Protective role of Zinc in liver cirrhosis: study in rats

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ABSTRACT

This study was designed to evaluate the effects of Zinc supplementation on different biochemical parameters in thioacetamide induced cirrhotic rats. For this purpose, 24 male Albino wistar rats were divided into four groups (n=6). Group I, remained healthy control rats, Group II, received thioacetamide (at a dose of 200mg/kg b.w, i.p, for 12 weeks, twice a week) in first phase and saline in second phase, Group III, received thioacetamide (200mg/kg b.w, i.p for 12 weeks, twice a week) in first phase and zinc sulphate (intraperitoneally at a dosage of 6mg/kg b.w/ day for 6 weeks) in second phase and Group IV, received zinc sulphate (intraperitoneally at a dosage of 6mg/kg b.w/ day for 6 weeks) in first phase and saline in second phase. Biochemical analysis was evaluated by total and direct bilirubin (Sherlock, 1951), liver specific enzymes (Retiman and Franhel, 1957), antioxidant enzymes [SOD (Kono *et al*., 1978), Catalase (Sinha *et al*., 1979), Glutathione reductase (Calberg and Mannervik, 1985), and MDA (Okhawa *et al*., 1979)] and plasma and intraerythrocyte sodium and potassium. Marked increase in total and direct bilirubin and ALT activity was the indicative markers of liver cirrhosis while reduced antioxidant activity (SOD and GSH) and increased MDA and Catalase levels and disturbed electrolyte homeostasis were observed in cirrhotic group. Zinc supplementation markedly reduced total bilirubin and ALT activity and restored the antioxidant enzymes (SOD and GSH), MDA and catalase activity and electrolyte homeostasis. These results indicate that zinc successively attenuates the thioacetamide induced liver cirrhosis.

Keywords: Thioacetamide, Zinc Sulfate, Antioxidant Enzymes, Malondialdehyde, Liver Specific Enzymes

INTRODUCTION

Zinc is an essential trace mineral with important anti-inflammatory (Himoto et al., 2008), antiapoptotic (Wen-Lin et al., 2005), and antioxidant effects (Saul, 2000). Various biological effects of zinc depend on its catalytic, structural and regulatory role in a large number of enzymes and zinc finger motifs (Christianson, 1991). Nearly 300 different enzymes contain zinc as an integral component (Keith et al., 2000). Zinc finger motif stabilizes the structure of a number of proteins which are involved in various cellular processes such as replication and repair, transcription and translation, metabolism and signaling, cell proliferation and apoptosis (Jennifer., 2007). Zinc plays central role in aging (Eugenio Mocchegiani, 2007) as it is required for cell cycle progression (Paul Smith, 2008) DNA repair (Yang Song and Emily, 2009) and for the prevention of neoplastic cell growth (Tony et al., 2011).

Zinc exerts a considerable effect in the stress response, affects the compensatory capacity, and acts as a neuromodulator (Eugenio Mocchegiani et al., 2005). The role of zinc in antioxidant defense mechanism includes the protection due to redox active transition metals such as copper and iron, and the protection of sulfhydryl groups of protein from oxidative damage. The chronic antioxidant effects of zinc results in the induction of metellothionein synthesis and the Cu/Zn superoxide dismutase stabilization. Metallothionein act as scavengers of toxic metals and a number of antioxidant molecules and make a connection between cellular zinc and its redox state (Wolfgang

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Maret, 2000). Other antioxidant effects exerted by zinc, include its protective role against vitamin E depletion (Goode et al., 1991) and apoptosis, induction of cell-proliferation and inhibition of NADPH oxidases (Milos Chvapil et al.,1976).

Effects of zinc deficiency include growth failure, reduced gonadal development in male, cell-mediated immune disorders, mental lethargy, skin changes, delayed wound healing,poor appetite and neurosensory disorders (Parsad ., 1995). Zinc deficiency causes the tissue oxidative damage and modulation of seleted signaling cascades in the liver (Patricia Oteiza et al., 2000). Zinc deficiency causes impairment of cellular immunity (Bogden, 2004), susceptibility to infections, induction of oxidative stress and its related conditions such as susceptibility to hepatitis, reduced acute response protection against hepatitis, and lipid oxidation, by changing the cellular redox state which results in activation of oxidant-sensitive transcription factors that affects the cell function and leads to induction of disease.

The role of zinc is very important in antioxidant defense mechanism as well as in regeneration of damaged cells. In views of abovementioned previous studies, it is hypothesized that cirrhosis of the liver could be prevented by the supplementation of zinc. The present study was designed to examine the protective role of zinc in thioacetamide induced liver cirrhosis in experimental rat's model.

MATERIALS AND METHODS

Animals and diet: 24 male Albino Wistar rats weighing 200-250gm were purchased from the animal house of ICCBS (International center for chemical and biological sciences, Karachi, Pakistan) for the study. Animals were acclimatized to the laboratory conditions before the start of experiment and caged in a quiet temperature-controlled animal room (23+4ºC). Rats had free access to water and standard rat diet. The experiments were conducted with ethical guidelines of internationally accepted principles for laboratory use and care in animal research (Health research extension Act of 1985).

Study design: The rats were randomly divided into four groups, each of six rats. The duration of the study was 18 weeks, divided into two phases. Thioacetamide and zinc sulphate were administered in either phase.
Group I: the control (remained untreated).
Group II: TAA-treated
Group III: TAA+ Zinc Sulphate treated
Group IV: Zinc Sulphate treated

In Phase I, TAA-treated and TAA+Zinc Sulphate groups received TAA, dissolved in 0.9% NaCl and was injected intraperitoneally at a dosage of 200mg/kg b.w, twice a week for 12 weeks. Zinc Sulphate group received zinc sulphate (intraperitoneally at a dosage of 6mg/kg b.w/ day for 6 weeks). In phase II, TAA-treated group received saline, TAA+Zinc Sulphate group received Zinc Sulphate (intraperitoneally at a dosage of 6mg/kg b.w/ day starting from 13th week for 6 weeks) after TAA in first phase to study the hepatocorrective role of zinc and zinc sulphate group received saline. At the end of experimental period, rats from all the groups were decapitated. The blood was collected from the neck wound in the lithium heparin coated tubes and centrifuged to collect plasma. Liver was excised, trimmed of connective tissues, rinsed with saline to eliminate blood contamination, dried by blotting with filter paper and weighed. The tissues then kept in freezer at –70ºC until analysis.

Assessment of ALT and total and direct bilirubin: Plasma ALT (Retiman and Franhel, 1957) and total and direct bilirubin (Sherlock, 1951) were analyzed using commercially prepared reagent kits from Randox.

Preparation of post mitochondrial supernatant: Liver homogenate was prepared by taking 1g of liver tissue in 10ml of 5mM potassium phosphate buffer (pH 7.8) by using a homogenizer. The homogenates were centrifuged at 800g for five minutes at 4ºC to separate the nuclear debris. The supernatant so obtained was centrifuged at 10,500g for 20 minutes at 4ºC to get post mitochondrial supernatant which was used to assay SOD, Catalase, MDA, and glutathione reductase activity.

Estimation of thiobarbituric acid substances: The malonyldialdehyde (MDA) content, a measure of lipid peroxidation, was assayed in the form of thio-barbituric acid reacting substances (TBARS) by the lipid peroxidation method (Okhawa *et al.*, 1979). Briefly, the reaction mixture consisted of 0.2 ml of 8.1% sodium dodecyle sulphate, 1.5 ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5 ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2 ml of 10%(w/v) of PMS. The mixture was brought up to 4.0 ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0 ml distilled water and 5.0 ml of the mixture of n-butanol and pyridine (15:1 v/v) was added and centrifuged. The organic layer was taken out and its absorbance was measured at 532 nm and compared with those obtained from MDA standards. The concentration values were calculated from absorption measurements as standard absorption.

Estimation of catalase: Catalase activity was assayed by the method of Sinha (Sinha *et al.*, 1979). Briefly, the assay mixture was consisted of 1.96 ml phosphate buffer (0.01M, pH 7.0), 1.0 ml hydrogen peroxide (0.2 M) and 0.04 ml PMS (10%w/v) in a final volume of 3.0 ml. 2 ml dichromate acetic acid reagent was added in 1 ml of reaction mixture, boiled for 10 minutes, cooled. Changes in absorbance were recorded at 570 nm.

Estimation of SOD: Superoxide dismutase levels in the cell free supernatant were measured by the method of (Kono *et al.*, 1978). Briefly 1.3ml of solution A (0.1 m EDTA containing 50 mM Na_2CO_3, pH 10.0), 0.5 ml of solution B (90µmNBTnitro blue tetra zolium dye) and 0.1 ml of solution C (0.6%Triton X-100 in solution A), 0.1 ml of solution D (20 mM Hydroxyl amine hydrochloride, pH 6.0) were mixed and the rate of NBT reduction was recorded for one minute at 560 nm. 0.1 ml of the supernatant was added to the test cuvette as well as reference cuvette, which do not contain solution D. Finally, the percentage inhibition in the rate of reduction of NBT was recorded as described above. One enzyme unit was expressed as inverse of the amount of protein (mg) require in one minute.

Estimation of glutathione reductase: GSH activity was determined by continuous spectrophotometric rate determination (Calberg and Mannervik, 1985). In a clean glass test tube, 0.3 mL of 10% BSA, 1.5mL of 50mM potassium phosphate buffer (pH 7.6), 0.35mL of 0.8mM ß-NADPH and 0.1mL of 30mM oxidized glutathione was taken and finally added 0.1mL of homogenate, mixed well by inversion. Absorbance was recorded at 340nm at 25°C for 5 minutes on kinetic spectrophotometer PRIM 500 (Germany) with automatic aspiration and thermostat. The activity was calculated using the molar coefficient for NADPH of 6.22 $\mu mol^{-1} \times cm^{-1}$ and expressed in unit/gram tissue.

Estimation of plasma sodium and potassium: Plasma was diluted 1:100 with 0.1N HCl and was used for simultaneous determination of sodium and potassium. The emission intensities of standards and samples were recorded against the respective blank solutions. The emission intensities of sodium, potassium was recorded at 589 and 768nm respectively.

Erythrocyte membrane preparation: The packed red cells extracted by centrifugation at 4°C, 450g for 15 minutes were resuspended and diluted in 25 volumes of 0.011 mol/L Tris-HCl buffer at p H 7.4. The hemolyzed cells were then centrifuged for 30 min at 12,000 rpm at 4°C and the membrane pellet was resuspended in 30 ml of 0.0ll mol/L Tris-HCl

buffer. This centrifugation step was repeated three times. The final concentration of the membrane suspension was ~4 mg protein/ml of Tris buffer. The membrane suspension was stored at -80°C until the assay was performed.

Estimation of intraerythrocyte sodium and potassium: Heparinized blood was centrifuged and plasma was separated. Buffy coat was aspirated and discarded. Erythrocytes were washed three times at room temperature by suspension in the magnesium chloride solution (112mmol/L), centrifugation at 450g at 4°C for 5 minutes and aspiration of the supernatant as described earlier (Fortes and Starkey, 1977). Final supernatant was retained for the estimation of intraerythrocyte sodium and potassium concentration. Neither electrolyte was detectable in the final wash. Washed erythrocytes were then used for the estimation of intraerythrocytes sodium and potassium (Tabssum et al., 1996).

Histopathological examination: Left lobe of liver was removed quickly and immersed in 10% formalin. Slices of liver then fixed in a solution containing ethanol (150ml), formaldehyde (60ml), acetic acid (15ml) and picric acid (1 g) for 2 hours. Then the samples were incubated in phosphate buffered formaldehyde until embedded in paraffin, sectioned at 5 µm and stained with hematoxylin and eosin (H/E), and analyzed by light microscopy.

STATISTICAL ANALYSIS

Results are presented as mean ± SD. Statistical Significance and difference from control and test values evaluated by Student's t-test. Statistical probability of **$P<0.05$, *$P<0.01$ were considered to be significant.

RESULTS

Effect of thioacetamide and zinc sulphate treatment on body weight in control and treated rats: Decreased body weight was observed after chronic administration of TAA in TAA and TAA+Zinc Sulphate groups. Rats of TAA+Zinc Sulphate group regained their body weight after zinc sulphate treatment in second phase. Rats of TAA group continuously lost their body weights. Rats of zinc sulphate group and control group gained their body weights throughout the treatment (fig.1).

Effect of thioacetamide and zinc sulphate treatment on liver weight and liver to body weight ratio in control and treated rats: Increased liver weight and liver-body weight ratio was observed in TAA group after 12 weeks

administration of TAA as Compare to control (7.11+1.36 P<0.01) (0.033+0.008 P<0.01) (Table-1) where as reduction in the liver weight and liver-body weight ratio was observed in TAA+ Zinc Sulphate group as compare to control (6.8+0.72 P<0.01) (0.036+0.001 P<0.01) respectively.

Effect of thioacetamide and zinc sulphate treatment on plasma total and direct bilirubin and ALT activity in control and treated rats: Table.2 & figure.2 show a marked increase in total bilirubin level in TAA-treated group as compare to control (3.19±0.2 P<0.01) whereas, in TAA+Zinc Sulphate treated group, zinc sulphate supplementation brought those increased levels to the normal concentrations as compare to control (0.75±0.01 P<0.01). Increased levels of direct bilirubin were shown by TAA-treated group as compare to control (3.31±0.4 P<0.01) whereas zinc sulphate supplementation brought those higher levels to normal levels as compare to control (1.15±0.02 P<0.01) (table .2, figure .3).

Plasma Alanine aminotransferase level was markedly increased in TAA-treated group as compare to control (1021.3±68.19 P<0.01). Alanine amino transferase level was decreased in TAA+Zinc Sulphate group as compare to control (250.8±13.6 P<0.01) (table .2, figure .4).

Effect of thioacetamide and zinc sulphate treatment on hepatic concentration of Glutathione reductase in control and treated rats: Hepatic concentration of glutathione reductase was significantly reduced in TAA-treated group as compare to control (0.031±0.001 P<0.01). TAA+Zinc sulphate group, after zinc sulphate supplementation, showed increased level of glutathione reductase as compare to control (0.7±0.02 P<0.01) Table.3, figure.5. Glutathione reductase was almost normal in zinc sulphate group as compare to control (0.79±0.03).

Effect of thioacetamide and zinc sulphate treatrment on hepatic concentration of superoxide dismutase in control and treated rats: Table-3 & figure-6 showed a significant decrease in SOD activity in TAA group as compare to control (430±1.5 P<0.01). TAA + Zinc Sulphate group, after zinc sulphate supplementation, showed almost normal levels of SOD activity (968.2±2.4 P<0.05) as compare to control. SOD activity was almost normal in Zinc Sulphate group (631.5±3.1 P<0.01) as compare to control.

Effect of thioacetamide and zinc sulphate treatment on hepatic concentration of MDA in control and treated rats: Level of MDA was markedly increased in TAA-treated animals as compare to control (128.8±1.8 P<0.01). Zinc sulphate administration in TAA+Zinc Sulphate group decreased the concentration of MDA as compare to control (46.1±1.2 P<0.01) while rats of Zinc Sulphate group showed normal range of MDA level as compare to control (58.2±1.5 P<0.01) after zinc sulphate treatment (Table-3, figure-7).

Effect of thioacetamide and zinc sulphate treatment on hepatic concentration of catalase in control and treated rats: Concentration of catalase was significantly increased in TAA group (40.1±0.01 P<0.01) as compare to control. Administration of zinc sulphate in second phase in TAA+Zinc Sulphate group brought these higher levels to normal limits (9.2±0.01 P<0.01) as compare to control. Level of catalase was normal (8.5±0.3 P<0.01) in Zinc Sulphate group as compare to control (Table-3, figure-8).

Effect of thioacetamide and zinc sulphate treatment on intra-erythrocytes sodium and potassium in control and treated rats: Decreased levels of intra erythrocyte sodium was observed in TAA group (3.9±0.2 P<0.01), whereas zinc sulphate supplementation significantly increased intra erythrocyte sodium in TAA+Zinc Sulphate group (5.2±0.2 P<0.01) as compare to control. Zinc Sulphate group showed significantly increased concentration of intraerythrocyte sodium as compare to control (8.1±0.5 P<0.01) (Table.4, figure.9).

Decreased intra erythrocyte potassium level was observed in TAA group as compare to control (72.8±1.5 P<0.01). Whereas increased intra-erythrocyte potassium level was observed in TAA+Zinc Sulphate group (131.2±2.3 P<0.01) as compare to control. A reduction in intraerythrocyte potassium was observed in Zinc Sulphate group as compare to control (74.8±3.1 P<0.01) (Table.4, figure. 10).

Effect of thioacetamide and zinc sulphate treatment on plasma sodium and potassium in control and treated rats: Plasma sodium was decreased in TAA group (136±1.7 P<0.01) as compare to control where as it was increased in TAA+Zinc Sulphate group (148.5±1.3 P<0.01) as compare to control. A slight increase in plasma sodium was observed in zinc sulphate group as compare to control (144.3±1.1 P<0.01) (Table.4, figure 11).

Table.4 & figure 12. showed decreased plasma potassium in TAA group (4.0±0.1 P<0.01) as compare to control where as zinc sulphate treatment in TAA+Zinc Sulphate group restored plasma potassium as compare to control (4.7±0.1

P<0.01). Alone zinc sulphate showed no effect on plasma potassium concentration as compare to control (5.1±0.1 P<0.01).

Histological examination: After 12 weeks administration of thioacetamide in TAA-treated rats, histological examination showed last stage of liver cirrhosis, amount of fibrosis was (++++++) maximum. Supplementation of zinc sulphate in TAA+zinc sulphate group reduces the amount of fibrous tissue (++) and the stage of nodule formation was (+) minimum (figure 4.9.4).

DISCUSSION

In the present study, long term administration of thioacetamide resulted in the development of sever liver injury in rats. Administration of zinc in cirrhotic rats causes a significant decrease in the level of plasma bilirubin and ALT activity. Our results are in agreement with the studies of Hussein Dashti who found that zinc supplementation normalizes the plasma bilirubin and ALT activity in cirrhotic rats (Dashti H et al., 1989).

We found that repeated thioacetamide treatment resulted in increased lipidperoxidation and decreased antioxidant enzyme levels. Dashti reported that thioacetamide administration is easy and reliable for the induction of liver cirrhosis in experimental animal models and Muller reported that resulting disease resembles the human cirrhosis. Thioacetamide exihibits its toxicity through its reactive metabolites, thioacetamide sulfoxide and thioacetamide-s, s-oxide (Chilakapati et al 2007).

Previous studies showed that thioacetamide induced liver cirrhosis can be prevented by the use of radical scavangers and antioxidants. The property of Zinc to stop oxidative reactions has been known for years. Zinc exerts its antioxidant action through acute and chronic effects. Acute effects include stabilization of protein sulfhydryls or reduction in the formation of hydroxyl from oxidative stress caused by reactive oxygen and nitrogen species, have ability to neutralize hydroxyl radicals, function as strong copper chelators hydrogen peroxide through the antagonism of redox active transition metals.Chronic effects of zinc results in induction of metallothioniens in many body organs such as liver, kidney and intestine. Metallothioniens make a connection between cellular zinc and redox state of the cell and show antioxidant properties under different circumtences such as toxicity caused by the use of some anticancer drugs or others, toxicity induced by ethanol, exposure by radiations, and oxidatively mediated mutagenesis. A longterm zinc deprivation results in increased susceptibility to injury or disease due to a number of oxidative stresses. Liver cirrhosis is found to have a poor zinc status.M.Ozaslan reported ability of zinc to regenerate damaged cells and its important antioxidant property. In our study, data of increased liver weight, liver to body weight ratio, decrease and increase in body weights with respect to the treatments of thioacetamide and zinc sulphate administration, the restorage of the level of total and direct bilirubin, ALT activity and restorage of plasma and intraerythrocyte electrolytes are in agreement with the studies of Dr. Mona Dameghindd. Furthermore, the reversal of levels of glutathione reductase and MDA and the catalase and superoxide dismutase activity indicate that zinc may play important role in treatment of liver cirrhosis.

Figure.1. Effect of thioacetamide and zinc sulphate treatment on body weight in control and treated rats:
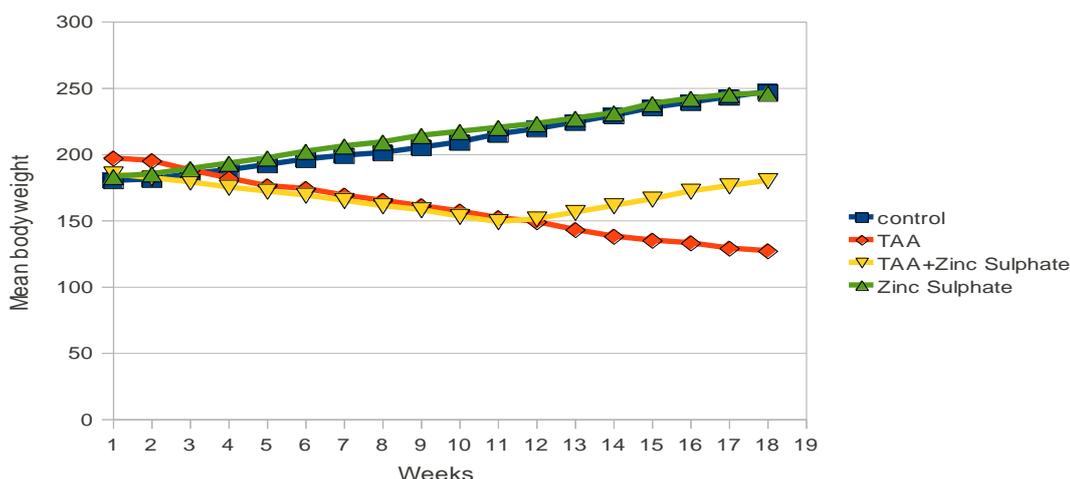
Table-1: Effect of thioacetamide and zinc sulphate treatment on liver weight, liver to body weight ratio in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats

| Groups	Liver Weights	Liver body-weight ratio
Control	6.33±1.31*	0.033±0.008*
TAA-treated	7.11±1.36*	0.341±0.004*
TAA+ZincSulphate treated	6.8±1.1*	0.036±0.001*
Zinc sulphate treated	7.1±1.4*	0.013±0.003*

n=6 Values are mean ± SD. Significant difference among control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and Zinc sulphate-treated groups by t-test *P<0.01,**P<0.00

Table.2: Effect of thioacetamide and zinc sulphate treatment on plasma total and direct bilirubin and ALT activity in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats:

Parameters	Control	TAA-treated	TAA+Zinc Sulphate-treated	Zinc Sulphate
Total bilirubin(unit/L)	0.58±0.04*	3.19±0.2*	0.75±0.08*	0.87±0.1*
Direct bilirubin(unit/L)	1.50±0.2*	3.31±0.2*	1.15±0.02*	1.36±0.02*
Alanine-amino transferase(unit/L)	10.5±0.15*	49.13+0.1*	12.2±0.1*	10.24+0.05

Values are mean ± SD. Significant difference among control, thioacetamide and zinc sulphate treated groups by t-test *P<0.01, **P<0.05.

Figure.2: Effect of thioacetamide and zinc sulphate treatment on plasma total bilirubin in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats
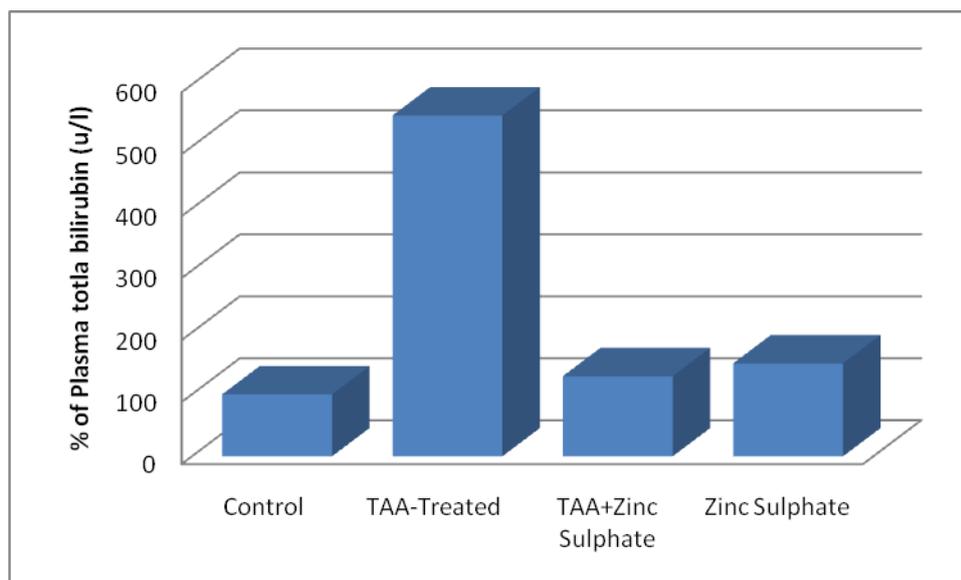
Figure .3: Effect of thioacetamide and zinc sulphate treatment on plasma direct bilirubin in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats
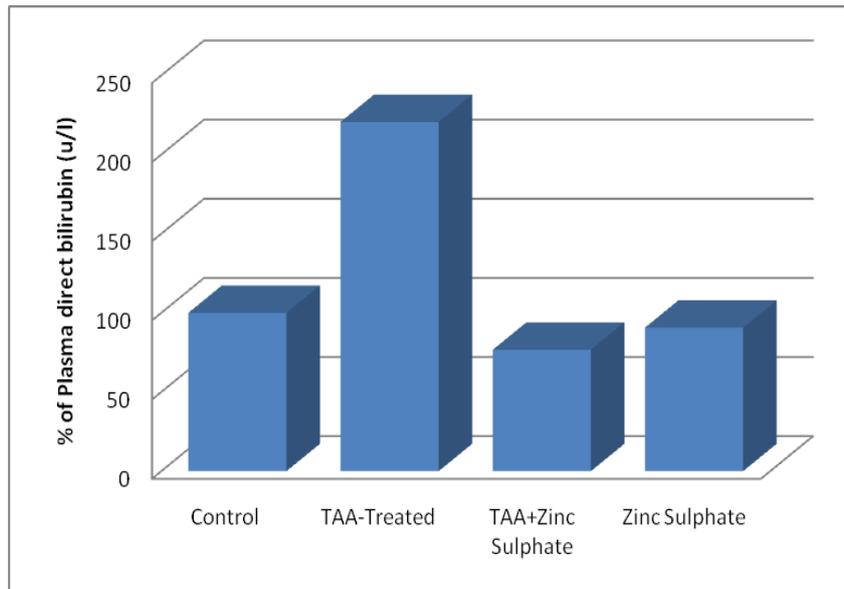
Figure .4: Effect of thioacetamide and zinc sulphate treatment on plasma Alanine-amintransferase in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats
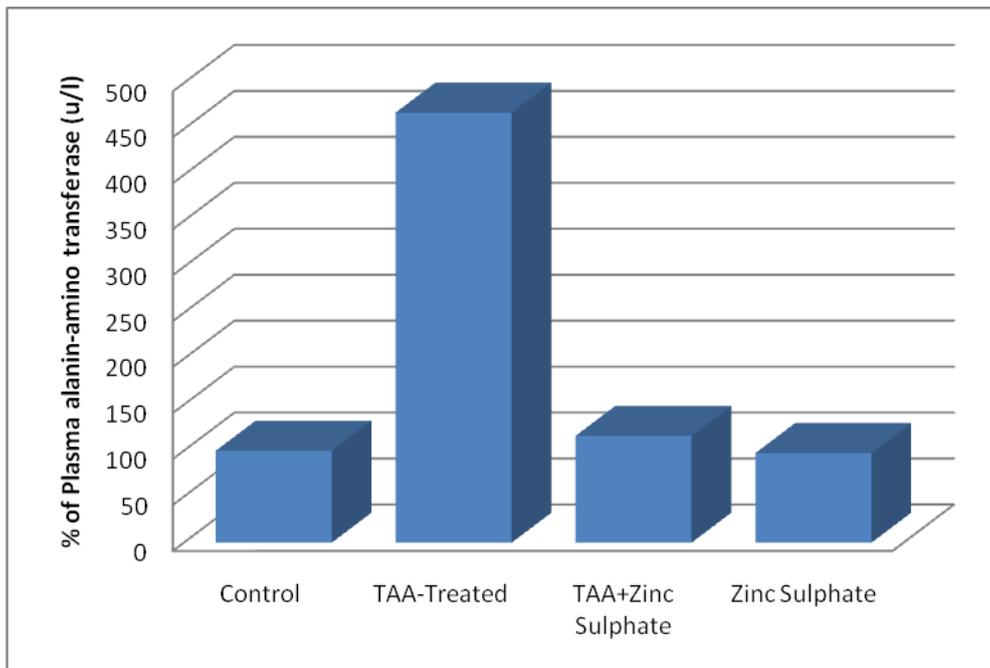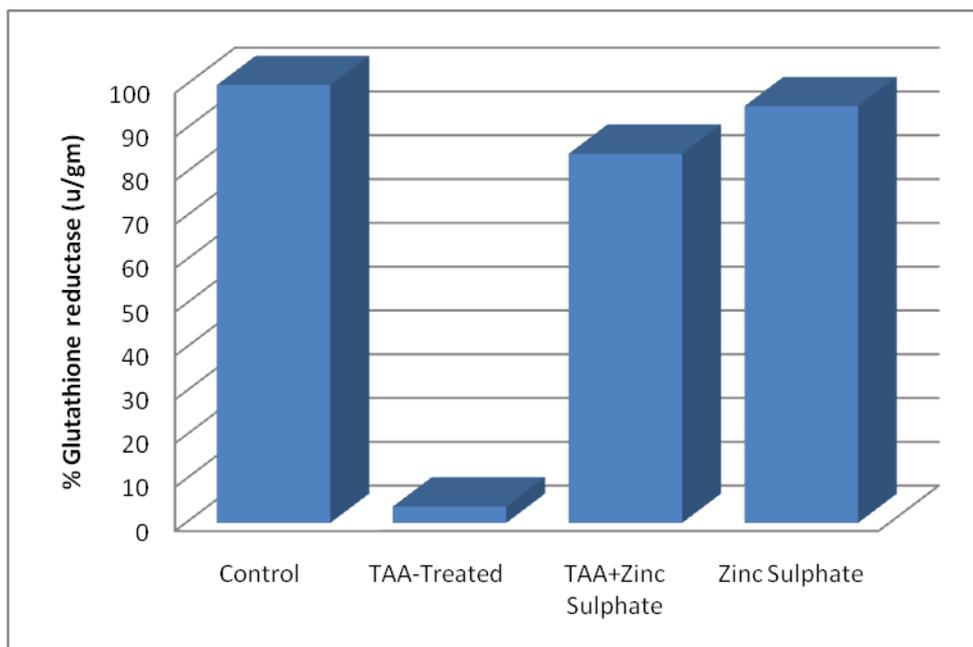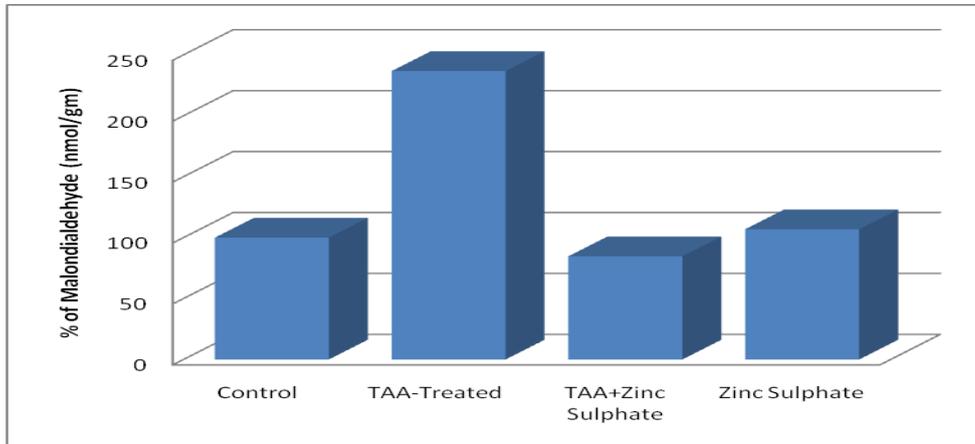
Table.3:Effect of thioacetamide and zinc sulphate treatment on hepatic concentration of glutathione reductase, superoxide dismutase, malondialdehyde and catalase in thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulpha-treated rats

Parameters	Control	TAA-treated	TAA+Zinc sulphate	Zinc Sulphate
Glutathione reductase (unit/gm of tissue)	0.83±0.0*4	0.031±0.001*	0.7±0.02*	0.79±0.01
Superoxide dismutase unit/gm of tissue.	961.4±2.*1	430±1.5*	968.2±2.4**	631.5±3.1*
Malondialdehyde nmol/gm of tissue.	54.4±1.4*	128.8±1.8*	46.1±1.2*	58.2±1.5*

n=6; Values are mean ± SD. Significant difference among control, thioacetamide, thioacetamide and zinc sulphate and zinc sulphate treated groups by t-test *$P<0.01$, **$P<0.05$.

Figure.5: Effect of thioacetamide and zinc sulphate treatment on Glutathione reductase in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats
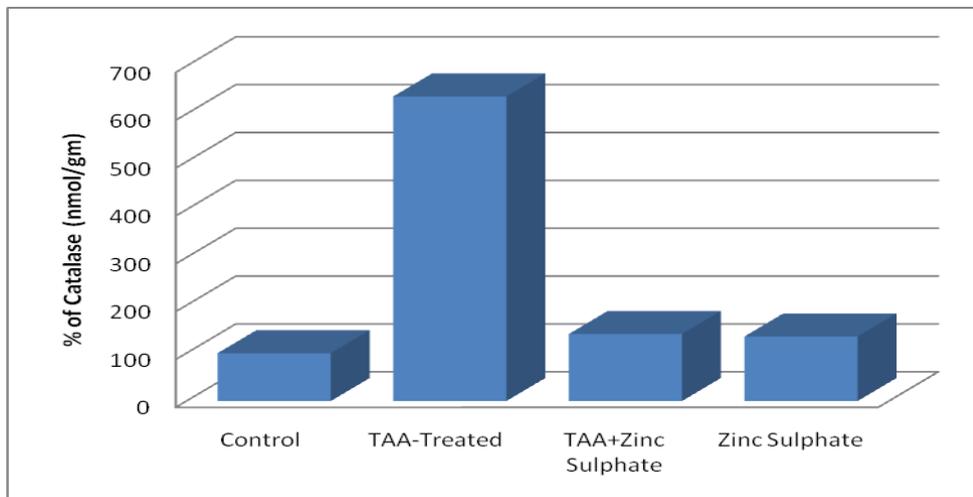
Figure .6: Effect of thioacetamide and zinc sulphate treatment on Superoxide dismutase in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats
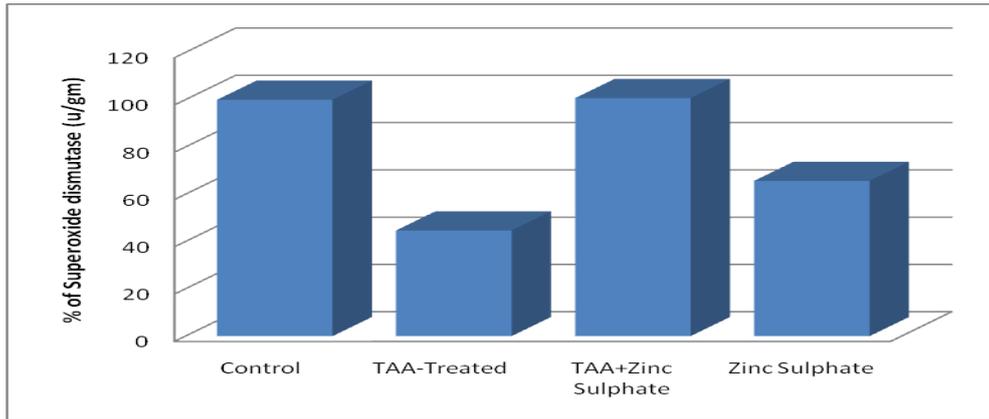
Figure -7: Effect of thioacetamide and zinc sulphate treatment on malondialdehy control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats
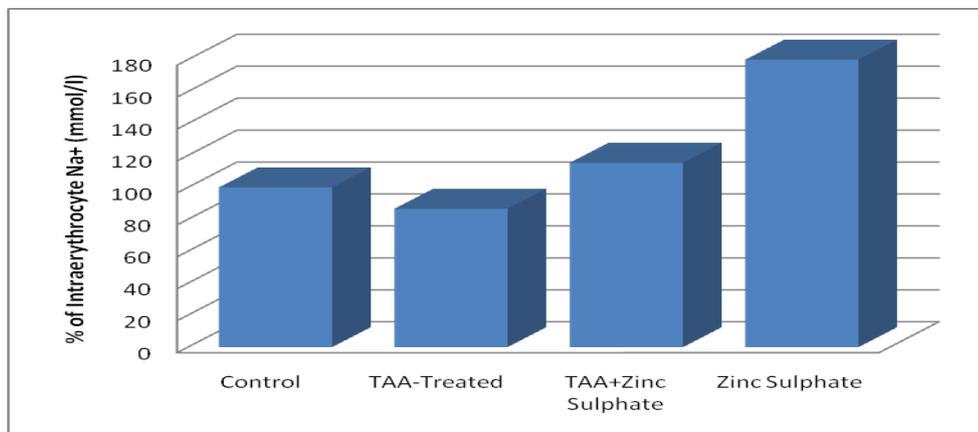

Figure .8: Effect of thioacetamide and zinc sulphate treatment on Catalase in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats

Table.4: Effect of thioacetamide and Zinc Sulphate treatment on intraerythrocyte sodium and potassium and on plasma sodium and potassium in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats:

Parameters	Control	TAA-treated	TAA+Zinc Sulphate	Zinc Sulphate-treated
IntraerythrocyteNa$^+$ mmol/L	4.5±0.4*	3.9±0.2*	5.2±0.2*	8.1±0.5*
Intraerythrocyte K$^+$ mmol/L	85.1±1.1*	72.8±1.5*	131.2±2.3*	74.8±3.1*
Plasma Na$^+$mmol/L	140.5±1.4*	136±1.7*	148.5±3.6*	144.3±2.6*
Plasma K$^+$mmol/L	5.0±0.1*	4.0±0.1*	4.7±0.1*	5.1±0.1*

n=6; Values are mean ± SD. Significant difference among control, thioacetamide, thioacetamide and zinc sulphate and sodium selenite treated groups by t-test *P<0.01, **P<0.05.
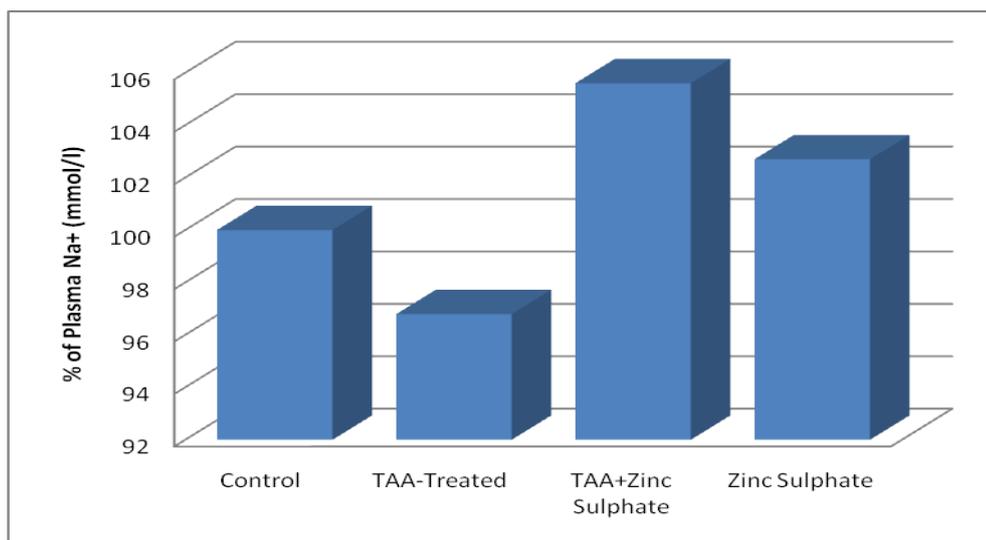
Figure .9: Effect of thioacetamide and zinc sulphate treatment on Intraerythrocyte Na$^+$ in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats
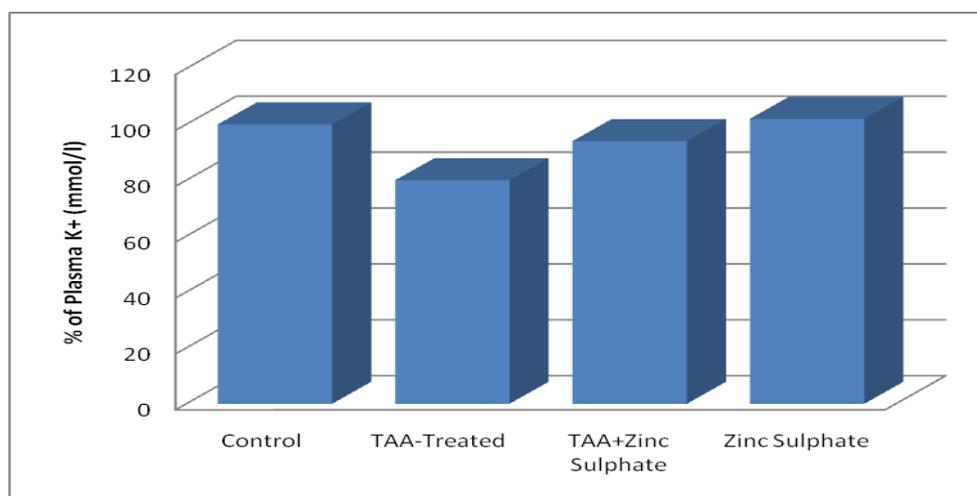

Figure 10: Effect of thioacetamide and zinc sulphate treatment on Intraerythrocyte K$^+$ in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats
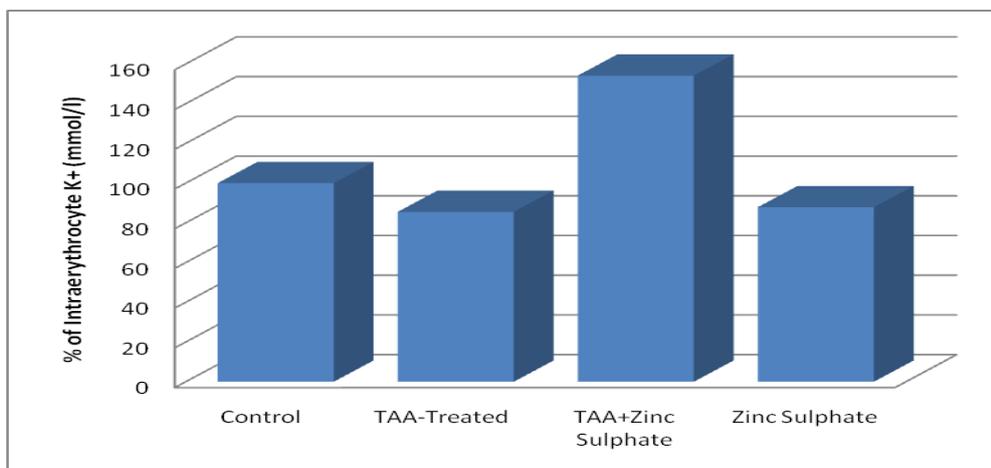
Figure 11: Effect of thioacetamide and zinc sulphate treatment on Plasma Na$^+$ in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats
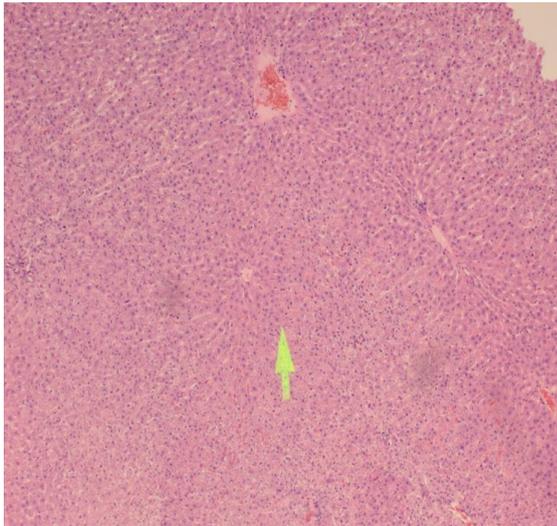

Figure 12: Effect of thioacetamide and zinc sulphate treatment on plasma K$^+$ in control, thioacetamide-treated, thioacetamide+zinc sulphate-treated and zinc sulphate-treated rats


Table .5. Histological examination of thioacetamide and zinc sulphate treatment on liver of control, thioacetamide treated, thioacetamide+zinc sulphate treated and zinc sulphate treated rats

| Group	Amount of fibrosis	Disorganization of liver architecture	Stage of nodule formation and disorientation of vascular architechture
Control	-	-	-
Thioacetamide	++++++	+++++	++++++
Thioacetamide+zinc sulphate	++	++	+
Zinc sulphate	-	-	-

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Figure 13: Histology of liver in control, thioacetamide-treated, thioacetamide+zinc sulphate treated and zinc sulphate treated rats
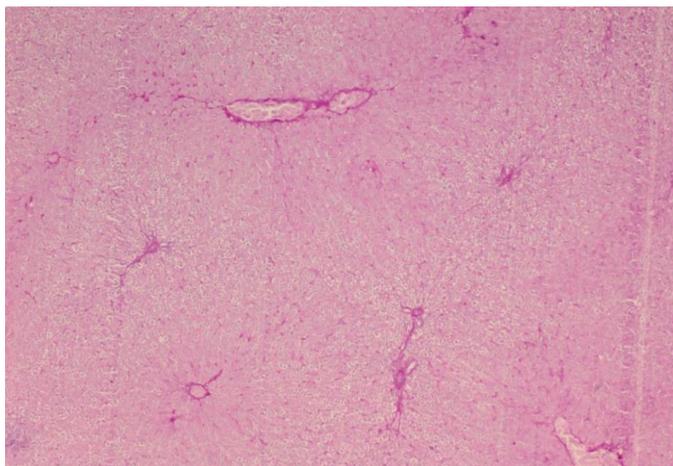

Figure 13A: Normal liver histology from control rats.
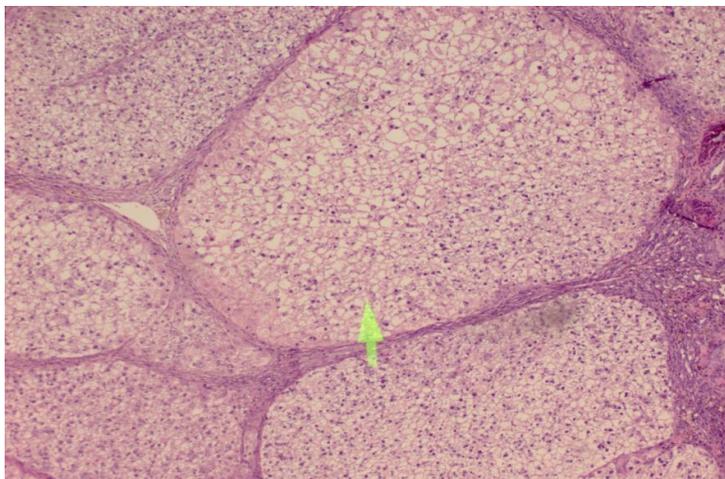
Figur13 B: Shows histological abnormalities after 12 administration of thioacetamide in TAA-treated rats.
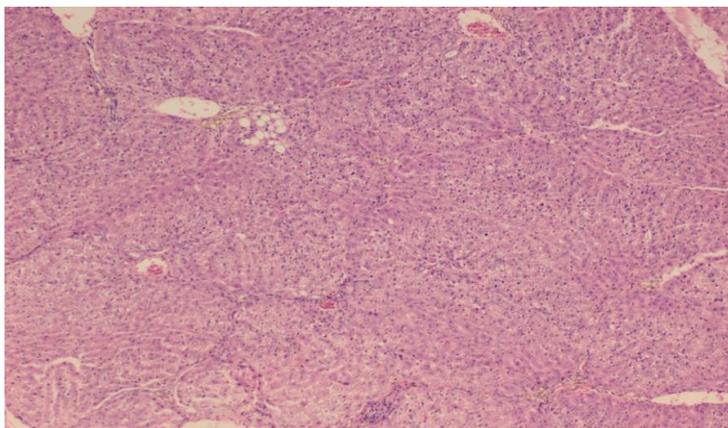
Figure 13C: Shows effect of zinc sulphate treatment which reduces degree of fibrosis in TAA+zinc sulphate treated rats.

Figure 13D: Shows normal lobular architecture in zinc sulphate-treated rats.

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