Hepatoprotective Activity and Lipid Peroxidation of *Boerhavia repens* L. against Carbon Tetrachloride-Induced Toxicity

Amit Kumar Nigam^{1,2}, Phool Chandra¹, Zeashan Hussain³, Neetu Sachan¹

¹School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Delhi Road (NH-24), Moradabad (UP)-244192, India; ²Goel Institute of Pharmacy & Sciences, Faizabad Road, Near Indira Canal, Lucknow, Uttar Pradesh, India; ³Mahatma Gandhi Institute of Pharmacy, Lucknow, Uttar Pradesh, India.

ABSTRACT

Introduction: Hepatic disease stand as one of the foremost health troubles worldwide with liver cirrhosis and drug-induced liver injury accounting 9th leading cause of death in western and developing countries.

Objective: Hepatoprotective activity of the aerial part of *Boerhavia repens* was investigated against the carbon tetrachloride-induced hepatotoxicity model in Wistar albino mice.

Methods: The acute toxicity study of the selected plant showed that there is no mortality or adverse reaction at the fixed dose of 2000 mg/kg. The methanolic extract at 200 and 400 mg/kg body weight respectively were administered daily to study the Hepatoprotective effect in the carbon tetrachloride-induced hepatotoxic model for 7 days. It was observed that there was a remarkable decrease in bilirubin, ALP, SGOT and SGPT levels in the treatment group as compared to the hepatotoxic group. In a histopathological study, hepatocytic necrosis and inflammation in the centrilobular region with portal triaditis was found in the hepatotoxicity induced group where as minimal inflammation with moderate portal triaditis and normal lobular architecture observed in extract treated groups. At the end of the experiment, Liver and blood samples were collected for biochemical measurements. Parameters measured were GSH, GPx, GR, GST, SOD, MOD.

Results: Results showed that methanol extract scavenged free radicals. Results of *in-vivo* experiments showed that the methanol extracts inhibited lipid peroxidation, protected the experimental animals from hepatic toxicity, and maintained the levels of antioxidants in a dose-dependent manner.

Conclusion: From the observation, it can be concluded that the methanolic extract of *Boerhavia repens* has the potential to revert the hepatic injury induced by carbon tetrachloride. Comparatively, the methanolic extract at 400 mg/kg w/w resulted in a significant Hepatoprotective effect. The standard drug silymarin was used for comparison.

Key Words: Hepatoprotective activity, lipid peroxidation, *Boerhavia repens*, Silymarin, *Carbon tetrachloride*, SGOT

INTRODUCTION

It is been recorded in history that Medicinal herbs have been used as a form of therapy for the treatment of various systemic disorders. The searching of the chemical constituents from plants, pharmacological and phytochemical screening would provide the basis for developing the new lead molecules in strategic favour of new drug discovery. The objective of researchers is the discovery and development of active and less toxic molecule for systemic activities. The novel medicinal agents from plant sources have always been of great interest to work on hepatic disease.¹

Medicinal plants possess Hepatoprotective activity through many mechanisms, included antioxidant properties of medicinal plants, enhancement of antioxidant defence.² Apart from medicinal uses phytochemical components which are environment friendly, economical and effectively shows anti-corrosive properties^{3,4} and also Phyto-compounds are used as biofuels.^{5,6}

Boerhavia repens L. (Nyctaginaceae), commonly known species of 'Punarnava' in the Indian system of medicine, is a perennial creeping herb found throughout the wasteland of India.⁷ The *Boerhaavia* sp. has ancient medicinal use in different societies from the times of the B.C. Herbal medicine

Corresponding Author:

Amit Kumar Nigam, School of Pharmaceutical Sciences, IFTM University, Lodhipur Rajput, Delhi Road (NH-24), Moradabad, Uttar Pradesh, India; Phone: +91-7905148344; Email: amitnigam2511@gmail.com

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has evolved and changed through the years. Several plant products have been identified through phytochemistry and the extract of their different plant parts are useful in various diseases without side effects.⁸

Various observational studies indicate that regular consumption of foods containing flavonoids may reduce the risk of several chronic conditions, including neurodegenerative diseases, atherosclerosis, and certain forms of cancer. These results have generated considerable interest in flavonoids as it is associated with specific health outcomes.^{9,10}

The interest of the present work is to enrich the flavonoids in methanolic extract in the roots of *Boerhavia repens* and determine its presence by chemical tests such as the Shinoda test, lead acetate test and sodium hydroxide test. The enriched portions can be used for further studies such as the phytochemical entities containing flavonoids in the plant *Boerhavia repens* and studies relating to Hepatoprotective activity of the plant.

MATERIALS AND METHODS

Collection and Authentication of Selected Plant

Boerhavia repens is a herbaceous plant that grows in clusters. The branches stand with many large cleft dark green leaves, woody stem and whitish purple flowers. The plant grows profusely in the rainy season and mature seeds are

formed in October-November. Due to its sticky nature, the plant gets stuck on the clothes of human beings and on the legs of animals, which helps in its dispersal from one place to another. Fresh aerial parts of *B. repens* were collected along with a herbarium specimen during November 2018 from the surrounding of Almora and Nainital, Uttarakhand, India. The samples were authenticated by National Botanical Research Institute, Lucknow with reference number NBRI/CIF/580/2018.

Preliminary Phytochemical screening¹¹⁻¹³

The methanolic extract of *BRME* was subjected to qualitative chemical tests for the detection of various plant constituents like carbohydrates, glycosides, proteins and amino acids, fixed oils and fats, gums and mucilage, alkaloids, phytosterols, flavonoids, tannins and phenolic compounds, saponins, triterpenoids, etc (Table 1-3).

Table 1: Physical characteristics of Methanolic plant extract

Parameters	Physical properties of BRME
Colour	Dark Brownish Green
Odour	Characteristic
Taste	Tasteless
Consistency	powder
Sense of touch	Smooth

Table 2: Preliminary phytochemical screening

S. No.	Chemical Constituents	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Ethanol extract	Aqueous extract
1.	Carbohydrates	-	-	-	+	+
2.	Proteins	-	-	-	+	+
3.	Amino acids	-	-	-	+	+
4.	Fats & Oils	+	+	-	-	-
5.	Glycosides	-	-	-	+++	++
6.	Flavonoids	-	+	+	++	-
7.	Alkaloids	-	-	-	++	+
8.	Phenolic Compounds	-	-	-	++	+++

Table 3: Physicochemical parameters

Parameters	Value
Moisture content (% w/w)	9.6
Foreign matters (% w/w)	0.2
Total ash (% w/w)	2.32
Acid insoluble ash (% w/w)	0.22
Water-soluble ash (% w/w)	0.30
Alcohol soluble extractive value (% w/w)	22.00
Water-soluble extractive value (% w/w)	16.12

Extraction

BRME were dried under shade and then powdered to get a coarse powder. Powdered materials were extracted with methanol by continuous hot extraction method for 72 hrs by using Soxhlet apparatus. The methanol extract was concentrated to a dry mass by vacuum distillation. After complete drying, the extracted material was weighed and the extractive value in percentage was calculated regarding the air-dried sample.

Experimental animals

Albino mice of weight ranges from 25-30 gm of female mice were used for toxicity studies. Institutional Animal Ethics Committee approved the experimental protocol and animals were maintained under standard conditions in an animal house. All the experiments were carried out as per CPCSEA guidelines (Committee for Control and Supervision of Experiments on Animals) after obtaining approval (Ethical committee No 2014/PO/Re/S/18/CPCSEA) from the Institutional animal ethical committee.

Acute oral toxicity study

The acute toxicity study was carried on albino mice as per the guidelines No: 423 given by the Organization for Economic Co-operations and Development, Paris [OECD 423, 2001]. Three Albino mice have fasted overnight and the test sample was given orally at a starting dose of 5mg/kg b.wt. Animals were observed for a period of 2h, then occasionally for 4hrs for the severity of any toxic signs and mortality. Since no mortality was observed, the same dose was repeated with another group of animals. The procedure was repeated for doses of 50, 300 and 2000mg/kg in a separate group of animals. The starting dose level should be that which is most likely to produce mortality in some of the dosed animals. From the maximum dose of 2000mg/kg, 1/10th and 1/5th of the values were taken as treatment dose for further studies. Behaviour as well as other toxic symptoms if any was observed for 24, 48 and 72h. The animals were kept under observation up to 14days after drug administration to find out delayed mortality if any.¹⁴

The acute toxicity study was done according to OECD 423 guidelines. In acute toxicity study, BRME did not show any mortality or toxic effect up to the administration of the single dose of 2000 mg/kg., body weight per oral during the observational period of 24 hr. It did not produce any significant changes in behavioural profile like; alertness, restlessness, irritability and fearfulness. No changes in neurological profile; spontaneous activity, reactivity, touch response & pain response and no effect on autonomic profile. BRME did not show any lethality or death after 72h of observation.

Carbon tetrachloride-induced liver toxicity

Animals were divided into five groups (n = 6). Group, I served as vehicle control, which received liquid paraffin.

Groups II-V were treated with CCl₄ in liquid paraffin (1:2) at the dose of 1 ml/kg body weight intraperitoneal once every 72 h for 14 days. Groups IV and V were treated with BRME at the doses of 200 and 400 mg/kg body weight, respectively, and Group III was administered with Silymarin at a dose of 25 mg/kg body weight orally. BRME (*Boerhavia repens* methanolic extract) treatment was started 10 days before CCl₄ administration and continued until the end of the experiment.

Evaluation of liver damage

At the end of the experiment blood was collected from retro-orbital plexus from the overnight fasted animals, after anaesthetized with 100 mg/kg ketamine, i.p. The blood samples were taken with 20 µl EDTA (5%) in each Eppendroff and centrifuged at 5000 rpm for 20 min (Sigma 3K30, UK). The supernatant (serum) was separated with the help of a micro-pipette and placed in a new Eppendroff well labelled and stored in -80°C for further analysis. The homogenate was centrifuged at 4°C for 5 min at 3000 rpm and the supernatant used for estimation of viscous oxidative stress markers (Biochemical and antioxidant estimation). The opposite liver tissue specimens were used for histopathological examination (Table 4).^{15, 1}

Table 4: Effect of BRME on liver weight in CCl₄ induced hepatotoxicity

Groups ^a	Dose (mg/kg)	Liver weight g /100g of body weight
Vehicle Control	5 ml	3.50 ± 0.042
CCl ₄	1 ml/kg	5.92 ± 0.05
Silymarin + CCl ₄	25 mg/kg	4.84 ± 0.058
BRME + CCl ₄	200 mg/kg	5.56 ± 0.06
BRME + CCl ₄	400 mg/kg	4.28 ± 0.07

Biochemical studies

After 48 h carbon tetrachloride intoxication, the animals were sacrificed by mild anaesthesia. Blood samples were collected in glass test tubes and allowed to coagulate for 20 min. Serum was separated by centrifugation at 3000 rpm for 20 min and used for evaluating biochemical parameters and liver tissue was sliced for histopathological studies. Biochemical parameters like Serum glutamate oxaloacetate transferase (SGOT), Serum glutamate pyruvate transferase (SGPT), alkaline phosphatase (ALP) and Serum bilirubin were estimated by reported methods.^{17, 18} (Table 5).

Table 5: Effect of BRME on serum bilirubin (Total and Direct), SALP, SGOT, GGTP, TP and SGPT in carbon tetrachloride treated Albino mice

Groups	Dose (per kg)	SGPT (u/l)	SGOT (u/l)	SALP (u/l)	GGTP (u/l)	Total Bilirubin (mg/dl)	TP (mg/dl)
Normal Control	5 ml	72±0.48	57±0.89	66±1.75	68±0.85	0.68±0.04	6.8±0.04
CCl ₄	1 ml	148±0.60	170±2.45	118±0.06	145±2.45	2.38±0.14	6.6±0.07
Silymarin + CCl ₄	25 mg	85±1.18	68±1.52	78±1.04	82±1.02	0.70±0.04	6.2±0.04
BRME + CCl ₄	200 mg	70±1.62*	92±1.34*	98±1.25*	105±1.03*	0.75±0.05*	6.4±0.06*
BRME + CCl ₄	400 mg	80±0.082**	89±0.48**	82±1.42**	102±1.04**	0.62±0.03**	7.0±0.04**

Antioxidant Activity

A marked reduction in hepatic GSH and antioxidant enzymes (GPx, GR, GST, and SOD) was observed in CCl₄ intoxicated mice. In contrast, a significant increase in the MDA level was evident compared to the normal control group (Table 6). Administration of BRME at the two treatment doses (200 and 400 mg/kg/day) produced a marked increase in GSH and improved the activity of all the antioxidant enzymes relative to the CCl₄ intoxicated group. In addition, the elevated hepatic level of MDA was reduced at the tested doses, respec-

tively, compared to the CCl₄ intoxicated group. The higher dose of BRME was more effective in reducing the MDA level compared to silymarin. Notably, the GSH, GPx, GR, GST, and SOD levels in the group treated with 400 mg/kg of BRME were markedly higher compared to the silymarin-treated group. Moreover, BRME treatment at a dose of 200 mg/kg induced a marked increase in the GR and GST levels compared to the silymarin treated group. These results indicated the strong antioxidant activity provided by BRME.^{19, 20, 21}

Table 6: Effect of BRME on lipid peroxidation (MDA), GSH and antioxidant enzymes after 6 weeks of CCl₄ intoxication in mice

Animal group	GSH (µm/g liver)	GPx (µm/g liver)	GR (µm/g liver)	GST (µm/g liver)	SOD (µm/g liver)	MDA (nm/g liver)
Control	3.70 ± 0.32	3.30 ± 0.31	2.46 ± 0.24	47.12 ± 4.40	356 ± 7.10	34.12 ± 2.98
CCl ₄	2.45 ± 0.32	2.10 ± 0.18	1.58 ± 0.35	22.24 ± 2.34	235.25 ± 3.10	69.20 ± 5.10
BRME (200 mg/kg/day)	3.35 ± 0.25	2.38 ± 0.45	2.65 ± 0.18	40.02 ± 3.47	300.10 ± 5.10	40.15 ± 2.75
BRME (400 mg/kg/day)	3.68 ± 0.22	3.45 ± 0.32	2.55 ± 0.38	48 ± 2.55	342.47 ± 5.42	38.02 ± 3.44
Silymerin (25 mg/kg/day)	3.48 ± 0.45	2.84 ± 0.28	2.45 ± 0.32	38 ± 4.65	324 ± 4.34	39.46 ± 2.22

Histopathological Investigations

Control (Group I): Hepatocytes of the normal control group showed normal lobular architecture of the liver (Figure 1a). Toxic Control Groups (II): Hepatocytic necrosis and inflammation region were observed in liver treated with carbon tetrachloride (Figure 1b). Silymarin 25 mg/kg (Groups III): Silymarin pretreated group showed normal hepatocytes normal architecture (Figure 1c). Methanolic extract 200 mg/kg and 400 mg/kg (Groups IV & V): Methanolic extract 200 mg/kg and 400 mg/kg treated group showed recovery of hepatic parenchyma, mild congestion and microvesicular changes. (Figure 1d & 1e).

Figure 1: Represents hepatocytes in the normal control (A), Silymarin (25 mg/Kg) (B), CCl₄ (2000 mg/Kg) (C), BRME (200 mg/kg) (D), BRME (400 mg/kg) (E) treatment group.

DISCUSSION

CCl₄ intoxication is a widely used experimental model for liver injury. The highly hepatotoxic metabolites, namely, trichloromethyl radicals (CCl₃^{*} and CCl₃O₂^{*}) are generated during the metabolic activation of CCl₄ by cytochrome P-450. These radicals have a central role in the initiation of lipid peroxidation, inflammation and fatty changes of the liver.^{22,23} Moreover, CCl₄ intoxication is associated with oxidative stress since the CCl₃^{*} and CCl₃O₂^{*} radicals alter the antioxidant state of the liver by deactivating the hepatic antioxidant enzymes including SOD, GPx, GR, and GST. Trichloromethyl radicals also react with the sulfhydryl groups of GSH leading to its deactivation.²² In the present study, CCl₄ treatment markedly increased the levels of AST, ALT, and ALP. The leakage of the marker enzymes into the blood was associated with marked necrosis, loss of hepatic architecture, hydropic degeneration, fatty changes, Kupffer cell hyperplasia, central vein congestion and infiltration of the liver by lymphocytes. The MDA level in the liver tissue was markedly increased in response to CCl₄ intoxication, indicating oxidative damage of the liver. CCl₄ administration also reduced the levels of GPx, SOD, GST, GSH, and GR in the liver tissue compared to the normal mice. The results of the present study demonstrated that treatment with BRME returned the increased MDA to its normal level. The inhibitory effect against lipid peroxidation suggested that BRME could prevent the liver injury induced by free radicals along with the subsequent pathological changes in the liver.

The marked reduction in the leakage of liver enzymes into the serum also confirmed the inhibitory effect of BRME against lipid peroxidation. In contrast, the GSH, GPx, SOD, GST, and GR levels were markedly improved compared to the silymarin-treated group. Modulation of these antioxidant defences contributed to the antioxidant and hepatoprotective activity of BRME. The remarkable hepatoprotective and antioxidant effect of BRME may be attributed to a synergistic effect between these compounds.²⁴

CONCLUSION

Based on the results of this study, the hepatoprotective effect of BRME is attributed to its ability to reduce the rate of lipid peroxidation, enhance the antioxidant defence status and guard against the pathological changes of the liver induced by CCl₄ intoxication. The Hepatoprotective activity of BRME is concluded to be partly mediated by the antioxidant effect of the extract.

ABBREVIATIONS

ALP, Alkaline phosphatase; BRME, *Boerhavia repens* Methanolic extract, CCl₄, Carbon tetrachloride; GPx, Glutathione Peroxidase; GSH, Glutathione; GST, Glutathione

S-transferase; MOD, Multiple Organ Dysfunction Syndrome; SGOT, Serum Glutamic Oxaloacetic Transaminase; SGPT, Serum Glutamic Pyruvic Transaminase; SOD, Superoxide dismutase.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

HUMAN AND ANIMAL RIGHTS

Animals were used for studies that are the basis of this research.

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Conflict of Interest

The authors declare that they have no financial conflict of interest.

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Nil

Authors Contribution

Amit Kumar Nigam has generated ideas and wrote the manuscript whereas Phool Chandra and Zeashan Hussain are rectifying the plagiarism. Neetu Sachan draws the entire graphical abstract.

REFERENCES

1. Rajavel R, Mallika P, Rajesh V, PavanKumar K, Moorthy Krishna S, Sivakumar T. et al. Anti-inflammatory Effects of the Methanolic extract of *Oscillatoria annae*. Res J Chem Sci. 2012;2:53-61.
2. Al-Snafi AE, Mousa HN, Majid WJ. Medicinal plants possessed hepatoprotective activity. IOSR J Pha. 2019;9:26-56.
3. Patil HM. Ethnobotanical Notes on Satpura Hills of Nandurbar District, Maharashtra, India. Res J Recent Sci. 2012;1:326-328.
4. Sulaiman S, Nor-Anuar A, Abd-Razak AS, Chelliapan S. A Study of Using *Allium cepa* (Onion) as Natural Corrosion Inhibitor in Industrial Chill Wastewater System. Res J Che Sci. 2012;2:10-16.
5. Nutan K, Chaturvedi A, Upadhyay RK. Corrosion Inhibition of Mild Steel by Alkaloid Extract of *Ocimum sanctum* in HCl and HNO₃ Solution. Res J Che Sci. 2012;2:51-56.
6. Bobade SN, Khyade VB. Preparation of Methyl Ester (Biodiesel) from Karanja (*Pongamia pinnata*) Oil. Res J Che Sci. 2012;2:43-50.

7. Deshpande DP, Urunkar YD, Thakare PD. Production of Biodiesel from Castor Oil using acid and Base catalysts. Res J Che Sci. 2012;2:51-56.
8. Rawat AKS, Mehrotra S, Tripathi SC, Shome U. Hepatoprotective activity of *Boerhaavia diffusa* L. roots a popular Indian ethnomedicine. J Ethnopharmacol. 1997;56:61-66.
9. Patil KS, Bhalsing SR. Ethnomedicinal uses phytochemistry and pharmacological properties of the genus *Boerhavia*. J Ethnopharmacol. 2016;182:200-220.
10. Mahesh AR, Kumar H, Ranganath MK, Devkar RA. Detail Study on *Boerhaavia diffusa* Plant for its Medicinal Importance- A Review. Res J Pha Sci. 2012;1:28-36.
11. Dantu PK, Chaudhary G. Morphological, Phytochemical and Pharmacological, Studies on *Boerhaavia Diffusa* L. J Med Plant Res. 2011;5:2125-2130.
12. Murti K, Panchal MA, Lambole V. Pharmacological Properties Of *Boerhaavia diffusa* - A Review. Int J Pha Sci Rev Res. 2010;5: 107-110.
13. Bruce RD. An up-and-down procedure for acute toxicity testing. Fun App Tox. 1985;5:151-157.
14. Kind PRN, King EJ. Estimation of plasma phosphatase by determination of hydrolysed phenol with amino-antipyrine. J Clin Pat. 1954;7:322-326.
15. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol. 1957;28:56-63.
16. Asha VV, Sheeba MS, Suresh V, Wills PJ. Wills PJ. Hepatoprotection of *Phyllanthus maderaspatensis* against experimentally induced liver injury in rats. Fitotherapy 2007;7:134-135.
17. Malloy HT, Evelyn KA. The determination of bilirubin with the photoelectric colourimeter. J Biol Che. 1937;11:481-490.
18. Paglia DE, Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Cli Med. 1967;70:158-169.
19. Zanetti G. Rabbit liver glutathione reductase. Purification and properties. Arc Bio Biop. 1979;198:241-246.
20. Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferases: The first enzymatic step in mercapturic acid formation. J Bio Chem. 1974;249:7130-7139.
21. Srivastava A, Shivanandappa T. Hepatoprotective effect of the root extract of *Decalepis hamiltonii* against carbon tetrachloride-induced oxidative stress in rats. F Che. 2010;118:411-417.
22. Lee SJ, Lim KT. Glycoprotein of *Zanthoxylum piperitum* DC has a hepatoprotective effect via an anti-oxidative character in vivo and in vitro. Tox Vit. 2008;22:376-385.
23. Han X, Shen T, Lou H. Dietary polyphenols and their biological significance. Int J Mol Sci. 2007;8:950-988.