Prevention of liver cirrhosis by Silymarin

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Abstract: This study was designed to evaluate the effects of sylimarin 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 silymarin (orally at a dosage of 200mg/kg b.w, twice a week, for 8 weeks) in second phase and Group IV, received silymarin (orally at a dosage of 200mg/kg b.w, twice a week, for 8 weeks) in first phase and saline in second phase. Biochemical analysis was evaluated by total and direct bilirubin (Retiman and Franhel, 1957, Sherlock, 1951), liver specific enzymes, 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 (Tabssum *et al*., 1996). 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. Silymarin 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 silymarin successively attenuates the thioacetamide induced liver cirrhosis.

Keywords: Liver cirrhosis, Silymarin, Thioacetamide, Liver enzymes, SOD, GSH, Catalase, MDA, Plasma sodium and potassium, Intraerythrocyte sodium and potassium.

INTRODUCTION

Silymarin mostly used for hepatoprotection is a flavonolignan from milk thistle Silybum marianum plant. Silymarin is a combination of four-flavonolignan isomer called as silybin, isosilybin, silydianin and silychristin. Silybin appears as the most active principle of silymarin (Pradhan and Girish. 2006). Because of its antioxidant properties, use of silymarin is beneficial in various chronic liver diseases caused by oxidative stress. Silymarin acts as a membrane stabilizer, has antioxidant properties, promotes regeneration of hepatocytes, decreases inflamation and prevents liver fibrosis. Survival period of alcoholic liver cirrhotic individuals can be increased by long-term silymarin administration (Feher and Lenqyel, 2012). Wellington and Jarvis reported increased activity of SOD in lymphocytes and erythrocytes and increased SOD expression in lymphocytes, increase in serum glutathione and glutathione peroxidase in liver disease (Wellington and Jarvis. 2001). Silymarin facilitates protein synthesis because of its structural similarity to steroid hormones. Pretreatment of silymarin to rats (with partial hepactomy) resulted in increased DNA, RNA, protein and cholesterole synthesis indicating its role in the liver regeneration (Srivastav *et al*., 1994). Protein synthesis mechanism may take place with the initiation of a physiologic regulator by silymarin. Which helps the silybin to locate itself on a specific binding site on the polymerase, results in the stimulation of ribosome synthesis. Structural similarity of silymarin to steroides enables it to enter the nucleus and stimulating RNA polymerase I (Schopen *et al*., 1969). Silymarin administration to CCL_4 induced cirrhotic rats resulted in complete reversal of increased levels of transaminases (Sharma *et al*., 1991). Silynmarin pretreatment to carbontetrachloride induced cirrhotic rats resulted in the prevention of extra accumulation of cholesterole and in reduction of liver collagen content (Favari and Perez. 1997).

Medicinally, silymarin has been utilized for the treament of liver diseases such as viral/drug-induced hepatitis, cirrhosis and alcoholic liver disorders. Silymarin has also been shown an efficient role in the treatment of cancers. Mechanism of silymarin action includes prevention of binding of hepatotoxin to the specific receptor site on the membrane of hepatocytes, decrease in oxidation of glutathione to increase its concentration in liver and intestine, antioxidant property, stimulation of rRNA polymerase and increased protein formation, resulted in increased regeneration of hepatocytes (Nitin Dixit *et al*., 2007).

Zahra *et al*. (2012) reported the substantial role of silymarin against thioacetamide induced progression of liver cirrhosis in rats. Silymarin administration of liver

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cirrhosis to TAA induced cirrhotic rats resulted in reduction of increased levels of ALT, ALP, AST, albumin, bilirubin and total protein activities (Zahra *et al*., 2012). In views of above-mentioned previous studies it is hypothesized that cirrhosis of liver can be attenuated by silymarin supplementation. Thus, the present study was designed to evaluate the hepatoprotective role of silymarin in thioacetamide induced liver cirrhosis in experimental rats.

MATERIALS AND METHODS

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+4°C). Rats had free access to water and standard rat diet.

Ethical guidelines

The experiments were conducted with ethical guidelines of institutional ERB (Ethical Review Board) and 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 20 weeks, divided into two phases. Thioacetamide (Sigma) and sylimarin were administered in either phase as described below. Thioacetamide and sylimarin were purchased from Merck and the other chemicals used in present study were purchased from BDH laboratory supplies, Fisher Scientific UK limited and Fluka AG.

Group I: The control (remained untreated)
Group II: TAA-treated
Group III: TAA+ silymarin treated
Group IV: Sylimarin treated

In Phase I, TAA-treated and TAA+ Sylimarin treated groups received TAA, dissolved in 0.9% NaCl and were injected intraperitoneally at a dosage of 200mg/kg b.w, twice a week for 12 weeks. Silymarin group received Silymarin (orally at a dosage of 200mg/kg b.w, twice a week, for 8 weeks). In phase II, TAA-treated group received saline, TAA +Silymarin group received Silymarin (orally at a dosage of 200mg/kg b.w, twice a week, starting from 13th week for eight weeks) after TAA in first phase to study the hepatocorrective role of Silymarin and Silymarin 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 phosphatebuffer (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 per oxidation, was assayed in the form of thio-barbituric acid reacting substances (TBARS) by the lipid per oxidation method (Okhawa *et al*., 1979). Briefly, the reaction mixture consisted of 0.2ml of 8.1% sodium dodecyle sulphate, 1.5ml of 20% acetic acid solution adjusted to pH 3.5 with sodium hydroxide and 1.5ml of 0.8% aqueous solution of thiobarbituric acid was added to 0.2ml of 10%(w/v) of PMS. The mixture was brought up to 4.0ml with distilled water and heated at 95°C for 60 minutes. After cooling with tap water, 1.0ml 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 1.96ml phosphate buffer (0.01M, pH 7.0), 1.0 ml hydrogen peroxide (0.2M) and 0.04ml PMS (10%w/v) in a final volume of 3.0ml. 2ml dichromate acetic acid reagent was added in 1ml 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 (0.1m EDTA containing 50 mM Na_2CO_3, pH 10.0), 0.5ml of solution B (90µmNBTnitro blue tetra zolium dye) and 0.1ml of solution C (0.6%Triton X-100 in solution A), 0.1ml of

solution D (20mM Hydroxyl amine hydrochloride, pH 6.0) were mixed and the rate of NBT reduction was recorded for one minute at 560nm. 0.1ml 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, 0.3mL 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 25ºC 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 were recorded at 589 and 768nm respectively.

Erythrocyte membrane preparation

The packed red cells extracted by centrifugation at 4ºC, 450g for 15 minutes were resuspended and diluted in 25 volumes of 0.011mol/L Tris-HCl buffer at p H 7.4. The hemolyzed cells were then centrifuged for 30min at 12,000 rpm at 4ºC 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 ~4mg 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 electrolytes were 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 (1g) 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 Silymarin treatment on body weight in control and treated rats

Decreased body weight was observed after chronic administration of thioacetamide in TAA and TAA+ Silymarin groups. Animals of TAA+ Silymarin treated group regained their body weight after Silymarin treatment in second phase. An increase in body weight was observed in control and Silymarin treated groups (fig. 1)

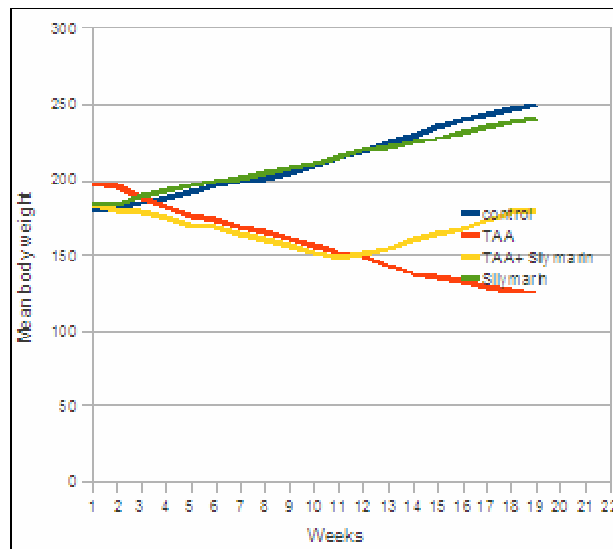

Fig. 1: Effect of thioacetamide and Silymarin treatment on body weight in control and treated rats

Effect of thioacetamide and Silymarin treatment on liver weight and liver to body weight ratio in control and treated rats

Increased liver weight and relative liver weight was observed in TAA group after 12 week administration of TAA as compare to control (7.11±1.36 P<0.01)

(0.041±0.004, P<0.01) (table 1). Whereas an increase in the liver weight and relative liver weight was also observed in TAA+ Silymarin group as compare to control (7.94+0.15 P<0.01) (0.035+0.005, P<0.01) respectively. Silymarin-treated group showed increased liver and relative liver weights (8.34±0.40 P<0.01) (0.036±0.001 P<0.01).

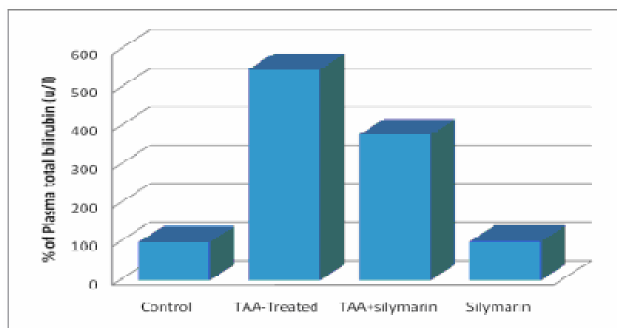

Fig. 2: Effect of thioacetamide and Silymarin treatment on Plasma total bilirubin in control and treated rats

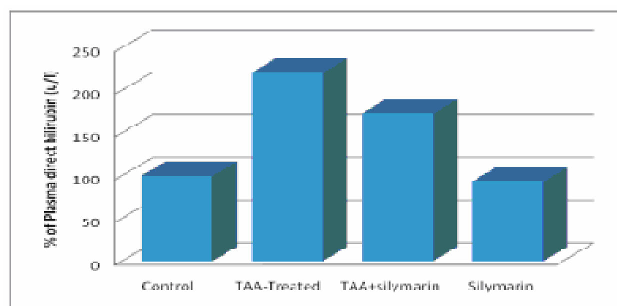

Fig. 3: Effect of thioacetamide and Silymarin treatment on Plasma direct bilirubin in control and treated rats

Effect of thioacetamide and Silymarin treatment on total and direct bilirubin and ALT activity in control and treated rats

Table 2 (fig. 2) shows 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 +Silymarin treated group, a significant decrease in total bilirubin was found as compare to control (2.2±0.06 P<0.01). Silymarin treated group showed normal concentration of total bilirubin as compare to control (0.59±0.02 P<0.01). Increased levels of direct bilirubin was shown by TAA-treated group as compare to control (3.31±0.04 P<0.01) whereas Silymarin supplementation significantly reduced direct bilirubin level in TAA +Silymarin group as compare to control (2.6±0.1 P<0.01) (table 2, fig. 3).

Plasma Alanine aminotransferase level was markedly increased in TAA group showed normal concentration of direct bilirubin as compare to control (1.4±0.01 P<0.05). TAA +Silymarin group showed a reduction in plasma ALT as compare to control (750±40.21 P<0.01). Silymarin group showed the normal concentration of plasma ALT as compare to control (205.3±15.6 P<0.01) (table 2, fig. 4).

Effect of thioacetamide and Silymarin 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 +Silymarin-treated group showed an increase in glutathione reductase level as compare to control (0.21±0.01 P<0.01) (table 3, fig. 5). Silymarin group showed normal level of glutathione reductase as compare to control (0.81±0.02 P<0.01).

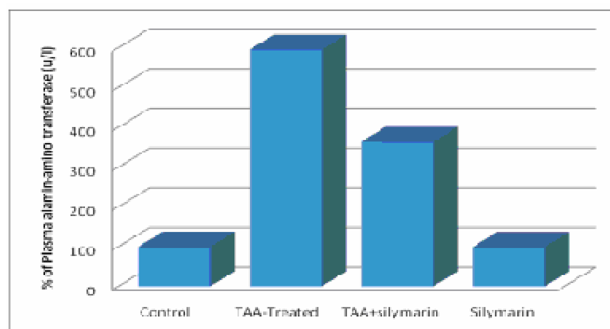

Fig. 4: Effect of thioacetamide and Silymarin treatment on Plasma alanine-amino transferase in control and treated rats

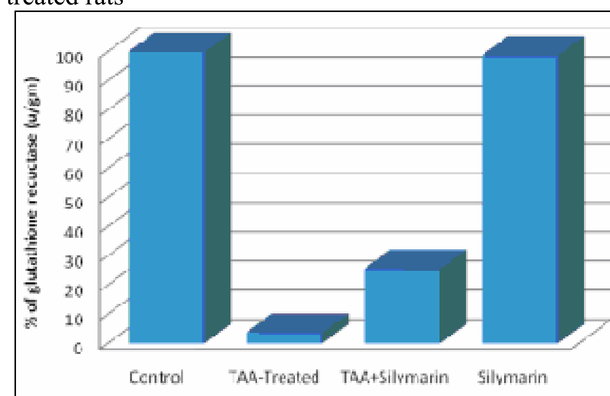

Fig. 5: Effect of thioacetamide and Silymarin treatment on glutathione reductase level in control and treated rats

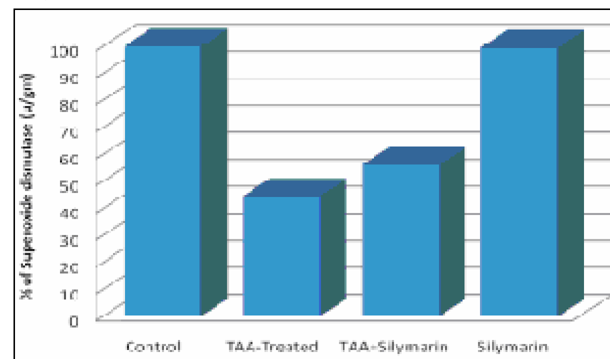

Fig. 6: Effect of thioacetamide and Silymarin treatment on Superoxide dismutase level in control and treated rats.

Effect of thioacetamide and Silymarin treatrment on hepatic concentration of super oxide dismutase in control and treated rats

Table 3 showed a significant decrease in SOD activity in TAA-treated group as compare to control (430±1.5 P<0.01). TAA +Silymarin treated group showed an increase in SOD activity as compare to control (540±1.3 P<0.01). Silymarin group showed normal range of SOD activity as compare to control (958±2.2 P<0.01) (fig. 6).

Effect of thioacetamide and Silymarin treatment on hepatic concentration of MDA in control and treated rats

Level of MDA was markedly increased in TAA-treated group as compare to control (128.8±1.8 P<0.01). AA+ Silymarin group showed a reduction in MDA level as compare to control (105.2±1.5 P<0.01) while Silymarin group showed almost normal range of MDA level as compare to control (55.1±1.2 P<0.01) (table-3, fig. 7).

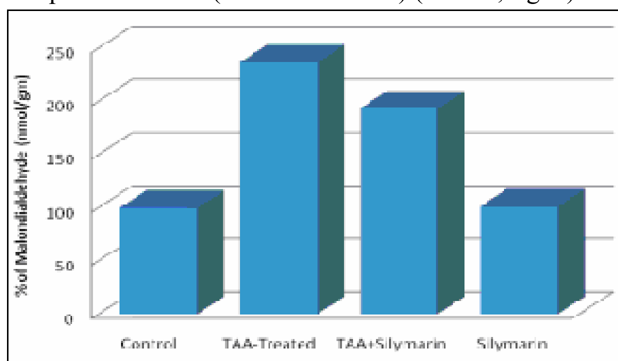

Fig. 7: Effect of thioacetamide and Silymarin treatment on Malondialdehyde level in control and treated rats

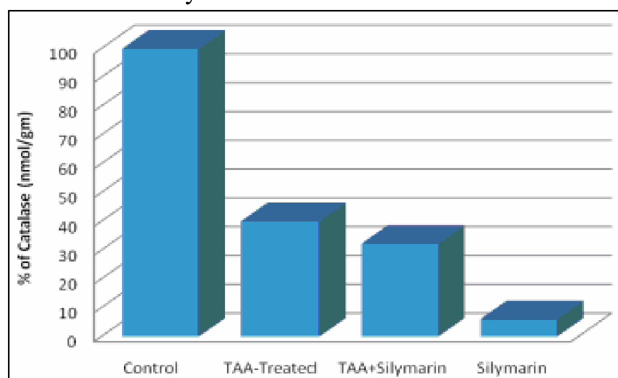

Fig. 8: Effect of thioacetamide and Silymarin treatment on Catalase in control and treated rats

Effect of thioacetamide and Silymarin treatment on hepatic concentration of catalase in control and treated rats

Concentration of catalase was significantly increased in TAA -treated group (40.1±0.01 P<0.01) as compare to control. TAA +Silymarin group showed a reduction in catalase level as compare to control (32.3±1.2 P<0.01). Silymarin group showed normal concentration of catalase as compare to control (6.1±0.01 P<0.05) (table 3, fig. 8).

Effect of thioacetamide and Silymarin treatment on plasma sodium and potassium in control and treated rats

Plasma sodium was decreased in TAA-treated group (136±1.7 P<0.01) as compare to control where as it was increased in TAA +Silymarin-treated group (142.6±4.49 P<0.01) as compare to control (table4, fig. 9).

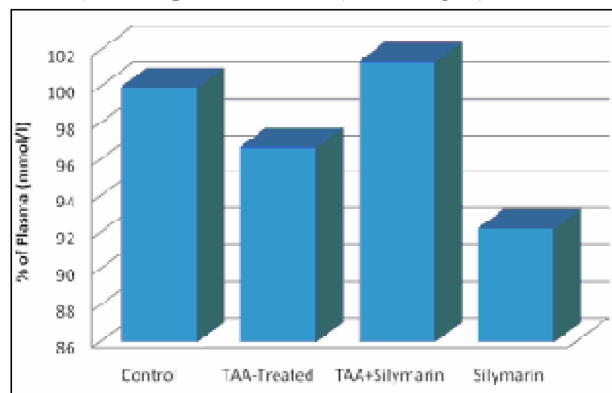

Fig. 9: Effect of thioacetamide and Silymarin treatment on Plasma sodium in control and treated rats

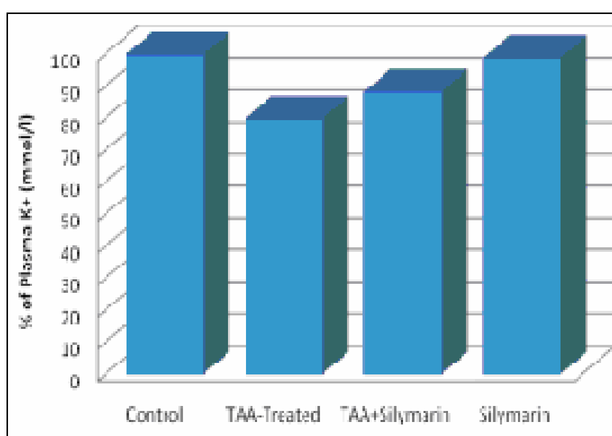

Fig. 10: Effect of thioacetamide and Silymarin treatment on Plasma K^+ in control and treated rats

Table 4 and fig. 10 showed decreased plasma potassium in TAA-treated group (4.0±0.1 P<0.01) as compare to control whereas Silymarin treatment in TAA +Silymarin treated group restored plasma potassium as compare to control (4.8±0.34 P<0.01).

Decreased levels of intraerythrocyte sodium was observed in TAA-treated group (3.9±0.2 P<0.01), whereas Silymarin supplementation significantly increased intraerythrocyte sodium in TAA +Silymarin-treated group (5.58±0.67 P<0.01) as compare to control (table 4, fig. 11).

Decreased intra erythrocyte potassium level was observed in TAA-treated group as compare to control (72.8±1.5 P<0.01). Whereas increased intraerythrocyte potassium level was observed in TAA +Silymarin-treated group (86.6±7.2 P<0.01) as compare to control (table 4 and fig. 12).

Table 1: Effect of thioacetamide and silymarin treatment on liver weight and liver to body weight ratio in control and treated rats

Groups	Liver Weights	Relative Liver Weights
Control	6.33±1.31*	0.033+0.008*
TAA-treated	7.11±1.36*	0.041±0.004*
TAA+Silymarin treated	7.94±0.15*	0.035+0.005*
Silymarin treated	8.34±0.40*	0.036+0.001*

Table 2: Effect of thioacetamide and Silymarin treatment on total and direct bilirubin and ALT activity in control and treated rats

Parameters	Control	TAA-treated	TAA+Silymarin treated	Silymarin treated
Total bilirubin (unit/L)	0.58±0.04*	3.19±0.2*	2.2±0.06*	0.59±0.02*
Direct bilirubin (unit/L)	1.50±0.03*	3.31±0.04*	2.6±0.1**	1.4±0.01**
Alanin-amino transferase (unit/L)	206±11.70*	1021.3±68.19*	750±40.21*	205.3±15.6*

Table 3: Effect of thioacetamide and Silymarin treatment on hepatic concentration of glutathione reductase, Superoxide dismutase, Malondialdehyde and Catalase in control and treated rats

Parameters	Control	TAA-treated	TAA+Silymarin-treated	Silymarin-treated
Glutathione Reductase (unit/gm of tissue)	0.82±0.02*	0.031+0.001*	0.21±0.01*	0.81±0.02*
Superoxide dismutase unit/gm of tissue.	961.4±2.1*	430±1.5*	540±1.3*	958±2.2*
Malondialdehyde nmol/gm of tissue.	54.4±1.4*	128.8±1.8*	105.2±1.5*	55.1±1.2*
Catalase nmol/gm of tissue.	6.3±0.02*	40.1±0.01*	32.3±1.2*	6.1±0.**

Table 4: Effect of thioacetamide and Silymarin treatment on plasma and intraerythrocyte sodium and potassium in control and treated rats

Parameters	Control	TAA-treated	TAA +Silymarin-treated	Silymarin-treated
Intraerythrocyte Na^+ mmol/L	4.5±0.4*	3.9±0.2*	5.58±0.67*	3.44±0.37*
Intraerythrocyte K^+ mmol/L	85.1±1.1*	72.8±1.5*	66.6±7.2*	102.8±4.8*
Plasma Na^+mmol/L	140.5±1.4*	136±1.7*	142.6±4.49*	129.6±0.94*
Plasma K^+mmol/L	5.0±0.1*	4.0±0.1*	4.42±0.34**	4.95±0.17*

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

Table 5: Histological examination of thioacetamide and Silymarin treatment on liver of control and treated rats

Group	Amount of fibrosis	Disorganization of liver architecture	Stage of nodule formation and disorientation of vascular architecture
Control	-	-	-
Thioacetamide	++++++	+++++	++++++
Thioacetamide+Silymarin	+++	++	+++

Histology of liver in control and treated rats

After 12 week administration of thioacetamide in TAA-treated rats, histological examination showed last stage of liver cirrhosis, amount of fibrosis was (+++++) maximum. Supplementation of silymarin in TAA +Silymarin group reduces the amount of fibrous tissue and the stage of nodule formation was (+++) minimum (fig. 13).

DISCUSSION

In present study, 12 week administration of thioacetamide resulted in development of severe cirrhosis in experimental rats. Thioacetamide treated rats showed increased concentrations of total and direct bilirubin and increased ALT activity while supplementation with silymarin resulted in a significant decrease in the level of total and direct bilirubin and ALT actitivity (table 2). Cirrhosis is actually progressive fibrosis and resulted in severe destruction of liver normal lobular architecture. Oral administration of thioacetamide to experimental animals resulted in induction of liver cirrhosis (Thomson and Robinson, 1980). Longterm thioacetamide administration resulted in hyperplastic liver nodules, adenomas of liver cells, cholangiomas and hepatocarcinomas (Gupta, 1956; Grant and Rees, 1958).

Fitzhugh and Nelson first time reported thioacetamide as a hepatotoxic agent (Fitzhugh and Nelson., 1948). Mangipudy reported production of centrilobular necrosis having a subsequent regenerative property resulted in administration of a single dose of thioacetamide to experimental animals (Mangipudy *et al*., 1995). Mehendale and Chilakapati reported use of thioacetamide as a model hepatotoxin because of its high liver specifity, regiospecifity for the perineous area and a sufficient time period between necrogenic effects of thioacetamide and failure of liver (Mehendale, 2005). CYP2EI and FAD monoxygenases are involved in the bioactivation of thioacetamide, which leads to its toxic effects (Zaragoza *et al*., 2000). Mangipudy *et al* reported cell death by apoptosis and necrosis as a result of thioacetamide administration to rodents (Mangipudy *et al*., 1998). Reactive metabolites are formed as a result of metabolic activation of thioacetamide (Tse Min chen *et al*., 2008). which bind covalently to macromolecules of cells or induce oxidative stress (Pallottini *et al*., 2006). Reactive oxygen species formation resulted from administration of thioacetamide leads to lipidperoxidation, depletion of glutathione and SH-thiol group's reduction (Zaragoza *et al*., 2000; Sanz *et al*., 2002).

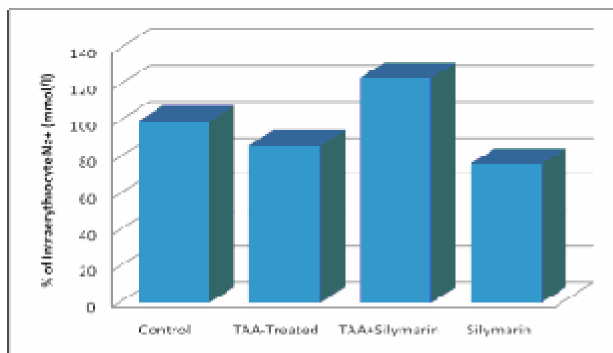

Fig. 11: Effect of thioacetamide and Silymarin treatment on Intraerytyrocyte Na^+ in control and treated rats

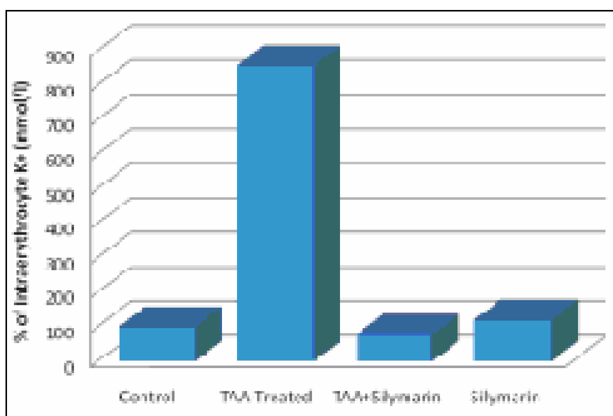

Fig 12: Effect of thioacetamide and Silymarin treatment on Intra-erytyrocyte potassium in control and treated rats

Silymarin, extracted from milk thistle (Silybum marianum), a mixture of polyphenolic flavonoids (silibin, isosilibin, silychristin and silydianin) has been utilized for centuries for the treatment of hepatic disorders (Jhy-Wen Wu *et al*., 2009). Silymarin exerts a regulatory action on permeability of mitochondrial and cellular membranes and enhances membrane stability against xenobiotic injury (Munter *et al*., 1986) Silymarin prevents the entry of a number of transport proteins at membranes and also occupies the binding sites so that inhibits the entry of toxins into hepatocytes (Faulstich *et al*., 1980). Silymarin with such effects and with its antioxidant property plays an important role in the treatment of iatrogenic and toxic liver disorders. Anti-inflammatory, antioxidant and diuretic properties of silymarin constituents enable the silymarin to protect the liver from dangerous toxins (Kiruthiga *et al*., 2007). Silymarin works as antilipid peroxidation, in detoxification reactions, reduces leukotiene synthesis from unsaturated free acids, increases synthesis of proteins, stabilizes mast cells and also function in the regulation of immune system. It prevents detoxification of cytochrome p^{450} and also inhibits toxic compounds metabolism such as thioacetamide (Papetti *et al*., 2002).

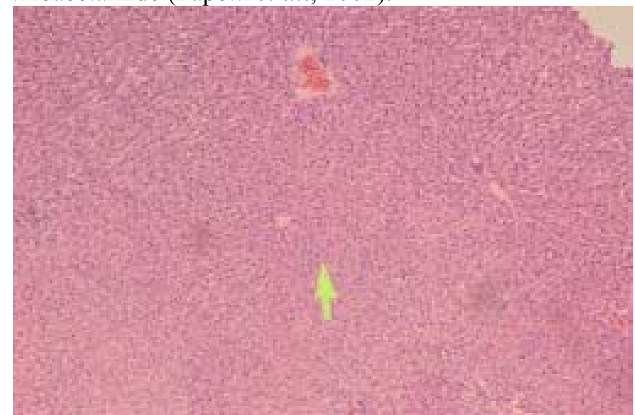

Fig. 13A: Normal liver histologyfrom control rats.

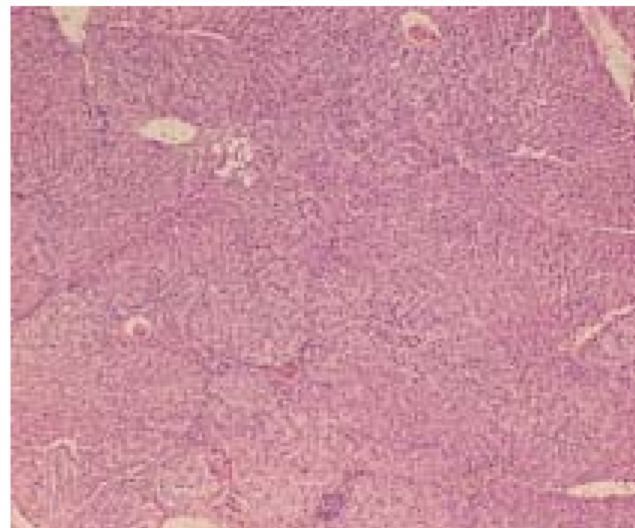

Fig. 13B: shows histological abnormalities after12 week administration of thioacetamide.

In present study, protective role of silymarin against thioacetamide induced liver cirrhosis was also reconfirmed. Silymarin markedly reduced liver pathology indicated by reduced levels of ALT activity and total bilirubin as also reported by Zahra (Zahra *et al*., 2012). A significant reduction in MDA and catalase level and a marked increase in GSH and SOD activities (table. 3), and the reversal of electrolyte homeostasis also indicates protective actions of silymarin against thioacetamide induced cirrhosis. Furthermore, data of increased liver weight, liver to body weight ratio and increased body weight after silymarin supplementation to thioacetamide administered cirrhotic rats confirms the role of silymarin in attenuation of liver cirrhosis.

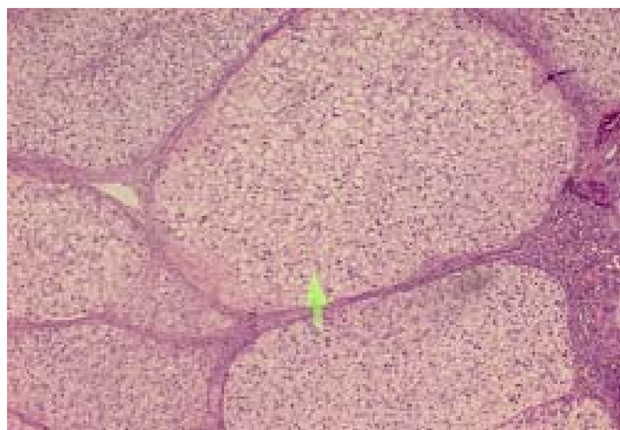

Fig. 13C: Shows effect of silymarin treatment, which reduces degree of fibrosis

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

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