Research Article

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CURATIVE EFFECT OF *BUTEA MONOSPERMA* (LAM.) FLOWERS ON CCL_4 INDUCED SUBCHRONIC HEPATIC INJURY IN RAT MODEL

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ABSTRACT

Butea monosperma (*B. monosperma*) *Lam.* is commonly known as Palash in Hindi and their flower are used to treat leprosy, gout, skin, eye diseases and has been reported to be associated with various remedial properties such as antihepatotoxic, antistress, antiestrogenic and chemopreventive. In the present study, hepatoprotective effects of *B. monosperma* (800 mg/kg *p.o.*) was studied against biochemical, light and ultrastructural and genotoxic changes induced by carbon tetrachloride (CCl_4, 0.15 ml/kg, *i.p.*). Subchronic exposure to CCl_4 for 3 weeks caused sharp elevation in the activity of liver marker enzymes (serum transaminases, alkaline phosphatase and lactate dehydrogenase), albumin, bilirubin, creatinine and urea level in serum. Tissue biochemistry revealed significant reduction in antioxidant status *i.e.* glutathione reductase, glutathione peroxidase, super oxide dismutase and catalase. Oxidative stress was measured by estimating reduced glutathione level and amount of thiobarbituric acid reactive substances. CCl_4 administration significantly decreased aniline hydroxylase activity and increased microsomal lipid peroxidation. A 5-day treatment of *B. monosperma* extract after toxicant administration reversed alterations in blood and tissue biochemical variables almost as same as in silymarin treated positive control. In conclusion, data suggest aqueous extract of *B. monosperma* prevent hepatic damage and improved cellular architecture through its antioxidant activity.

Keywords: Hepatoprotective; carbon tetrachloride, oxidative stress, *Butea monosperma.*

INTRODUCTION

Liver is the main organ responsible for drug metabolism and appears to be sensitive target site for substances modulating biotransformation. Herbs have recently attracted attention as health beneficial food and as source materials for drug development. Herbal medicines derived from plant extracts are being increasingly utilized to treat a wide variety of clinical diseases including liver diseases[1] with relatively little knowledge regarding their modes of action [2].

Butea monosperma Lam. is commonly known as Palash in Hindi and widely distributed in India. Its flowers are used to treat leprosy, gout, skin and eye diseases and has been reported to be associated with various remedial properties such as[3] antistress[4], antiestrogenic[5] and chemopreventive[6]. Its flowers contain various flavonoids like butein, butin, isobutrin, isomonospermoside and steroids [7].

We have already reported the dose dependent hepatoprotective activity of *Butea monosperma* extract against CCl_4 induced acute liver damage[8]. In the present study, an attempt has been made to confirm hepatoprotective efficacy of *Butea monosperma* against biochemical, light and ultra-structural and genotoxic alterations induced by subchronic exposure to CCl_4

MATERIALS AND METHODS

Preparation of plant extract

Flowers of *Butea monosperma* (*B. monosperma*) was generously obtained from Central Council for Research in Unani Medicine, India and identified by the Dr.A.K. Jain, Professor, School of Studies in Botany, Jiwaji University, Gwalior (M.P.). Flowers were dried in shade, powdered and extracted with distilled water (250 g/4 l) for 18 h with concomitant shaking. Filtrate was evaporated in vacuum to yield a yellow powder, which was administered orally according to body weight of animals. Silymarin (50 mg/kg, p.o.) was used as a positive control during experimental regimen [9].

Animals and Chemicals

Adult female albino Swiss mice (30±5 g body weight) and albino rat of Sprague–Dawley strain (160±10 g body weight) were randomly selected from departmental animal facility where they were housed in polypropylene cages under uniform husbandry conditions of light (14 h) and dark (10 h) with temperature (25±2 ◦C) and relative humidity (60–70%). Animals were fed on commercially available standard animal diet (Pranav Agro Industries Ltd., New Delhi, India) and drinking water ad libitum. Experimental protocols were approved by the Institutional Ethical Committee (CPCSEA/501/01/A) of Jiwaji University, Gwalior, India, following guidelines set by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Chennai, India. All chemicals were procured from Sigma–Aldrich (USA), E- Merck (Germany), Ranbaxy Pvt. Ltd. and BDH Company (India).

Preparation of doses and treatments

The CCl_4 was administered at the dose of 0.15 ml/kg, *i.p.* with vehicle (olive oil)[10]. An aqueous suspension of extract was prepared in distilled water and selected optimum dose was administered (800 mg/kg, *p.o.)* to the animals on previous study[8]. Silymarin (50 mg/kg), a known hepatoprotective agent was administered as positive control.

Experimental procedure

Female rats were divided into five groups of six animals each. Group 1 received olive oil as vehicles and served as normal control. Groups 2-4 were administered CCl_4 (0.15 ml/kg, *i.p.*) for 3 weeks. Animals of group 2 treated as experimental control. Groups 3 and 4 were orally administered *B. monosperma* aqueous extract (800 mg/kg) and silymarin (50 mg/kg) respectively for 5 days after CCl_4 administration. Animals of all the groups were sacrificed after 48 h of the last treatment.

Liver marker enzymes

Blood was withdrawn by puncturing retro-orbital venous sinus, centrifuged and serum was isolated to determine aspartate aminotransferase (AST) and alanine aminotransferase (ALT)[11], serum alkaline phosphatase (Kit No.11767700011730) and lactate dehydrogenase (Kit No. 11760200011730) were assessed by kit methods as per instructions provided by the company E - Merck (Germany).

Serological estimation

Albumin (Kit No.1118275), bilirubin (Kit No. 103333.0001), urea (Kit No.11761800011730) and creatinine (Kit No.10338500011730) were assessed by kit methods as per instructions provided by the company E -Merck (Germany).

Markers of oxidative stress

Lipid peroxidation (LPO) was determined by measuring thiobarbituric acid reactive substances (TBARS) [12]. Reduced glutathione (GSH) level was determined by dithionitrobenzoic acid (DTNB) [13].

Metabolic enzymes

The activities of adenosine triphosphatase (ATPase) [14] and glucose-6-phosphatase (G-6-Pase) [15] were determined in liver.

Antioxidant defense system

The activities of glutathione peroxidase (GPx) [16], glutathione reductase (GR) [17], super oxide dismutase (SOD) [18] and catalase (CAT) [19] were determined in liver.

Microsomal fraction

Liver microsomes were prepared by calcium precipitation method [20] for the estimation of MDMEs. Activity of AH was assayed by measuring the intensity of blue colored conjugate of phenol and *p*-amino phenol (PAP) at l630 nm [21] and expressed as nM PAP/min/g liver. Malondialdehyde (MDA) contents, a measure of LPO, were assayed in the form of thiobarbituric acid reactive substance (TBARS) in microsomal fraction[12].

Genotoxicity evaluation: Comet assay

Comet assay was performed according to Singh *et al*., 1988 [22]

Histopathological observations

Liver samples were fixed in Bouin's fixative and processed to obtain 5 µm thick paraffin sections and stained with hematoxylene and eosine (H&E) and observed under photo micrographic attachment.

Transmission electron microscopy

1 mm^3 pieces of liver were fixed in Karnovsky's fixative (3.2% glutaraldehyde prepared in 0.1 M phosphate buffer) at 4°C for 18 h followed by washing the samples with phosphate buffer. Osmium tetraoxide (1%) was used for post fixation, then tissues were dehydrated in acetone series and subsequently embedded in epon resin and polymerized at 70°C for 20 h. Ultrathin sections were cut on Reichert Jung Ultra cut-E Microtome using glass knives. The sections were placed on uncoated grids, stained with uranyl acetate and lead citrate and examined under JEOL JEM 1200 EX transmission electron microscope at 80 KV [23]

Statistical analysis

Data were subjected to statistical analysis through one-way analysis of variance (ANOVA) significant at 5 % followed by student's t-test at $P \leq 0.05$ [24]. Results are presented as mean $\pm$ S.E. of six animals used in each group.

RESULTS

Liver marker enzymes

Liver marker enzymes studies represented hepatoprotective efficacy of *B. monosperma* aqueous extract against subchronic injury induced by CCl_4. *B. monosperma* treatment for five days reversed varying degree of changes in serological alteration. CCl_4 elicited toxic response, thereby; a significant enhanced release of AST, ALT, SALP and LDH was observed (Fig 1A-D). *B. monosperma* aqueous extract protected against altered enzymatic activities and prevented their leakage, conferring its hepatoprotective efficacy ($p \leq 0.05$). On the basis of % protection, *B. monosperma* showed more than 50% recoupment in diagnostic enzymes of liver dysfunction. It exhibited its better hepatoprotective effect in recouping activities of ALP.

Serological estimation

Toxicant exposure caused significant increase in albumin, bilirubin, urea and creatinine ($P \leq 0.05$). On the basis of % protecttion, *B. monosperma* aqueous extract showed more than 80% protection in bilirubin and creatinine level. Treatment showed 60% and 50% protection in albumin and urea respectively (Figure 2A-D).

Markers of oxidative stress

Effect of CCl_4 and *B. monosperma* on LPO and GSH level are presented in figure 3A-B. CCl_4 administered in experimental group showed increased oxidative stress as assessed by increased MDA production and diminished GSH contents ($P \leq 0.05$). Treatment with *B. monosperma* LPO was significantly decreased with the increase in GSH contents. More than 60% protection was observed in LPO and GSH level with therapy.

Metabolic enzymes

Treatment of *B. monosperma* aqueous extract for 5 consecutive days significantly decreased G-6pase and ATPase activity as compared to CCl_4 intoxicated groups ($P \leq 0.05$). *B. monosperma* therapy proved to be effective in these variables and significantly recovered the activity of metabolic enzymes. Analysis of variance showed significantly improved activities of G-6pase and ATPase with *B. monosperma* therapy ($P \leq 0.05$; Figure3C-D).

Antioxidant defense system

Figure 4A-D present influence of *B. monosperma* aqueous extract on tissue non-enzymatic antioxidative status. CCl_4 exposure significantly lowered the GR, GPx, SOD and CAT ($P \leq 0.05$). *B. monosperma* treatment increased GR, GPx, SOD and CAT status in target organ showing recoupment as compared to silymarin treated positive control ($P \leq 0.05$). % protection showed 56%, 35%, 50% and 30% recovery in antioxidant status with *B. monosperma* treatment.

Microsomal fraction

Activity of AH was significantly diminished and level of microsomal LPO was increased by CCl_4 administration ($P \leq 0.05$). *B. monosperma* therapy showed improvement in the AH activity and microsomal LPO respectively at 5% level of probability. Percent protection showed 39% recovery in AH and 62% in LPO. *B. monosperma* therapy proved to be effective in these variables and significantly recovered the activity of microsomal AH and LPO level (Figure 4E-F).

Genotoxicity evaluation

In all CCl_4 treated groups (0.15 ml/kg), hepatocytes showed an increase of 2 folds percent damage (23.8%) and tail length 17.3 µm. Therapy with BM showed 79% protection in tail length, 86% in tail moment and 56% in tail DNA as compared to that of control DNA (Figure 5A-G).

Light microscopic study

Light microscopic studies of control liver showed normal parenchymal architecture with well formed cords of hepatocytes and sinusoidal spaces (Figure 6A). Administration of CCl_4 produced enlargement in the whole liver with obvious pathological changes. Heavy lymphocytic infiltration around the central vein, vacuolation, swelling in hepatocytes and disturbed cord arrangement due to hepatocytic degeneration were observed (Figure 6B). Therapy with *B. monosperma* aqueous extract at 800 mg/kg showed well formed cord arrangement of polygonal hepatocytes and almost normal sinusoids (Fig. 6C). Silymarin treatment improved the histoarchitecture of liver same as control with well formed hepatocytes arranged in cords with remarkable nuclei (Figure 6D).

Ultra-structural studies:

Ultrastructure of control liver showed uniformly distributed mitochondria with smooth and rough ER at proximity to the nucleus (Figure 7A). The CCl_4 administration showed extensive cellular damage with marked dilation in ER and loss of cell cytoplasm at several places as a result of which a number of vacuoles appeared with poor distribution of glycogen. Loss in organization of rough ER was seen with detached ribosomes and heavy lipid accumulation on CCl_4 administration (Figure 7B). *B. monosperma* aqueous extract treatment provided overall improvement in the ultrastructure when compared with experimental control group as seen the nucleus has normal appearance with intact nuclear envelop and nucleolus (Figure 7C). After silymarin treatment, rough ER showed linear attachment of ribosomes with small glycogen rosettes and well formed mitochondria in cytoplasm (Figure 7D).

DISCUSSION

Hepatic cells participate in a variety of metabolic activities and contain a host of enzymes. In liver injury; the transport function of the hepatocytes is disturbed, resulting in the leakage of plasma membrane, thereby causing an increased enzyme level in serum. The elevated activities of AST and ALT in serum are indicative of liver injury [25, 26]. Alkaline phosphatase mainly arises from the lining of canaliculi in hepatocytes and also brush border of the renal tubules. It is excreted normally via bile through liver and involves in active transport across the capillary wall. Increased activity of alkaline phosphatase, which occurs due to *de novo* synthesis by liver cells, is a reliable marker of hepatobiliary dysfunction due to damage [27]. More than 10 fold increase in the activity of this enzyme after CCl_4 intoxication clearly indicated severity of damage in liver. LDH is an intracellular enzyme and also is used as a marker of liver injury. In this investigation, leakage of LDH from injured hepatocytes was obvious after subchronic administration of CCl_4. *B. monosperma* therapy attenuated increased level of these enzymes and caused a subsequent recovery towards normalization that might be due to recoupment in cell membrane. Stabilization of AST, ALT, SALP and LDH activities by *B. monosperma* treatment clearly indicated improvement in the functional status of liver cells, which may be due to free radical scavenging action of active constituents of *B. monosperma i.e.* butrin and isobutrin. Investigators have previously demonstrated antioxidative and hepatoprotective effect of the extract of *Bauhinia racemosa* [28] and *Emblica officinalis* [29] against CCl_4 induced toxicity.

Generation of reactive oxygen species via LPO is one of the mechanisms involved in tissue damage [30]. Significant increase in hepatic and microsomal LPO confirms damage in these organs due to increased oxidative insult [31]. Administration of *B. monosperma* at 800 mg/kg doses inhibited LPO as an effective chain breaking antioxidant and helped in preventing CCl_4 induced peroxidative damage. Decrease in GSH level might be due to its increased utilization by the hepatocytes in scavenging toxic radicals of CCl_4. It has been reported that most covalent binding of toxicant to hepatic protein occurs only after depletion of GSH, and the severity of hepatic necrosis is related to the degree of covalent binding [32]. Several flavonoids are found in *B. monosperma* those are known to increase the expression of c-glutamyl cysteine synthetase resulting increased production of glutathione [33]. A possible mechanism of the protective effects of *B. monosperma* in maintaining the GSH towards control might be due to several bioactive compounds and active constituents *i.e.* butrin and isobutrin present in it that might protect oxidative damage by directly neutralizing reactive oxidants, increase the capacity of endogenous antioxidant defense and increase the steady state of GSH and/ or its rate of synthesis that confers enhanced protection against oxidative insult.

ATPase is a mitochondrial lipid-dependent membrane-bound enzyme. Any alteration in membrane lipid leads to change in membrane fluidity, which in turn alters ATPase activity and subsequently energy dependent cellular function. CCl_4 exposure provoked significant loss of ATPase activity in liver, which might be due to dysfunctional changes in mitochondria and cell membrane permeability. Inhibition of ATPase after CCl_4 exposure has also been confirmed in other studies [34]. *B. monosperma* aqueous extract prevented membrane lesion to a large extent with concomitant recovery in enzymatic activity by maintaining cell membrane permeability. The G-6-Pase is a crucial enzyme of glucose homeostasis and plays an important role in the regulation of the blood glucose level. Administration of *B. monosperma* restored this enzyme activity of G-6-Pase due to membrane stabilization and improvement in metabolism. Our findings substantiated the therapeutic effect of *Rhoicissus tridentate* [29].

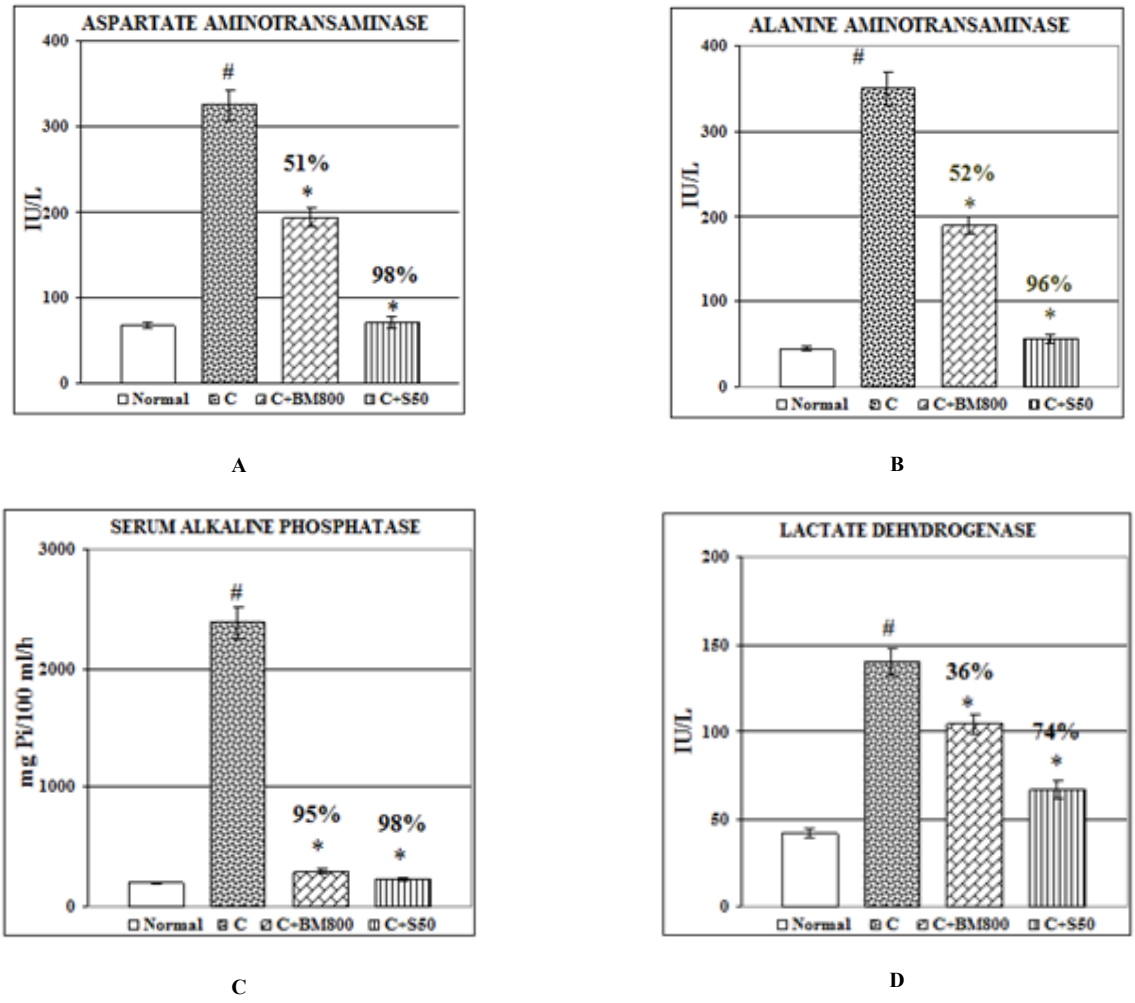
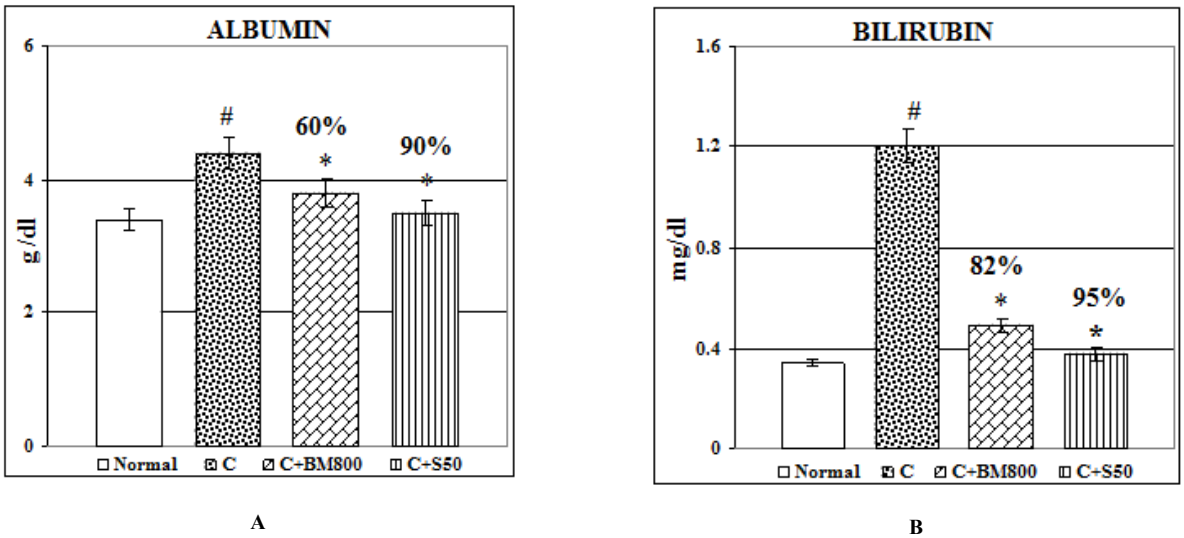
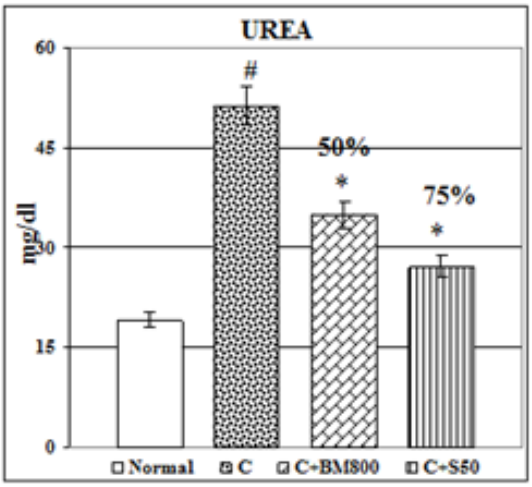
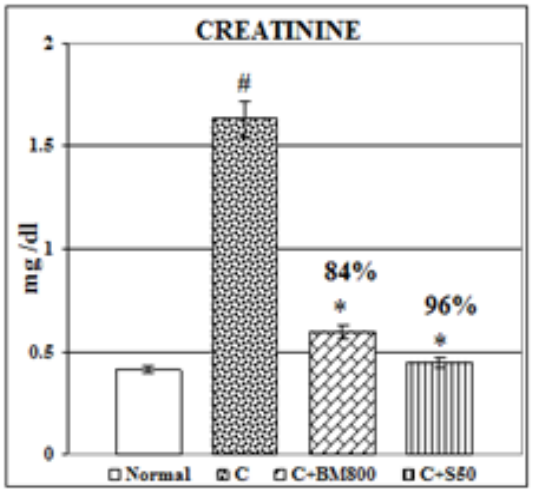


Figure 1 (A–D): Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), serum alkaline phosphatase (SALP) and Lactate dehydrogenase (LDH). C = Carbon tetrachloride (CCl_4), BM = *Butea monosperma* (800 mg/kg), S = Silymarin (50 mg/kg). CCl_4 *vs* normal at **P*≤0.05; treatments *vs* CCl_4 at **P*≤0.05. [@] Significant for ANOVA at *P*≤0.05; [@]AST = 136, [@]ALT = 180, [@]SALP = 306; [@]LDH= 60.1



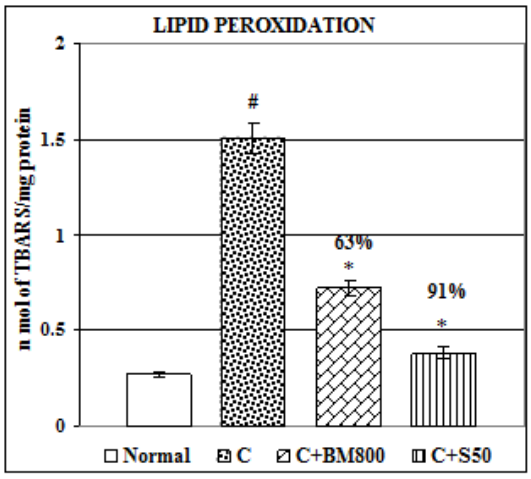


C

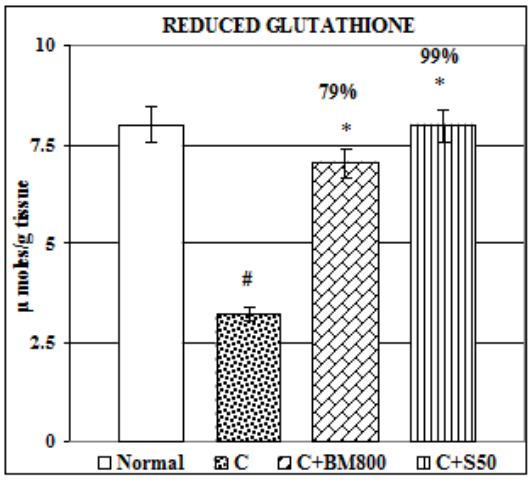


D

Figure 2 (A–D): Activities of albumin, bilirubin, urea and creatinine. C = Carbon tetrachloride (CCl_4), BM = *Butea monosperma* (800 mg/kg), S = Silymarin (50 mg/kg). CCl_4 *vs* normal at **P*≤0.05; treatments *vs* CCl_4 at **P*≤0.05. @ Significant for ANOVA at *P*≤0.05; @albumin = 4.39, @bilirubin = 114, @urea = 141; @creatinine= 47.4



A



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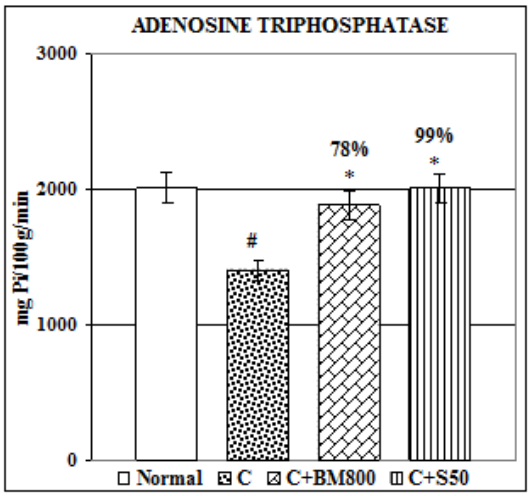

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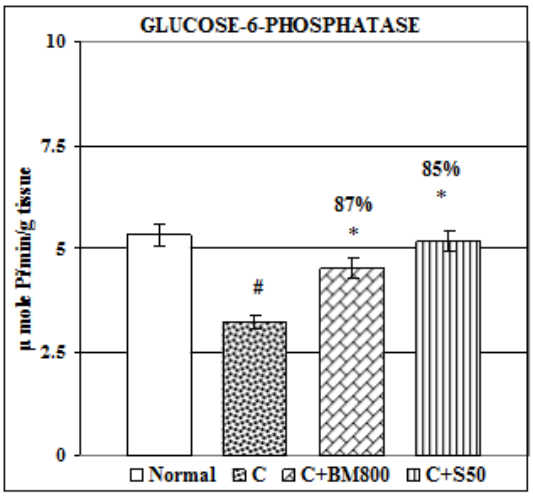
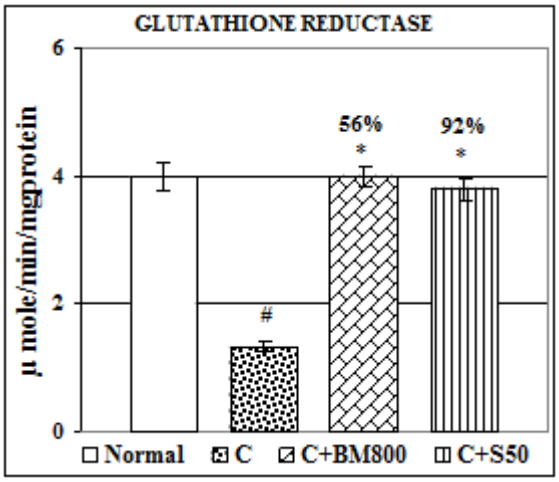


D

Figure 3 (A–D): Activities of Lipid peroxidation (LPO), Reduced glutathione (GSH), Adenosine triphosphatase (ATP) and Glucose-6-phosphatase (G-6-pase). C = Carbon tetrachloride (CCl_4), BM = *Butea monosperma* (800 mg/kg), S = Silymarin (50 mg/kg). CCl_4 *vs* normal at **P*≤0.05; treatments *vs* CCl_4 at **P*≤0.05. @ Significant for ANOVA at *P*≤0.05; @ LPO = 141, @ GSH = 32.9, @ ATP = 141; @ G-6-pase = 47.4.



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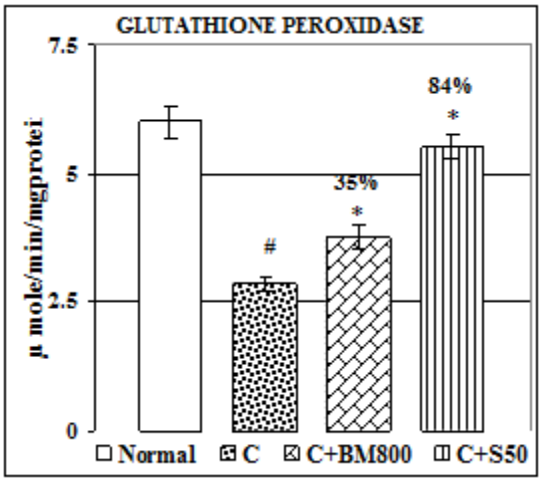

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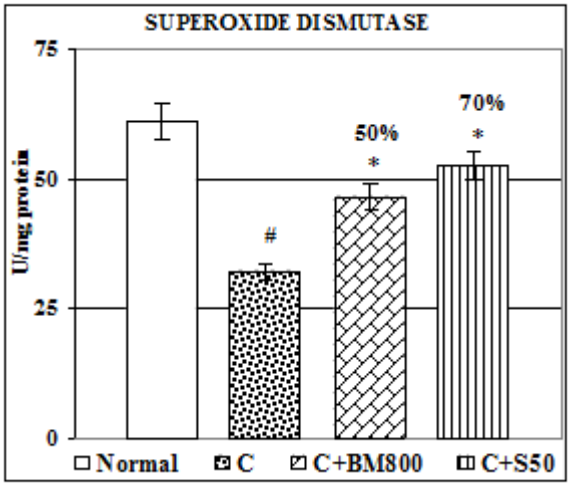

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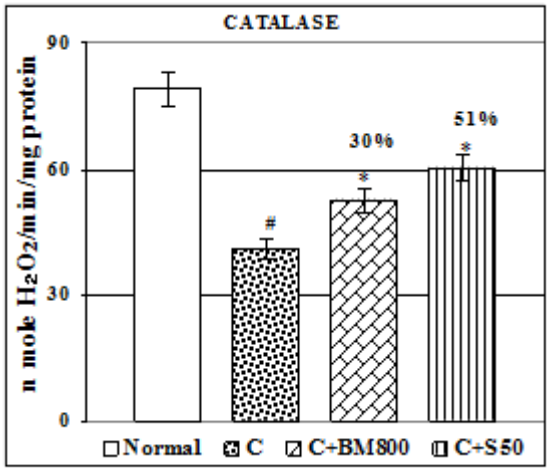

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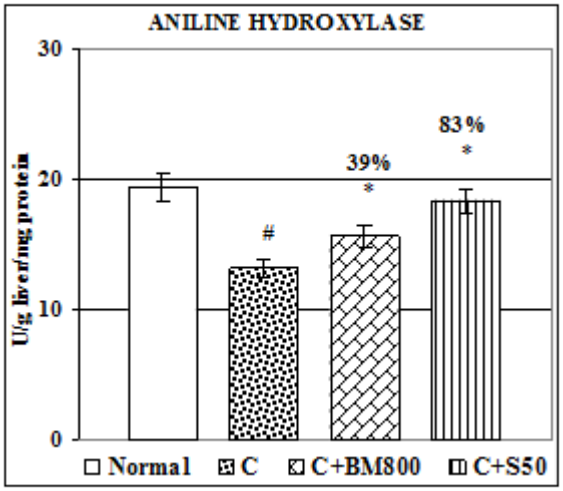

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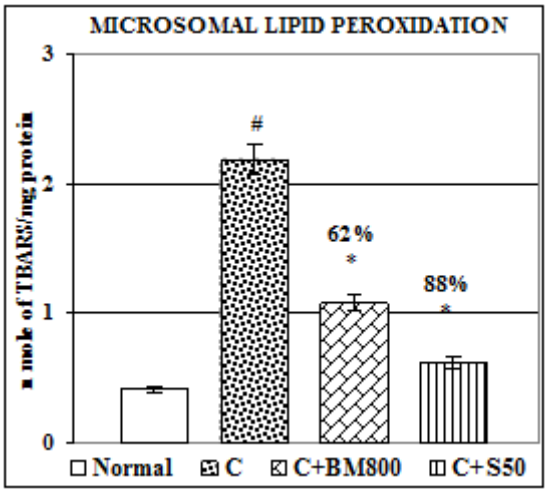
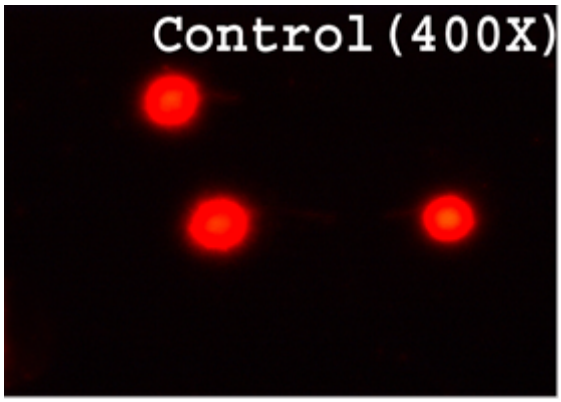
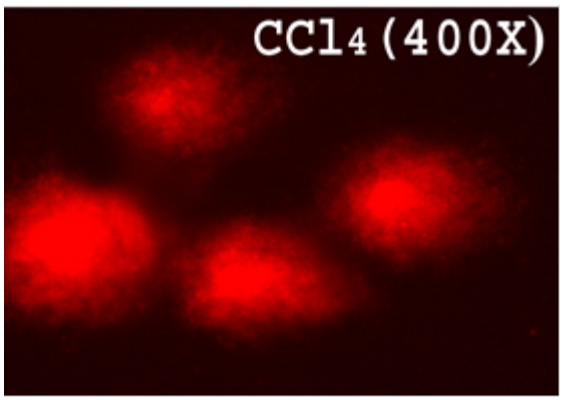


F

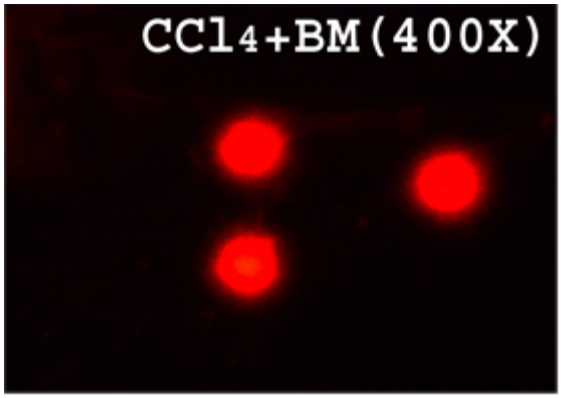
Figure 4 (A–F): Activities of Glutathione reductase (GR), Glutathione peroxidase (GPx), Superoxide dismutase (SOD), Catalase (CAT), Aniline hydroxilase (AH) and microsomal lipid peroxidation (MLPO) C = Carbon tetrachloride (CCl_4), BM = *Butea monosperma* (800 mg/kg), S = Silymarin (50 mg/kg).
CCl_4 *vs* normal at **P*≤0.05; treatments *vs* CCl_4 at **P*≤0.05. [@] Significant for ANOVA at *P*≤0.05; [@] GR = 43.7, [@] GPx = 27.5,[@] SOD = 18, [@] CAT = 21.3, [@] AH = 8.62; [@] MLPO = 133


Control (400X)

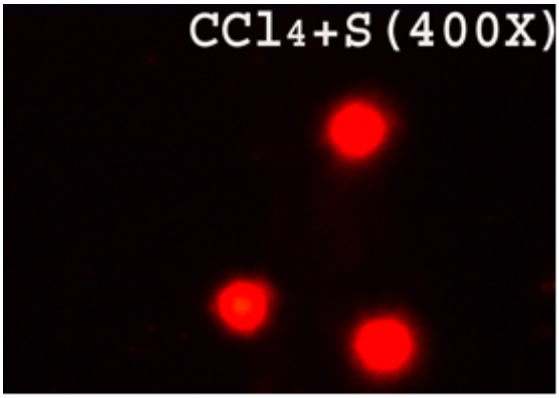
A


CCl4 (400X)

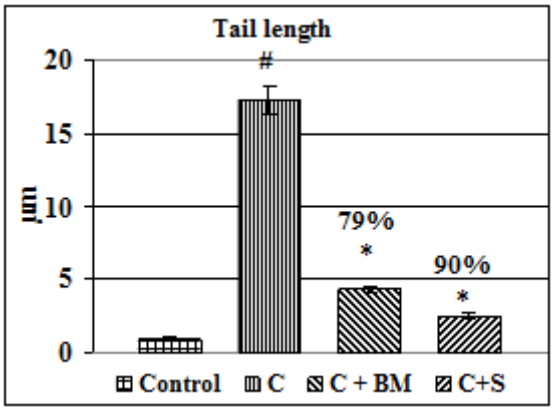
B


CCl4+BM (400X)

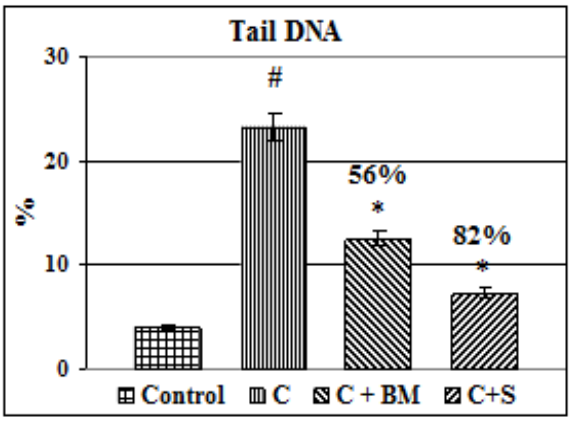
C


CCl4+S (400X)

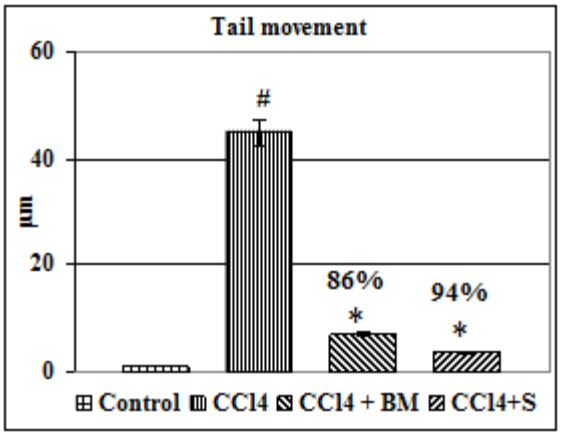
D


Tail length
20
15
10
5
0
μm
#
79%
*
90%
*
Control
C
C + BM
C+S

E

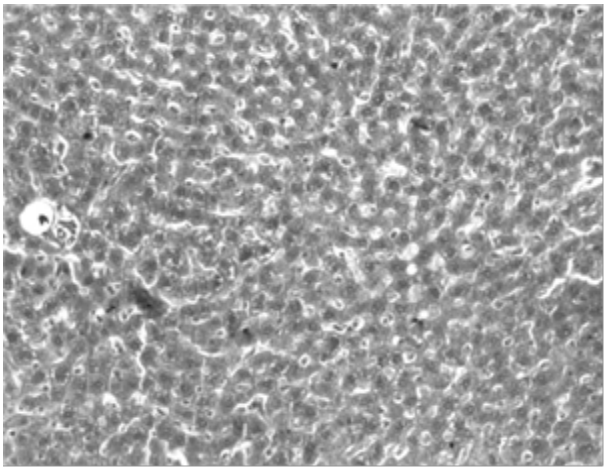


F

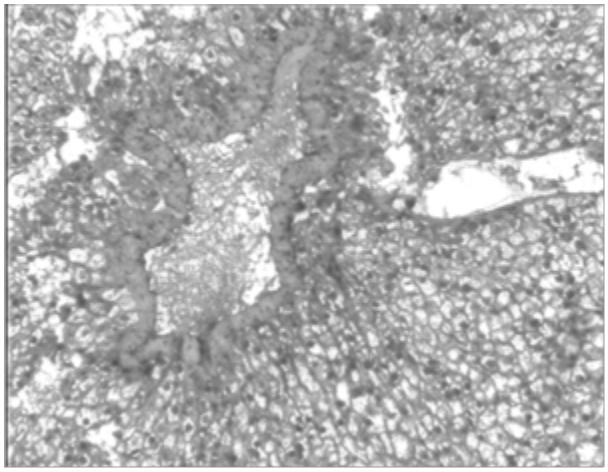


G

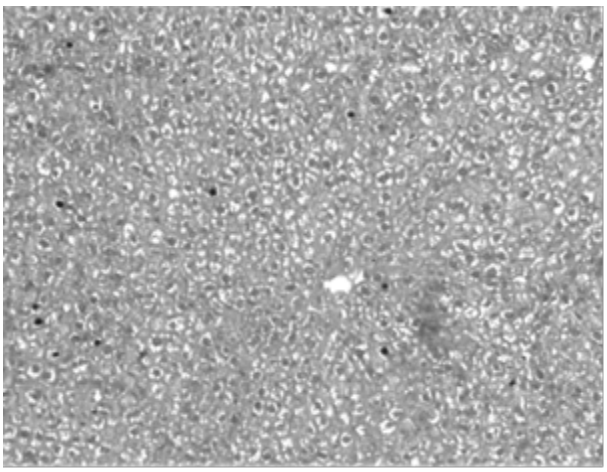
Figure 5 (A-G): CCl_4 = Carbon tetrachloride (CCl_4), BM = *Butea monosperma* (800 mg/kg), S = Silymarin (50 mg/kg). CCl_4 *vs* normal at **P*≤0.05; treatments *vs* CCl_4 at **P*≤0.05. [@] Significant for ANOVA at *P*≤0.05; [@]Tail length=258, [@] Tail DNA=122,[@]Tail movement=318



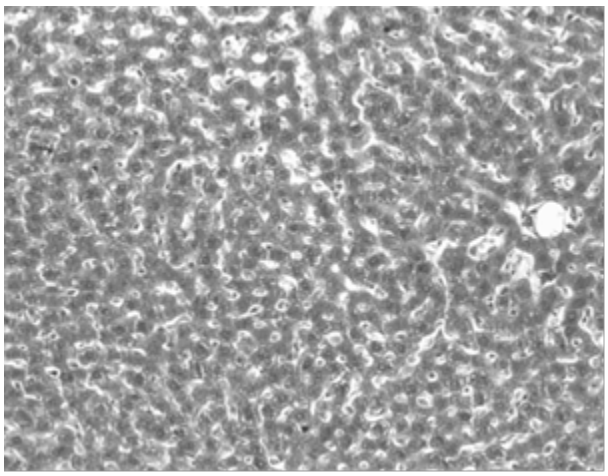
A



B



C



D

Figure 6:
A: Photomicrographs of liver of control rats showing normal hepatic cells with well prominent nucleus; well-formed central vein; preserved cytoplasm (x-100).
B: Liver section of CCl_4 treated rats showing massive fatty changes, loss of cellular boundaries and collapsed cell, membrane with hyperchromatic nuclei (x-100).
C: Therapy with BM showing well-formed central vein with chord arrangement (x-100).
D: Therapy with silymarin showing better formed hepatocytes (x-100).

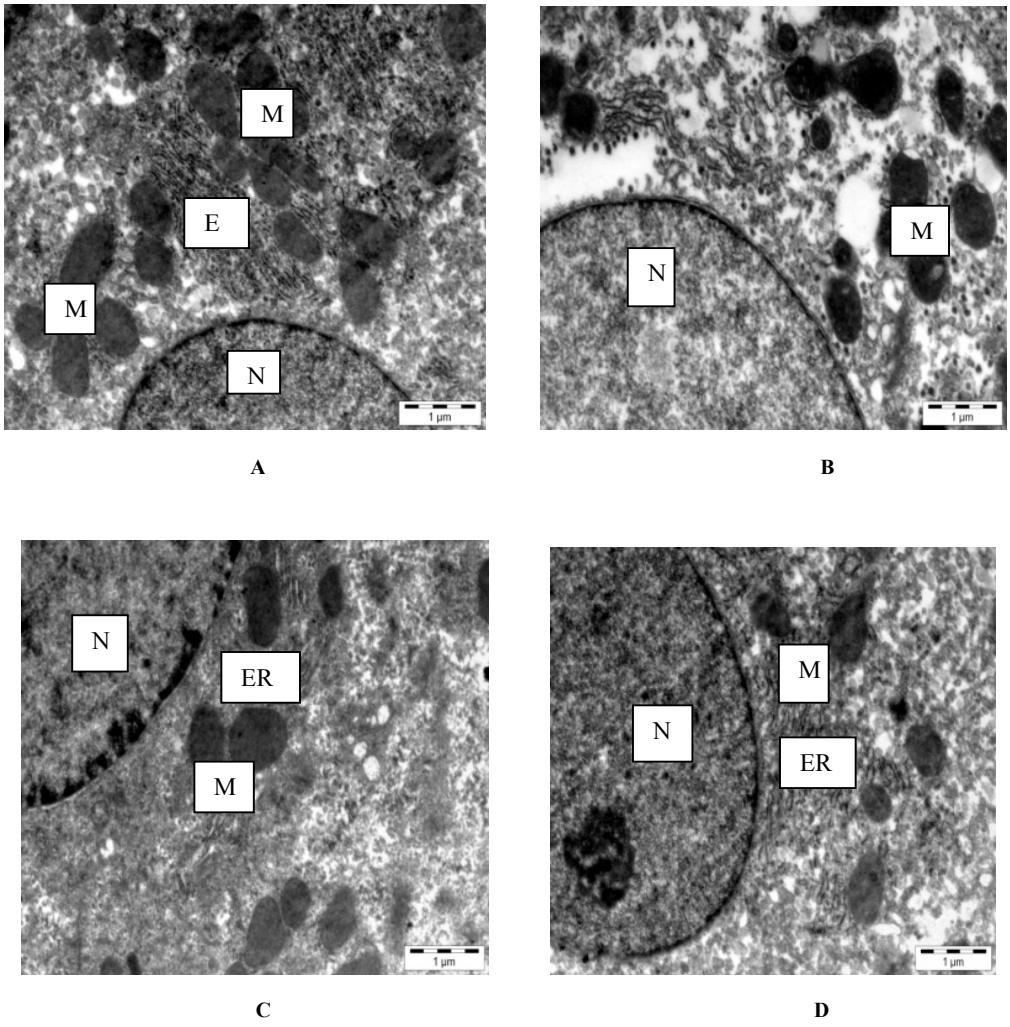


Figure 7:
A: TEM of control liver showing prominent nucleus (N), along with intact nuclear envelop, well-formed extensive endoplasmic reticulum (ER) and mitochondria (M) with distinct cristae (x-5600).
B: Micrographs showing CCl_4 intoxication. Swelling with fragmentation of mitochondrial cristae (M), endoplasmic reticulum is disarrayed and shows bizarre arrangement (ER) (x-5600)
C: Micrographs showing treatment with BM. Well-formed intact nucleus (N) was visible, mitochondria with clear cristae (M) (x 5600), endoplasmic reticulum (ER) were persist (x-5600).
D: Therapy of Silymarin showing improved structure of hepatocyte, mitochondria (M), endoplasmic reticulum (ER) and prominent nucleus (N) (x-5600).

Hypoalbuminemia and reduction in hepatic protein content due to CCl_4 intoxication threw the light on cellular damage, which decreased functional efficiency of liver. It is assumed that *B. monosperma* stimulated protein synthesis, which eventually induced the repair of tissue damage and the replacement of enzymes and structural components damaged by toxic reactions. Bilirubin is one of the most useful clinical parameters to know the severity of hepatic necrosis. It is an important degradation product of hemoglobin and is normally excreted into the bile. If hepatic parenchymal damage is severe, less bilirubin will be excreted and hyperbilirubinemia is observed that reflects pathophysiology of liver damage [35]. Increase in total serum bilirubin concentration after CCl_4 administration might be attributed to the failure of normal uptake, conjugation and excretion by the damaged hepatic parenchyma. A noticeable observation with *B. monosperma* was that this compound profoundly decreased the elevated level of serum bilirubin, which suggests that it can be used in the condition of jaundice. In the present investigation there was a significant rise in serum urea and creatinine concentration after toxicant administration. It may be due to dysfunctional and dystrophic changes in the liver and kidney. Due to severe renal impairments, urea excretion falls and its concentration in serum rises rapidly [36]. Accumulation of the urea has been reported in liver diseases like cirrhosis and encephalopathy. Our experiment revealed that *B. monosperma* significantly provided protective effects on serum urea and creatinine, leading to normal hepatic physiology as well as improved glomerular filtration rate.

To protect themselves against free radicals, cells have developed antioxidant defense and repair systems, which prevent accumulation of oxidatively damaged molecules. The antioxidant defense system includes enzymes *i.e.* glutathione peroxidase, catalase, glutathione reductase, glutathione-S-transferase, superoxide dismutase. These radical scavenging enzymes provide the first-line of defense against oxidizing species [37]. Excessive production of free radicals might result in alterations in the biological activity of cellular macromolecules. Therefore,

reduction in the activity of these enzymes might result in a number of deleterious effects due to accumulation of superoxide radicals, hydrogen peroxide and depletion in GSH level. Administration of *B. monosperma* thus, reduced the generation of free radicals significantly in the liver. Similar findings were also reported with plant extracts of *Momordica dioica* Roxb [38].

During the process of evolution, the liver developed DMEs, which are different from the enzymes of intermediate metabolism [39]. CYP2E1 metabolizes and activates many toxicologically important substrates including CCl4 to more toxic product in endoplasmic reticulum [40]. Activities of AH in hepatocytes were significantly decreased after CCl_4 administration, which clearly indicated damage in endoplasmic reticulum. *B. monosperma* restored this enzymatic activity more towards control by modulating MDMEs and indicated its hepatoprotective efficacy. This is probably due to the fact that flavonoids, which are the main constituents of *B. monosperma* have membrane permeation properties that might influence CYP450 enzyme system by modulating the DMEs [41, 42].

Comet assay has been found highly effective in biomonitoring of natural compounds. Natural compounds in herbal extracts possess varied antioxidative efficiency to reserve the DNA damage. Damaged DNA strands were broken into fragments and migrated towards the anode during electrophoresis [43]. The extent of DNA damage was then quantified using normalized tail length. This normalized tail length of the comet indicated the amount of damage as longer tails indicated that the strand breaks were frequent and that the DNA was fragmented to several smaller molecules. One hundred comets on each slide were scored according to the length and relative intensity of fluorescence in the tail was compared to the head. Lower ratio suggested lower level of DNA damage or higher antioxidant activity [43]. Subchronic exposure to CCl_4 could produce a genotoxic response with the metabolic activation. The protective effects against DNA damage was very clear when compared with the damage level of the positive control; *B. monosperma* depicted over 60% reduction in the level of DNA damage. These results might suggest that there may be more than one compound that exhibited antioxidation activity and these compounds have different polarity *B. monosperma* aqueous extract suggesting its genoprotective effect on DNA damage.

Light and ultrastructural observations were also consistent with the biochemical findings of this study. Electron micrographs of liver after CCl_4 administration exhibited fatty degeneration, dilated endoplasmic reticulum and inconsistency in nuclear envelop. Damage in ER and inhibition of AH activity evidenced that CCl_4 inactivated CYP system. The reversal of alterations after treatment with *B. monosperma* might be due to the presence of OH functionality in its structure that inhibited free radical formation or propagation of lipid peroxyl radicals in biomembranes preventing mitochondria and other cellular damage along with normalization of metabolizing enzymes.

B. monosperma aqueous extract was found to possess butrin and isobutrin as its major active constituents. Butrin and isobutrin has been reported to act as strong antihepatotoxic agents. It is reported that approximate yield 5mg of isobutrin and 4 mg butrin from 24 mg butanol fraction. This corresponds to 20-83 % isobutrin and 16.6 % butrin in the methanolic extract [44]. Since the active constituents of *B. monosperma* extract have known protective effects in other studies, so the observed hepatoprotective activity of *B. monosperma* extract in our study may be suggested due to the presence of these compounds.

CONCLUSION

Thus, it can be concluded that *B. monosperma* aqueous extract possesses strong potential as an emerging hepatoprotective agent by reversing hepatic oxidative insult, CYP enzymatic activity and ultrastructural changes may be due to the presence of its active constituents, *viz*., butrin and isobutrin in addition with other minor constituents and by the antioxidant effect of flower aqueous extract.

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