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Hibiscus Rosa Sinensis alleviates Thioacetamide induced Acute Hepatotoxicity in Wistar Rats

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Abstract

The plant phenolic compounds such as flavonoids play an important role in the protection of several disorders. Some of the plant derived compounds possess potent hepatoprotective efficacy. The present study was designed to assess the prophylactic effect of *Hibiscus rosa sinensis (HRS)* extract against thioacetamide (TAA) induced hepatotoxicity in male wistar rats. TAA treated showed noticeable hepatotoxicity symptoms marked by suppression of antioxidant armoury and destruction of liver morphology. It resulted in an elevation of serum hepatotoxicity markers such as aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase activity. However, oral pretreatment with *HRS* at lower (100 mg/kg b.wt.) and higher dose (200 mg/kg b.wt.), was given to rats for 14 consecutive days, showed significant reduction in the activity of enzymes viz; catalase, xanthine oxidase, glutathione peroxidase, glutathione reductase, quinone reductase and in the levels of reduced glutathione. Moreover, *HRS* extract significantly ameliorated TAA mediated liver injury possibly by inhibition of lipid peroxidation, restoration of endogenous enzymatic defense system and effective in preventing the development of hepatotoxicity. Histopathological examination also revealed marked recovery in pretreated groups. The protective effects of the extract could be due to the presence of polyphenolic content. Quercetin being one of its major components therefore the protective effect may be attributed on the quercetin as well as its synergistic effect with other polyphenols. The results indicate the antioxidant properties of *HRS* extract against TAA induced biochemical alterations.

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INTRODUCTION

Liver is one of the major target organ in the human body and the main site for metabolism and excretion of the substance. It is involved in most of the biochemical pathways for growth, fight against disease, supply of the nutrients, energy provision,

and reproduction [1]. Hepatic injury is associated with alteration of these metabolic functions [2] and sometimes, leads to severe health problems.

Human is exposed to a variety of foreign chemicals such as drugs, food additives and environmental pollutants in their daily life. Various drugs like acetaminophen, alcohol, ibuprofen, CCl4, lead and contraceptives are some of the drugs that cause liver injury. Most of these compounds are known to cause chemical alteration in the human body. The liver plays a significant role in this process [3].

Acute liver failure is a major problem for hepatologists to face. Multiorgan failure and death are associated with failure of liver. The most common cause of acute liver failure includes acute viral hepatitis and drug induced hepatic injury. Acute liver failure is associated with high rates of mortality inspite advances in intensive care and the progress of new treatment methods. Liver transplantation remains the only effective treatment [4].

Since past several years thioacetamide (TAA) has been used as a model to induce acute liver toxicity in rats [5]. TAA is a potent centrilobular liver toxicant which undergoes a two-step bioactivation mediated by microsomal CYP2E1 to thioacetamide sulphoxide, and lead to formation of a reactive metabolite thioacetamide-*S*, *S*-dioxide [6]. Toxic effects of TAA are due to the production of (thioacetamide-S-oxide which reacts with proteins resulting in their denaturation. It has been evaluated that the administration of TAA leads to the cell death by necrosis as well as apoptosis in experimental animals [7].

Disorders of Liver are a great problem in the world today. In spite of its recurrent incidence, high morbidity and high mortality, its medicinal management is inadequate at present; no such therapy has effectively prevented the development of liver disorders [8]. There is a long history that environment has provided a remarkable number of modern drugs obtained from natural sources and are used in traditional medicine. Since time various medicinal plants have been used in daily life to protect against several diseases and also to improve health and therefore have been used as a source of medicine. The extensive uses of herbal preparation which are described in ancient period have been traced to the incidence of herbal products with medicinal properties. Plants are the main source of the production of various chemical compounds which are used as a source of diverse type of medicines and provide protection against various diseases [9]. Although crude drugs are not accepted across the world but their assessment has revealed their efficacy in assured health problems [10, 11]. The edible plants contains various bioactive compounds acting together to prevent the necrotic changes [12].

Hibiscus rosa sinensis is a medicinal plant, belongs to family Malvaceae and used in Ayuvedic preparation for a long period of time described in Indian literatures. Quercetin, anthocyanins, cyclopeptide alkaloid and several vitamins e.g. thiamine, riboflavin, niacin, ascorbic acid are the main constituents of *HRS* [13]. Previous studies have shown that *HRS* possesses antioxidant, antispermatogenic, androgenic, antitumor and anticonvulsant activities [14]. Therefore, the main aim of our study was to investigate the hepatoprotective effect of *HRS* against TAA induced liver toxicity.

MATERIALS AND METHODS

Chemicals and reagents

Glutathione Reductase (GR), oxidized (GSSG) and reduced glutathione (GSH), 1,2-dithio-bis-nitrobenzoic acid (DTNB), 1-chloro-2, 4-dinitrobenzene (CDNB), bovine serum albumin (BSA), oxidized and reduced nicotinamide adenine dinucleotide phosphate (NADP), (NADPH), flavine adenine dinucleotide (FAD), glucose-6-phosphate, 2,6- dichlorophenolindophenol (DCPIP), thiobarbituric acid (TBA), quercetin etc were obtained from Sigma-Aldrich, USA. Sodium hydroxide, ferric nitrate, trichloroacetic acid (TCA)

and perchloric acid (PCA). All other reagents used are of highest purity and commercially available.

Animals

Male Wistar rats (150–200 g), 6–8 weeks old were obtained from the Central Animal house Facility of Hamdard University. Rats were housed in animal care facility under room temperature 25±1°C with 12-h light/dark cycle and have given free assess to standard pellet diet and tap water. Before the treatment rats were left for seven days to acclimatize. Research committee of Hamdard University ethically approved protocols undertaken.

Collection and preparation of extract

Hibiscus rosa sinensis were collected from the herbal garden of Hamdard University, New Delhi, India. Freshly collected plant material was shade-dried and coarsely powdered in a grinder. The extraction procedure was followed as described by Didry *et al*. Briefly, 100gm dried powdered parts of *Hibiscus rosa sinensis* were extracted with methanol in a soxhlet for 72hrs.Then by removing the solvent under reduced pressure in rotatory evaporator (Buchi Rotavapour, Switzerland), the concentrated methanolic fraction obtained was stored at 4°C and was dissolved in distilled water to make the required doses.

Treatment regimen

Group I served as a control group and was given saline. Group II was given distilled water by oral gavage for 14 consecutive days and was given a dose of TAA (300 mg/kg b.wt.i.p) on 14th day. Groups III was given *HRS* at a dose (D1) 100 (mg/kg b.wt.) dissolved in distilled water for 14 consecutive days followed by TAA (300 mg/kg b.wt.i.p) on 14th day. Groups IV was given *HRS* at a dose (D2) 200 (mg/kg body weight) dissolved in distilled water for 14 consecutive days followed by TAA (300 mg/kg b.wt.i.p) on 14th day. Group V was given *HRS* (D2) 200 (mg/kg body weight) only. All animals were sacrificed on 15th day. Liver tissue was processed for biochemical estimations. Blood was collected and serum was separated out and processed for serological parameters.

Post Mitochondrial Supernatant preparation (PMS)

Liver was removed and cleaned with ice-cold saline (0.85% sodium chloride). Liver tissues were homogenized in chilled phosphate buffer (0.1 M, pH 7.4) using a Potter Elvehjen homogenizer and were centrifuged at 3000 rpm for 10 min at 4°C by Eltek Refrigerated Centrifuge to separate the nuclear debris. The aliquot so obtained was centrifuged at 12000 rpm for 20 min at 4°C to obtain PMS, which was used as a source of enzymes [15].

Biochemical parameters in liver tissue of wistar rats

Estimation of lipid peroxidation (LPO)

Membrane lipid peroxidation LPO was done by the method of Wright et al [16] with minute modification. The reaction mixture in a total volume of 1.0 ml contained 0.60 ml phosphate buffer (0.1 m, pH 7.4), 0.2 ml microsomes and 0.2 ml ascorbic acid (100 mm). The reaction mixture was incubated at 37°C in a shaking water bath for 1 h. The reaction was stopped by adding 1.0 ml 10% trichloroacetic acid. Following the addition of 1.0 ml 0.67% thiobarbituric acid, all tubes were placed in a boiling water bath for 20 min and then transferred to a crushed ice-bath before centrifuging at 2500g for 10 min. The amount of malondialdehyde (MDA) formed in each of the samples was assessed by measuring the optical density of the supernatant at 535 nm against a reagent blank. The results were expressed as nmol of MDA formed per minutes per gram of tissue using molar extension coefficient 1.56 X $10^5 M^{-1} cm^{-1}$.

Estimation of reduced glutathione (GSH)

Reduced glutathione was assessed by the method of Jollow et al [17]. 1.0-ml of 10% PMS mixed with 1.0 ml of 4% sulphosalicylic acid, Then incubated at 4°C for a minimum time period of 1 h and then centrifuged at 4°C at 1200×*g* for 15min.Briefly reaction mixture have 0.4 ml supernatant, 2.2 ml phosphate buffer (0.1M, pH 7.4) and 0.4 ml DTNB (4 mg/ml) in a total

volume of 3.0 ml. The yellow color developed was read immediately at 412 nm on spectrophotometer (Perkin Elmer, lambda EZ201). The reduced glutathione concentration was calculated as nmol GSH conjugates/g tissue.

Assay for catalase activity

Catalase activity was done by the method of Claiborne [18]. In short the reaction mixture comprised of 0.05 ml PMS, 1.0ml hydrogen peroxide (0.019M), 1.95 ml phosphate buffer (0.1M, pH 7.4), in a total volume of 3 ml. Changes in absorbance were recorded at 240 nm and the change in absorbance was calculated as nmol H_2O_2 consumed /min/mg protein.

Assay for glutathione peroxidase (GPx) activity

The activity of glutathione peroxidase was calculated by the method of Mohandas et al [19]. A total of 2 ml volume consisted of 0.1ml EDTA (1 mM), 0.1ml sodium azide (1 mM), 1.44ml phosphate buffer (0.1M, pH 7.4), 0.05 ml glutathione reductase (1 IU/ml), 0.05 ml reduced glutathione (1 mM), 0.1 ml NADPH (0.2mM) and 0.01ml H_2O_2 (0.25mM) and 0.1ml 10% PMS. The depletion of NADPH at 340 nm was recorded at 25°C. Activity of the enzyme was calculated as nmol NADPH oxidized/min/mg protein with the molar extinction coefficient of 6.22 X $10^3 M^{-1}$ $cm.^{-1}$

Assay for glutathione reductase (GR) activity

The activity of glutathione reductase was measured by the method of Carlberg and Mannervik [20]. The reaction mixture consisted of 1.65 ml phosphate buffer (0.1 M, pH 7.6), 0.1 ml NADPH (0.1 mM), 0.05 ml oxidized glutathione (1 mM), 0.1 ml EDTA (0.5 mM) and 0.1 ml 10% PMS in a total volume of 2 ml. Enzyme activity was assessed at 25°C by measuring disappearance of NADPH at 340 nm and was calculated as nmol NADPH oxidized/min/ mg protein using molar extinction coefficient of 6.22 $X10^3$ M^{-1} $cm.^{-1}$

Assay for quinone reductase (QR) activity

The activity of quinine reductase was measured by the method of Benson et al [21]. The 3-mL reaction mixture consisted of 2.13 ml Tris-HCl buffer (25 mM, pH 7.4), 0.7 mL BSA, 0.1 mL FAD, 0.02 mL NADPH (0.1 mM), and 50 µl (10%)PMS. The reduction of DCPIP was recorded calorimetrically at 600 nm and enzyme activity was calculated as nmol of DCPIP reduced $min^{-1}mg$ $protein^{-1}$ using molar extinction coefficient of 2.1×10^4 $M^{-1}cm^{-1}$.

Assay for xanthine oxidase (XO) activity

The activity of xanthine oxidase was measured by the method of Stripe et al [22]. The reaction mixture consisted of 0.2 ml PMS that was incubated for 5 min at 37°C with 0.8 ml phosphate buffer (0.1 M, pH 7.4). The reaction was started by adding 0.1 ml xanthine (9 mM) and kept at 37°C for 20 min. The reaction was terminated by adding of 0.5 ml ice-cold perchloric acid (PCA) (10% v/v). After 10 min, 2.4 ml of distilled water was added and centrifuged at 4000 rpm. for 10 min and mg uric acid formed per minute per mg protein was recorded at 290 nm.

Measurement of liver toxicity markers serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT)

AST and ALT activity were determined by the method of Reitman and Frankel [23]. Each substrate, 0.5 ml (2mM α-ketoglutarate and either 200 mM. L-alanine or L-aspartate) was incubated for 5 min at 37°C in a water bath. 0.1 ml serum was then added and the volume was adjusted to 1.0 ml with 0.1 M, pH 7.4-phosphate buffer. The reaction mixture was incubated for exactly 30 and 60 min at 37°C for ALT and AST, respectively. Then to the reaction mixture, 0.5ml of 1mM DNPH was added, after another 30 min at room temperature, the colour was developed by addition of 5.0 ml of 0.4N NaOH and the product read at 505nm.

Assay for lactate dehydrogenase (LDH) activity

LDH activity has been estimated in serum by the method of Kornberg [24]. The assay mixture consisted

of 0.2 ml serum, 0.1ml 0.02 M NADH, 0.1ml 0.01 M sodium pyruvate, 1.1ml 0.1 M phosphate buffer PH 7.4 and distilled water in a total volume of 3ml. Enzyme activity was recorded at 340 nm and activity was calculated as nmol NADH oxidized/min/mg protein.

Estimation of protein

The protein concentration in all samples was determined by the method of Lowry et al using bovine serum albumin (BSA) as standard [25].

Histopathological evaluation

After blood sampling, the rats were dissected and their livers were separated. The livers were fixed with 10% formalin solution. Histolologic sections were prepared from the livers, stained with haematoxylin and eosin. Analysis was done on 400x magnification.

Statistical analysis

Differences between groups were analyzed using analysis of variance (ANOVA) followed by Dunnet's multiple comparisons test. All data points are presented as the treatment groups mean ±Standard error of the mean (S.E.).

RESULTS

***Hibiscus rosa sinensis* pretreatment attenuates the serum LDH, AST and ALT level**

Protective effect of *HRS* extract on serum LDH, AST and ALT level was observed. Significant change in these parameters was found in TAA treated groups as compared to control group ($p < 0.001$). Pretreatment with *HRS* was found significantly effective at lower dose (100 mg/kg b.wt.) LDH ($p < 0.01$), AST ($p < 0.01$) and ALT ($p < 0.001$) and at higher dose (200 mg/kg b.wt.) LDH ($p < 0.001$), AST ($p < 0.001$), ALT ($p < 0.001$) in the normalization of these markers when compared to TAA treated group. *HRS* alone pretreated group did not show any significant difference when compared with control group.

***Hibiscus rosa sinensis* pretreatment decreased XO activity and MDA level**

MDA formation was measured to reveal the oxidative damage on lipid peroxidation on TAA induced liver damage of wistar rats. A significant increase in the XO ($p < 0.001$) activity and MDA ($p < 0.001$) level was found in TAA treated group as compared to control group. It has been observed that pretreatment with *HRS* extract at lower dose (100 mg/kg b.wt.) leads to the significant restoration of XO activity ($p < 0.05$) and MDA level ($p < 0.01$) and at higher dose (200 mg/kg b.wt.) leads to the significant restoration of XO activity ($p < 0.01$) and MDA level ($p < 0.001$) when compared with TAA treated group. No significant difference was found in the XO activity and MDA level between control and only D2 group.

***Hibiscus rosa sinensis* pretreatment restored the hepatic GSH level**

Protective effect of *HRS* extract on hepatic GSH level was marked. The level of GSH was depleted significantly ($p < 0.001$) in TAA treated group as compared to control group. *HRS* pretreatment increased its level significantly ($p < 0.001$) in both lower (100 mg/kg b.wt.) and higher (200 mg/kg b.wt.) doses as compared to TAA treated group. Only *HRS* pretreated group exhibited no significant changes in GSH level as compared to control group.

Effects of *Hibiscus rosa sinensis* pretreatment on the activities of hepatic antioxidant enzymes

TAA administration was found to deplete hepatic antioxidant enzymes GPx ($p < 0.01$), GR ($p < 0.001$), QR ($p < 0.01$) and catalase ($p < 0.001$) significantly as compared to control. Pretreatment with *HRS* extract before TAA administration has been found significantly effective in restoring the activities of these enzymes at lower dose (100 mg/kg b.wt.) GR ($p < 0.05$) and QR ($p < 0.05$) but non significant for GPx and at higher dose (200 mg/kg b.wt.) GPx ($p < 0.05$), GR ($p < 0.001$), QR ($p < 0.001$) and catalase ($p < 0.001$). It has been observed that there is no

significant difference in the activity of these antioxidant enzymes between control and only *HRS* treated groups.

Histopathological analysis

Histological evaluation (Figure1) reveals that liver sections from control rats showed normal architecture, characterized by polyhedral shaped hepatocytes and cytoplasm granulated with small uniform nuclei. Hepatocytes were arranged in well-organized hepatic cords and separated by narrow blood sinusoids. The liver sections of rats treated with TAA showed centrolobular necrosis and inflammatory cell infiltration. Pretreatment of rats receiving TAA with *HRS* extract at lower dose (100 mg/kg b.wt.) showed less disarrangement and degeneration of hepatocytes. In contrast, rats treated with higher dose (200 mg/kg b.wt.) showed restoration of the liver morphology.

DISCUSSION

Liver has multiple important functions in which one of the major functions is detoxification of drugs and toxic compounds [26]. It has been investigated that in many cases free radicals are produced during detoxification of the drugs [27]. Over dose of drugs or long time use of various drugs might produce large amounts of free radicals that cause oxidative stress and hepatic injury [28]. TAA is known to cause apoptosis as well as necrosis in the hepatocytes by the production of free radicals during TAA metabolism [29]. TAA is a potent hepatotoxicant and its toxic effects have been attributed to the results from its bioactivation which produces toxic reactive intermediate called thioacetamide S-oxide [30, 31]. Thioacetamide S-oxide causes oxidative stress in the hepatocytes [32]. It cause changes in permeability of the cell, increase in Ca^{++} concentration level in intracellular region, as a result nuclear volume increases, enlargement in the size of the nucleoli and inhibition of mitochondrial activity which causes cell death [33, 34].

In the recent times consideration has been given to herbal products for the prevention of human diseases. It has been reported that *HRS* possess multiple biological activities including antioxidant, antispermatogenic, androgenic, antitumor and anticonvulsant suggesting the hepatoprotective effect of *HRS* may be due to its antioxidant activity [14]. Lipid peroxidation in liver caused by TAA administration is responsible for oxidative stress which leads to liver damage in experimental animals and also through clinical trials in human studies [35]. MDA is produced as a result of lipid peroxidation during the conversion of polyunsaturated fatty acid or lipid peroxides [36]. In the present study, we found that pretreatment with *HRS* extract significantly decreased MDA formation and XO activity due to ROS in rats treated with TAA. Pretreatment with *HRS* extract restored the MDA level signifying that the extract might be successful in quenching the free radicals, thus inhibiting LPO and protect the membrane damage from oxidative damage in rats and also restored the XO activity. Various studies of rats and cultured cells have reported that oxidative stress is responsible for TAA-induced liver damage which leads to lipid peroxidation [37, 38, 39]. Amelioration of antioxidant enzymes activity was also observed. *HRS* restore the antioxidant enzymes activity of GR, GPx, QR, catalase and GSH possibly by quenching excessive ROS and free radicals generated by TAA. Catalase is an enzymatic antioxidant distributed extensively in all animal tissues, and its activity is found to be highest in liver and erythrocytes. It decomposes hydrogen peroxide and help in the protection of the tissues from highly reactive hydroxyl radicals [40]. Thus reduction in the catalase activity might result in lethal effects due to the assimilation of superoxide radical and hydrogen peroxide. *HRS* pretreatment restored the activity of catalase. Reduced glutathione is a first line of defense neutralizes the hydroxyl radical and plays a major role against inflammatory responses and oxidative stress [41]. A significant dose dependent restoration of

glutathione and dependent enzymes, namely glutathione reductase and glutathione peroxidase, to normal levels in *HRS* pretreated groups was found. Simultaneously the *HRS* pretreatment was found to restore the depleted level of quinone reductase. Good correlation is found between cellular damage and leakage of enzymes as supported by the elevated levels of serum marker enzymes [42]. It has been previously reported that TAA caused elevations in the levels of serum marker enzymes AST, ALT and LDH as seen in this study [43]. The level of serum toxicity marker enzymes (AST, ALT and LDH) were increased in the TAA treated group and were restored in the *HRS* extract pretreated group. Histopathological findings also suggested that of *HRS* have ameliorated effect on TAA-induced hepatic necrosis. Hepatic injury induced by TAA administration in the experimental animals cause histopathological changes in the liver which shows similarities with the human diseases [44]. The present findings showed that liver section from control group showed radial arrangement of hepatocytes around the central vein, granulated cytoplasm having uniform nuclei. In contrast to this liver section from TAA treated group showed loss of hepatic architecture and necrosis in the centrilobular area of hepatocytes. These histological changes were further restored and showed normal architecture of hepatocytes in the experimental animals that received *HRS* pretreatment.

CONCLUSION

It can be concluded that the results we have obtained reveal that of *HRS* possess hepatoprotective and antioxidant action on TAA induced liver toxicity in rats. The hepatoprotective effect of may be due to its ability to block the bioactivation of thioacetamide and by scavenging the free radicals and inhibition of lipid peroxidation. TAA administration caused oxidative stress in the liver. Pretreatment of *HRS* stabilize that stress in the liver. Further work is however needed to define the exact mechanisms which will explain the hepatoprotective action of *HRS*. The biochemical and histopathological findings obtained from the present study showed that *HRS* can distinctly reduce hepatic injury induced by TAA administration in experimental rats.

Table 1: Results of pretreatment of *Hibisus rosa sinensis* on serum markers enzymes like AST, ALT and LDH on thioacetamide administration in liver of wistar rats.

Treatment regimen per group	AST (IU/L)	ALT (IU/L)	LDH (n mol NADH oxidised / min/ mg protein)
Group I (control)	46.44 ± 0.47	45.85 ±0.75	216.89 ± 12.06
Group II (only TAA)	101.27 ±1.76***	104.30±0.80***	544.87 ± 29.73***
Group III (D1+ TAA)	76.41 ±0.70##	76.50±0.95###	306.82 ± 20.35##
Group IV (D2 + TAA)	51.27± 0.46###	55.64±0.08###	232.76 ± 18.62###
Group V (only D2)	50.54 ± 4.87	51.85±1.71	227.47 ± 3.34

Results represent mean ± SE of six animals per group. Results obtained are significantly different from control group (***P < 0.001). Results obtained are significantly different from (TAA) thioacetamide treated group (##P < 0.01) and (###P < 0.001). *Hibiscus rosa sinensis*; D1= 100 mg/kg b.wt; D2 = 200 mg/kg b.wt.

Table 2: Results of pretreatment of *Hibiscus rosa sinensis* on antioxidant enzymes like catalase, XO and LPO on thioacetamide administration in liver of wistar rats.

Treatment regimen per group	Catalase (nmol H_2O_2 consumed/min/mg protein)	XO (µg uric acid formed/min/mg protein)	LPO (n mol MDA formed / hr/ g tissue)
Group I (control)	32.36 ± 1.729	0.22±0.01	10.51 ± 0.36
Group II (only TAA)	7.75 ± 0.934***	0.79±0.04***	31.19 ± 3.30***
Group III (D1+ TAA)	17.1 ± 0.213##	0.46±0.07#	21.69 ±0.45##
Group IV (D2 +	25.13 ± 0.104###	0.30±0.03##	15.06± 0.72###

TAA)			
Group V (only D2)	29.23 ± 0.103	0.25±0.05	12.26 ± 0.32

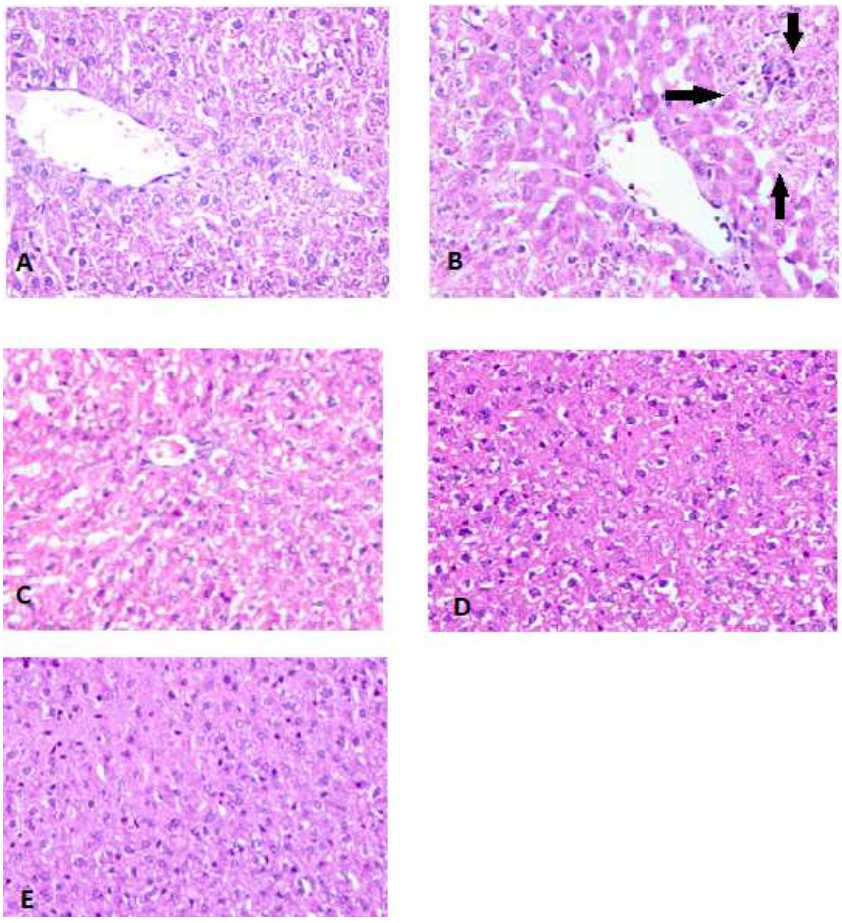
Results represent mean ± SE of six animals per group. Results obtained are significantly different from control group (***P < 0.001). Results obtained are significantly different from (TAA) thioacetamide treated group ($^{\#}$P < 0.05, $^{\#\#}$P < 0.01) and ($^{\#\#\#}$P < 0.001). *Hibiscus rosa sinensis*; D1= 100 mg/kg b.wt; D2 = 200 mg/kg b.wt.

Table 3: Results of pretreatment of *Hibiscus rosa sinensis (HRS)* on antioxidant enzymes like GSH, QR, GR and GPX on thioacetamide administration in liver of wistar rats

Treatment regimen per group	GSH (n mol CDNB Conjugate formed /g tissue)	QR (n mol dichloroindophenol reduced/min/mg protein)	GR (n mol NADPH Oxidized/min/ mg protein)	GPX (n mol NADPH Oxidized/min/ mg protein)
Group I (control)	0.58±0.004	521.33±13.16	506.19±28.31	223.86±16.92
Group II (only TAA)	0.206±0.01***	255.48±8.75**	155.54±4.90***	104.65±13.19**
Group III (D1+ TAA)	0.381±0.01$^{\#\#\#}$	415.76±11.37$^{\#}$	367.88±3.97$^{\#}$	141.09±13.8^{ns}
Group IV (D2 + TAA)	0.47±0.02$^{\#\#\#}$	499.9±20.92$^{\#\#}$	487.88±38.90$^{\#\#\#}$	195.2±5.37$^{\#}$
Group V (only D2)	0.49±0.01	515.24±42.18	492.96±30.20	201.61±14.36

Results represent mean ± SE of six animals per group. Results obtained are significantly different from control group (***P < 0.001, **P < 0.01). Results obtained are significantly different from (TAA) thioacetamide treated group ($^{\#}$P < 0.05, $^{\#\#}$P < 0.01) and ($^{\#\#\#}$P < 0.001). Whereas, Results obtained are not significant from TAA treated group (ns) for GPx (D1). *Hibiscus rosa sinensis*; D1= 100 mg/kg b. wt.; D2 = 200 mg/kg b. wt.

Figure 1. Histopathological examination. Histological slide (A) represents control group, slide (B) represent TAA group. (C) and (D) are (D1+TAA) and (D2+TAA) groups (100 and 200 mg/kg b.wt. respectively), slide (E) represent only D2 group. (A) There were no changes observed in liver architecture. (B) TAA treated rats show loss of cellular structure, centrilobular necrosis, inflammatory cells infiltration. Slide (C) and (D) showing less disruption and deterioration of hepatocytes. Slide (E) shows normal architecture, granulated cytoplasm and uniform nuclei.



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Conflict of interest declaration

The authors of the present research work do not have any conflict of interest to present this research work.

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