± 5.54) in group IV and from (38.0 – 47.0 mg/dl) with a mean value of (41.65 ± 2.65) in group V.

The cholesterol level was significantly increased in groups II, IV and V as compared to the control group (P_1 <0.001, <0.001, 0.049), respectively. It didn't show any significant difference in group III as compared with the control group (P_1 = 0.716).

The cholesterol level was significantly decreased in groups III, IV and V as compared to group II (P_2 <0.001).On the other hand it was significantly increased in groups IV and V as compared to group III (P_3<0.001, 0.001), respectively.

The cholesterol level was significantly decreased in groups V as compared to group IV (P_4 = 0.045).

Table (9a): Individual data of serum cholesterol levels (mg/dl) for all studied groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	37	84	28	56	44
2	29	65	33	49	38
3	36.5	87	27	50	42
4	35	69	29	56	43
5	38	75	24	42	39
6	32	80	30	44	40
7	31	63	32	45	42.5
8	32	82	29	53	47
9	36	73	35	43	40
10	34.5	84.5	39	55	41
Min. – Max.	29.0 – 38.0	63.0 – 87.0	24.0 – 39.0	42.0 – 56.0	38.0 - 47.0
Mean ± SD	34.10 ± 2.95	76.25 ± 8.55	30.60 ± 4.30	49.30 ± 5.54	41.65 ± 2.65
Median	34.75	77.50	29.50	49.50	41.50

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (9b): The statistical analyses of serum cholesterol levels (mg/dl) for all studied groups of rats.

Cholesterol	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	29.0 – 38.0	63.0 – 87.0	24.0 – 39.0	42.0 – 56.0	38.0 - 47.0
Mean ± SD	34.10 ± 2.95	76.25 ± 8.55	30.60 ± 4.30	49.30 ± 5.54	41.65 ± 2.65
Median	34.75	77.50	29.50	49.50	41.50
F(p)	119.873*(<0.001)				
P_1		<0.001*	0.716	<0.001*	0.049*
P_2			<0.001*	<0.001*	<0.001*
P_3				<0.001*	0.001*
P_4					0.045*

[F]P: P value for F test f (ANOVA) for comparing between the different studied groups.
P1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

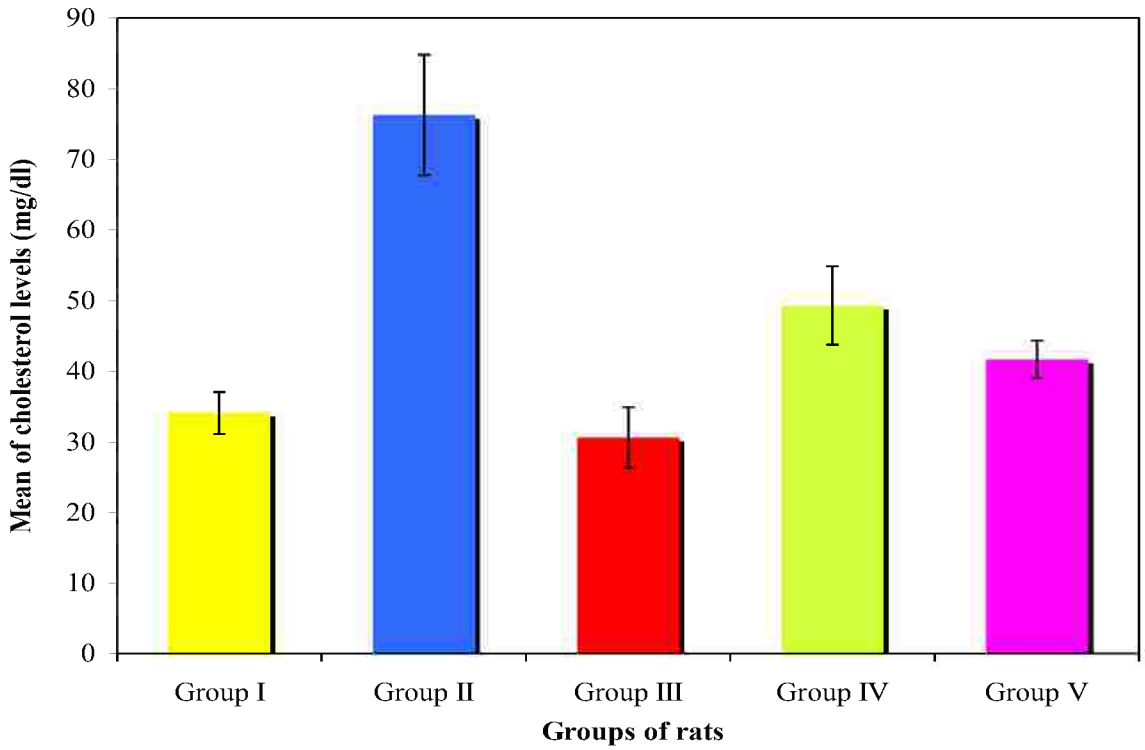

Figure (15): Serum cholesterol levels (mg/dl) for all studied groups of rats.

(3) Serum triglycerides levels (mg/dl):

The TG level in group I ranged from (50.0 – 61.0 mg/dl) with a mean value of (56.60 ± 3.18), from (82.0 - 130.0 mg/dl) with a mean value of (103.60 ± 15.83) in group II, from (47.0 – 55.0 mg/dl) with a mean value of (50.89 ± 2.90) in group III, from (58.0 -102.0 mg/dl) with a mean value (79.50 ± 17.11) in group IV and from (64.0 – 95.0 mg/dl) with a mean value of (73.50 ± 10.71) in group V.

The TG level was significantly increased in groups II, IV and V as compared to the control group (P_1 <0.001, 0.001, 0.046), respectively. It didn't show any significant difference in group III as compared with the control group (P_1 = 0.853).

The TG level was significantly decreased in groups III, IV and V as compared to group II (P_2<0.001, 0.001, <0.001) respectively, while it was significantly increased in groups IV and V as compared to group III (P_3<0.001, 0.003) respectively.

No significant change was observed in the TG level in group V as compared to group IV (P_4 = 0.855).

Table (10a): Individual data of total serum TG levels (mg/dl) for studied all groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	59	109	55	58	64
2	60	96	49	61	74
3	56.5	91	52	68	73
4	54	92	50.4	102	72
5	57.5	122	47	99	64
6	61	130	49.5	87	90
7	50	94	53	97	65
8	55	120	51	66	68
9	57	100	47	90	95
10	56	82	55	67	70
Min. – Max.	50.0 - 61.0	82.0 – 130.0	47.0 – 55.0	58.0 – 102.0	64.0 - 95.0
Mean ± SD	56.60 ± 3.18	103.60 ± 15.83	50.89 ± 2.90	79.50 ± 17.11	73.50 ± 10.71
Median	56.75	98.0	50.70	77.50	71.0

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (10b): The statistical analyses of total serum TG levels (mg/dl) for all studied groups of rats.

Triglycerides	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	50.0 - 61.0	82.0 – 130.0	47.0 – 55.0	58.0 – 102.0	64.0 - 95.0
Mean ± SD	56.60 ± 3.18	103.60 ± 15.83	50.89 ± 2.90	79.50 ± 17.11	73.50 ± 10.71
Median	56.75	98.0	50.70	77.50	71.0
F(p)	32.079*(<0.001)				
p_1		<0.001*	0.853	0.001*	0.046*
p_2			<0.001*	0.001*	<0.001*
p_3				<0.001*	0.003*
p_4					0.855

[F]P: P value for F test f (ANOVA) for comparing between the different studied groups.
P_1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P_3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P_4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

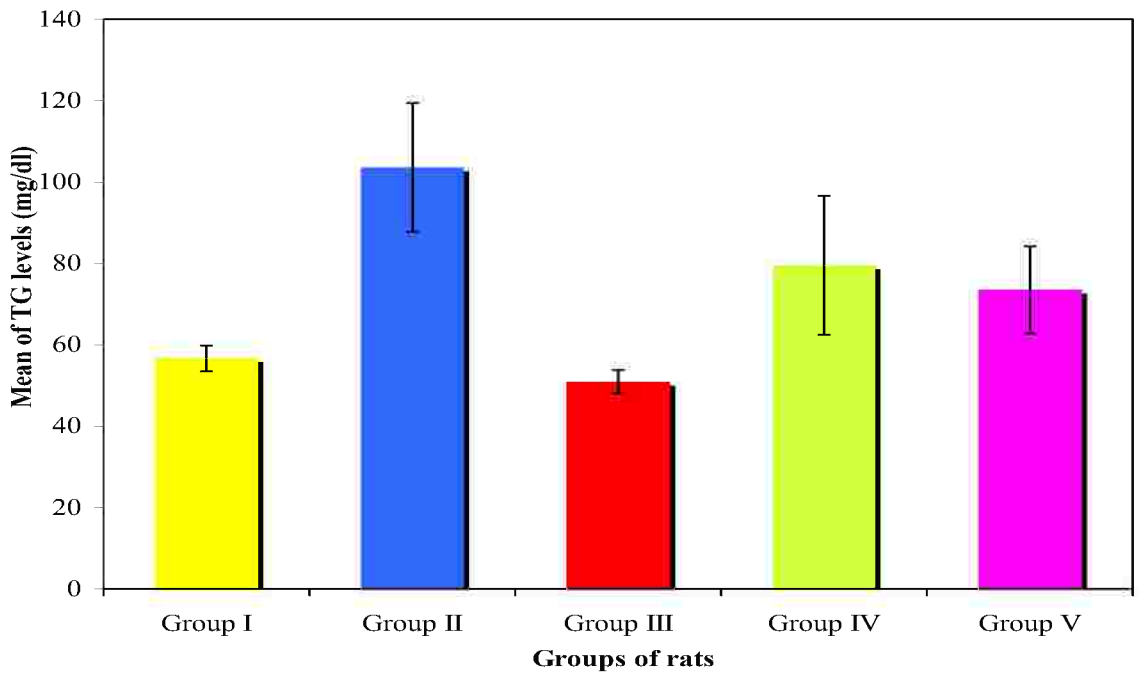

Figure (16): Serum TG levels (mg/dl) for all studied groups of rats.

(4) Serum ALT activity (U/L):

The ALT activity in group I ranged from (27.0 – 35.0 U/L) with a mean value of (30.54 ± 2.95), from (55.0 - 68.0 U/L) with a mean value of (60.55 ± 3.69) in group II, from (23.60 – 32.0 U/L) with a mean value of (28.50 ± 3.06) in group III, from (43.0 – 52.30 U/L) with a mean value (47.52 ± 3.14) in group IV and from (30.0 – 41.0 U/L) with a mean value of (36.34 ± 3.86) in group V.

The mean values of ALT activity were significantly increased in groups II, IV and V as compared to the control group (P1 <0.001, <0.001, 0.011), respectively. It didn't show any significant difference in group III as compared to the control group (P_1 = 0.805).

The mean values of ALT activity were significantly decreased in groups III, IV and V as compared to group II (P_2 <0.001), but they were significantly increased in groups IV and V as compared to group III (P_3<0.001).

The mean values of ALT activity were significantly decreased in group V as compared to group IV (P_4 <0.001).

Table (11a): Individual data of serum ALT activity (U/L) for all studied groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	30.4	62	31	46.3	36.7
2	28.2	59	32	48	39
3	31	60	30.5	43	41
4	29	68	28	50	38.2
5	34.4	57	23.6	49	30
6	35	55	31.5	47.3	35
7	33.9	64	24	52.3	38.5
8	27.5	62	30	43.8	35
9	29	60	26	44.5	30
10	27	58.5	28.4	51	40
Min. – Max.	27.0 – 35.0	55.0 – 68.0	23.60 – 32.0	43.0 – 52.30	30.0 – 41.0
Mean ± SD	30.54 ± 2.95	60.55 ± 3.69	28.50 ± 3.06	47.52 ± 3.14	36.34 ± 3.86
Median	29.70	60.0	29.20	47.65	37.45

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (11b): The statistical analyses of serum ALT activity (U/L) for all studied groups of rats.

ALT	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	27.0 – 35.0	55.0 – 68.0	23.60 – 32.0	43.0 – 52.30	30.0 - 41.0
Mean ± SD	30.54 ± 2.95	60.55 ± 3.69	28.50 ± 3.06	47.52 ± 3.14	36.34 ± 3.86
Median	29.70	60.0	29.20	47.65	37.45
F(p)	157.613* (<0.001)				
p_1		<0.001*	0.805	<0.001*	0.011*
p_2			<0.001*	<0.001*	<0.001*
p_3				<0.001*	<0.001*
p_4					<0.001*

[F]P: P value for F test f (ANOVA) for comparing between the different studied groups.
P_1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P_3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P_4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

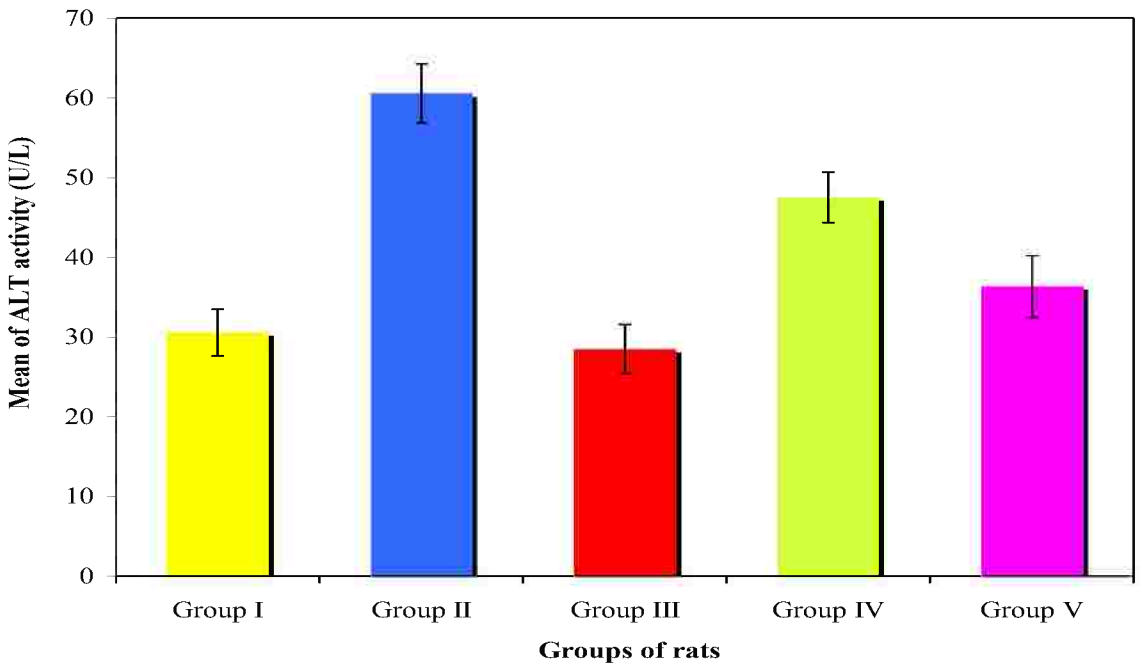

Figure (17): Serum ALT activity (U/L) for all studied groups of rats.

(5) Serum AST activity (U/L):

The AST activity in group I ranged from (50.0 – 64.0 U/L) with a mean value of (57.31 ± 4.50), from (73.0 - 92.0 U/L) with a mean value of (83.09 ± 7.29) in group II, from (40.90 – 58.0 U/L) with a mean value of (49.99 ± 5.42) in group III, from (66.0 – 81.80 U/L) with a mean value (74.92 ± 5.55) in group IV and from (58.0 – 71.50 U/L) with a mean value of (66.24 ± 4.59) in group V.

The mean values of AST activity were significantly increased in groups II, IV and V as compared to the control group (P_1 <0.001, <0.001, 0.021), respectively. It didn't show any significant difference in group III as compared to the control group (P_1 = 0.088).

The mean values of AST activity were significantly decreased in groups III, IV and V as compared to group II (P_2<0.001, 0.042, <0.001), respectively. They were significantly increased in groups IV and V as compared to group III (P_3<0.001).

The mean values of AST activity were significantly decreased in group V as compared to group IV (P_4 =0.026).

Table (12a): Individual data of serum AST activity (U/L) for all studied groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	51.5	92	55	70	67
2	55.8	74	48.6	81	69.5
3	62	85.4	40.9	66	58
4	59	87	54	74	70
5	64	91.5	52.8	69	68
6	55	73	58	76.4	65
7	58	75	53	80	69.4
8	56.4	90	45	72	71.5
9	61.4	83	48	81.8	59
10	50	80	44.6	79	65
Min. – Max.	50.0 – 64.0	73.0 – 92.0	40.90 – 58.0	66.0 – 81.80	58.0 - 71.50
Mean ± SD	57.31 ± 4.50	83.09 ± 7.29	49.99 ± 5.42	74.92 ± 5.55	66.24 ± 4.59
Median	57.20	84.20	50.70	75.20	67.50

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (12b): The statistical analyses of serum AST activity (U/L) for all studied groups of rats.

AST	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	50.0 – 64.0	73.0 – 92.0	40.90 – 58.0	66.0 – 81.80	58.0 - 71.50
Mean ± SD	57.31 ± 4.50	83.09 ± 7.29	49.99 ± 5.42	74.92 ± 5.55	66.24 ± 4.59
Median	57.20	84.20	50.70	75.20	67.50
F(p)	56.829* (<0.001)				
p_1		<0.001*	0.088	<0.001*	0.021*
p_2			<0.001*	0.042*	<0.001*
p_3				<0.001*	<0.001*
p_4					0.026*

[F]P: P value for F test f (ANOVA) for comparing between the different studied groups.
P_1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P_3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P_4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

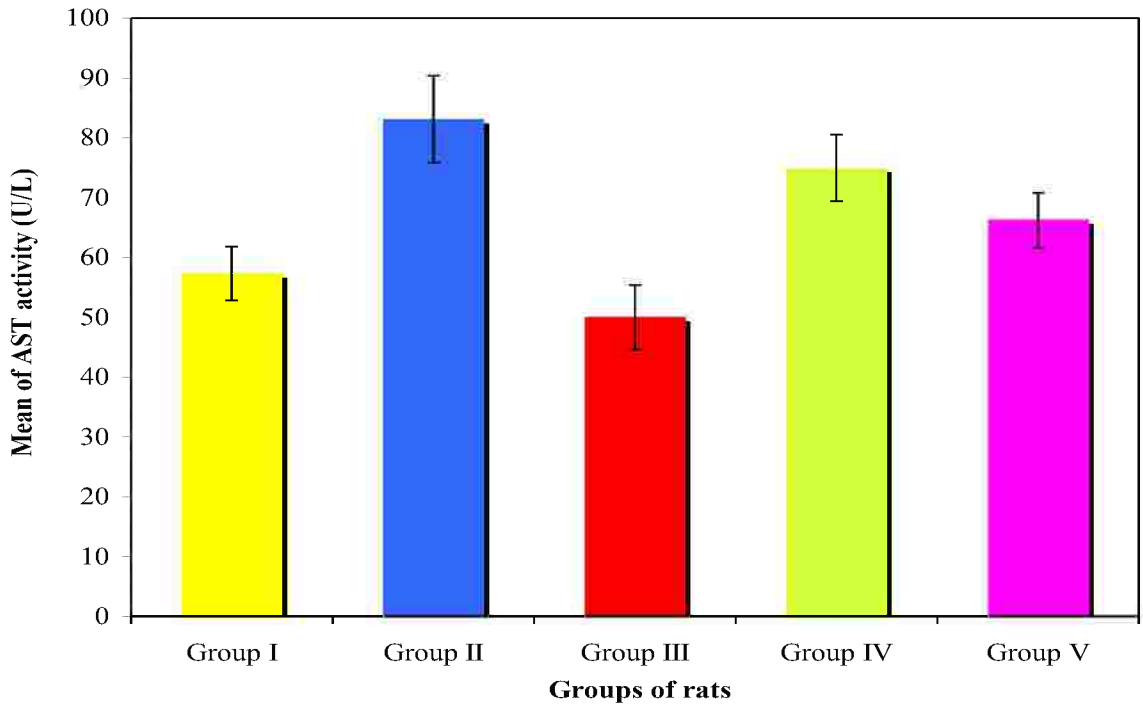

Figure (18): Serum AST activity (U/L) for all studied groups of rats.

(6) Hepatic protein content (mg/ml):

The protein level in group I ranged from (13.53 – 17.15 mg/ml) with a mean value of (14.97 ± 1.21), from (5.31 – 10.62 mg/ml) with a mean value of (7.75 ± 1.66) in group II, from (12.24 – 15.59 mg/ml) with a mean value of (14.15 ± 1.21) in group III, from (9.34 – 14.40 mg/ml) with a mean value (12.56 ± 1.81) in group IV and from (10.49 – 17.53 mg/ml) with a mean value of (12.85 ± 2.07) in group V.

As compared with the control group, the protein level in group II and IV was significantly decreased (P_1 <0.001, 0.039), respectively. While it did not show any significant difference in groups III and V (P_1 = 0.864, 0.093), respectively.

The mean values of protein level were significantly increased in groups III, IV and V as compared to group II (P_2 <0.001).

The protein content didn't show any significant difference in groups IV and V as compared to group III (P_3=0.326, 0.532), respectively. As well as in group V as compared to group IV (P_4 =0.997).

Table (13a): Individual data of hepatic protein content (mg/ml) for all studied groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	15.400	7.431	13.181	13.056	11.338
2	15.650	5.306	15.119	14.244	11.025
3	13.650	7.338	13.806	14.400	12.213
4	17.150	7.963	14.650	13.306	13.900
5	13.525	7.681	12.244	14.275	11.869
6	14.150	9.775	14.775	9.556	12.463
7	15.338	8.588	15.494	9.338	13.213
8	15.994	5.463	12.400	12.650	14.463
9	13.556	7.369	15.588	12.525	17.525
10	15.306	10.619	14.244	12.244	10.494
Min. – Max.	13.53 – 17.15	5.31 – 10.62	12.24 – 15.59	9.34 – 14.40	10.49 – 17.53
Mean ± SD	14.97 ± 1.21	7.75 ± 1.66	14.15 ± 1.21	12.56 ± 1.81	12.85 ± 2.07
Median	15.32	7.56	14.45	12.85	12.34

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (13b): The statistical analyses of hepatic protein content (mg/ml) for all studied groups of rats.

Protein	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	13.53 – 17.15	5.31 – 10.62	12.24 – 15.59	9.34 – 14.40	10.49 – 17.53
Mean ± SD	14.97 ± 1.21	7.75 ± 1.66	14.15 ± 1.21	12.56 ± 1.81	12.85 ± 2.07
Median	15.32	7.56	14.45	12.85	12.34
F(p)	29.769*(<0.001)				
p_1		<0.001*	0.864	0.039*	0.093
p_2			<0.001*	<0.001*	<0.001*
p_3				0.326	0.532
p_4					0.997

[F]P: P value for F test f (ANOVA) for comparing between the different studied groups.
P_1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P_3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P_4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

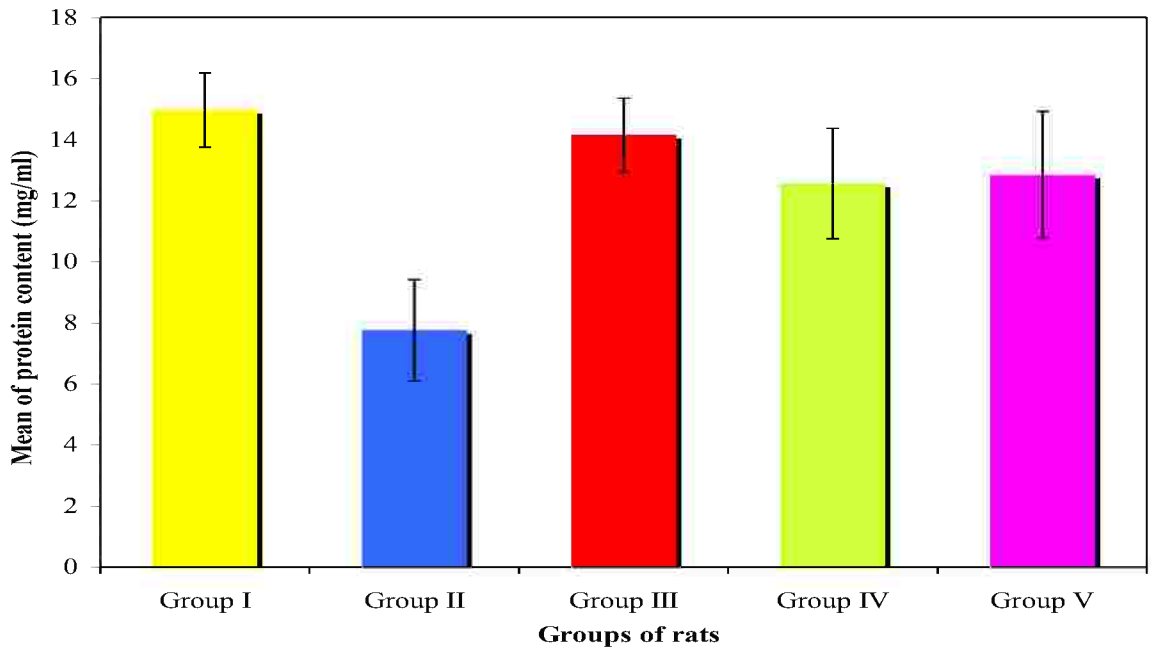

Figure (19): The protein content (mg/ml) for all studied groups of rats.

(7) Hepatic glutathione content (mg/g tissue):

The GSH content in group I ranged from (2.12 – 3.12 mg/g tissue) with a mean value of (2.72 ± 0.37), from (1.81 - 2.18 mg/g tissue) with a mean value of (1.97 ± 0.14) in group II, from (2.47 – 3.20 mg/g tissue) with a mean value of (2.68 ± 0.27) in group III, from (1.95 - 2.67 mg/g tissue) with a mean value (2.30 ± 0.25) in group IV and from (1.98 - 2.59 mg/g tissue) with a mean value of (2.33 ± 0.16) in group V.

The GSH content was significantly decreased in groups II, IV and V as compared to the control group (P_1<0.001, 0.001, 0.001), respectively. While it didn't show any significant difference in group III as compared to the control group (P_1 = 0.750).

The GSH content was significantly increased in groups III, IV and V as compared to group II (P_2 <0.001, 0.005, 0.003), respectively.

The GSH content was significantly decreased in group IV and V as compared to group III (P_3= 0.002, 0.004), respectively. While no significant difference in group V was shown as compared to group IV (P_4 =0.800).

Table (14a): Individual data of GSH content (mg/g tissue) for all studied groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	2.985	1.951	2.485	2.468	2.417
2	3.031	1.968	2.781	2.445	2.263
3	2.911	2.178	3.1	2.673	2.303
4	2.303	1.815	2.480	1.991	2.587
5	2.122	2.093	2.542	1.951	1.979
6	2.156	1.809	3.2	2.1	2.2
7	3.116	2.042	2.519	2.531	2.417
8	2.815	2.139	2.661	2.360	2.462
9	2.809	1.809	2.474	2.076	2.337
10	2.906	1.9	2.548	2.417	2.336
Min. – Max.	2.12 – 3.12	1.81 - 2.18	2.47 – 3.20	1.95 - 2.67	1.98 - 2.59
Mean ± SD	2.72 ± 0.37	1.97 ± 0.14	2.68 ± 0.27	2.30 ± 0.25	2.33 ± 0.16
Median	2.86	1.96	2.54	2.39	2.34

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (14b): The statistical analyses of GSH content (mg/g tissue) for all studied groups of rats.

GSH	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	2.12 – 3.12	1.81 - 2.18	2.47 – 3.20	1.95 - 2.67	1.98 - 2.59
Mean ± SD	2.72 ± 0.37	1.97 ± 0.14	2.68 ± 0.27	2.30 ± 0.25	2.33 ± 0.16
Median	2.86	1.96	2.54	2.39	2.34
F(p)	14.675*(<0.001)				
p_1		<0.001*	0.750	0.001*	0.001*
p_2			<0.001*	0.005*	0.003*
p_3				0.002*	0.004*
p_4					0.800

[F]P: P value for F test f (ANOVA) for comparing between the different studied groups.
P_1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P_3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P_4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

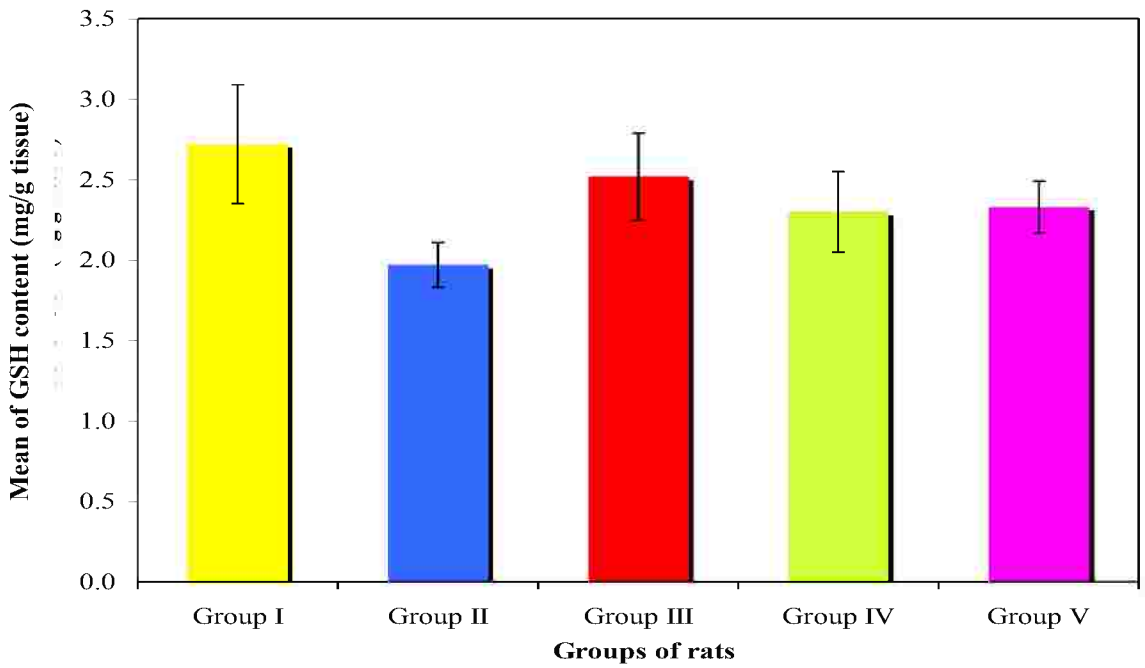

Figure (20): The GSH content (mg/g tissue) for all studied groups of rats.

(8) The malondialdehyde level (nmol/ml):

The MDA level in group I ranged from (7.39 – 11.60 nmol/ml) with a mean value of (9.12 ± 1.39), from (14.17 – 18.49 nmol/ml) with a mean value of (16.69 ± 1.68) in group II, from (6.93 – 9.26 nmol/ml) with a mean value of (8.08 ± 0.74) in group III, from (8.80 – 14.52 nmol/ml) with a mean value (12.28 ± 2.17) in group IV and from (8.33 – 14.29 nmol/ml) with a mean value of (11.74 ± 2.07) in group V.

As compared with the control group, the mean values of MDA were significantly increased in groups II, IV and V (P1 <0.001, 0.002, 0.028), respectively. It didn't show any significant difference in group III (P_1 = 0.680).

The MDA level was significantly decreased in groups III, IV and V as compared to group II (P_2 <0.001). On the other hand, in groups IV and V the mean values of MDA were significantly increased as compared to group III (P_3<0.001).

No significant change was observed in MDA level in group V as compared with group IV (P_4 =0.901).

Table (15a): Individual data of MDA levels (nmol/ml) for all studied groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	8.213	18.259	8.446	13.470	10.315
2	8.797	17.208	7.862	14.404	11.717
3	10.549	17.675	7.629	12.301	14.287
4	10.666	17.442	6.928	14.521	13.52
5	11.600	14.171	8.446	12.58	11.951
6	7.395	15.456	9.264	12.9	14.171
7	7.629	14.988	7.745	14.404	13.34
8	9.264	18.493	7.862	10.549	9.614
9	8.329	14.755	9.147	8.914	8.329
10	8.797	18.493	7.512	8.797	10.199
Min. – Max.	7.39 – 11.60	14.17 – 18.49	6.93 – 9.26	8.80 – 14.52	8.33 – 14.29
Mean ± SD	9.12 ± 1.39	16.69 ± 1.68	8.08 ± 0.74	12.28 ± 2.17	11.74 ± 2.07
Median	8.80	17.32	7.86	12.74	11.83

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (15b): The statistical analyses of MDA levels (nmol/ml) for all studied groups of rats.

MDA	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	7.39 – 11.60	14.17 – 18.49	6.93 – 9.26	8.80 – 14.52	8.33 – 14.29
Mean ± SD	9.12 ± 1.39	16.69 ± 1.68	8.08 ± 0.74	12.28 ± 2.17	11.74 ± 2.07
Median	8.80	17.32	7.86	12.74	11.83
F(p)	47.847*(<0.001)				
P_1		<0.001*	0.680	0.002*	0.028*
P_2			<0.001*	<0.001*	<0.001*
P_3				<0.001*	<0.001*
P_4					0.901

FP: P value for F test f (ANOVA) for comparing between the different studied groups.
P_1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P_3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P_4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

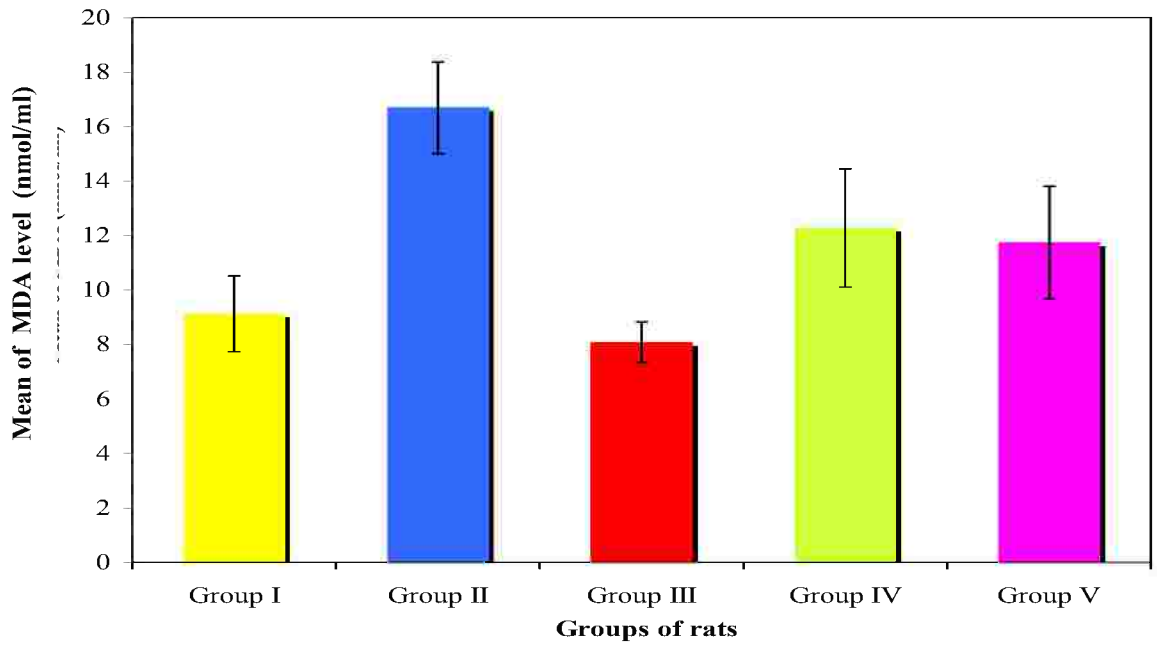

Figure (21): MDA levels (nmol/ml) for all studied groups of rats.

(9) Hepatic glutathione peroxidase specific activity (IU/mg protein):

The GPx specific activity in group I ranged from (0.133 – 0.223 IU/mg protein) with a mean value of (0.185 ± 0.030), from (0.045 - 0.143 IU/mg protein) with a mean value of (0.084 ± 0.034) in group II, from (0.142 – 0.273 IU/mg protein) with a mean value of (0.190 ± 0.035) in group III, from (0.091 - 0.141 IU/mg protein) with a mean value (0.114 ± 0.018) in group IV and from (0.110 - 0.195 IU/mg protein) with a mean value of (0.143 ± 0.024) in group V.

The GPx specific activity was significantly decreased in groups II, IV and V as compared to the control group (P1 <0.001, <0.001, 0.002), respectively. It didn't show any significant difference in group III (P_1 = 0.663).

The GPx specific activity was significantly increased in groups III, IV and V as compared to group II (P_2 <0.001, 0.027, <0.001), respectively. It was significantly decreased in groups IV and V as compared to group III (P_3<0.001, 0.001), respectively.

The specific activity of GPx was significantly increased in group V as compared to group IV (P_4 =0.028).

Table (16a): Individual data GPx specific activity (IU/mg protein) for all studied groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	0.207	0.09	0.171	0.106	0.148
2	0.185	0.055	0.221	0.105	0.149
3	0.210	0.055	0.185	0.113	0.153
4	0.133	0.045	0.142	0.091	0.121
5	0.223	0.106	0.193	0.093	0.149
6	0.199	0.133	0.177	0.135	0.154
7	0.138	0.072	0.190	0.104	0.195
8	0.171	0.068	0.273	0.141	0.117
9	0.198	0.143	0.166	0.139	0.110
10	0.182	0.073	0.185	0.110	0.135
Min. – Max.	0.133 – 0.223	0.045 - 0.143	0.142 – 0.273	0.091 - 0.141	0.110 - 0.195
Mean ± SD	0.185 ± 0.030	0.084 ± 0.034	0.190 ± 0.035	0.114 ± 0.018	0.143 ± 0.024
Median	0.192	0.073	0.185	0.108	0.149

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (16b): The statistical analyses of GPx specific activity (IU/mg protein) for all studied groups of rats.

GPx specific activity	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	0.133 – 0.223	0.045 – 0.143	0.142 – 0.273	0.091 - 0.141	0.110 - 0.195
Mean ± SD	0.185 ± 0.030	0.084 ± 0.034	0.190 ± 0.035	0.114 ± 0.018	0.143 ± 0.024
Median	0.192	0.073	0.185	0.108	0.149
F(p)	24.756* (<0.001)				
P_1		<0.001*	0.663	<0.001*	0.002*
P_2			<0.001*	0.027*	<0.001*
P_3				<0.001*	0.001*
P_4					0.028*

[F]P: P value for F test f (ANOVA) for comparing between the different studied groups.
P_1: P value for Post Hoc test (Scheffe) for comparing all groups with group I.
P2: P value for Post Hoc test (Scheffe) for comparing groups III, IV and V with group II.
P_3: P value for Post Hoc test (Scheffe) for comparing groups IV and V with group III.
P_4: P value for Post Hoc test (Scheffe) for comparing group V with group IV.
*: Statistically significant at $p \leq 0.05$.

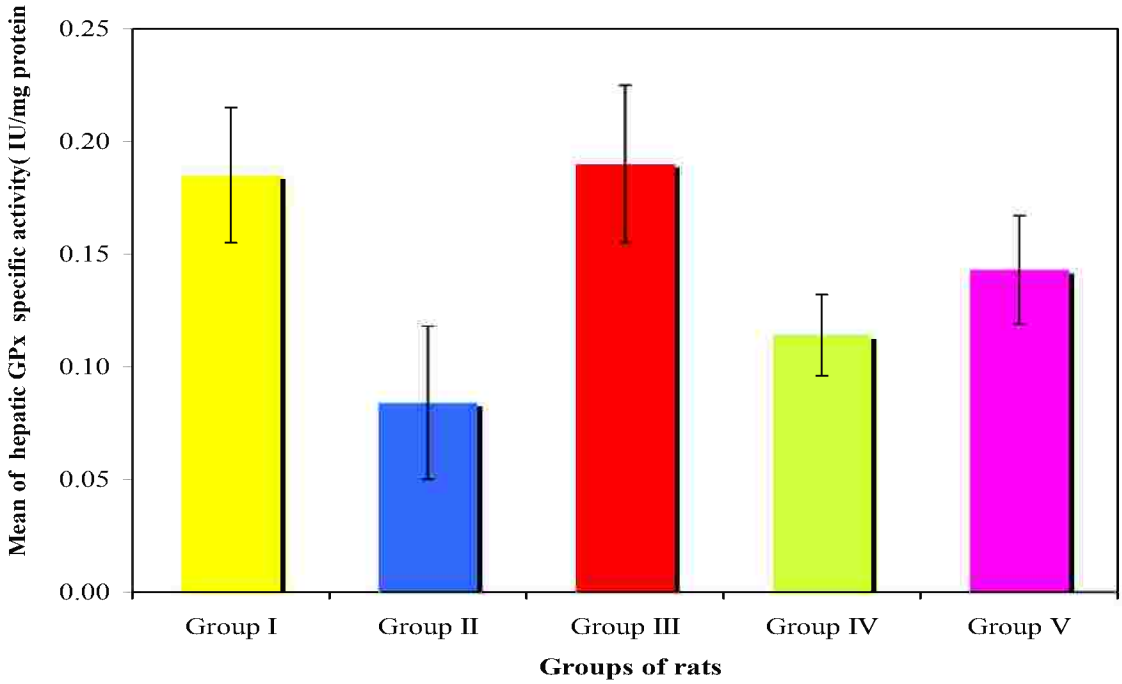

Figure (22): Hepatic GPx specific activity (IU/mg protein) for all studied groups of rats.

Molecular results:

1- Cytochrome P450 2E1 mRNA gene expression levels (copies):

The CYP2E1 mRNA gene expression level in group I ranged from (0.21 – 28.64 mg/dl) with a mean value of (4.25 ± 8.80),from (276.3- 23493.9 mg/dl)with a mean value of (4394.1 ± 6979.0) in group II, from (0.15 - 34.8 mg/dl) with a mean value of (7.10 ± 10.96) in group III,from (14.12 – 2721.15 mg/dl) with a mean value (741.78 ± 1003.2) in group IV and from (1.18 – 4576.4 mg/dl) with a mean value of (868.4 ± 1364.96) in group V.

The CYP2E1 mRNA gene expression level was significantly increased in groups II, IV and V as compared to the control group (P1 <0.001, <0.001, 0.001), respectively. It didn't show any significant difference in group III as compared to the control group (P_1 = 0.384).

The mean values of CYP2E1 mRNA gene expression level were significantly decreased in groups III, IV and V as compared to group II (P_2 <0.001, 0.013, 0.010), respectively. While they were significantly increased in groups IV and V as compared to group III (P_3<0.001, 0.004), respectively.

No significant change was observed in CYP2E1 mRNA gene expression level in group V as compared to group IV (P_4 =0.940).

Table (17 a): Individual data of CYP2E1 mRNA gene expression levels (copies) for all studied groups of rats.

Number of rats	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
1	0.76	276.28	3.36	328.56	4576.4
2	28.64	2194.99	3.84	2721.15	781.44
3	0.28	1184.45	2.06	306.55	5.74
4	0.36	23493.92	9.19	14.12	588.13
5	0.21	2556.58	0.58	34.3	885.27
6	0.89	6608	34.78	2401.97	4.92
7	0.41	1408.55	0.57	38.32	40.5
8	5.98	820.29	0.15	37.79	1.18
9	0.51	1002.93	16.11	735	860
10	4.5	4394.50	0.36	800	940
Min. – Max.	0.21 – 28.64	276.3- 23493.9	0.15 - 34.8	14.12 – 2721.15	1.18 – 4576.4
Mean ± SD	4.25 ± 8.80	4394.1 ± 6979.0	7.10 ± 10.96	741.78 ± 1003.2	868.4 ± 1364.96
Median	0.64	1801.78	2.71	317.56	684.79

n = number of rats in each group.
Group I : control group.
Group II : diabetic group.
Group III : ginseng group.
Group IV : ginseng-pretreated diabetic group.
Group V : ginseng-treated diabetic group.

Table (17b): The statistical analyses of CYP2E1 mRNA gene expression levels (copies) for all studied groups of rats.

CYP2E1	Group I (n= 10)	Group II (n= 10)	Group III (n=10)	Group IV (n=10)	Group V (n = 10)
Min. – Max.	0.21 - 28.64	276.3- 23493.9	0.15 - 34.8	14.12 – 2721.15	1.18 – 4576.4
Mean ± SD	4.25 ± 8.80	4394.1 ± 6979.0	7.10 ± 10.96	741.78 ± 1003.2	868.4 ± 1364.96
Median	0.64	1801.78	2.71	317.56	684.79
χ^2 (p)	34.285* (<0.001)				
P_1		<0.001*	0.384	<0.001*	0.001*
P_2			<0.001*	0.013*	0.010*
P_3				<0.001*	0.004*
P_4					0.940

χ^2P P: p value for Kruskal Wallis test for comparing between the different studied group
P1: p value for Mann Whitney test for comparing all groups with group I.
P2: p value for Mann Whitney test for comparing groups III, IV and V with group II.
P3: p value for Mann Whitney test) for comparing groups IV and V with group III.
P4: p value for Mann Whitney test for comparing group V with group IV.
*: Statistically significant at P ≤ 0.05

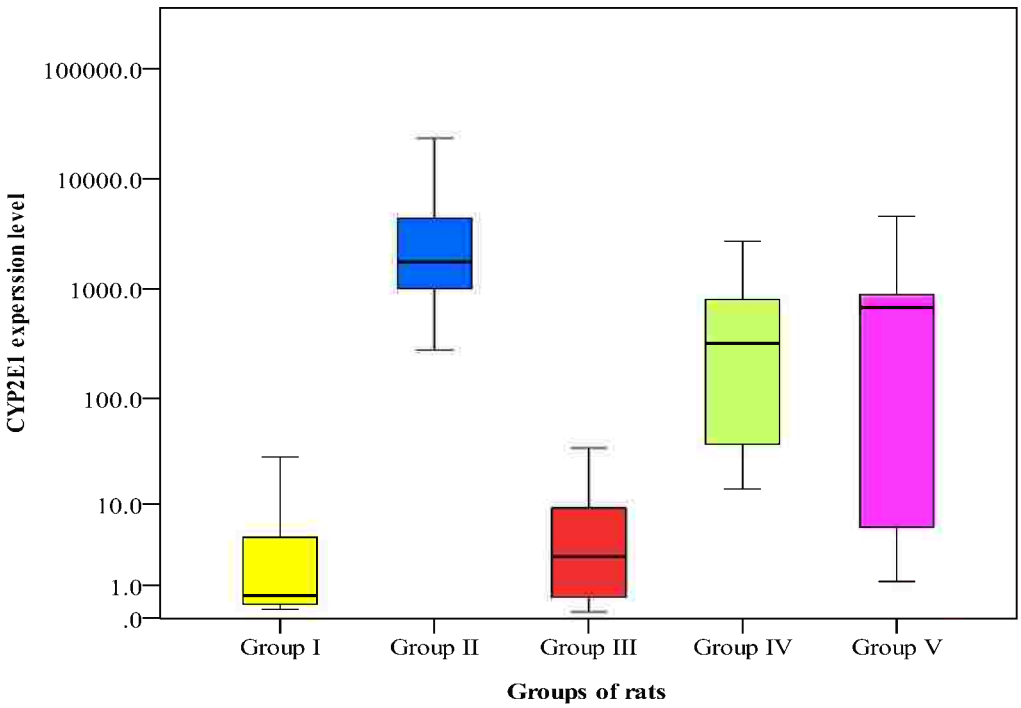

Figure (23): CYP2E1 mRNA gene expression levels (copies) for all studied groups of rats.

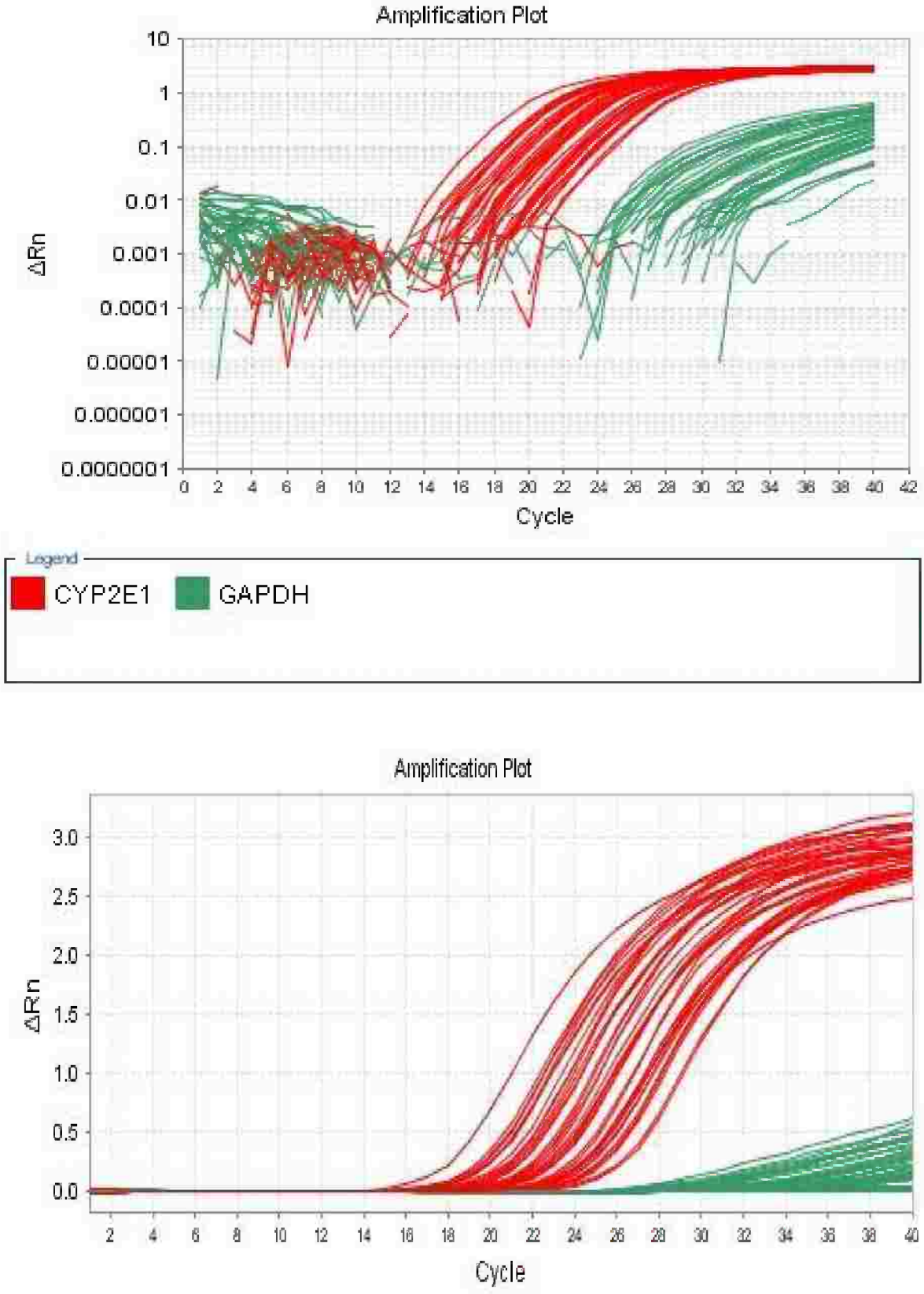

Figure (24): Amplification plot curves of CYP2E1 mRNA gene and the control gene (GAPDH) expression.

Correlation results:

The statistical correlation between all different parameters was shown in Table (18).

There were significant positive correlations between:

- The level of CYP2E1mRNA gene expression and each of ALT activity, AST activity, FPG levels, TG levels, cholesterol levels and MDA levels.
- The activity of ALT and activity of AST.
- The GSH content and GPx specific activity.
- The FPG levels and both TG levels and cholesterol levels.
- The TG levels and cholesterol levels.

There were significant negative correlations between:

- The level of CYP2E1 mRNA gene expression and each of final body weight, GSH content, GPx specific activity and protein content.
- The MDA levels and both GSH content and GPx specific activity.

Table (18): Correlations between all different studied parameters in all studied groups of rats.

		Final Weight	FPG	Cholesterol	TG	ALT	AST	Protein	GSH	MDA	GPx	CYP2E1
Initial Weight	**Coff.**	0.547*	0.209	0.151	0.188	0.121	0.043	-0.200	-0.182	0.188	-0.073	0.227
	P	<0.001	0.146	0.294	0.190	0.404	0.769	0.165	0.205	0.191	0.615	0.113
Final Weight	**Coff.**		-0.476*	-0.427*	-0.416*	-0.408*	-0.526*	0.363*	0.315*	-0.488*	0.437*	-0.282*
	P		<0.001	0.002	0.003	0.003	<0.001	0.010	0.026	<0.001	0.002	0.047
FPG	**Coff.**			0.869*	0.843*	0.888*	0.895*	-0.746*	-0.735*	0.836*	-0.780*	0.760*
	P			<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Cholesterol	**Coff.**				0.788*	0.899*	0.828*	-0.765*	-0.665*	0.854*	-0.736*	0.727*
	P				<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
TG	**Coff.**					0.802*	0.768*	-0.703*	-0.714*	0.743*	-0.667*	0.668*
	P					<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
ALT	**Coff.**						0.873*	-0.821*	-0.684*	0.857*	-0.791*	0.701*
	P						<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
AST	**Coff.**							-0.729*	-0.666*	0.801*	-0.761*	0.733*
	P							<0.001	<0.001	<0.001	<0.001	<0.001
Protein	**Coff.**								0.621*	-0.758*	0.552*	-0.594*
	P								<0.001	<0.001	<0.001	<0.001
GSH	**Coff.**									-0.668*	0.573*	-0.596*
	P									<0.001	<0.001	<0.001
MDA	**Coff.**										-0.731*	0.656*
	P										<0.001	<0.001
GPx	**Coff.**											-0.703*
	P											<0.001

*: Statistically significant at $p \leq 0.05$ and $r > 0.3$.

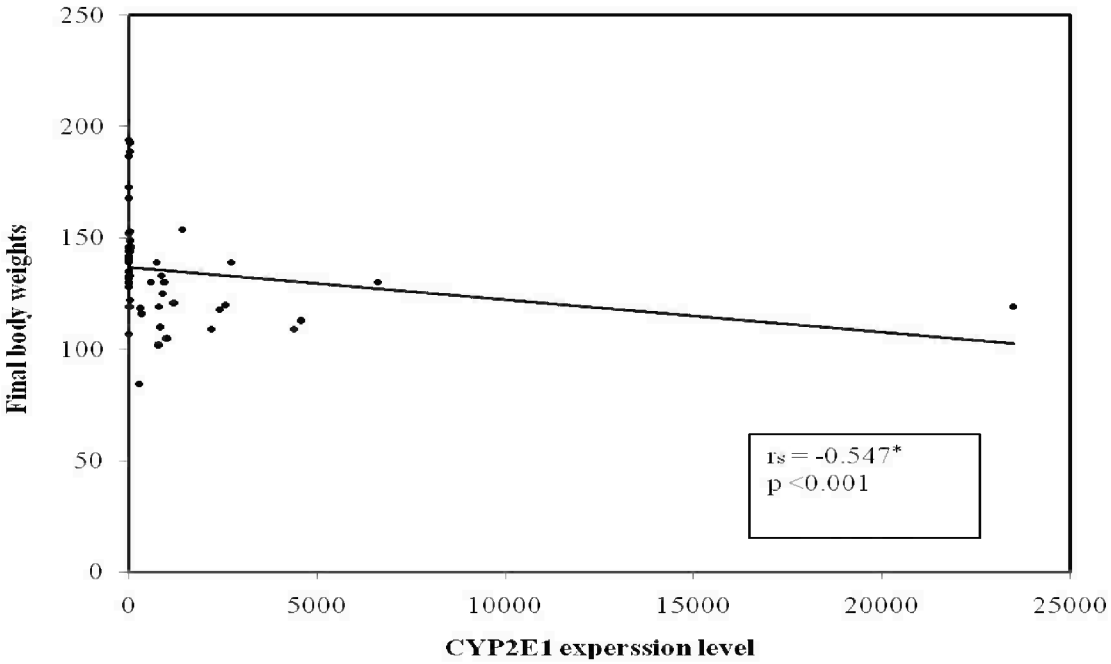

Figure (25): Correlation between CYP2E1 mRNA with final body weights of rats.

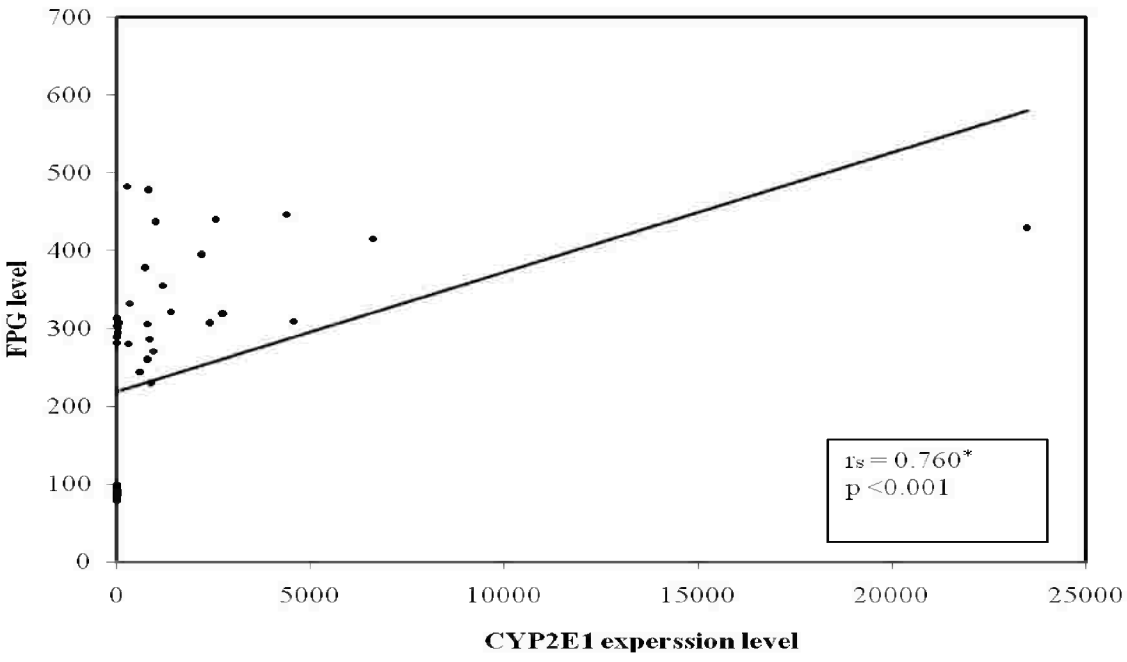

Figure (26): Correlation between CYP2E1 mRNA with FPG level.

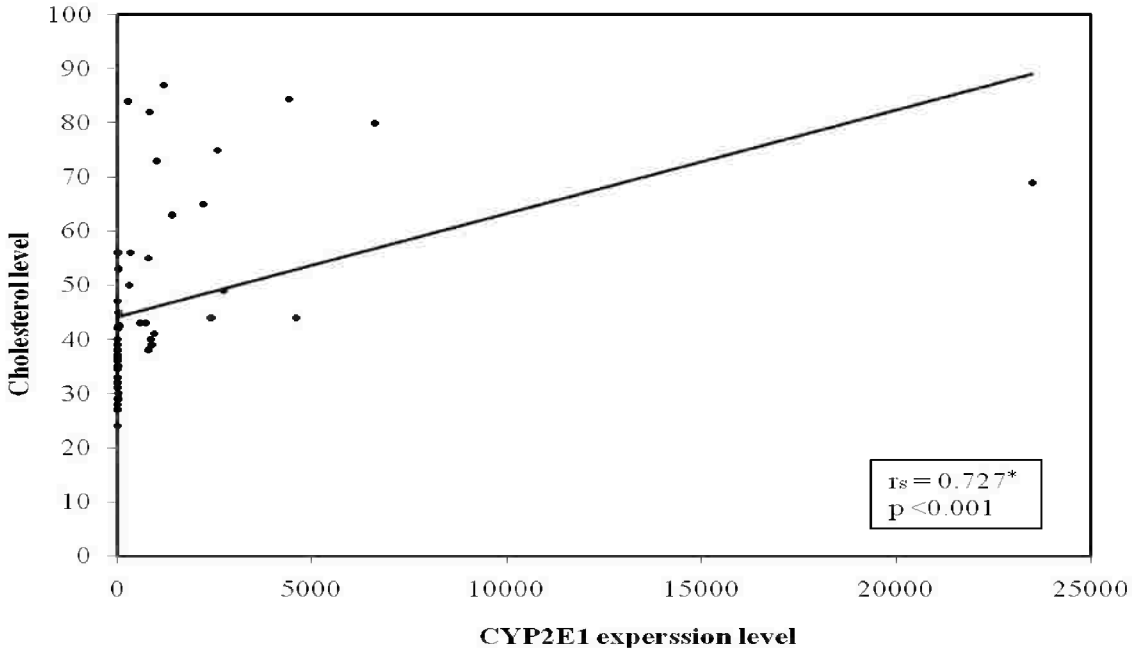

Figure (27): Correlation between CYP2E1 mRNA with cholesterol level.

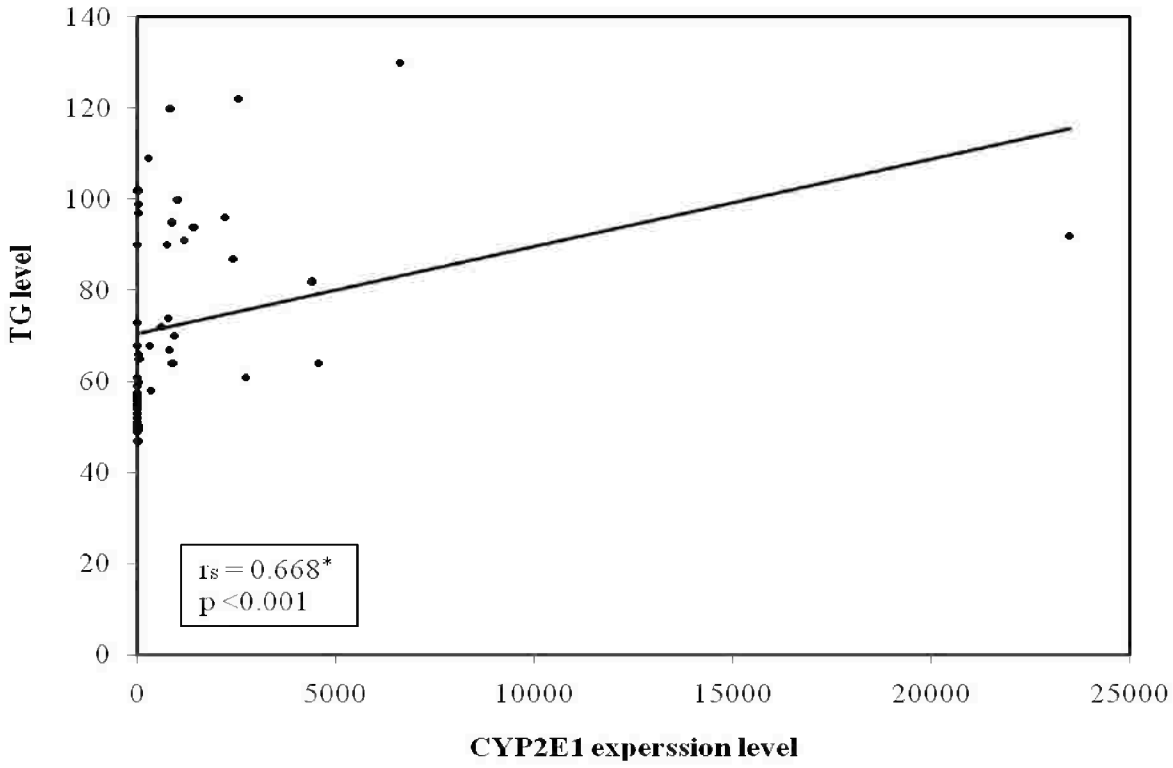

Figure (28): Correlation between CYP2E1 mRNA with serum TG level.

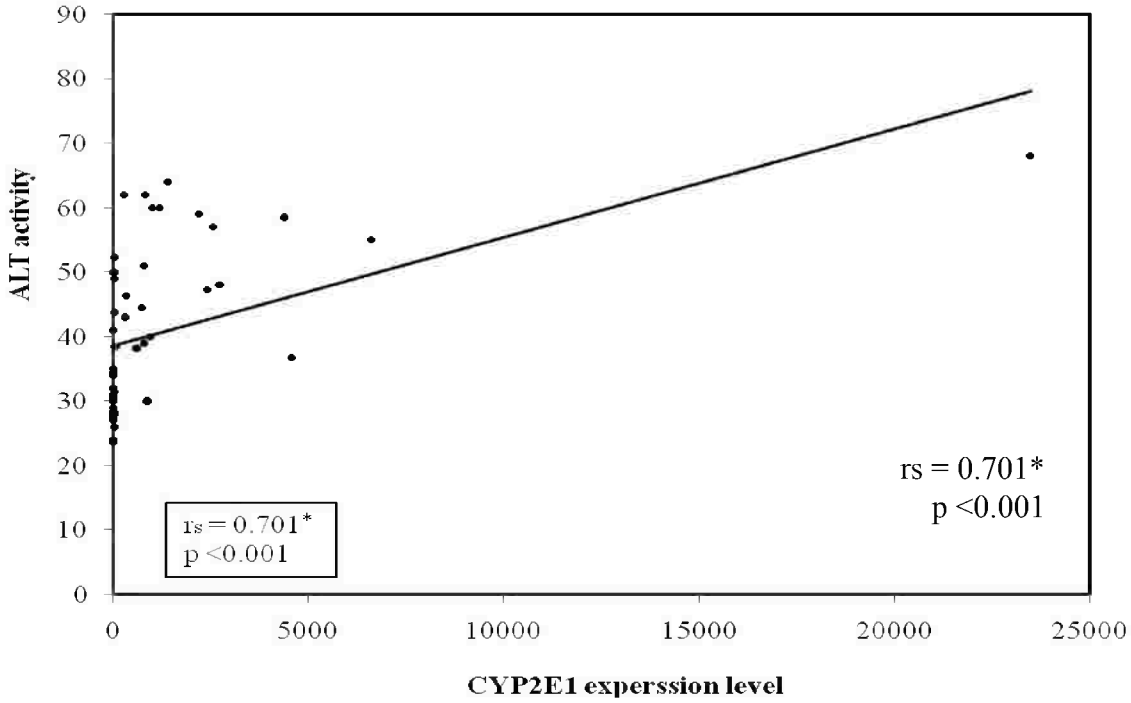

Figure (29): Correlation between CYP2E1 mRNA with ALT activity.

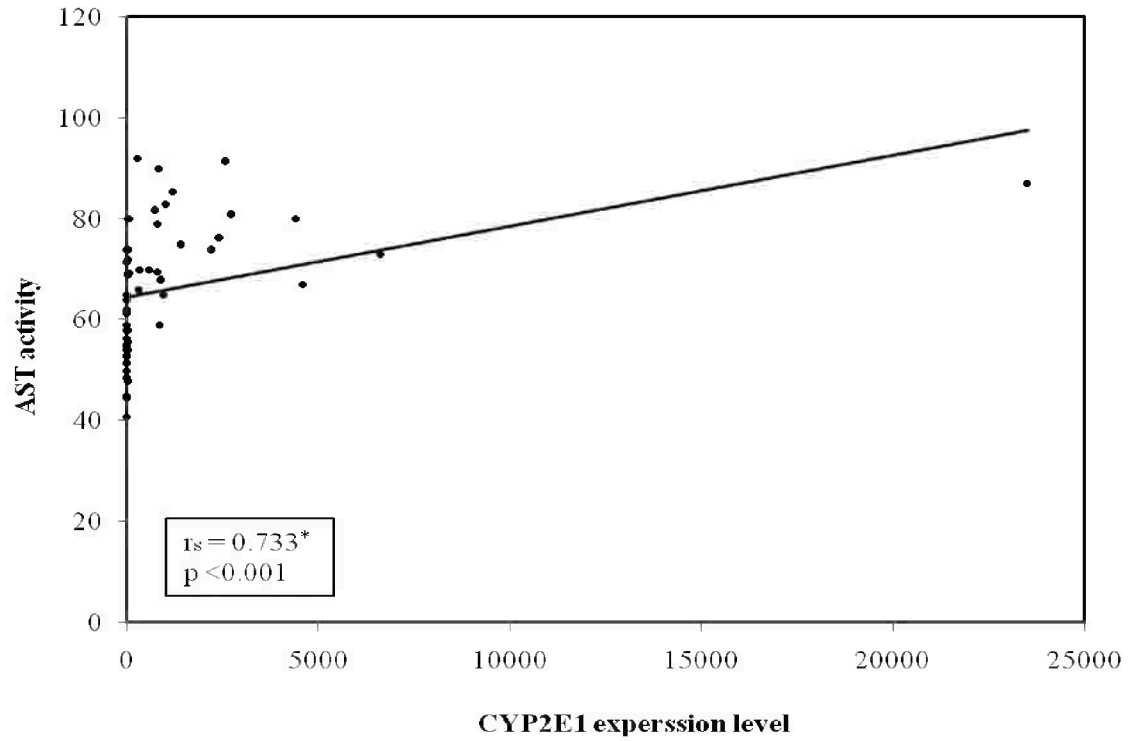

Figure (30): Correlation between CYP2E1 mRNA with AST activity.

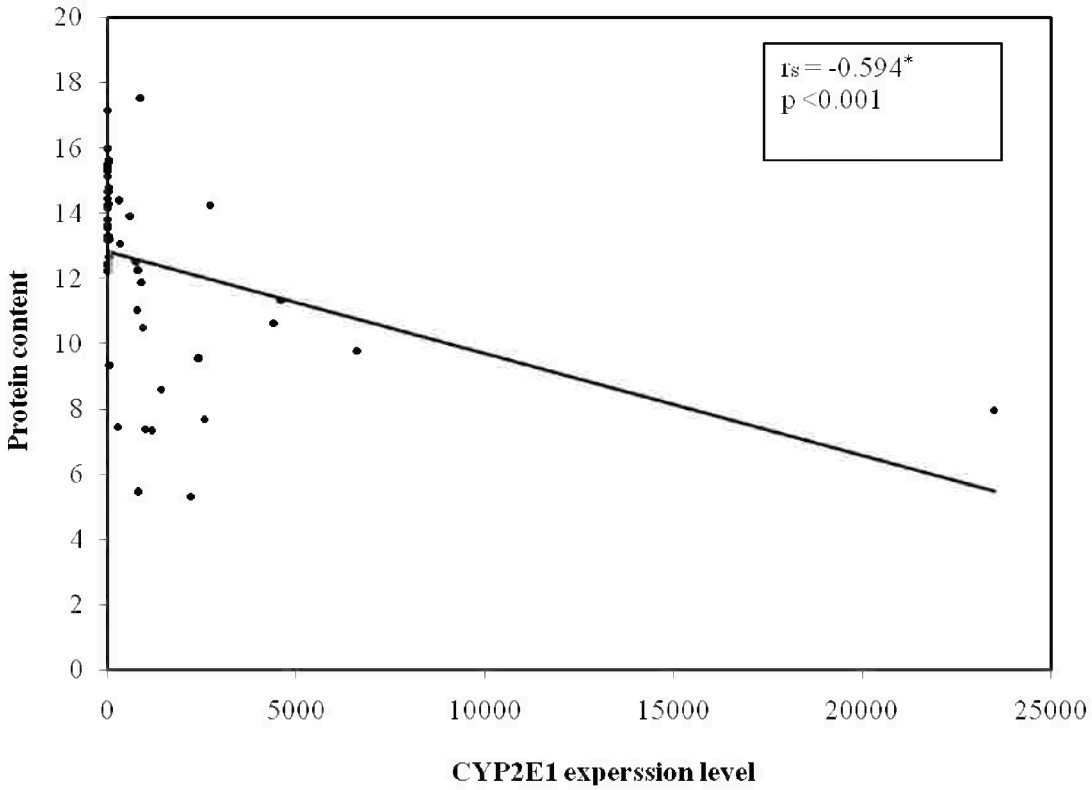

Figure (31): Correlation between CYP2E1 mRNA with protein content

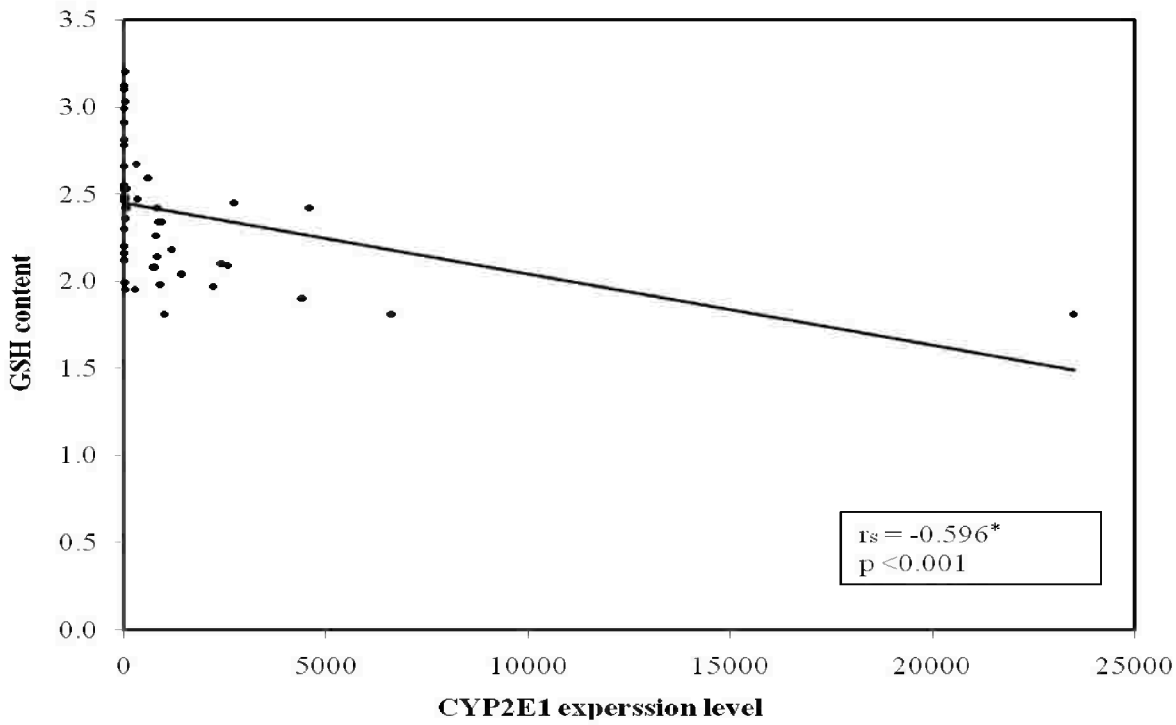

Figure (32): Correlation between CYP2E1 mRNA with GSH content.

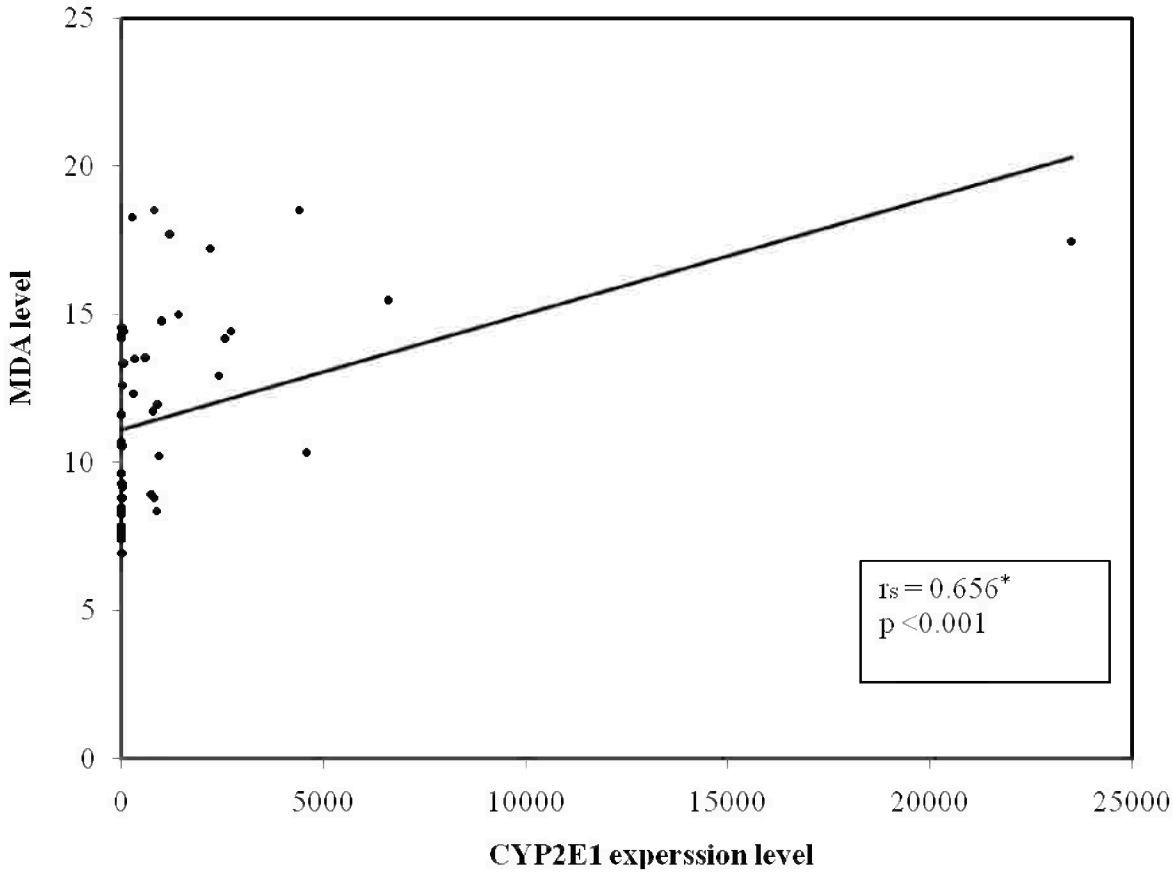

Figure (33): Correlation between CYP2E1 mRNA with MDA level.

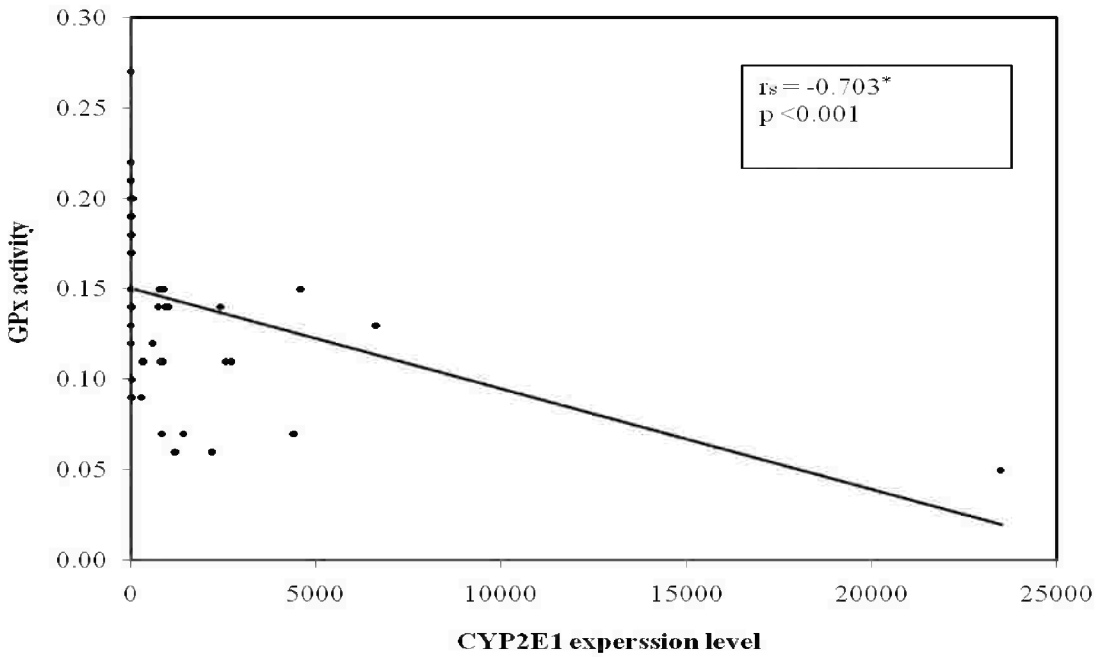

Figure (34): Correlation between CYP2E1 mRNA with GPx activity.

(C) Histopathological results:

A – Liver

Group I: (control group).

Control normal liver sections showed preserved hepatic lobular architecture with central vein (CV), sinusoids radiate out from the central vein (S), hepatocytes with central rounded nuclei (HC) and kupffer cells (KC) (Figure 35).

Group II: (diabetic group).

Liver sections of rat of this group after STZ injection showed severe dilatation and congestion of the portal tract, with degeneration in the surrounding hepatocytes with kupffer cells activation and reduction in the number of nuclei, also necrotic cells and edema which is characterized by distinct eosinophilic cytoplasm with pyknotic or absent nuclei. Also, an inflammatory cells infiltration and cytoplasmic vacuolization in most cells were seen (Figure 36a and 36b).

Group III: (ginseng group).

Liver sections of rat after administration of ginseng revealing normal architecture, with regenerating hepatocytes (Figure 37).

Group IV: (ginseng-pretreated diabetic group).

Liver section of diabetic rat pretreated with ginseng showed nearly normal histological appearance, in spite it was accompanied by vacuolization in some cells and pyknotic nuclei were still observed (Figure 38).

Group V: (ginseng-treated diabetic group).

Liver sections of diabetic rats treated with ginseng, revealed nearly normal restoration of hepatocytes and sinusoids. Although dilatation in sinusoids were observed (Figure 39).

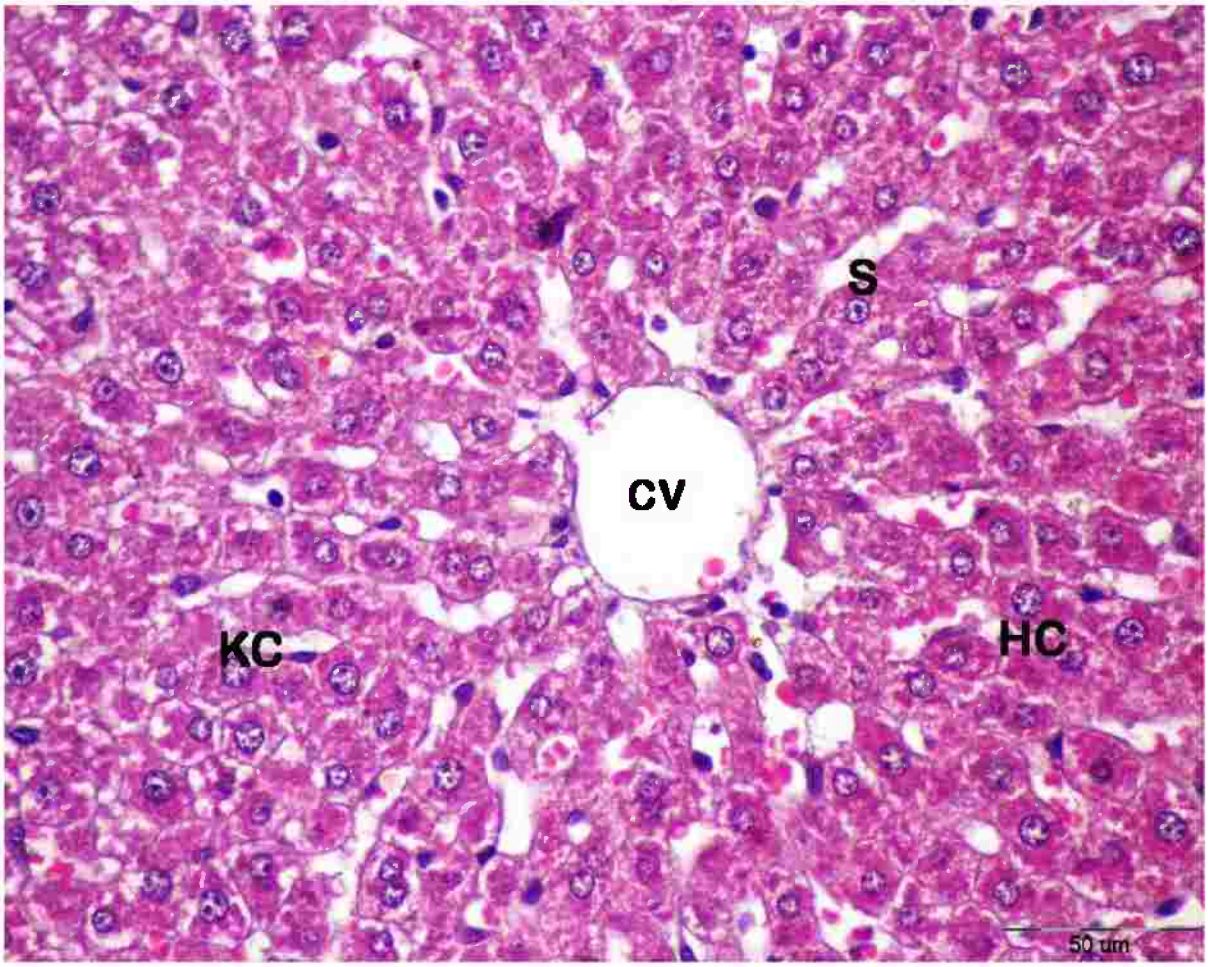

Figure (35): (Group I) Section of control rat liver showing normal histological appearance including central vein (CV), sinusoids radiate out from the central vein (S), hepatocytes with central rounded nuclei (HC), and kupffer cell (KC). H&E 50 X.

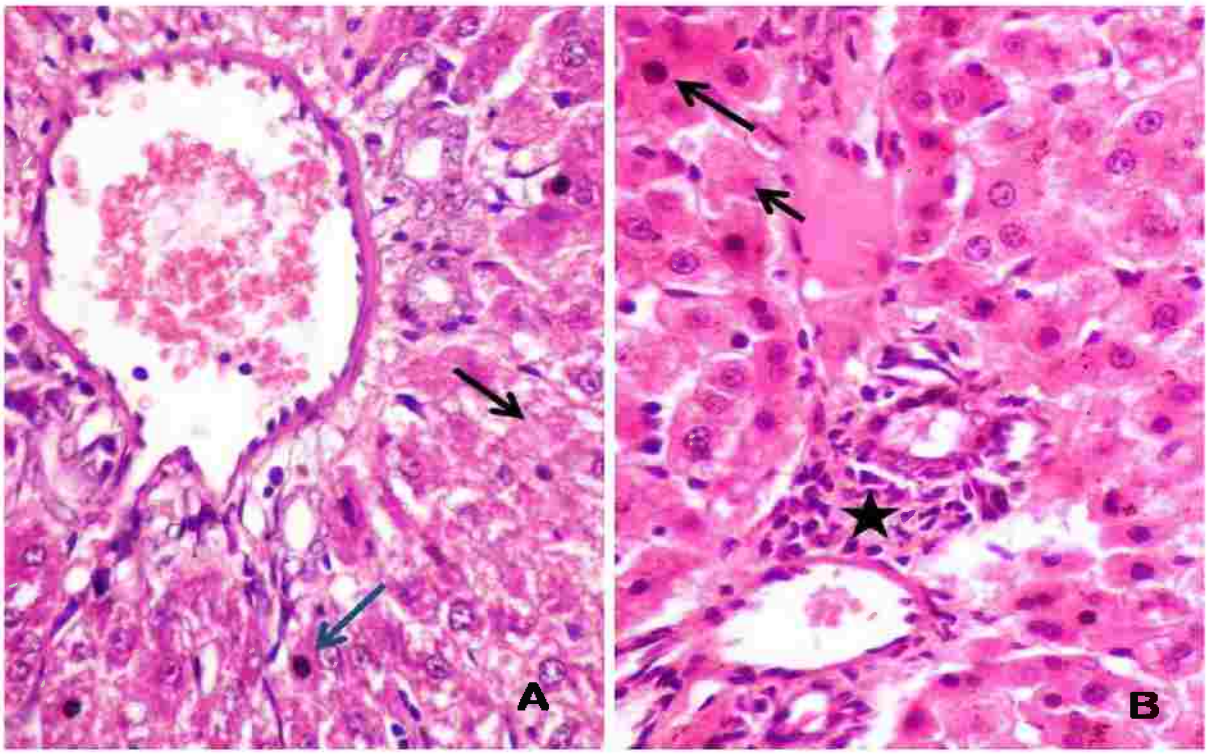

Figure (36a): (Group II) Sections of rat liver administrated STZ showed:

A : severe dilatation and congestion of the portal tract,with degeneration in the surrounding hepatocytes and reduction in the number of nuclei (black arrow), also pyknotic nuclei were observed (blue arrow).

B : Necrotic cells and edema which is characterized by distinct eosinophilic cytoplasm with pyknotic or absent nuclei (Arrows), also an inflammatory cells infiltration were seen (star). H&E 50 X.

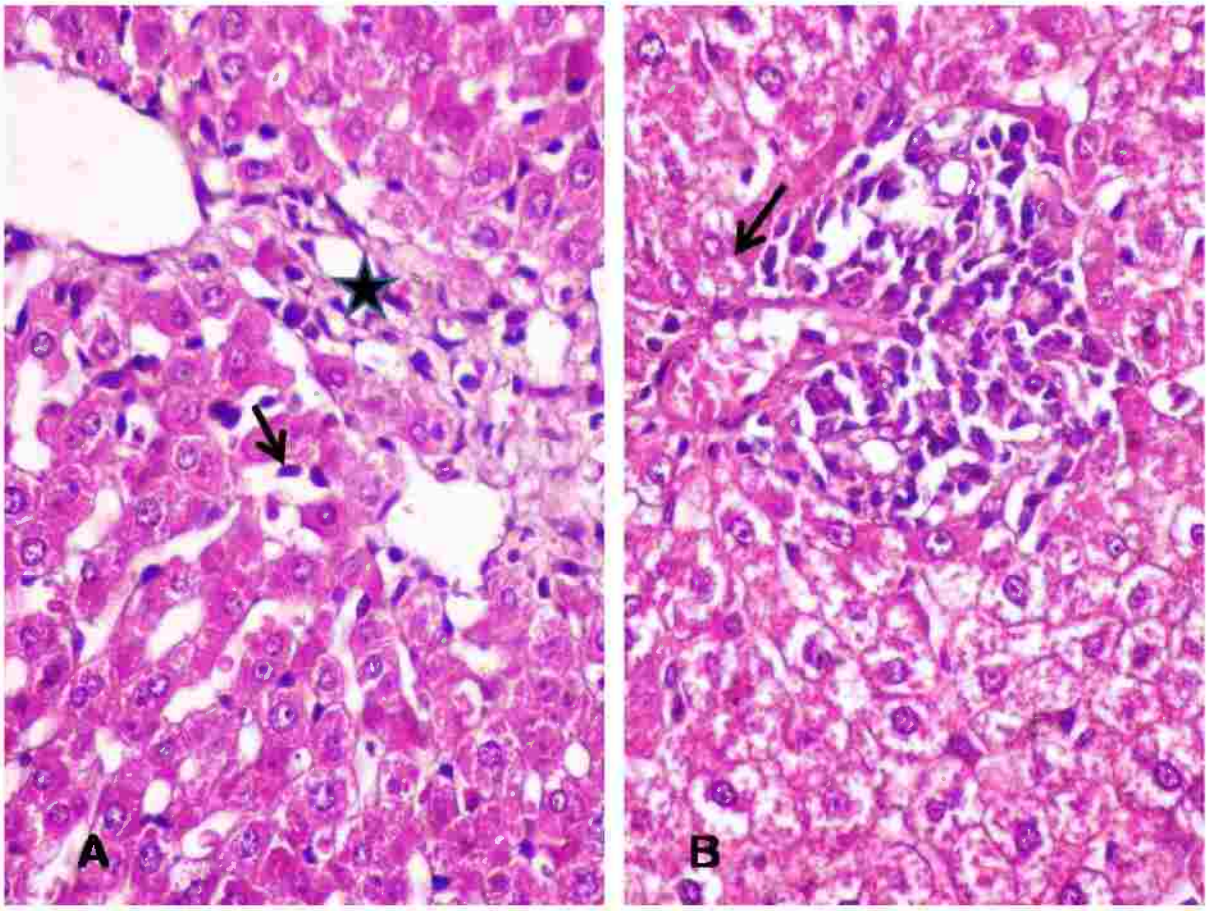

Figure (36b): (Group II) Sections of rat liver administered STZ showed:

A : sinusoidal dilations (star), in addition to kupffer cells activation (arrow)

B: hepatocytes, and sinusoids arrangement were congested, beside the presence of an inflammatory Infiltrate cells in the portal area, also there was cytoplasmic vacuolization in most cells (arrow). H&E 50 X.

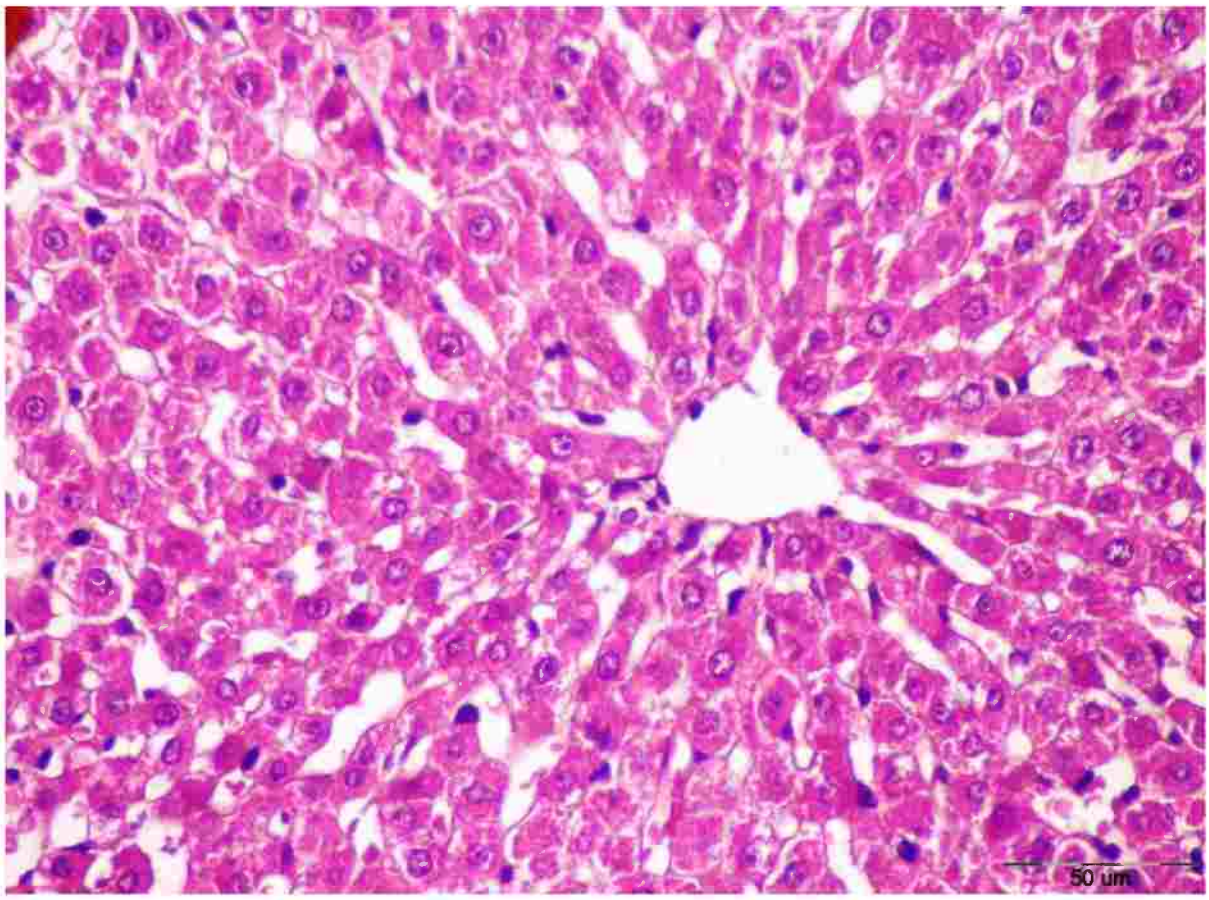

Figure (37): (Group III) Liver section of rat received ginseng revealing normal architecture, with regenerating hepatocytes. H&E 50 X.

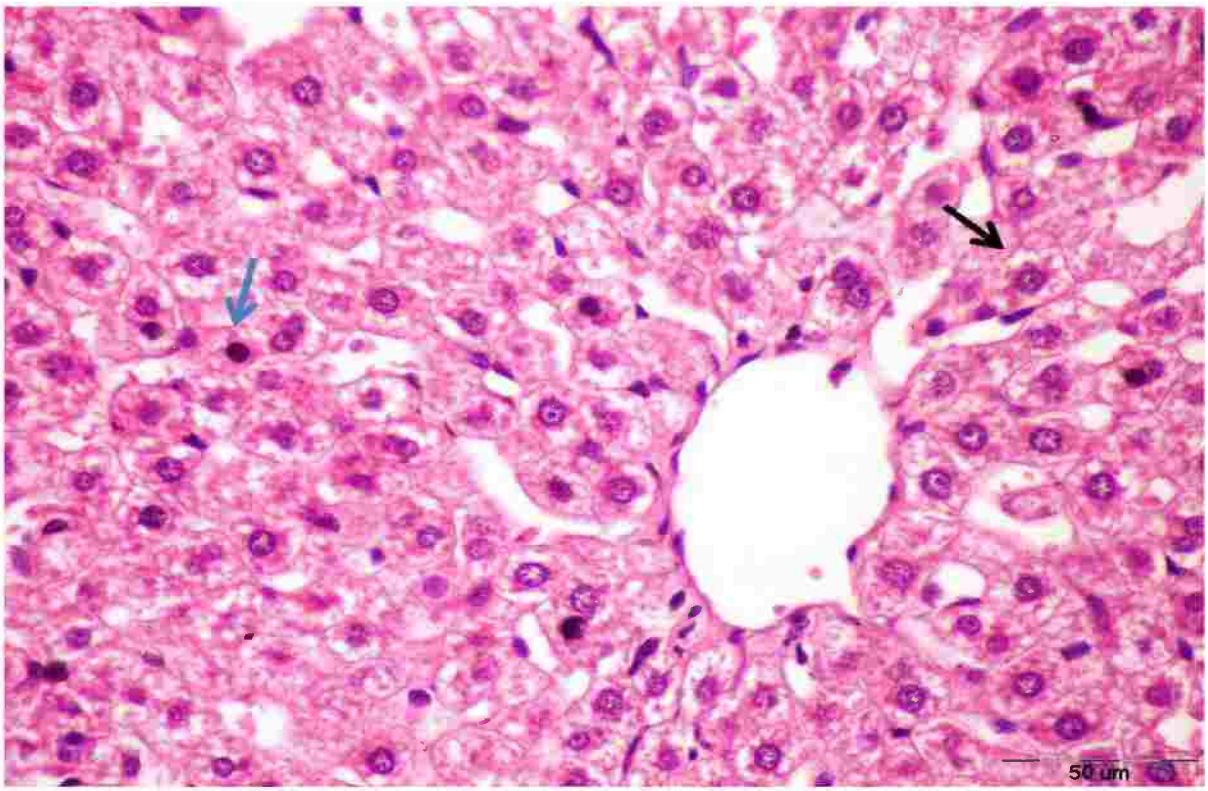

Figure (38): (Group IV) Liver section of rat pretreated with ginseng showing nearly normal histological appearance, in spite it was accompanied by vacuolization in some cells(black arrow) and pyknotic nuclei were still observed (blue arrow). H&E 50X

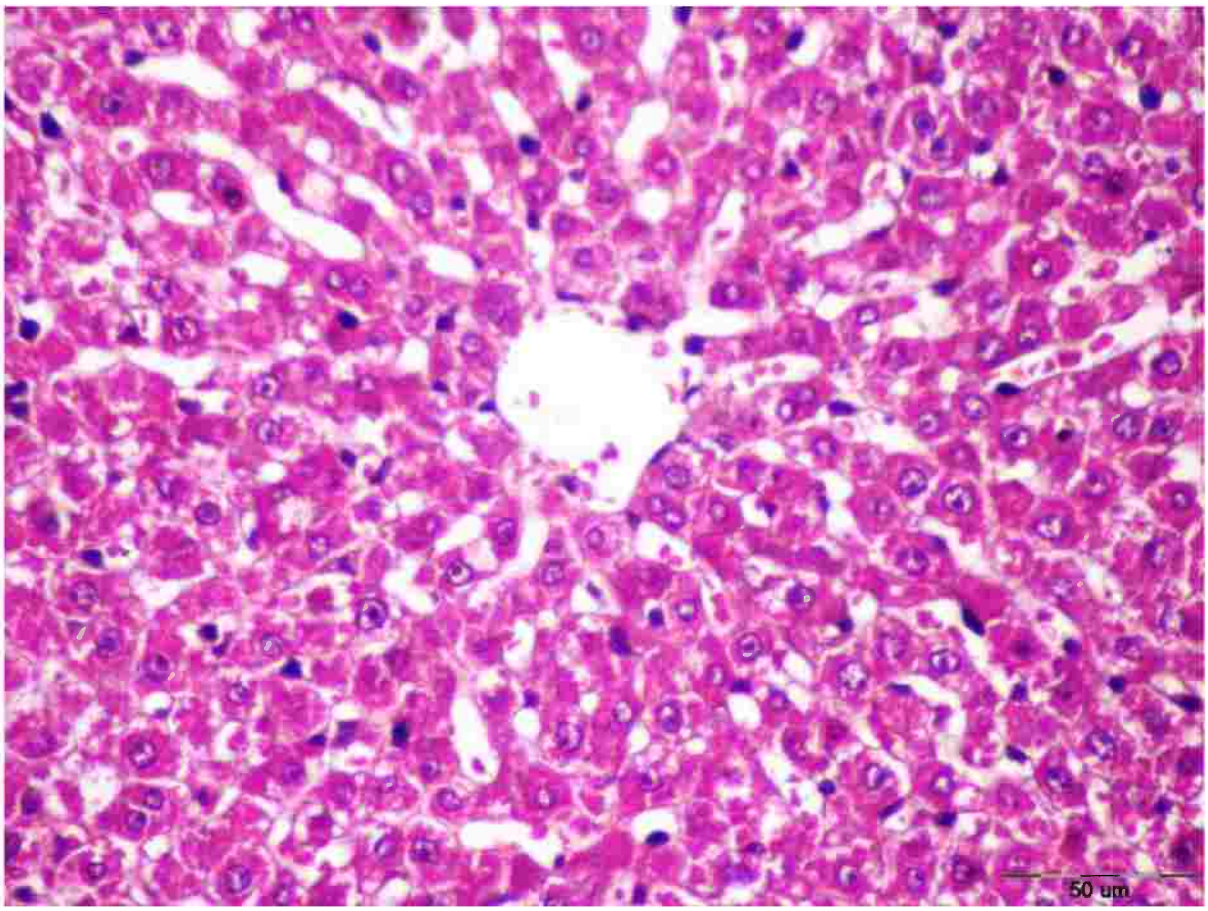

Figure (39): (Group V) Liver section of diabetic rat treated with ginseng, revealed nearly normal restoration of hepatocytes and sinusoids. Although dilatation in sinusoids were observed. H&E 50X.

B- Pancreas

Group I: (Control group)

Control normal pancreas sections showed normal islet of langerhans (L) and normal acini tissues (A), islets were regular with well defined boundaries. Their cells had oval or rounded nuclei. Note the clusters of centrally placed β-cells and peripherally placed alpha-cells (α-cells) (Figure 40).

Group II: (diabetic group)

Pancreas sections of rat of this group after STZ injection showed shrinkage in the pancreatic islet of langerhans as well as reduction and necrosis in the islet cells and severe vacuolation, with β-cell degranulation in the islet of langrehans, though the pancreatic acinar epithelium appeared normal, also disorganization of the structure of the endocrine and exocrine cells illustrated in completely damage of Langerhans cells (Figure 41a and 41b).

Group III: (ginseng group)

Pancreas sections of rat of this group after administration of ginseng showed islet cells and acini were look like normal (Figure 42).

Group IV: (ginseng-pretreated diabetic group)

Pancreas sections of diabetic rat of pretreated with ginseng showed nearly normal islets of langerhans while degeneration of some cells was still observed (Figure 43).

Group V: (ginseng-treated diabetic group)

Pancreas sections of diabetic rat of treated with ginseng, revealed remarkable improvement in the islet of Langerhans. There was an increase in the islet cellular density (Figure 44).

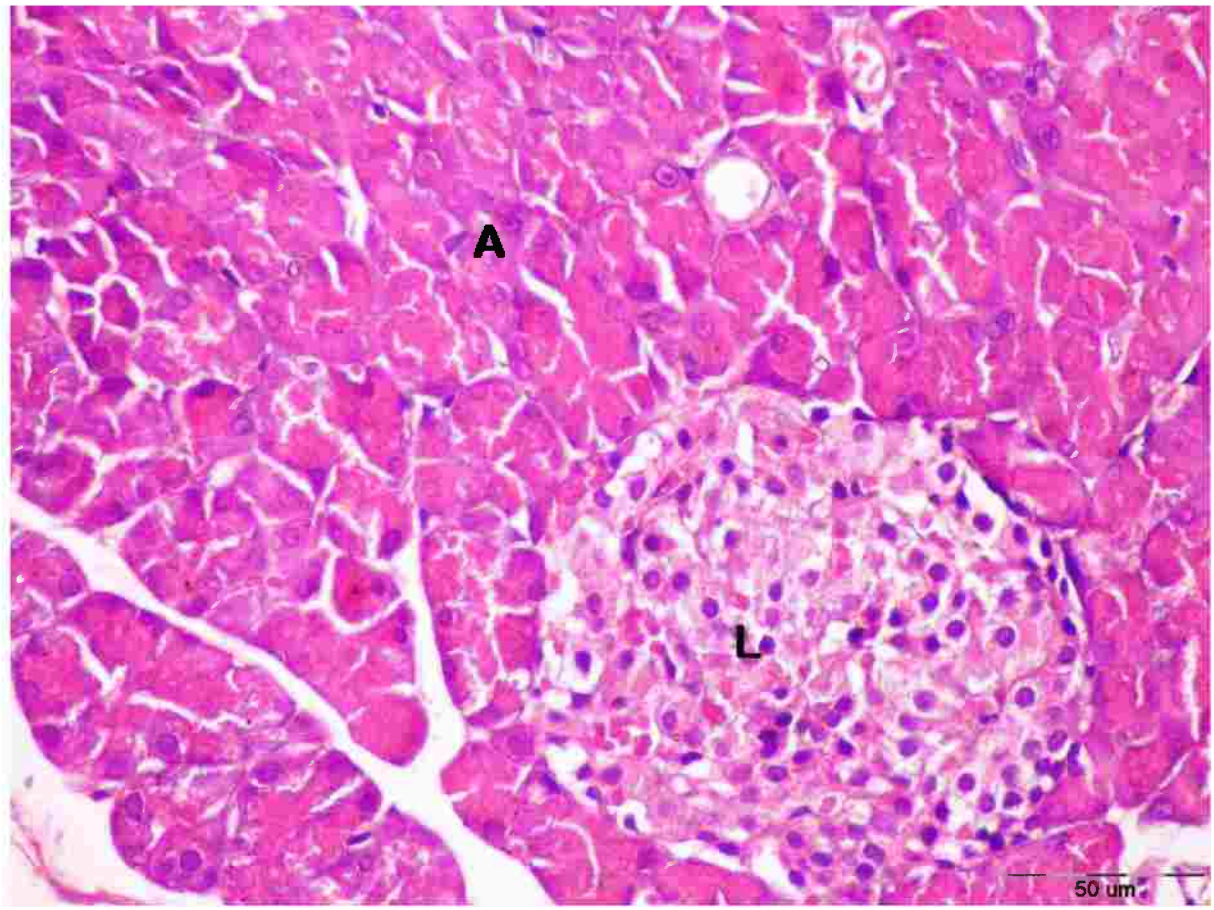

Figure (40): (Group I) Control rat pancreatic tissue, showed normal islet of langerhans and normal acini tissues , islets were regular with well defined boundaries. Their cells had oval or rounded nuclei. Note the clusters of centrally placed β-cells and peripherally placed α-cells. H&E 50x

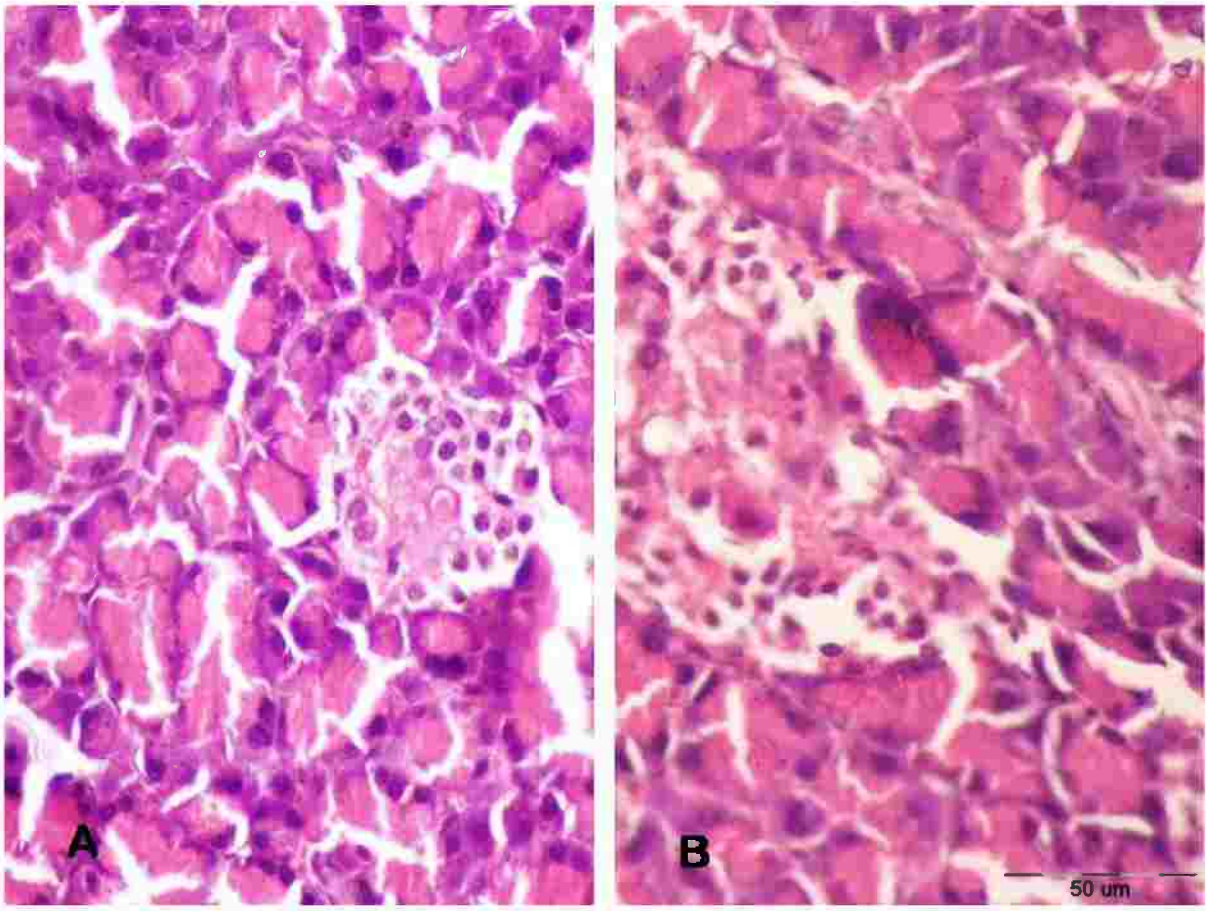

Figure (41a): (Group II) Sections of rat pancreas administered streptozotocin showed:

A : Shrinkage in the pancreatic islet of langerhans as well as reduction in the number of cells in the islet.

B : Signs of necrosis in the islet, and severe reduction in the number of cells, H&E 50x.

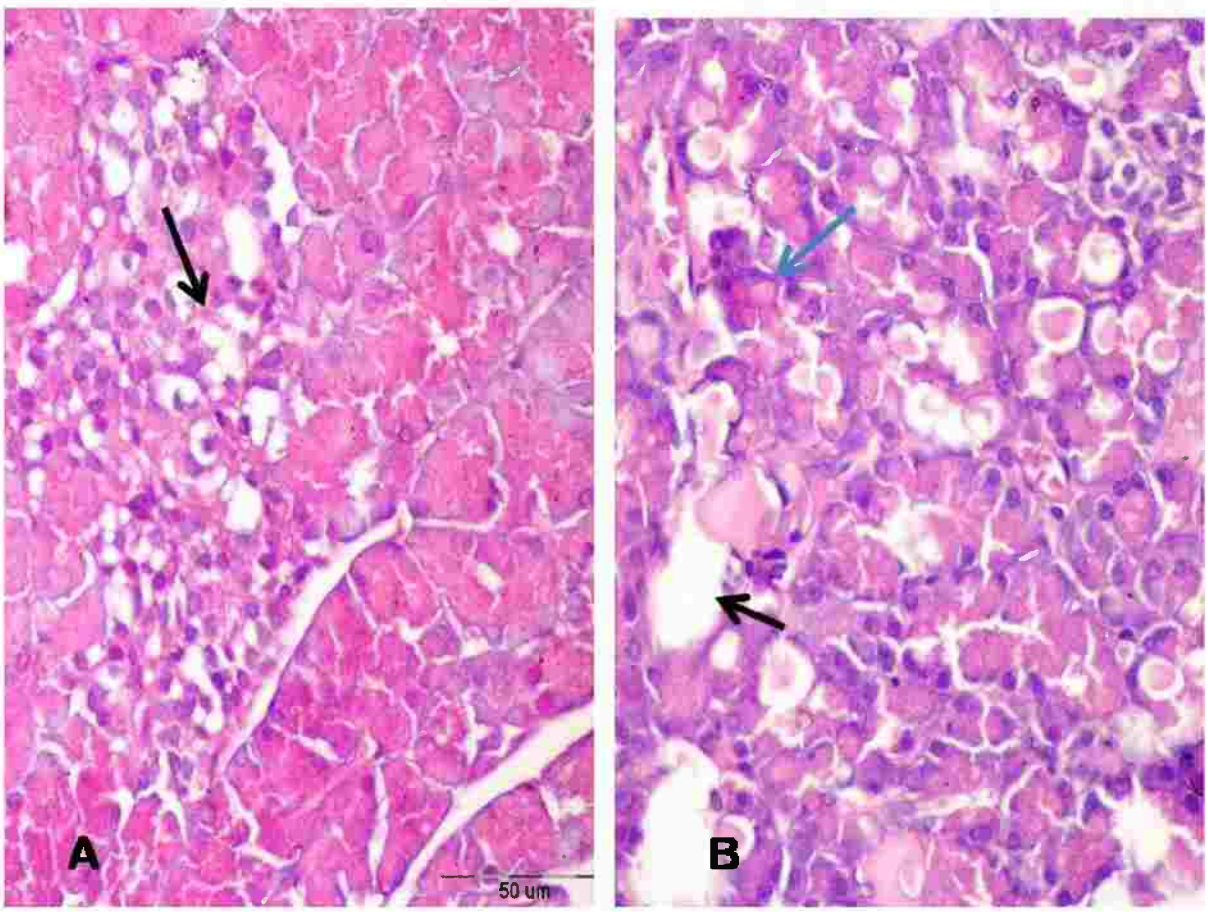

Figure (41b): (Group II) Sections of rat pancreas administered streptozotocin showed :

A: Severe vacuolation, with β-cell degranulation in the islet of langrehans, though the pancreatic acinar epithelium appeared normal.

B: Disorganization of the structure of the endocrine and exocrine cells illustrated in completely damage of langerhans cells (black arrow) with damaged and necrotic pancreatic acini (blue arrow).H&E 50 X.

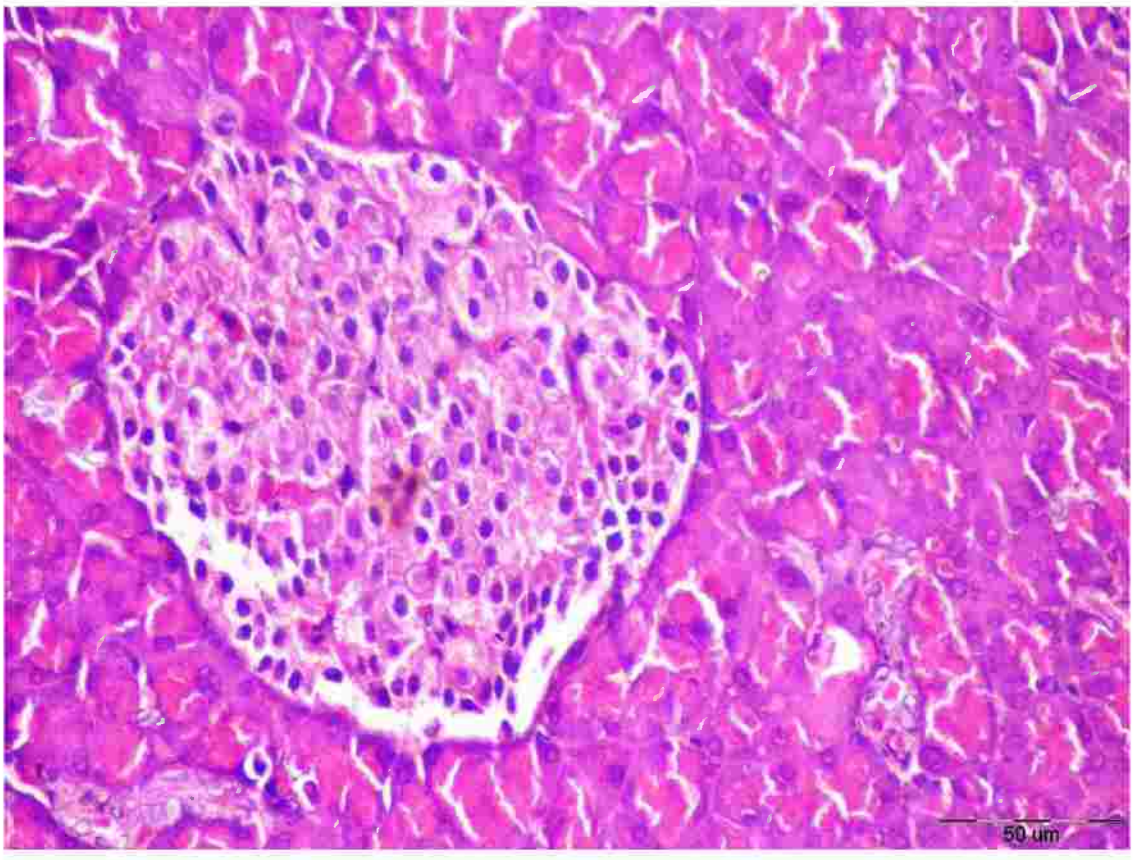

Figure (42): (Group III) Pancreatic sections of rats administered ginseng showed islet cells and acini were look like normal. H&E 50x.

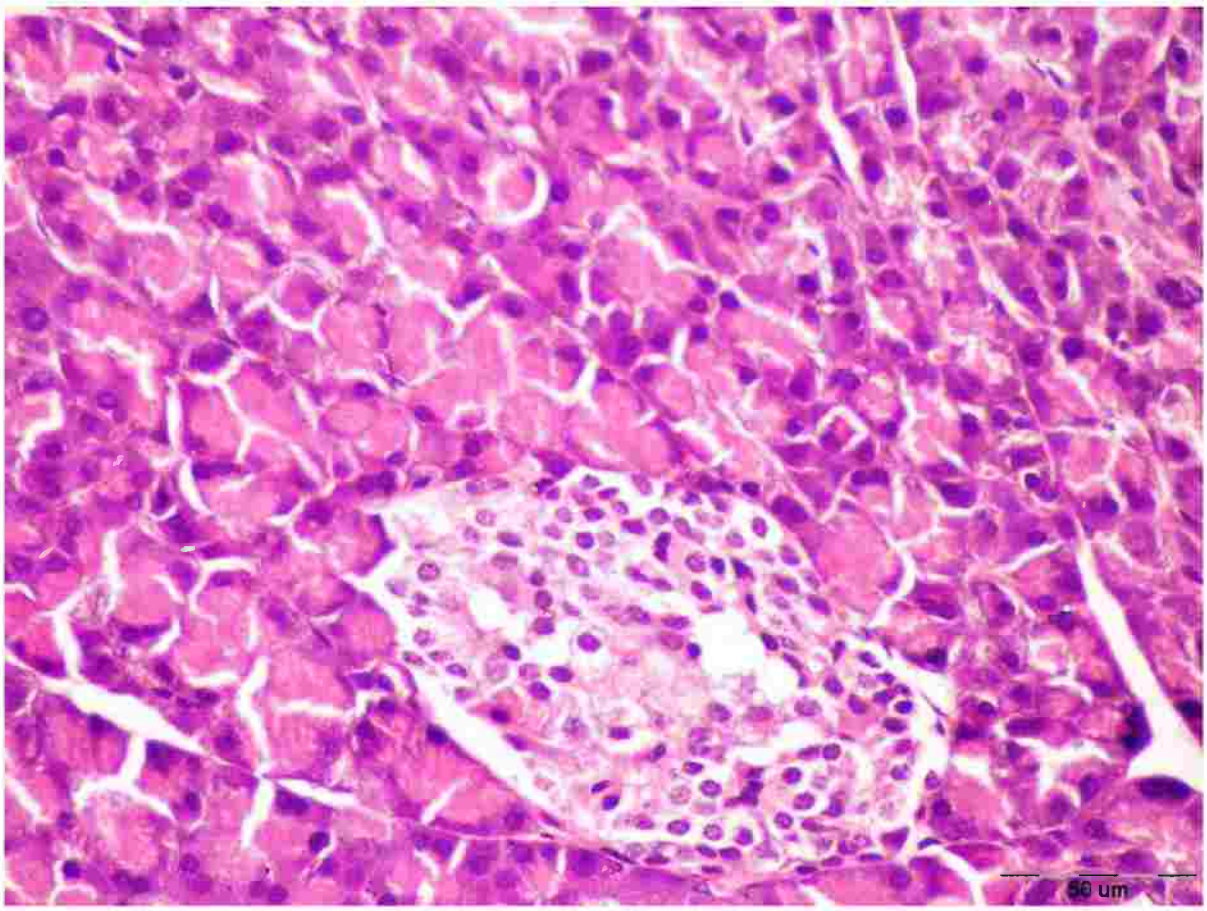

Figure (43): (Group IV) Pancreatic section of diabetic rat pretreated with ginseng showing nearly normal islets of langerhans while degeneration of some cells was still observed. H&E 50 X.

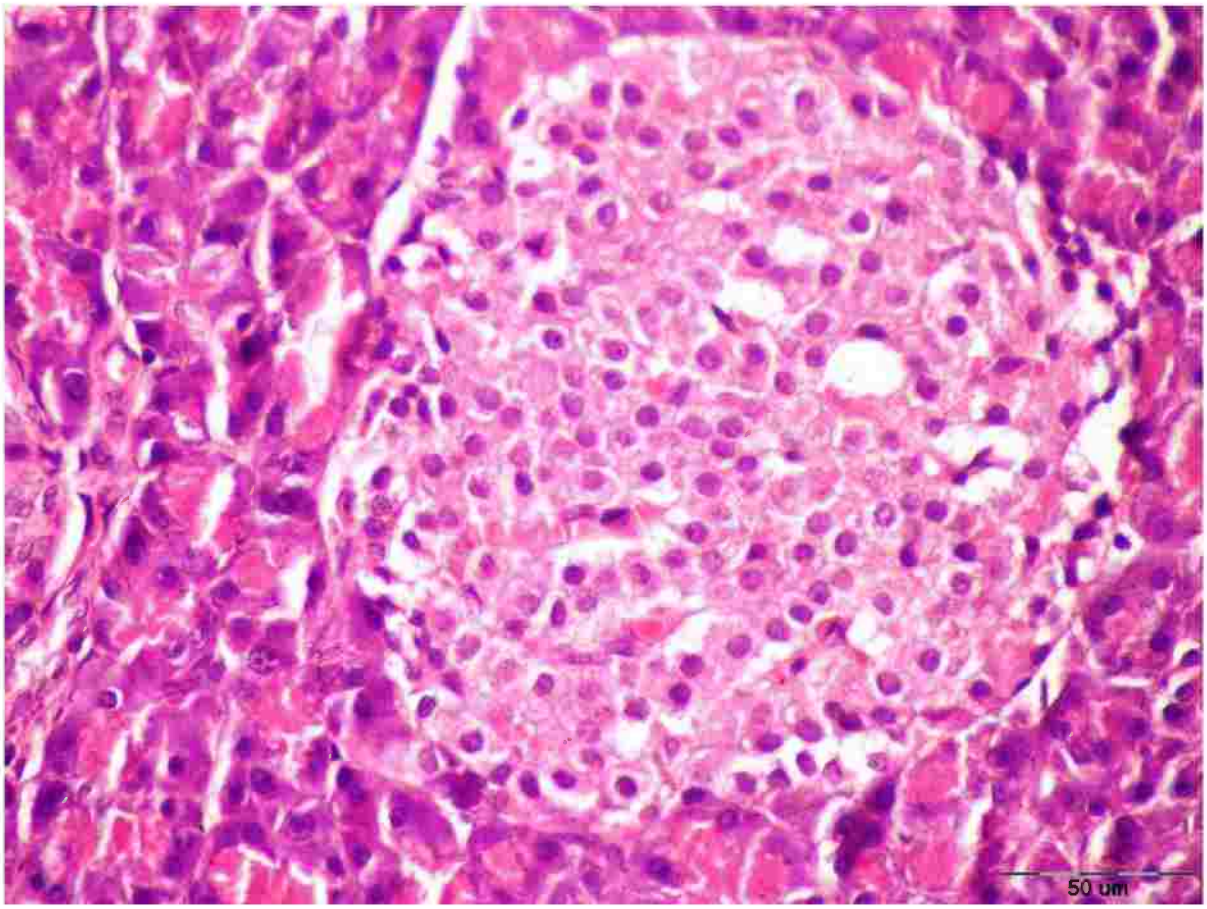

Figure (44): (Group V) Section of diabetic rat pancreas treated with ginseng, revealed remarkable improvement in the islet of Langerhans. There was an increase in the islet cellular density, H&E 50 X.

DISCUSSION

DM is a metabolic disorder characterized by a loss of glucose homeostasis with the disturbance of carbohydrates, fat and protein metabolism resulting from defects in insulin [201]. In the present study, diabetes was induced in rats by a single intraperitoneal injection of STZ (60 mg/kg body weight). The hepatoprotective and antidiabetic activity of *Panax ginseng* extract was determined.

Treatment of DM with oral hypoglycemic agents like sulphonylurea and biguanide is associated with severe adverse effects [202]. Therefore, herbal drugs are gaining importance in the treatment of various diseases.

Ginseng has been used as a medicinal plant for more than 2,000 years; it has a wide range of pharmacological and physiological actions, such as anti-aging, anti-stress, anti-fatigue and anti-tumor activities [203]. In addition, several studies indicated the properties of ginseng in lowering blood glucose level [204] and stimulating sugar metabolism [205].

CYP2E1 is one of the CYP450 isoforms. Overexpression of CYP2E1 is of direct importance to human health and has been associated with a range of diseases, including diabetes [206–208], alcoholic liver disease and cancer [209–213].

The present results reveled that in groups II, IV and V the mean value of FPG was significantly increased concomitant with a significant decrease in the final body weight of rats compared to the control group.

The administration of STZ to the normal rats results in the destruction of β-cells of islets of langerhans and malfunctioning of the pancreas causing diabetic condition. This leading to the increase in the blood glucose levels and decreased body weight in the untreated diabetic rats. These results are in accordance with the results of Szkudelski in (2001) who showed that the β-cells undergo destruction and necrosis due to the action of STZ [214]. Also, the elevation of blood glucose in STZ-induced diabetic rats may be due to lower levels of plasma insulin[215].

These results are confirmed by the histopathological findings which revealed that shrinkage in the pancreatic islet of langerhans as well as reduction in the number of cells in the islet and necrosis formation in group II received STZ. These findings consistent with the results of several studies detected the same histological changes [216,217].

In this study, the rats received ginseng (group III) exhibited a much high body weight over that of the control and the diabetic groups,while in group V the body weight decreased than the control group. The weight-enhancing effect of the ginseng may be related to the proposed peroxisome proliferator-activated receptors gamma (PPARγ) agonist the effect of ginseng extracts [218-220]. Ginsenosides have been proposed to work through PPAR alpha and gamma (PPARα and γ) [218, 221, 222]. The PPARs are members of the nuclear hormone receptor family and are distributed in a variety of tissues [223,224]. PPARγ has a large distribution in adipocytes and plays an important role in the adipogenesis and stimulates production of small

insulin-sensitive adipocytes. Also, PPARγ induce the expression of genes involved with the insulin signaling cascade [219].

In group IV and V there was insignificant increase in the final body weight as compared to the diabetic group, these increase in body weight may be due to the effect of ginseng treatment. While there was significant decrease in body weight as compared to the ginseng group, these results may be due to the effect of STZ-administration. These biochemical observations are supported by the histopathological examination of pancreas in group IV which showed nearly normal islets of langerhans while degeneration of some cells was still observed and in group V it revealed remarkable improvement in the islet of Langerhans. There was an increase in the islet cellular density.

The current investigation reveled that the rats administrated ginseng (group III) had no effect on all studied parameters (FPG, cholesterol, TG, ALT, AST, GSH, GPx, MDA and CYP2E1mRNA gene expression) as compared to the control group indicating the safety of ginseng. In accordance with El-Khayat et al. in (2011) who study the role of *Panax ginseng* is protecting to renal function in diabetic rats by STZ where the mean values of fasting blood sugar and antioxidant parameters were not changed in the ginseng group indicating the safety of ginseng [225].

These biochemical observations are supported by the histopathological examination of the rat liver and also pancreas which indicated that both organs in group III were look like normal.

The present investigation showed that, there was a significant decrease in FPG in group III, IV and V as compared to the diabetic group.

The reduction of the elevated FPG by ginseng was attributed to enhancement of glucose uptake through stimulating translocation of glucose transporter GLUT4, inhibition of intracellular inflammatory molecules as Jun N-terminal kinase which caused serine phosphorylation to insulin receptor substrate. Consequently, this leads to interruption of signal transduction from insulin receptor to downstream molecules and insulin resistance [226,227]. Additionally, other investigators recorded a definite insulinogenic properties of ginseng [228] or direct and indirect stimulatory on β-cell secretion of insulin [229].

The present work revealed that the mean values of FPG level in group IV and V showed insignificant differences when compared with each other but statistically higher than FPG level in ginseng group. These results may be due to the effect of STZ-administration.

In comparison with the control group, the groups II, IV and V had a significantly increase in cholesterol, TG levels, ALT and AST activities due to STZ-administration which produce several side effects including liver and kidney dysfunction [230].The increased level of cholesterol may be due to the inability of the rats to metabolize carbohydrates as energy source, and the subsequent use of FFA for energy and cholesterol synthesis [231].

The higher level of TG may be due to that the hyperglycemia which a major complication of diabetes. In hyperglycemia, the rate at which plasma fatty acids become TG is greater than normal, leading to an increase in the plasma TG concentration [232].

Serum transaminases are responsible for producing ketone bodies from amino acids and produce increased concentration of glucose levels [233]. Increase in ALT and AST results in increased glucose levels [234].The present study illustrated a significant increase in serum AST and ALT activities in groups II, IV and V as compared to the control group. These results were agreed with the findings recorded by Maritim et al in (2003) and Jung et al in (2006). They reported that the rise in the activity of these enzymes is mainly due to their leakage from liver into blood stream [235,236]. Experimental studies have shown that subtle membrane changes are sufficient to allow passage of intracellular enzymes to the extracellular space [237].Very large concentration gradient between the hepatocytes and the sinusoidal space usually exists for enzymes. Cell damage increases permeability causing cytosolic isoenzymes to spill into the sinusoids and from there into the peripheral blood [238].

These results are confirmed with the histopatholgical results which revealed that the liver portal tract showed severe dilatation and congestion, with degeneration in the surrounding hepatocytes and reduction in the number of nuclei, also pyknotic nuclei were observed in group II. These findings are in agreement with the findings of Das et al. in (1996) and Degirmenchi et al.in (2002) who showed dilatation of veins, loss of usual concentric arrangement of hepatocytes, liver fibrosis and decreased in glycogen activity [238,239].

The present results revealed that cholesterol, TG levels and ALT, AST activities in groups III, IV and V were significantly decreased as compared to the diabetic group. These results may be attributed to ginseng treatment since saponins components in ginseng stimulated the decline and excretion of cholesterol, improved lipid metabolism [240] and inhibited the intestinal absorption of cholesterol [241]. Also, the ginseng extracts decreased aminotranferase activity, thus having a protective effect against STZ-induced liver damage [242]. The mechanism of hepatoprotection of ginseng saponins was assumed to be through an inhibition of the activity of CYP450 enzymes in the rat liver microsomes [243].

The significantly increased cholesterol, TG levels and ALT, AST activities in groups IV and V as compared to the ginseng group (group III) may be due to the effect of STZ-toxicity [242].

In the current study there were a significant decrease in cholesterol level, ALT and AST activities, while insignificantly decreased in FPG and TG levels in the ginseng-treated diabetic group (group V) as compared to that of the ginseng-pretreated diabetic group (group IV), indicating that the therapeutic effect of ginseng is more pronounced than the protective effect. These results are confirmed with the histolopatholgical results which revealed that the liver showed nearly normal histological appearance, in spite it was accompanied by vacuolization in some cells and pyknotic nuclei were still observed in group IV and also revealed nearly normal restoration of hepatocytes. Although dilatation in sinusoids were still observed in group V.

There is increasing evidence showing that diabetes is associated with increased oxidative stress [244]. Hyperglycemia may cause increased production of free radicals which is related to glucose auto-oxidation that has been linked to non enzymatic glycation and glycated proteins which are a source of free radicals [245]. GSH, the primary endogenous antioxidant, has a multifaceted role in antioxidant defense and it is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by GPx [247].

In terms of changes in hepatic GSH content and GPx specific activity, the groups II, IV and V had a significant decrease compared to the control group and a significant increase were shown in groups III, IV and V as compared with the diabetic group (group II).

The decrease in GSH content is thought to be due to STZ's absorption into the body, inducing an oxidative stress environment and hence GSH is used up to buffer the oxidative effects leading to decreased GSH content.

However, with the administration of the ginseng extract, this reduced the need for GSH, leading to an increase in its content [242].

GPx are a class of selenoproteins that use two molecules of GSH to facilitate the reduction of oxidants. There are several isoforms that differ with respect to the oxidants used [243]. The effect of diabetes on GPx activity is highly variable with respect to the model of diabetes used and even the tissue type studied [248,249].

A decrease in GPx specific activity due to the direct attack of STZ-administration-produced ROS against endogenous antioxidant enzymes [250]. Upon administration of the ginseng extract, there was an increase in the activity of this antioxidant enzyme. In particular, it is thought that ginsenosides such as Rh2 that are present in ginseng induce the expression of antioxidant enzymes [242] by stimulating their gene expression [251].

The level of GSH content in groups IV and V showed insignificant difference when compared with each other but statistically lower than the ginseng group (group III). These results may be to the effect of STZ-administration [242].

The specific activities of GPx in groups IV and V were significantly lower than that of ginseng group and this may be due to the effect of STZ-administration. On the other hand, the GPx activity in group V was significantly higher than that of group IV indicating that the therapeutic effect of ginseng is more pronounced than the protective effect.

The MDA content, a measure of lipid peroxidation, is parallel with the degree of oxidative stress. Therefore, the assay of MDA could be a maker of cell damage [252]. In the present study, we observed that MDA levels in groups II, IV and V was significantly higher than the control group indicating a generation of free radicals and increase oxidative damage in liver. El-Khayat et al. in (2011) found the same results but on the kidney [225]. We also found a reduction of MDA in group III, IV and V than diabetic group.This observation is in accordance with Liu et al. in (2003) who found that ginseng extracts scavenge oxidative species [253], also Surh et al. in (2001) indicated that ginseng extracts attenuate lipid

peroxidation. That is, it may be related to saponins which play a major role in antioxidant activities [254].

The level of MDA in groups IV and V was significantly increased as compared with the ginseng group. These results may be due to the effect of STZ-administration [242]. On the other hand, the MDA level in group V was insignificantly decreased than in group IV indicating that the therapeutic effect is more pronounced than the protective effect.

This study is concentrated on the regulation of the hepatic drug-metabolizing CYP gene (CYP2E1) in diabetes and showed the effect of administrated ginseng. We found that there was an elevation in CYP2E1 mRNA gene expression in group II, IV and V as compared to the control group. These results are in agreement with various studies carried out previously [251,252]. It is clear that chemically STZ-induced DM consistently results in an increased expression of hepatic and lymphocytic CYP2E1 protein and mRNA, and alterations in other drug-metabolizing enzymes in rodents, which are partly a result of increased concentrations of circulating ketones [257-261]. Elevated oxidative stress and ROS production in diabetes often parallels the increased expression of CYP2E1 mRNA [262].

CYP2E1 is known to metabolize endogenous compounds such as fatty acids, lipid hydroperoxides and ketone bodies into aldehyde and many xenobiotics and carcinogens into nucleophilic reactive species. Diabetes, fasting and long-term alcohol consumption all result in altered nutritional status and metabolism (increased ketone body, glucose and fatty acid levels) as well as altered hormone (insulin, glucagon and growth hormone) secretion and so affect CYP2E family [263].

Current literature about CYP2E1 in diabetes states that the enzyme is regulated at transcriptional, translational or posttranslational mechanism. The induction of CYP2E1 mRNA is not accompanied by an increase in CYP2E1 gene transcription, rather than mRNA is selectively stabilized in diabetes due to lack on insulin action. Since, mRNA of CYP2E1 was found to be destabilized by insulin [264], the elevation of CYP2E1 mRNA levels in the diabetic state has been attributed to mRNA stabilization [263]. These results are confirmed with the histolopatholgical results which revealed that the liver sections in group II showed severe dilatation and congestion of the portal tract, with degeneration in the surrounding hepatocytes and reduction in the number of nuclei, also pyknotic nuclei were observed.

A significant reduction of CYP2E1 mRNA gene expression in groups III, IV and V was observed when compared to the diabetic group (group II). These reductions may be attributed to ginseng treatment. Our observation is consistent with a previous study which stated that ginseng root has been observed to supress the enzyme activity and mRNA expression of CYP450 isoenzyymes [265].

Treatment with ginseng led to a significant reduction in CYP2E1 mRNA gene expression suggesting that the suppression of CYP450 by ginseng leads to reduction of reactive metabolites and tissue injury. Several studies suggested that natural compounds that reduce chemical activating enzymes are useful for protection against chemically induced toxicity. Therefore, inhibition of CYP2E1 by ginseng may be important role in both protection

against hepatotoxicity of STZ and chemoprevention via decreasing metabolic activation of other xenobiotics [266].

The mechanisms which provide ginseng hepatoprotective effects are closely attributed to antioxidant properties [267]. So the reduction of this induction by antioxidant treatment suggests some other mechanisms which also regulate the mRNA levels of CYP2E1. Effects of antioxidants on CYP2E1 mRNA gene expression may be mediated by the reduced levels of circulating ketone bodies; which has a direct effect on CYP2E1 mRNA gene expression in diabetes since elevated expression of CYP2E1 mRNA in these pathophysiological states in rats has been attributed to elevated ketone body levels [263]. Also, further investigation into ginseng metabolism suggested that ginsenoside metabolites like compound K, produced after oral administration, are responsible for the inhibition of the CYP450-mediated metabolism rather than naturally occurring ginsenosides [268]. This evidence may explain some of the hepatoprotective effects of ginseng against hepatotoxins.

The present work revealed that the level of CYP2E1 mRNA gene expression in groups IV and V showed insignificant difference when compared with each other but higher than the ginseng group (group III) these results may be due to the effect of STZ-administration.These findings are confirmed with the histolopatholgical results which revealed that the liver sections in group IV were nearly normal but with vacuolization and pyknotic nuclei in some cells and in group V, there were normal restoration of hepatocytes . Although dilatation in sinusoids were seen.

There were a significant positive correlation between CYP2E1 mRNA gene expression and each parameters of FPG, Cholesterol, TG, MDA levels, ALT and AST activites.

Chemically induced diabetes in laboratory animals can cause changes in expression, protein level and enzymatic activity of various CYP450s [269]. So our observation of positive correlation may be due to that CYP2E1 has been identified as a source of ROS, which are thought to cause lipid peroxidation resulting in liver damage [270-272].

Furthermore our correlation study indicated that there was a significant negative correlation between CYP2E1 mRNA gene expression and each of GSH content and GPx activity. It is important to note that elevation of CYP2E1 mRNA gene expression level in DM generates oxidative stress, which could contribute to increase in GSH utilization and also the reduction of GPx enzyme is due to increase in GSH concentration.

In the current study it was found that ginseng is safe since the values of all parameters are the same and not changed when compared to their corresponding levels in the control group.

The therapeutic effect of ginseng is more pronounced than the protective effect where (FPG, Cholesterol, TG and MDA) levels and (ALT and AST) activity were decreased, GSH content and GPx specific activity were increased in the ginseng-treated diabetic group (group V) than the ginseng-pretreated diabetic group (group IV). These results were confirmed by the histopathological study.