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Research Article

Hepatoprotective Activity of Boerhavia diffusa Extract

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ABSTRACT

Boerhavia diffusa Linn. (Nyctaginaceae), commonly known as 'Punarnava' is a perennial creeping herb widely studied and has a long history of uses by the tribal people and in Ayurvedic and Unani medicines. Present study was carried out to evaluate the ameliorative effect of the Boerhavia diffusa extract against carbon tetrachloride (CCl4) – induced hepatotoxicity in mice. Swiss albino female mice were cotreated with Boerhavia diffusa extract at three different doses (100, 200 and 300 mg/kg body weight/day) alongwith CCl4 (1/10 of the LD50 value). Serum marker enzymes, lipid peroxidation and histopathological analysis were carried out to evaluate the hepatoprotective effect of the Boerhavia diffusa plant extract. Cotreatment of Boerhavia diffusa extract (100, 200 and 300 mg/kg bw/day) along with CCl4 caused significant ($p<0.05$) decrease in the activities of serum marker enzymes (ALT, AST, ALP, ACP, LDH and γ-GT) as well as bilirubin contents and lipid peroxidation in a dose-dependent manner. Also cotreatment of Boerhavia diffusa extract significantly ameliorated CCl4 – induced histopathological changes and preserved the histoarchitecture of the liver tissue to near normal. Results of the present study indicate that the Boerhavia diffusa extract possess potent hepatoprotective activity against CCl4 – induced hepatic damage in Swiss albino mice which is mainly due to its antioxidative properties.

Key words: Boerhavia diffusa, carbon tetrachloride, hepatoprotective activity, lipid peroxidation, mice.

INTRODUCTION

About 80% of the world population rely on the use of traditional medicine which is predominantly based on plant materials1. It is been recorded in history that medicinal herbs have been used as form of therapy for the relief of pain. The exploration of the chemical constituents from plants, their pharmacological and phytochemical screening would provide the basis for developing the new lead molecules in strategic favour of natural product drug discovery. The biologically active agents from natural sources have always been of great interest to working on various diseases2. Boerhaavia diffusa (Nyctaginaceae), commonly known as 'Punarnava' in the Indian system of medicine is a perennial creeping herb found throughout the waste lands of India. Medicinal plants play a key role in the human health care. The Boerhaavia sp. has ancient medicinal use in different societies from the times of the B.C. A number of plant products have been identified through phyto-chemistry and the extract of their different plant parts are useful in various diseases without side-eff 3. Genus Boerhaavia, consisting places, ditches and marshy places during rains. The plant is also cultivated to some extent in West Bengal3. Pharmacological studies have demonstrated that Boerhavia diffusa extract known to possess anti-convusant, diuretic, anti-inflammatory, anti-fibrinolytic, antibacterial, antihelminthic, antileprosy, anti-asthamatic, anti-urethritis, anti-lymphoproliferative, anti-metastatic, anti-diabetic, immune-modulation, anti-nociceptive, nephroprotective, antiurolithiatic and antioxidant activities4. The main aim of the present study was to evaluate the hepatoprotective activity of Boerhavia diffusa extract against CCl4-induced hepatotoxicity in Swiss albino mice.

MATERIALS AND METHODS

Experimental Animals: All inbred adult healthy Swiss strain female albino mice (Mus musculus) weighing 30-35 gm, were obtained from Torrent Research Centre, Bhat, Gandhinagar – 382 428, India. Animals were kept in the Animal House of Zoology Department of Gujarat University, Ahmedabad, India under controlled conditions of 40 species is distributed in tropical and sub-tropical (temperature 25 ± 2 °, relative humidity 50-55% and 12 h regions and warm climate. Among 40 species of Boerhaavia, 6 species are found in India, namely Boerhavia diffusa, Boerhavia erecta, Boerhavia rependa, Boerhavia chinensis, Boerhavia hirsute and Boerhavia rubicunda. Boerhaavia diffusa in India is found in warmer parts of the country and throughout up to 2,000 m altitude in the Himalayan region. It is a perennial, spreading hogweed, commonly occurring abundantly in waste light/dark cycle). They were fed with certified pelleted rodent feed supplied by Amrut Feeds, Pranav Agro Industries Ltd., Pune, India and potable water ad libitum. All animal studies were sanctioned by Institutional Animal Ethics Committee of Gujarat University, Ahmedabad. All the experimental protocols were approved by the Committee for the Purpose of Control and Supervision of Experiment on Animals, New Delhi, India. Animals were handled according to the guidelines published by Indian National Science Academy, New Delhi, India (1991).

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Table 1: Effect of plant extracts on CCl_4 – induced biochemical changes in serum of mice

Experimental groups	ALT	AST	ALP	ACP	LDH	γ-GT
(I) CONTROL						
1. Untreated	9.40 ± 0.41	18.45 ± 0.67	0.34 ± 0.15	0.21 ± 0.01	0.83 ± 0.03	0.76 ± 0.04
2. Vehicle control	9.90 ± 0.40	18.40 ± 0.60	0.35 ± 0.01	0.20 ± 0.01	0.84 ± 0.02	0.74 ± 0.04
3. *Boerhavia diffusa*	10.05 ± 0.63	26.00 ± 0.57	0.31 ± 0.01	0.21 ± 0.01	0.84 ± 0.02	0.77 ± 0.04
4. Liv. 52	10.05 ± 0.60	24.40 ± 0.71	0.35 ± 0.01	0.22 ± 0.01	0.83 ± 0.02	0.77 ± 0.03
(II) CARBON TETRACHLORIDE (CCl_4) – TREATED						
5. CCl_4(826 mg/kg bw/day)	42.00 ± 0.89[a]	81.45 ± 1.09[a]	0.76 ± 0.01[a]	0.36 ± 0.01[a]	1.86 ± 0.04[a]	1.62 ± 0.07[a]
(III) CCl_4 + *BOERHAVIA DIFFUSA* (BD) EXTRACT – TREATED						
6. CCl_4 +BD100	32.55 ± 0.47[b]	67.45 ± 1.57[b]	0.65 ± 0.01[b]	0.33 ± 0.01[b]	1.61 ± 0.03[b]	1.45 ± 0.07
7. CCl_4 +BD200	24.10 ± 0.87[b]	52.15 ± 0.81[b]	0.61 ± 0.01[b]	0.28 ± 0.01[b]	1.45 ± 0.04[b]	1.29 ± 0.04[b]
8. CCl_4 +BD300	16.90 ± 0.56[b]	29.60 ± 0.86[b]	0.49 ± 0.01[b]	0.24 ± 0.01[b]	0.98 ± 0.02[b]	1.09 ± 0.04[b]
(v) CCl_4 + LIV. 52 (L) – TREATED						
9. CCl_4 + L300	18.75 ± 0.61[b]	32.55 ± 1.60[b]	0.47 ± 0.01[b]	0.26 ± 0.01[b]	1.03 ± 0.03[b]	1.12 ± 0.05[b]

Results are expressed as mean ±SEM; n = 10.
No significant difference was noted between groups 1-4.
[a] as compared between vehicle control (group 2) and toxin - treated (group 5),
[b] as compared between toxin - treated (group 5) and toxin + antidote - treated (groups 6-9).
Level of significance p<0.05.
Units: ALT – mU/mL; AST – mU/mL; ALP - µmoles of p-nitrophenol released/mg protein/ 30 min; ACP - µmoles of p-nitrophenol released/mg protein/30 min; LDH - µmoles pyruvate liberated/mg protein/min; γ-GT - µmoles p-nitroaniline liberated/mg protein/30 min.
BD 100, BD 200, BD 300 is 100, 200, 300 mg/kg body weight/day.
L300 is 300 mg/kg body weight/day.

Table 2: Effect of plant extracts on bilirubin content in serum of mice

Experimental groups	Total bilirubin	Direct bilirubin	Indirect bilirubin
(I) CONTROL			
1. Untreated	1.41 ± 0.04	0.81 ± 0.03	0.60 ± 0.05
2. Vehicle control	1.39 ± 0.03	0.80 ± 0.03	0.59 ± 0.05
3. *Boerhavia diffusa*	1.42 ± 0.03	0.82 ± 0.02	0.60 ± 0.02
4. Liv. 52	1.40 ± 0.03	0.80 ± 0.03	0.61 ± 0.05
(II) CARBON TETRACHLORIDE (CCl_4) – TREATED			
5. CCl_4 (826 mg/kg bw/day)	3.48 ± 0.11[a]	1.63 ± 0.04[a]	1.85 ± 0.13[a]
(III) CCl_4 + *BOERHAVIA DIFFUSA* (BD) – TREATED			
6. CCl_4 + BD100	3.20 ± 0.06[b]	1.61 ± 0.03	1.67 ± 0.05
7. CCl_4 + BD200	2.36 ± 0.05[b]	1.39 ± 0.03[b]	1.00 ± 0.05[b]
8. CCl_4 + BD300	1.83 ± 0.04[b]	1.06 ± 0.03[b]	0.76 ± 0.05[b]
(IV) CCl_4 + LIV. 52 (L) – TREATED			
9. CCl_4 + L300	1.96 ± 0.06[b]	1.08 ± 0.04[b]	0.88 ± 0.07[b]

Results are expressed as mean ±SEM; n = 10.
No significant difference was noted between groups 1-4.
[a] as compared between vehicle control (group 2) and toxin - treated (group 5),
[b] as compared between toxin - treated (group 5) and toxin + antidote - treated (groups 6-9).
Level of significance p<0.05.
Units: Total; direct and indirect bilirubin content – mg/100 mL.
BD 100, BD 200, BD 300 is 100, 200, 300 mg/kg body weight/day.
L300 is 300 mg/kg body weight/day.

Chemicals: The chemicals used in the entire study were purchased from Hi Media Laboratories Pvt. Ltd., Mumbai, India; Sisco Research Laboratories Pvt. Ltd., Mumbai, India and Sigma-Aldrich, St. Louis, MO, USA and was of analytical grade. HPLC grade CCl4 and other solvents were obtained from Merck Specialities Pvt. Ltd., Mumbai, India. Olive oil was obtained from Figaro Madrid, Spain.

Collection of plant materials and extract preparation: The extract was prepared according to World Health Organization protocol CG-06 (1983) with slight modifications 5.

Study design: The study was focused to determine the hepatoprotective activity of Boerhavia diffusa extract against CCl4 – induced hepatotoxicity in Swiss strain female albino mice. In the experiment, a total 90 animals were randomly divided into nine groups (10 animals per group) and caged separately. Group 1 (untreated control) animals were maintained without any treatment

and given free access to feed and drinking water. Animals of group 2 (vehicle control) received olive oil (0.2 mL/animal/day) for 30 days as olive oil was used as vehicle to dissolve CCl4. Antidote control group 3 animals were orally administered with 300 mg/kg body weight/day Boerhavia diffusa extract. Standard Liv. 52 control (Group 4) animals were given oral treatment of Liv. 52 (300 mg/kg body weight/day). Animals of group 5 received CCl4 (826 mg/ kg body weight/day, p.o.) for 30 days. Dose of CCl4 was based on LD50 value (1/10th of the LD50 value). Animals of groups 6, 7 and 8 were orally treated with 100, 200 and 300 mg/kg body weight/day of Boerhavia diffusa extract along with CCl4. Animals of group 9 were orally treated with 300 mg/kg body weight/day of Liv. 52, as standard along with CCl4. Mortality rate, behavioural and clinical changes were noted in the animals of all groups. Animals were given treatment for 30 days and humanly sacrificed on the 31st day. Fresh flowing blood of the animals were collected by cardiac puncture in a vial and allowed to clot. Serum was separated by centrifuging the blood at 1000 × g for 10 min collected serum samples were stored under refrigerated conditions and used within 24 h. Liver was dissected out quickly, blotted free of blood, weighed and used for histopathological and biochemical analysis.

Histopathological examination: The tissues for histopathological examinations were preserved in 10% expressed as µmoles p -nitrophenol released/mg protein/30 min.

Acid phosphatase (EC 3.1.3.2) activity: The acid phosphatase (ACP) activity was assayed in the serum of mice by the method as described in Sigma Technical Bulletin (Sigma Technical Bulletin, MO, USA)8. Acid phosphatase at the optimum pH 4.8 catalyzes the hydrolysis of p-nitrophenyl phosphate (disodium salt) to p-nitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with sodium hydroxide to form a yellow coloured complex which was measured at 420 nm. The acid phosphatase activity was expressed as µmoles p-nitrophenol released/mg protein/30 min.

Lactate dehydrogenase (EC 1.1.1.27) activity: Lactate dehydrogenase (LDH) activity was measured by the method of King (1965)9. This method is based on the ability of LDH to convert lactate to pyruvate with the help of coenzyme nicotinamide adenine dinucleotide (reduced). The pyruvate formed is made to react with 2, 4-dinitrophenyl hydrazine in hydrochloric acid and the hydrazine formed in alkaline medium was read at 540 nm. The LDH activity was expressed as µmoles pyruvate liberated/mg protein/min. γ-Glutamyl transpeptidase (EC 2.3.2.2) activity: The γ- glutamyl transpeptidase (γ-GT) activity in serum was analysed following the method of Orlowski and Meister 10 neutral buffered formalin immediately after autopsy. (1965). This enzyme catalyzes transfer of gamma glutamyl Standard technique for haematoxylin and eosin (H & E) staining was followed. The tissues were dehydrated by passing through ascending grades of alcohol, cleared in xylene and embedded in paraffin wax (58° - 60º C mp). The 5 µm thick sections were cut on a rotary microtome and stained in haematoxylin and eosin, dehydrated in alcohol, cleared in xylene, mounted in DPX and examined under a light microscope.

Serum analysis

Alanine transaminase (EC 2.6.1.2) activity: The alanine transaminase (ALT) activity in serum was assayed by the method of Reitman and Frankel (1957)6. A buffered solution of α-ketoglutarate and L- alanine were made to react with the serum for 30 min. The pyruvate formed from L-alanine by the enzymatic reaction reacts with 2, 4-dinitrophenyl hydrazine (DNPH) in alkaline medium and formazon formed was measured at 540 nm. The enzyme activity was expressed as mU/mL in case of serum. Aspartate transaminase (EC 2.6.1.1) activity: The aspartate transaminase (AST) activity was assayed by the method of Reitman and Frankel (1957)6. Assay is similar as mentioned in ALT activity except L-aspartate is used instead of L-alanine and incubation was carried out for 60 min. The enzyme activity was expressed as mU/mL in case of serum.

Alkaline phosphatase (EC 3.1.3.1) activity: Alkaline phosphatase (ALP) activity was assayed by the method of Bessey et al. (1946)7. Alkaline phosphatase at optimum pH 10.5 catalyzes the hydrolysis of p-nitrophenyl phosphate (disodium salt) to p-nitrophenol and inorganic phosphate. The liberated p-nitrophenol combines with sodium hydroxide to form a yellow coloured complex which was measured at 410 nm. The alkaline phosphatase activity was groups from gamma glutamyl peptides to suitable acceptor. The enzymatic reaction in the presence of substrate γ-glutamyl-p-nitroaniline results in the formation of p-nitroaniline whose release was monitored by noting increase in absorbance at 410 nm. The activity of enzyme was expressed as µmoles p -nitroaniline liberated/mg protein/min.

Bilirubin contents: The serum bilirubin content was determined by the method of Malloy and Evelyn (1937)11. Bilirubin couples with diazotized sulfanilic acid to form pink coloured azobilirubin. The intensity of this end product formed was directly proportional to the bilirubin content in serum which was read at 540 nm and expressed as mg/100 mL.

Lipid peroxidation (LPO): The lipid peroxidation (LPO) was measured in liver tissue by the method of Ohkawa et al. (1979)12. This method is based on the formation of red chromophore that absorbs light at 532 nm following the reaction with thiobarbituric acid (TBA) producing lipid peroxidation products like malondialdehyde (MDA) and others which are collectively called as thiobarbituric acid reactive substances (TBARS). The results were expressed as nmoles MDA formed/mg protein/60 min.

Hepatoprotective index: The liver protecting activity of the hydro-alcoholic extract of Boerhavia diffusa was expressed as hepatoprotective index (HPI) which was calculated using formula:

$HPI = (1 – T\text{-}V/ C\text{-}V) \times 100$

Where T is mean value of plant extracts along with CCl4, C is the mean value of CCl4 alone and V is the mean value of vehicle control animals (Prakash et al., 2008)13.

Table 3: Effect of plant extracts on CCl_4 – induced lipid peroxidation.

Experimental groups	LPO
(I) CONTROL	
1. Untreated	1.45 ± 0.02
2. Vehicle control	1.40 ± 0.03
3. *Boerhavia diffusa* (BD)	1.45 ± 0.05
4. Liv. 52 (L)	1.44 ± 0.06
(II) CARBON TETRAVHLORIDE (CCl_4) – TREATED	
5. CCl_4 (826 mg/kg bw/day)	4.99 ± 0.23[a]
(III) CCl_4 + *BOERHAVIA DIFFUSA* (BD) EXTRACT – TREATED	
6. CCl_4 + BD100	4.26 ± 0.05[b]
7. CCl_4 + BD200	3.36 ± 0.10[b]
8. CCl_4 + BD300	2.21 ± 0.05[b]
(IV) CCl_4 + Liv. 52 (L) – TREATED	
9. CCl_4 + L300	2.31 ± 0.10[b]

Results are expressed as mean ± SEM; n = 10.
No significant difference was noted between groups 1-4.
[a] as compared between vehicle control (group 2) and toxin - treated (group 5),
[b] as compared between toxin - treated (group 5) and toxin + antidote - treated (groups 6-9).
Level of significance p<0.05.
Units: LPO – nmoles MDA/mg protein/60 min.
BD 100, BD 200, BD 300 is 100, 200, 300 mg/kg body weight/day.
L300 is 300 mg/kg body weight/day.

Statistical analysis: The data were statistically analysed using one way Analysis of Variance (ANOVA) followed by Tukey's post hoc test. The results were expressed as means ± SEM; significance was accepted with $p < 0.05$. Pearson's correlation analysis was used to analyse the correlation between dose administration and alteration in enzymatic and non-enzymatic parameters in serum of mice. Pearson's correlation analysis was also used to find the correlation between lipid peroxidation and other parameters.

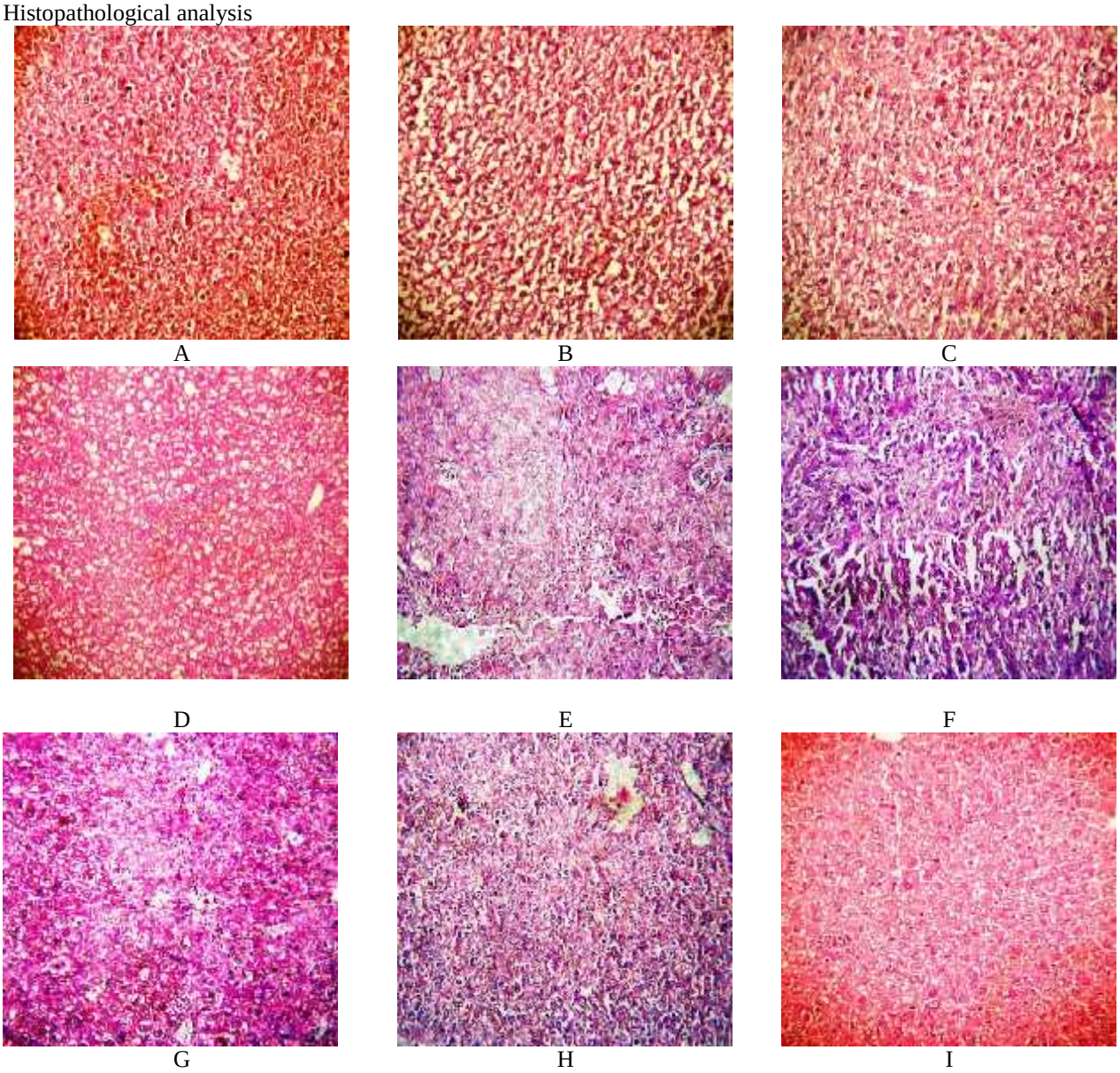
RESULTS

Serum enzymes: The CCl4 treatment (Group 5) for 30 days caused, significant ($p<0.05$) elevation in ALT (324.24%), AST (342.66%), ALP (117.14%), ACP (77.06%), LDH (122.09%) and γ-GT (117.99%) activities as compared to vehicle control. Also, total bilirubin (150.35%) as well as direct (103.75%) and indirect (213.55%) bilirubin contents were significantly ($p<0.05$) increased after CCl4 treatment. Co-treatment of CCl4 and hydro-alcoholic extract of Boerhavia diffusa significantly ($p<0.05$) ameliorated CCl4-induced changes in activities of ALT, AST, ACP, ALP, LDH and γ- GT as well as total, direct and indirect bilirubin contents in serum of mice, as compared to CCl4 alone treated group (Table 1 and 2). The hepatoprotective effects were dose-dependent (r = 0.9608–0.9989). Hepatoprotective index was maximum in high dose of Boerhavia diffusa plus CCl4 -treated group (ALT: 78.20%; AST: 82.24%; ALP: 68.86%; 75.00%; LDH: 86.28%; γ– GT: 65.69%; TB: 78.95%, DB: 68.68% and IB: 86.51%). Protection provided in plant extracts treated groups were compared with Liv, 52 - treated group (Group 9). Liv. 52 treatment along with CCl4 had significantly reduced activities of ALT, AST, ACP, ALP, LDH, γ-GT as well as total, direct and indirect bilirubin contents but to a lesser extent than that of Boerhavia diffusa extract.

Lipid peroxidation: No significant changes were observed between different control groups (Groups 1-4). However, CCl4 (826 mg/kg body weight/day, p.o.) (Group 5) treatment for 30 days caused significant ($p<0.05$) elevation in lipid peroxidation (256.42%) as compared to vehicle control in Swiss albino mice liver. Co-treatmentof Boerhavia diffusa extracts alongwith CCl4 (Groups 6-8) for 30 days caused significant amelioration, as compared to CCl4 alone treated mice (Group 5). The effect was dose-dependent (r = 0.9975). Hepatoprotective index was calculated for three doses of Boerhavia diffusa extract, which was 20.34% (BD 100), 45.41% (BD 200) and 77.44% (BD 300).

Histopathological examinations: Liver sections (H & E stained) of untreated control (Plate A) and vehicle control mice (Plate B) showed normal arrangement of hepatocytes with clearly visible nucleus, sinusoids, central vein and portal triad. Oral administration of Boerhavia diffusa extracts/ Liv. 52 alone treatment did not cause any alteration in normal architecture of hepatocytes and central vein (Plate C and Plate D). Oral administration of CCl4 for 30 days caused severe hepatocellular necrosis, cytoplasmic vacuolization, fatty infiltration like ballooning of hepatocytes, fibrosis, lymphocytic infiltration and loss of cellular boundaries (Plate E). Cotreatment of three different doses of Boerhavia diffusa extracts along with CCl4 caused amelioration of CCl4 – induced histopathological changes in a dose-dependent manner (Boerhavia diffusa F – H). Results revealed that oral administration of Boerhavia diffusa extracts (100, 200 and 300 mg/kg body weight) along with CCl4 caused significant, dose-dependent protection against the changes induced by CCl4. Oral administration of Boerhavia diffusa

Histopathological analysis



A B C

D E F

G H I

Plate A: Liver of untreated control mice showing normal arrangement of hepatocytes with clearly visible nucleus, sinusoids, central vein and portal triad (225X). Plate B: Liver of vehicle control mice showing normal arrangement of hepatocytes and central vein with clearly visible nucleus, sinusoids and portal triad (225X). Plate C: Liver treated with BD300 alone showing normal architecture of hepatocytes with clearly visible nucleus, sinusoids and central vein (225X). Plate D: Liver of L300 alone treated mice showing normal arrangement of hepatocytes and central vein with clearly visible nucleus, sinusoids and portal triad (225X). Plate E: Liver of mice treated with CCl_4 (826 mg/kg body weight/day) for 30 days showing severe hepatocellular necrosis, cytoplasmic vacuolization, fatty infiltration, fibrosis, lymphocytic infiltration and loss of cellular boundaries (225X). Plate F: BD100 along with CCl_4 treated group of mice showing moderate hepatocellular necrosis, cytoplasmic vacuolization, fatty infiltration and loss of cellular boundaries (225X). Plate G: BD200 along with CCl_4 treated group of mice showing mild hepatocellular necrosis, cytoplasmic vacuolization, fatty infiltration and loss of cellular boundaries (225X). Plate H: BD300 along with CCl_4 – treated group of mice showing normal arrangement of hepatocytes and central vein (225X). Plate I: L300 along with CCl_4 – treated group of mice showing normal arrangement of hepatocytes. Mild cytoplasmic vacuolization and hepatocellular necrosis is observed (225X).

(300 mg/ kg body weight/day) along with CCl4 resulted in restoration of normal liver architecture (Plate I). Results were compared with Liv. 52 (300 mg/kg body weight/day) treated group of animals (Group 9). Liv.52 administration along with CCl4 caused significant protection against the changes induced by the CCl4 but to a lesser extent as compared to Boerhavia diffusa - treated groups of mice (Plate I). On the basis of histopathological studies it could be concluded that Boerhavia diffusa extract treatment prevents CCl4-induced hepatotoxicity in mice.

DISCUSSION

Carbon tetrachloride treatment showed significant liver damage and it was well indicated by increased levels of hepato-specific enzymes like ALT, AST, ALP, ACP, LDH and γ-GT activities in serum (Table 1). The increased serum levels of hepatic markers have been attributed to the liver injury, because these enzymes are placed in the cytoplasmic area of the cell and are released into circulation in case of cellular damage14,15 . Any rise observed in the activities of these intracellular liver enzymes in serum indicates increased leakage due to membrane damage16. Oral administration of CCl4 caused a significant ($p<0.05$), dose-dependent increase in liver and serum ALT, AST, ALP and ACP enzyme activities17. Carbon tetrachloride causes oxidative stress by causing significant rise in LDH activity18. Ogeturk et al. (2004) have noted a significant increase in γ-GT after CCl4 administration19. Zimmerman et al. (1965) stated that the CCl4 - induced the increase of serum ALT and AST levels which source from cell membrane and mitochondrial damages in liver cells20. There are many authors' reports indicating that these enzyme activities were significantly elevated after CCl4 treatment21,22,23,24. The hepatic cells consist of higher concentrations of AST, ALT, LDH and γ- GT in cytoplasm and AST in particular exists in 25 are considered to be very sensitive and reliable for measuring hepatotoxicity as well as hepatoprotective effect of various compounds. The reduction levels of ALT and AST by the extracts is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl432. A significant

decrease in activities of LDH and γ-GT after Boerhavia diffusa extract treatments indicates subsequent recovery towards normalization which might be due to recoupment of the cell membrane. Lipids are more easily attacked by the activated metabolites of CCl4 resulting in damage to intracellular membranes and the plasma membrane24. Radical formation and lipid peroxidation are the predominant cellular mechanisms involved in the development of fatty liver caused by CCl421. Extensive accumulation of lipids is regarded as a pathological condition, and when the accumulation becomes chronic, fibrotic changes occur in the cells that progress to cirrhosis and impaired liver function33. The present study revealed significant protective effects of the Boerhavia diffusa extracts against CCl4-induced changes in LPO levels (Table 3) in liver of mice. Lipid peroxidation mitochondria. Due to the damage caused to hepatic cells, levels were found to be significantly reduced after treatment the leakage of enzymes in plasma causing an increased levels of hepato-specific enzymes in serum26. The elevated serum enzyme levels like AST, ALT, LDH and γ-GT are indicative of cellular leakage and functional integrity of cell membrane in liver27. γ - Glutamyl transpeptidase is a microsomal enzyme present in hepatocytes and its primary role is to metabolize extracellular glutathione allowing for precursor amino acids to be assimilated and reutilized for intracellular glutathione synthesis. An increase in serum γ-GT is a defense mechanism reflecting the induction of cellular γ-GT, when there is oxidative stress28. Monitoring level of LDH in liver tissue is an important marker as it is actively involved in glucose metabolism and alterations in its activity it generally indicates xenobiotics - induced hepatic injury29. The results of the present study revealed significant increase in the bilirubin content in CCl4 – treated mice (Table 2). Bilirubin assay is also considered to be a sensitive indicator as it substantiates the functional integrity of the liver with the severity of necrosis30; and thus any increase seen in its concentration in serum is an indicator of liver cell damage. Carbon tetrachloride has been previously reported to cause increase in serum bilirubin concentration31. Table 1 and 2 represents the results of plant extracts treatment on CCl4 -induced changes in activities of some liver enzymes and important marker enzymes in serum of the Boerhavia diffusa extracts along with CCl4 in a dose- dependent manner. Liver cell injury induced by CCl4 involves initially the metabolism of CCl4 to trichloromethyl free radical by the mixed-function oxidase system of the endoplasmic reticulum. It is postulated that secondary mechanisms link CCl4 metabolism to the widespread disturbances in hepatocyte function. These secondary mechanisms could involve the generation of toxic products arising directly from CCl4 metabolism or from peroxidative degeneration of membrane lipids34. In our study, elevations in the levels of end products of lipid peroxidation in liver of mice treated with CCl4 were observed. The increase in MDA level in liver suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Histopathological (H & E) examinations of CCl4 alone treated group of mice revealed severe hepatocellular necrosis and cytoplasmic vacuolization, fatty infiltration like ballooning of hepatocytes, fibrosis, lymphocytic infiltration and loss of cellular boundaries (Plate E). Carbon tetrachloride (CCl4) has been widely used in animal models to investigate chemical-induced liver damage. The most remarkable pathological characteristics of CCl4 - induced hepatotoxicity are fatty liver, cirrhosis and necrosis, which have been thought to result from the formation of reactive intermediates such as*) metabolized by the mice. A significant and dose-dependent decrease were observed in ALT, AST, ALP, ACP, LDH and γ-GT trichloromethyl free radicals (CCl3 mixed function cytochrome P450 in the endoplasmic 35 activities (Table 1) and bilirubin contents (Table 2) in reticulum. Eidi et al. (2012) have reported the acute serum when the Boerhavia diffusa extracts were administered along with CCl4. The results of the present study indicated that the Boerhavia diffusa extract reverse the increase seen in the activities of leakage enzymes and hepatotoxic effects induced by CCl4 administration were confirmed histopathologically, revealing extensive hepatocellular degeneration and necrosis, fatty changes, inflammatory cell infiltration, congestion, and sinusoidal 36 bilirubin contents. Boerhavia diffusa extracts provide dilatation. Cotreatment of Boerhavia diffusa extracts significant protection to the liver. Liver marker enzymes such as ALT, AST, ALP activities and bilirubin contents

along with CCl4 caused reversal of the histopathological alterations induced by CCl4 in a dose-dependent manner. Liv. 52 treatment along with CCl4 treatment also mitigate the toxic effect of CCl4 but to a lesser extent as compared to Boerhavia diffusa extract treated group of mice (Group I). Liv.52, used as a reference standard drug in present study, is a well-known hepatoprotective polyherbal formulation used in the treatment of liver diseases, evidenced by various experimental and clinical studies. Various animal experiments using different chemical toxicants have demonstrated the hepatoprotective effect of Liv. 5237,38,39,40,41.

CONCLUSIONS

The results obtained from this study indicates that the Boerhavia diffusa extract possess potent hepatoprotective activity against CCl4 – induced hepatic damage in Swiss albino mice which is mainly due to its antioxidative properties.

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DECLARATION OF INTEREST

The authors declare that there is no conflict of interest in respect of this study. This research received no specific grant from any funding agency in the public, commercial, or not for profit sectors.

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