Original Article

Hepatoprotective Potential of *Ziziphus oenoplia* (L.) Mill Roots against Paracetamol-Induced Hepatotoxicity in Rats

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ABSTRACT

Objective: To evaluate the hepatoprotective potential of ethyl acetate fraction of *Ziziphus oenoplia* (L.) Mill roots against paracetamol induced hepatotoxicity in Wistar albino rats.
Materials and Methods: Paracetamol-treated groups (II, III, IV and V) were administered orally single daily dose of fraction (150 and 300 mg/kg) for 14 days. The serum levels of glutamic oxaloacetic transaminase (SGOT), glutamate pyruvate transaminase (SGPT), alkaline phosphatase (SALP), γ-glutamyltransferase (γ -GT), and bilirubin were analyzed together with glutathione S-transferase (GST), glutathione reductase (GR), hepatic malondialdehyde formation and glutathione content.
Results and Discussion: The significantly high activity of serum enzymes, SGOT, SGPT, SALP, γ-GT, and bilirubin due to paracetamol, were regained to normal in a dose-dependent manner. Meanwhile the reduced activities of GST and GR were also regained to normal. Additionally, ethyl acetate fraction also extremely interrupted the increase of hepatic malondialdehyde formation and decrease of reduced glutathione content in the liver of paracetamol-intoxicated rats in a dose-dependent manner. Silymarin also showed well hepatoprotective activity on post-treatment against paracetamol-induced hepatotoxicity in rats. The biochemical observations play as supplementary component with histopathological assessment of rat liver sections. It is concluded that ethyl acetate fraction has an effective hepatoprotective action against paracetamol-induced hepatic injure in rats.

Keywords: Hepatoprotective, Glutamic oxaloacetic transaminase, Alkaline phosphatase, Glutathione reductase, Bilirubin.

INTRODUCTION

Liver diseases are a major global concern and this type of disorder still has extremely poor prognosis and high mortality because of the lack of effective preventive/treatment options. In the field of recent remedy, hepatoprotective is a chief fitness trouble in the field of recent remedy; consequently the exploration for novel efficient drug having no side effects is proceeding[1]. The development of hepatoprotective remedy is of vital essential due to role of liver cells metabolic. Natural medications are more efficient and safer option approach for hepatotoxicity. The previous investigations[2-6] has revealed that hepatoprotective effects are linked with natural antioxidants. Several plants bioactive components and extracts have been investigated as natural remedy for hepatotoxicity[7,8]. The reactive oxygen species (ROS) involves in numerous acute and chronic liver diseases, for example: acetaminophen overdose, haemochromatosis, alcoholic liver injury, toxin exposure and viral hepatitis[9-15]. ROS cause impairment of cellular membrane stability and cell death by lipid peroxidation[16].

A lot of hepatotoxins have need of metabolic activity mainly by the liver cytochrome P_{450} (CYP) enzymes, which damage liver in animals and humans[17]. Nevertheless paracetamol is well thought-out secure at curative dose but generate liver necrosis and malfunction at elevated dose[9,18-21]. Paracetamol is broadly metabolized by conjugation with sulphate and glucuronic acid at normal dose. N-acetyl-p-benzo-quinoneimine (NAPQI) produced in oxidation reactions catalyzed by CYP_{450} enzymes in the liver, is a highly electrophilic metabolite that induces hepatic injure. In general non-toxic metabolites, converted product of toxic oxidation metabolites, are excreted in urine via conjugation with glutathione (GSH) containing sulphydryl groups. Nevertheless high doses of paracetamol bound the capability of GSH to detoxify NAPQI, and end result in the utilization of liver GSH stores[22,23]. It has been revealed that CYP2E1, CYP1A2, and intracellular GSH take part in important role in the hepatotoxicity induced by paracetamol[24-27].

Ziziphus oenoplia (L.) Mill. (ZO) (Family-Rhamnaceae) commonly well-known as makai in hindi and jackal jujube in English, is a thorny sprawling straggling shrub often semi-scandent by its prickles, is distributed all over the hotter regions of Pakistan, Sri Lanka, India, Malaysia, Ceylon, Australia and Tropical Asia[28] (Fig. 1 & 2). It is one of the folk herbal plants believed to possess some pharmacological properties as blood purifier, febrifuge, abdominal pain killer, etc.[29,30] It is frequently used in central zone, Uttar Pradesh (India) for liver diseases[31]. The roots of the plant possess antiulcer, antioxidant,[32,33] anthelminthic,[34,35] anti-plasmodial,[36] angiogenic potential[37], anti-denaturation and antibacteial activity[38], wound healing activity[39], hepatoprotective potential against antitubercular drugs induced hepatotoxicity[40] and as an ingredient in the preparation of stomach ache pills among the Munda tribe[41].

Therefore, in continuation with the earlier observations, this study prompted us to investigate the hepatoprotective activity of ethyl acetate fraction of *Z. oenoplia* roots (ZO) against paracetamol induced liver damage in albino rats.

MATERIALS AND METHODS

Chemicals

All chemicals were of analytical grade. Silymarin was obtained from Ranbaxy Laboratories Limited, Gurgaon, India. Thiobarbituric acid, dithio-bis-2-

nitrobenzoic acid, corn oil and assay kit for lactate dehydrogenase were purchased from Sigma Chemical Co St Louis MO USA. Assay kit for serum aspartate amino-transferase and alanine aminotransferase were taken from Dialab, Austria. Paracetamol was acquired from SDFCL, Mumbai, India.

Plant material

The roots of *Z. oenoplia* were collected from District Hardoi, Uttar Pradesh, (India) in the month of July 2010 and authenticated with existed voucher specimen (NBRI/CIF/175/2010) at National Botanical Research Institute, Lucknow, Uttar Pradesh, India. The prepared herbarium was deposited in the laboratory for future reference.

Animals

Wistar albino rats (150-200 g) of either sex were selected for the study and maintained at a controlled temperature of 25 to 28^{0}C with a 12 hour light/dark cycle and fed a standard diet (Amrut, India) and water *ad libitum*. All experiments were performed in the morning according to current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals[42]. The protocols were approved by Institutional Committee for Ethical use of Animals and Review Board (Reg. No. 1732/ GO/Re/S/13/CPCSEA).

Extraction and fractionation

The air-dried powdered roots (200 g) were extracted with 50% ethanol at 50 ^{0}C on a water bath for 24 hours using Soxhlet apparatus and centrifuged at10,000 rev/min. The extract was separated by filtration and concentrated on rotavapour (Buchi R-200, USA) at 40^{0}C and then freeze-dried in lyophilizer (Labconco, USA) under reduced pressure to obtain solid residue (5.96 g practical yield). The extract thus obtained was partitioned with organic solvents to afford the n-hexane (33.09% yield), chloroform (37.41% yield), n-butanol (47.48% yield), and ethyl acetate fractions (70.14% yield).

Paracetamol-induced hepatotoxicity

Wistar albino rats were divided into five groups comprising six animals in each group.

Group I (NC): Normal control was administered a single daily dosage of liquid paraffin (1.0 ml/kg body weight, p.o.).

Group II (HC): Hepatotoxic control received Paracetamol (2.0 g/kg body weight, p.o.).

Group III (HCE1): Animals were given Paracetamol + ethyl acetate fraction of *Z. oenoplia* orally (150 mg/kg)

Group IV (HCE2): Animals were given Paracetamol + ethyl acetate fraction of *Z. oenoplia* orally (300 mg/kg)

Group V (HCSD): Animals were given Paracetamol + Silymarin (100 mg/kg)

All the treatments were given orally in CMC (1%) in distilled water (10 ml/kg) by means of orogastric cannula for 14 days. Paracetamol (2.0 g/kg body weight of animal) suspension prepared by using 0.5% Tween 80 and subjected for hepatoprotective activity against paracetamol-induced liver damage. Animals were sacrificed 48 hours after the last injection. Blood was taken, allowed to clot, and serum was separated. Liver was dissected out and used for biochemical studies.

Biochemical determinations

The Biochemical parameters like serum enzymes: serum glutamic oxaloacetic transaminase (SGOT), serum glutamate pyruvate transaminase (SGPT), serum alkaline phosphatase (SALP), and bilirubin were assayed according to standard

methods[43,44], using an assay kit. γ-glutamyltransferase (γ-GT) was determined by the method of Szasz[45]. The contents of glutathione (GSH)[46] and malondialdehyde (MDA) was determined respectively[47,48]. The activities of glutathione S-transferase (GST) and glutathione reductase (GR) were determined by the standard methods respectively[49-52]. The protein content was measured by the method of Lowry *et al*[53] with bovine serum albumin as standard.

Liver histopathological assessment

Instantly liver sections were put in 10% formalin, dehydrated in gradual ethanol (50-100%), cleared in xylene, and then embedded in paraffin. Sections (4-5 µm thick) were made and then stained with hematoxylin and eosin (H-E) dye for photomicroscopic examination including fatty changes, cell necrosis, lymphocytes, infiltration of Kupffer cells, hyaline and ballooning degeneration.

Statistical analysis

The data are expressed as mean ± S.E.M. The difference among means has been analyzed by student's *t*-test, method of Woolson[54]. A value of $P<0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

The results of hepatoprotective effects of ethyl acetate fraction of *Z. oenoplia* on Paracetamol intoxicated rats are shown in Table 1. In the paracetamol-treated control, serum GOT, GPT, ALP, γ-GT, and bilirubin were substantially (statistical significance vs. normal) greater than before. The groups treated with 150 and 300 mg/kg of fraction showed a significant decrease in the elevated levels of SALP, SGPT, SGOT, bilirubin and γ-GT in a dose-dependent manner to normal. Treatment with ethyl acetate fraction of ZO at a dose of 300 mg/kg showed highly significant activity which is almost comparable with the group treated with silymarin, a powerful hepatoprotective remedy.

Histopathological observations

The histological observations support the results obtained from serum enzyme assays. Histological architecture of the liver sections of normal control animals (Group I) showed normal hepatic cells with well-preserved prominent nucleus, nucleolus, cytoplasm and well brought out central vein [Fig. 3]. The liver sections of paracetamol intoxicated rats showed massive fatty changes, necrosis, broad infiltration of the lymphocytes, Kupffer cells around the central vein, ballooning degeneration, and loss of cellular boundaries [Fig. 4]. Paracetamol-induced group was more severe than the other groups. The histology of liver sections of rats treated with ethyl acetate fraction 150 and 300 mg/kg showed a more or less normal lobular pattern with a mild degree of fatty change, necrosis, and lymphocyte infiltration [Fig. 5-7].

Effects of ethyl acetate fraction on hepatic malondialdehyde and glutathione levels

The production of MDA in paracetamol-induced group was increased drastically as compared with the normal group [Fig. 8]. Treatment with 150 and 300 mg/kg of ethyl acetate fraction reduced paracetamol-induced MDA production in a dose-dependent manner significantly ($P<0.001$). Administration of paracetamol decreased the hepatic GSH level drastically. Treatment with 150 and 300 mg/kg of ethyl acetate fraction elevated the hepatic GSH levels toward normal in a dose-dependent manner [Fig. 9]. The results were comparable with silymarin.

Effects of ethyl acetate fraction on glutathione S-transferase and glutathione reductase activities

GST and GR activities in paracetamol-intoxicated rats were decreased drastically as compared with the normal group. Ethyl acetate fraction caused a recovery toward normal in a dose-dependent manner of treating. From these results, it is observed that ethyl acetate fraction 150 and 300 mg/kg showed highly significant activity (P<0.001) which is almost similar to the silymarin [Table 1].

Liver injury induced by paracetamol is the best characterized system of xenobiotic induced hepatotoxicity and is commonly used model for screening hepatoprotective drugs. Paracetamol a common analgesic and antipyretic drug causes induction of hepatocellular damage or necrosis in higher doses in experimental animals and humans revealed by several investigations[55]. Paracetamol produced hepatotoxicity due to reactive metabolite N-acetyl-p-benzo quinoneimine (NAPQI), which causes oxidative stress and glutathione depletion[56]. Paracetamol is metabolized primarily in the liver and eliminated by conjugation with sulfate and glucuronide, and then excreted through the kidney. Moreover, paracetamol hepatotoxicity has been attributed to the production of toxic metabolites, when a fraction of paracetamol is activated by hepatic cytochrome P-450 to a highly reactive metabolite N-acetyl-p-benzo-quinoneimine (NAPQI)[57]. N-acetyl-p-benzo-quineimine can alkylate and oxidise intracellular GSH, which results in liver GSH depletion subsequently leads to increased lipid peroxidation by abstracting hydrogen from a polyunsaturated fatty acid and ultimately, liver damage due to higher doses of paracetamol. Reactive metabolites can produce initial cell stress through a wide range of mechanisms including depletion of glutathione (GSH) or binding to lipids, nucleic acids, enzymes, and other cellular molecules[58]. AST predominantly found in mitochondria of hepatocytes. ALT is one of the most sensitive diagnostic tests of hepatic diseases and thus is a better parameter for detecting liver injury. Serum ALP is also associated with liver cells damage. The ALP, AST and ALT activity are widely used as most common biochemical markers to evaluate liver injury[59]. Administration of paracetamol caused a significant elevation in level of enzymes such as AST, ALT and ALP, it has been attributed to break structural integrity of liver, because those are cytoplasmic in location and released into circulation after cellular damages indicating development of hepatotoxicity[60]. The present study shows that ethyl acetate fraction of *Z. oenoplia* have hepatoprotective potential, as exhibited by the significant inhibition in the elevated levels of serum enzyme activities induced by Paracetamol. Ethyl acetate fraction of *Z. oenoplia* given orally (150 & 300 mg/kg), once daily for 14 days, showed dose-dependent hepatoprotective effect and its highly significant effect was observed with doses of 150 and 300 mg/kg body weight, against paracetamol-induced hepatotoxicity. Hepatotoxic compounds like paracetamol are known to cause marked elevation in serum enzyme levels. In the current study, treatment with ethyl acetate fraction of *Z. oenoplia* attenuated the increases in the activities of SGPT, SALP, SGOT, γ-GT, and bilirubin produced by paracetamol, demonstrating that ethyl acetate fraction of *Z. oenoplia* protects liver injury induced by paracetamol. This is in agreement with the commonly accepted view that serum levels return to normal with the healing of hepatic parenchyma and the regeneration of hepatocytes[61].

Reduced glutathione (GSH), a regenerator for alpha-tocopherol, is a

substrate for glutathione related enzymes, and, therefore it plays an important role in the antioxidant defense system. Reduced GSH removes free radical species such as superoxide, hydrogen peroxide radicals, and keeps up the membrane protein thiols. The reduced GSH depletion in hepatic mitochondria is considered the most important mechanism in the paracetamol induced hepatotoxicity[62]. Reduced GSH level was depleted in paracetamol treated group may be due to conjugation of reduced GSH with NAPQI to form mercapturic acid. In present study, administration of ethyl acetate fraction of *Z. oenoplia* showed great ability to reduce oxidative stress by increasing the levels of reduced glutathione as compared to paracetamol intoxicated group. The function of GST is divided into binding and catalysis. GST, a soluble protein, is present in cytosol, which plays an important role in the detoxification and excretion of xenobiotics[63,64]. The cytosolic GST catalyzes the conjugation reaction of GSH and has an integral role in the detoxification of electrophilic toxicants[65]. The activity of GST decreased drastically compared with the normal group in paracetamol intoxicated rats. The activity of GST improved significantly ($P<0.001$) at 150 and 300 mg/kg of ethyl acetate fraction of *Z. oenoplia* compared with that of paracetamol group. In contrast, the GST activity at 300 mg/kg was almost similar to that shown by silymarin, a potent hepatoprotective agent. These above observations strongly indicate the hepatoprotective activity of ethyl acetate fraction of *Z. oenoplia* against paracetamol intoxicated rats. The increase in MDA level in liver induced by paracetamol suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanism[57]. A highly significant decrease in lipid peroxidation in liver tissue was observed in ethyl acetate fraction of *Z. oenoplia* treated groups which prevents lipid peroxidation. The activity of GR is significantly decreased in paracetamol intoxicated rats,. However, ethyl acetate fraction of *Z. oenoplia* brought the activity of GR toward normal. GR is involved in diverse cellular events such as defensive actions against free radicals and reactive oxygen species (ROS) as well as protein and DNA biosynthesis, by maintaining a high ratio of GSH/GSSG[66-68].

CONCLUSION

Therefore, it is concluded that effects of ethyl acetate fraction of *Z. oenoplia* on liver protection may be due to the reduction of oxidative stress and its ability to reduce elevated levels of serum marker enzymes and are related to glutathione-mediated detoxification as well as free radical scavenging activity. These studies are in progress for better understanding of the mechanism of action and to evaluate the efficacy of the ethyl acetate fraction of *Z. oenoplia* on liver organelle that are possibly damaged during experimental hepatitis.

ACKNOWLEDGMENTS

Authors are much thankful to Dr C. S. Nautiyal, Director, CSIR-National Botanical Research Institute, Lucknow for providing necessary facilities and financial support from Council of Scientific Industrial Research, (OLP-089 and BSC-0106), New Delhi.

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Table 1. Effects of ethyl acetate fraction of *Ziziphus oenoplia (L.) Mill* on serum and liver biochemical indices in Paracetamol intoxicated rats

Group	Treatment	SGOT (U/L)	SGPT (U/L)	SALP (U/L)	SB (mg/dl)	γGT (IU/L)	GST (nmol/min /mg protein)	GR (u/mg Protein)
I	NC	68.24 ± 3.35	53.47 ± 2.12	80.96 ± 9.18	0.49 ± 0.02	32.31± 4.12	263.5 ± 10.12	30.49 ± 2.84
II	HC	122.26 ± 2.45[a]	135.98 ± 1.23[a]	185.73 ± 6.16[a]	1.16 ± 0.08[a]	208.36± 12.13[a]	156.48 ± 2.46[a]	13.73 ± 1.56[a]
III	HCE1	110.42 ± 5.31[b]	95.13 ± 5.01[b]	142.86 ± 2.02[b]	0.98 ± 0.05[b]	79.32 ± 3.09[b]	205.16 ± 1.32[b]	22.61 ± 1.89[b]
IV	HCE2	84.12 ± 1.45[c]	66.27 ± 4.62[c]	121. 56 ± 4.06[c]	0.76 ±0.04c	70.16 ± 9.87[c]	219.52 ±3.26[c]	25.19 ± 1.02[c]
V	HCSD	79.32 ± 7.74[c]	71.37 ± 0.59[c]	82.28 ± 3.02[c]	0.80 ± 0.07[c]	112.21± 6.86[c]	226.13 ± 3.26[c]	26.54 ± 1.2[c]

Data are expressed as mean ± S.E.M (n = 6). [a]$P < 0.01$ as compared with normal rats. [b]$P < 0.001$ and [c]$P < 0.0001$ as compared with Paracetamol treated group.

Figure 1. Dried roots of *Ziziphus oenoplia*

 ISSN 2321 – 2748

Figure 2. Plant of *Ziziphus oenoplia*

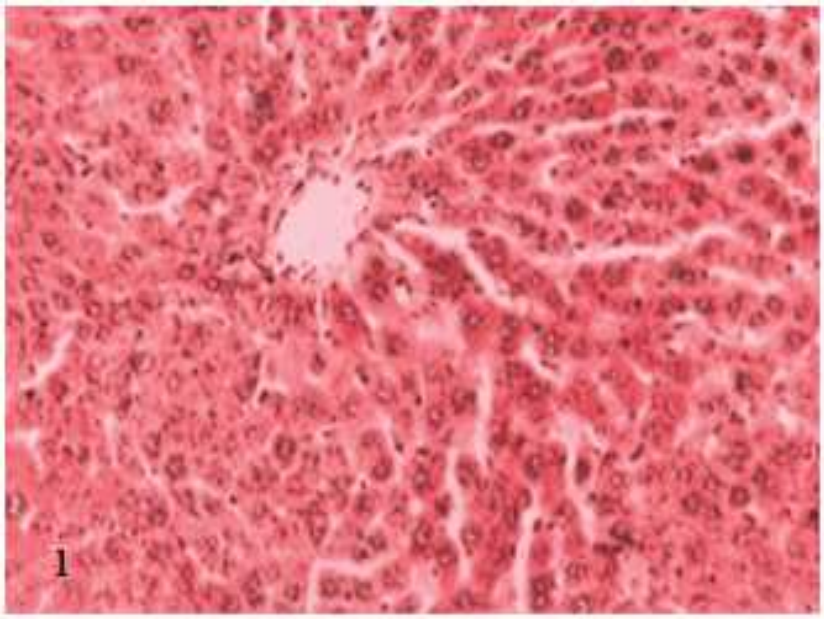

Figure 3. Normal rats (Group I) showed normal hepatocytes with well preserved cytoplasm with normal lobular structural design of the liver

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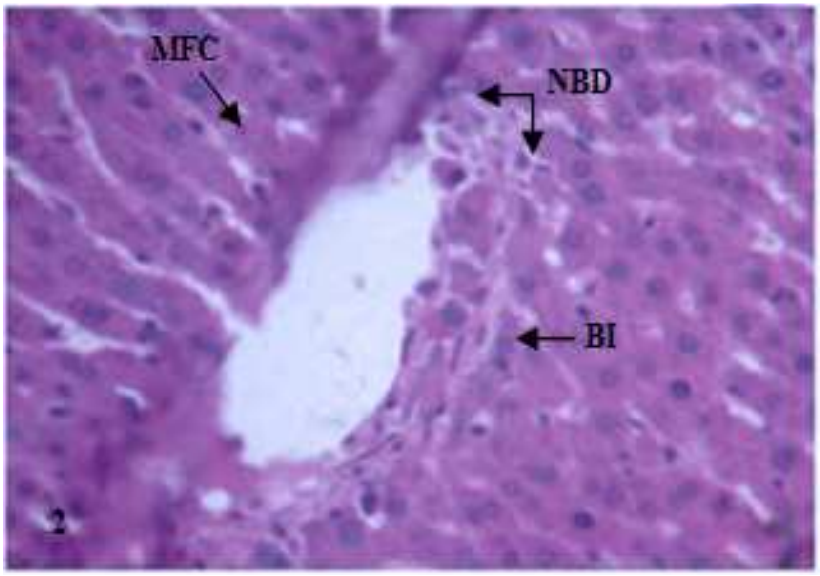

Figure 4. Hepatocytes of Paracetamol (2.0 g/kg, each p.o.) treated rats (Group II) showing massive fatty changes (MFC), necrosis ballooning degeneration (NBD), and broad infiltration of the lymphocytes and kupffer cells around the central vein and the loss of cellular boundaries (BI)

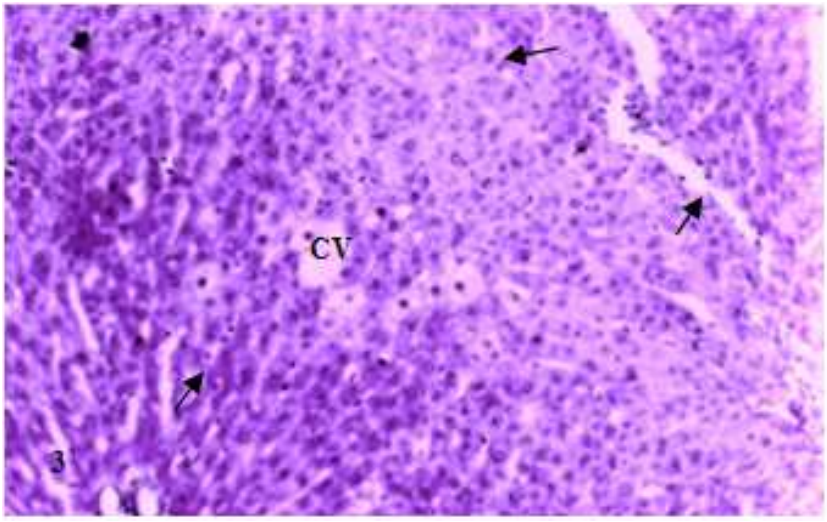

Figure 5. Hepatocytes of rats treated with paracetamol (2.0 g/kg, each p.o.) + ethyl acetate fraction of ZO (150 mg/kg, p.o.)×14 days (Group III) showing well brought out central vein (CV), hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus

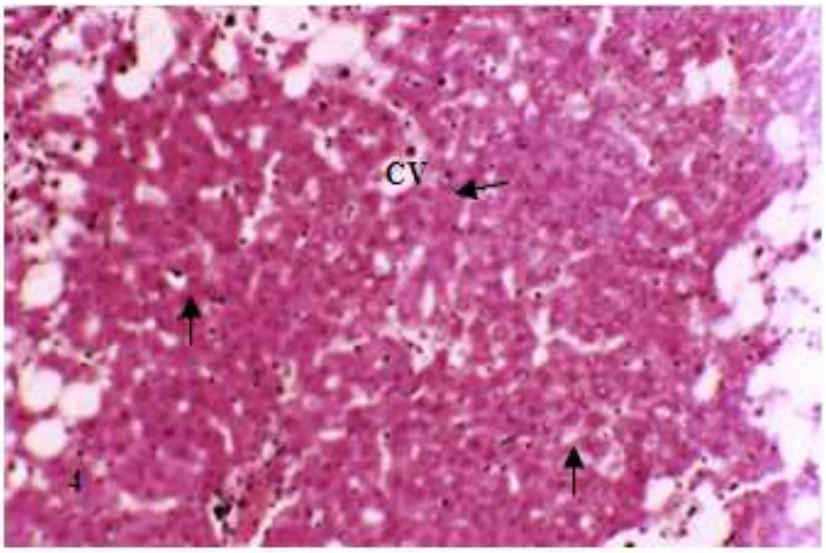

Figure 6. Liver section of rats treated with paracetamol (2.0 g/kg, each p.o.) + ethyl acetate fraction of ZO (300 mg/kg, p.o.)×14 days (Group IV) showing well brought out central vein (CV), hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus

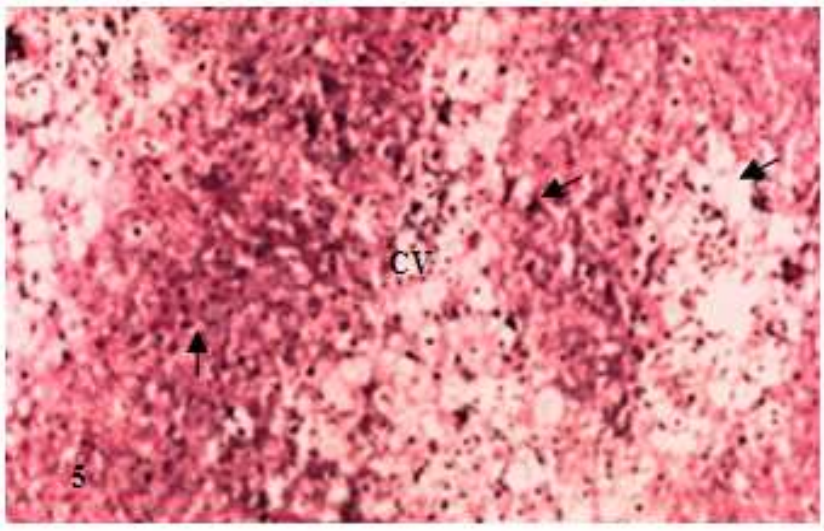

Figure 7. Liver section of rats treated with paracetamol (2.0 g/kg, each p.o.) + Silymarine (150 mg/kg, p.o.)×14 days (Group V) showing well brought out central vein (CV), hepatic cell with well preserved cytoplasm, prominent nucleus and nucleolus

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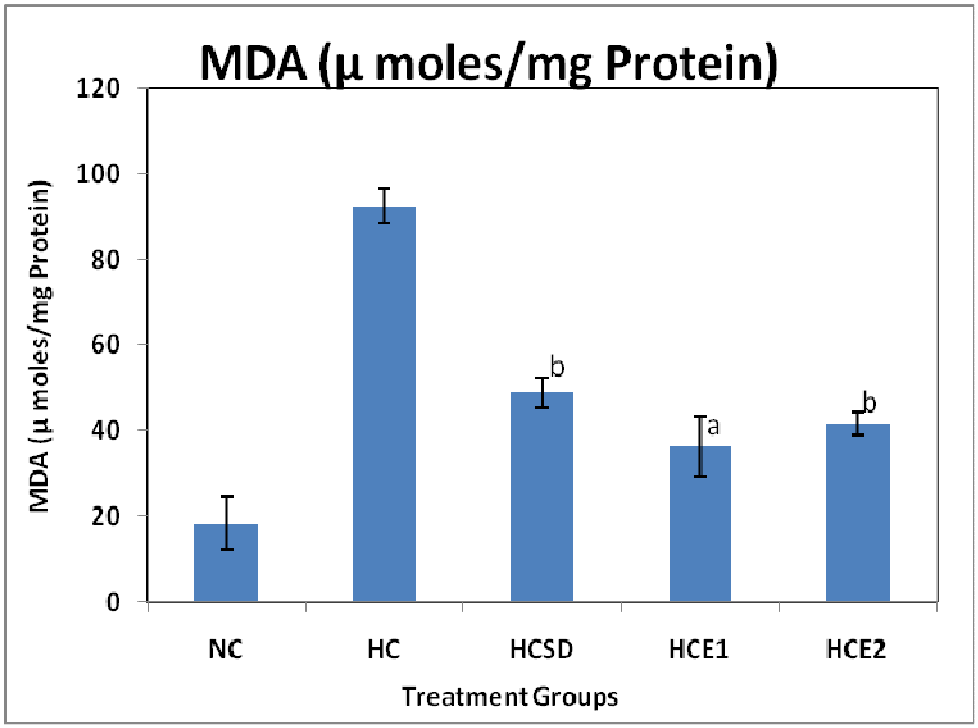

Figure 8. Rats were administered with ethyl acetate fraction 150, and 300 mg/kg orally once a day for 14 days, and then Paracetamol (2.0 g/kg body weight, p.o.) was injected four times at every 72 hours after the administration of ethyl acetate fraction. The rats were decapitated 48 hours after the final administration of paracetamol. The data are expressed as mean ± S.E.M (n = 6). $^{a}P<0.01$, $^{b}P<0.001$ as compared with the paracetamol-treated group by Duncan's new multiple range test

 ISSN 2321 – 2748

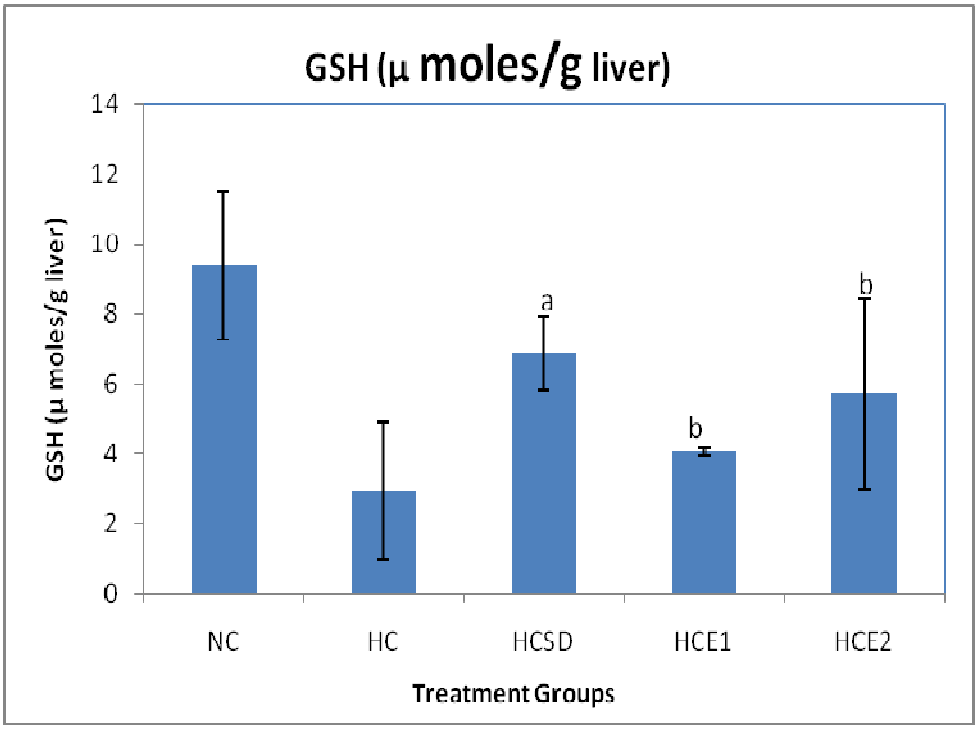

Figure 9. Rats were administered with ethyl acetate fraction 150, and 300 mg/kg orally once a day for 14 days, and then Paracetamol (2.0 g/kg body weight, p.o.) was injected four times at every 72 hours after the administration of ethyl acetate fraction. The rats were decapitated 48 hours after the final administration of paracetamol. The data are expressed as mean ± S.E.M (n = 6). $^{a}P<0.01$, $^{b}P<0.001$ as compared with the paracetamol-treated group by Duncan's new multiple range test