PharmacologyOnLine August 30, 2014

Archives • 2014 • vol.2 •18-28

PROTECTIVE EFFECT OF *BACOPA MONNIERA* METHANOL EXTRACT AGAINST CARBON TETRACHLORIDE INDUCED HEPATOTOXICITY AND NEPHROTOXICITY

Muhammad Shahid[1,2*], Fazal Subhan[2]

[1]Department of Pharmacy, Sarhad University of Science & Information Technology, Peshawar, Pakistan
[2]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan

*shahidsalim_2002@hotmail.com

Abstract

In the present study *Bacopa monniera* methanol extract (mBME) was evaluated against carbon tetrachloride induced hepatic and renal damage in rats. The in-vitro antioxidant activity of mBME was determined by DPPH free radical scavenging assay. Acute treatment with carbon tetrachloride (2 ml/kg) caused elevation of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and creatinine and induced histopathological changes as severe necrosis of centrilobular hepatocytes, microvesicular as well as macrovesicular steatosis in liver and tubular dilatation with epithelial cells necrosis and interstitial nephritis in kidneys. Pretreatment with mBME (40 mg/kg) for 14 days decreased the serum ALT, AST and creatinine levels and protected liver and kidneys from the toxicological influence of carbon tetrachloride. The EC_{50} for the DPPH free radical scavenging assay of mBME (19.20 µg/ml) as compared to ascorbic acid (4.510 µg/ml) and BHT (8.561 µg/ml) revealed that mBME had an efficient antioxidant potential. The protective effect of *Bacopa monniera* against carbon tetrachloride induced hepato-nephrotoxicity may be due to its strong antioxidant activity.

Key words: *Bacopa monniera*, carbon tetrachloride, hepatotoxicity, nephrotoxicity

Introduction

Liver diseases are the most common cause of death (1). The major etiologies of liver failure are viral hepatitis, ischemia and drug or toxin induced liver disease (2). Injury to hepatocytes results either directly from the disruption of intracellular function or membrane integrity or indirectly from immune-mediated membrane damage (3). Increase production of free radicals with deficiency of dietary antioxidants are important etiological factors in alcoholic liver diseases (4). Moreover increased generation of reactive oxygen and nitrogen species along with decreased antioxidant defense, promotes the development and progression of hepatic and extra-hepatic complications of hepatitis-C infection (5). Hepatotoxicity remains a major reason for drug withdrawal from pharmaceutical development and clinical use (6).

Kidney diseases are a major public health problem (7). The leading causes are diabetes mellitus, hypertension and glomerulonephritis (8). Tubular and vascular changes along with interstitial inflammation are responsible for acute renal injury (9). Reactive oxygen species play an important role in glomerulonephritis, acute or progressive renal failure and tubulointerstitial nephritis (10). Patients with end stage renal disease on hemodialysis have increase oxidation of lipids, thiols, proteins and DNA (11). The interplay among intra-renal hemodynamic changes, ischemic and toxic injuries to tubular cells form the basis for the acute decrease in glomerular filtration rate, which is the result of intra-renal vasoconstriction, with a decrease in glomerular filtration pressure, tubular obstruction, trans-tubular back-leakage of the filtrate and interstitial inflammation (12).

Bacopa monniera from Scrophulariaceae family has been traditionally used as memory-enhancing, anti-anxiety, diuretic, aphrodisiac, and antipyretic (13). The constituents responsible for *Bacopa monniera* pharmacological effects are bacoside A and B (14). *Bacopa monniera* has cognitive enhancing (15), antidepressant (16), antiepileptic (17), antiamnesic (18), antibacterial (19), antidiabetic (20), anti-inflammatory (21), antihypertensive (22), anticancer (23), antiasthmatic (24), spasmolytic (25), antiulcer (26), analgesic (27), antifungal (28), antioxidant (29), antiaddictive (30), neuroprotective (31), cardioprotective (32), hepatoprotective (33) and nephroprotective properties (34).

The hepatoprotective effect of *Bacopa monniera* is due to its strong antioxidant potential (35-36).

Keeping in view the pharmacological profile of *Bacopa monniera*, the present study was designed to evaluate the impact of *Bacopa monniera* methanol extract (mBME) against carbon tetrachloride induced hepatotoxicity and nephrotoxicity.

Materials and Methods

Animals

Male Sprague Dawley rats, weighing 150-200 gm and maintained in a 12 h light/dark cycle at 22 ± 2 ^{o}C were used in the experiments. Food and water were provided *ad libitum*. Experiments on animals were performed in accordance with the UK Animals (Scientific Procedures) Act 1986 and according to the rules and ethics set forth by the Ethical Committee of the Department of Pharmacy, University of Peshawar. Approval for the study was granted vides letter number Pharm/EC/446.

Chemicals and standards

Carbon tetrachloride (Obtained from Raziki Agency Corporation, Pakistan), 2,2-diphenyl-1-picrylhydrazyl (DPPH; Sigma-Aldrich, Germany), ascorbic acid (Sigma-Aldrich, Germany), butylated hydroxytoluene (BHT; Sigma-Aldrich, Germany) and olive oil (Spanish oil, Rafael Salgado, Spain).

Plant material

Bacopa monniera was collected in April from Ramali stream near Quaid-e-Azam University, Islamabad. After identification by Prof. Dr. Muhammad Ibrar (Pharmacognosist), a specimen was submitted with a voucher number 20016 (PUP) in the herbarium at the Department of Botany, University of Peshawar. The method of Rauf (37) was adapted for extraction and fractionation of *Bacopa monniera*. Briefly, the aerial parts were separated, shade dried and coarsely grinded. It was defatted with n-hexane and was further treated with acetone to remove chlorophyll type pigments. Extraction was done with methanol in Soxhlet apparatus and the extract was then filtered and concentrated under reduced pressure at 50^{o}C in a rotary evaporator. A semisolid mass (yield 6.5%) was obtained on drying the concentrated extract on a water bath at 50^{o}C.

In-vitro antioxidant activity of *Bacopa monniera* methanol extract

The in-vitro antioxidant activity was measured

according to Molyneux (38) with slight modifications. 1 ml of methanol 0.1 mM DPPH free radical solution was mixed with 1 ml of different concentrations (1, 10, 30, 50, 100, 200, 400, 600, 800 and 1000 µg/ml) of mBME or standards in methanol. The solutions were thoroughly mixed and incubated in dark at ambient temperature for 40 minutes. Absorbance was then measured at 517 nm using UV/visible spectrophotometer (Lambda 25, PerkinElmer, USA). Ascorbic acid and butylated hydroxytoluene were used as standards. Control was prepared by mixing 1 ml of 0.1 mM DPPH free radical solution with 1 ml of methanol. Blank consisted of methanol alone. The percent scavenging of the DPPH free radicals was calculated as follows.

Percent of DPPH free radicals scavenging activity = $[(A_I-A_{II}/A_I) \times 100]$

The absorbance of the control reaction was A_I while the absorbance in the presence of sample was A_{II}. The EC_{50} value, which is the concentration of antioxidant at which there is 50% loss of DPPH free radicals scavenging activity, was calculated from the graph of absorbance versus respective concentrations using nonlinear regression analysis. The antiradical power and stoichiometry was determined according to Mishra and others (39). The experiments were performed in triplicate.

Treatment groups

Carbon tetrachloride was administered according to Janakat and Merie (40) while mBME was administered according to Sumathi and Devaraj (34). Rats were divided into the following groups.

Group I received the vehicle and served as control. Group II received a single dose of carbon tetrachloride (1:1 in olive oil, 2 ml/kg, i.p.) on day 14. Group III was treated with mBME (40 mg/kg/day, p.o) for 14 days and on the last day; a single dose of carbon tetrachloride (2 ml/kg, i.p.) was administered two hours after administration of mBME. Group IV was treated with mBME alone (40 mg/kg/day p.o.) for 14 days. Animals were sacrificed after 24 hours of carbon tetrachloride treatment.

Biochemical analysis

At the end of experiment, blood samples from animals were collected in tubes by cardiac puncture, allowed to clot and serum was separated by centrifugation (K240R, Centurion scientific, UK) at 3000 rpm for 15 minutes at 37°C. All serum samples were stored at 4°C till the determination of biochemical parameters. Serum samples were assayed for alanine aminotransferase, aspartate aminotransferase (ALT, AST; GO F400 CH, Chema Diagnostica, Italy) and creatinine (CR 0500 CH, Chema Diagnostica, Italy).

Histological evaluation

At the end of experiment, all animals were sacrificed by cervical dislocation and liver and kidneys were removed, weighed and fixed immediately in 10% neutrally buffered formalin. The tissues were dehydrated in graded ethanol solutions (50, 70, 80, 90, two changes each of 100%), cleared in two changes each of 100% xylene and were infiltrated and embedded in paraffin wax. Tissue blocks were sectioned at 4 µm using a rotary microtome (SLEE Mainz CUT 5062, Germany) and were stained with Harris hematoxylin and eosin (H & E) for microscopic observation (Labomed Lx400 with digital camera iVu 3100, USA). Histological changes were scored as none (–), mild (+), moderate (++), or severe (+++) damage (41).

Statistical analysis

The difference of significance was calculated by one way ANOVA followed by Tukey's multiple comparison test using GraphPad Prism 5 (GraphPad Software Inc. San Diego CA, USA).

Results

Antioxidant potential of *Bacopa monniera* methanol extract

mBME or standards exhibited a concentration dependent scavenging of DPPH free radicals (table 1). The concentration dependent increase was up to 10 µg/ml for ascorbic acid and 50 µg/ml for BHT or mBME after which the percent inhibition remained constant. The maximum inhibition of DPPH free radical scavenging activity by mBME was 93.37 % at 50 µg/ml while that of ascorbic acid and BHT were 96.18 and 92.18 % at 100 and 800 µg/ml respectively. The percent of maximum DPPH free radical scavenging activity was in the order of:

Ascorbic acid > mBME > BHT

mBME or standards exhibited a concentration dependent decrease in absorption (figure 1). The EC_{50} for the DPPH free radical scavenging effect was in the order of: Ascorbic acid > BHT > mBME. The EC_{50}, antiradical power and stoichiometry of mBME, ascorbic acid and BHT are shown in table 2.

Effect of *Bacopa monniera* methanol extract or carbon tetrachloride alone or in combination on organs weight

No significant effect was found in the weight of liver and kidneys after treatment with mBME or carbon tetrachloride alone or in combination (table 3).

Effect of *Bacopa monniera* methanol extract on carbon tetrachloride induced elevation of serum ALT, AST and creatinine

Treatment with carbon tetrachloride (group II) caused significant elevation ($p < 0.001$) of serum ALT and creatinine as compared to control (group I). However the elevation of serum AST was statistically non-significant. Pretreatment with mBME (group III) significantly decreased the serum ALT ($p < 0.01$) and creatinine ($p < 0.001$) as compared to carbon tetrachloride treated rats (group II). The decrease in serum AST was statistically non-significant. The biochemical changes are shown in figure 2 (ALT), figure 3 (AST) and figure 4 (creatinine).

Carbon tetrachloride induced histopathological changes in liver and kidneys

In liver, treatment with carbon tetrachloride caused extensive necrosis throughout the hepatic lobule. The central veins were difficult to visualize and were severely congested. Sinusoidal spaces were dilated and were infiltrated with large number of mononuclear lymphocytes and red blood cells. The centrilobular hepatocytes showed severe necrosis and contained hyaline inclusions. Ballooning degeneration, microvesicular and macrovesicular steatosis were predominant. The histopathological changes in liver are shown in figure 5B.

In kidneys, treatment with carbon tetrachloride caused dilatation of renal tubules and widening of interstitial spaces. Some cuboidal epithelial cells of renal tubules were necrotic while others were swelled and contained clear vacuoles in their cytoplasm. The brush borer lining the epithelial cells was destroyed.

The interstitial spaces were infiltrated by large number of red blood cells and lymphocytes. There is widening of capsular space between the parietal and visceral layers of the Bowman's capsule. Glomeruli were congested. The histopathological changes in kidneys are shown in figure 6B.

Protective effect of *Bacopa monniera* methanol extract against carbon tetrachloride induced hepatotoxicity and nephrotoxicity

Pretreatment with mBME for 14 days preserved the liver histoarchitecture beside mild congestion of central vein, dilatation of sinusoids with infiltration of lymphocytes and microvesicular steatosis (figure 5C). Moreover in kidneys mBME provided full protection (figure 6C). Animals treated with mBME alone showed no significant histopathological effects in liver (figure 5D) and kidneys (figure 6D). The histopathological changes were scored in table 4 (liver) and 5 (kidneys).

Discussion

The toxic effect of carbon tetrachloride is due to its conversion by cytochrome P-450 to the highly reactive trichloromethyl radical ($\cdot CCl_3$) which rapidly react with oxygen to form trichloromethyl peroxyl radical. These and other radicals formed in subsequent steps of metabolism are involved in the lipid peroxidation processes (42). Lipid peroxidation causes membrane disruption, resulting in loss of membrane integrity and leakage of microsomal enzymes (43). In this study, treatment with carbon tetrachloride caused elevation of serum ALT, AST and creatinine. Histopathological examination showed that in liver carbon tetrachloride was associated with microvesicular as well as macrovesicular steatosis, ballooning degeneration, perivenular hepatocyte necrosis, infiltration of mononuclear lymphocytes and hyaline change (figure 5B). In kidneys the changes occurred as renal epithelial cells vacuolization, tubular dilatation and interstitial nephritis (figure 6B). These findings are in accordance with the previous studies on carbon tetrachloride induced hepatotoxicity (44) and nephrotoxicity (45-46).

Herbal medicines are increasingly being utilized to treat a wide variety of clinical diseases, with relatively little knowledge on their modes of action (47). Herbs have been used for the prevention and treatment of liver (48-49) and kidney diseases (50). In the acute toxicity study, pretreatment with mBME restored carbon tetrachloride induced elevation of

ALT, AST and creatinine which showed an improvement in the functional status of liver and kidneys.

These findings can be further corroborated with histopathological studies. The liver had a more or less normal lobular pattern with mild degree of microvesicular steatosis and sinusoidal dilatation (figure 5C) while the kidneys showed almost normal histoarchitecture (figure 6C). These results are in agreement with those previously reported (51).

Antioxidants from natural sources are effective in health maintenance by reducing the toxic effects of xenobiotics (52). DPPH antioxidant assay is considered as a standard method for the antioxidant activity of pure and natural compounds (39). Antioxidants with higher free radical scavenging capacity will scavenge DPPH free radicals efficiently while antioxidants having lower scavenging capacity will scavenge less avidly and take some time (53).

In the present study mBME exhibited a concentration dependant increase of percent scavenging of DPPH free radicals. The percent scavenging by mBME (93.37 %) was greater than BHT (92.18 %) but lower than ascorbic acid (96.18 %). The estimation of the quality of antioxidants present in an extract is determined by EC_{50} values with a low value indicates strong antioxidant activity in the extract (54). Similarly the larger the antiradical power, the more efficient is the antioxidant (39). The EC_{50} of mBME (19.20 µg/ml) as compared to BHT (8.561 µg/ml) and ascorbic acid (4.510 µg/ml) indicated that mBME was a strong scavenger of free radicals. Similar results were presented in previous studies (55-57). Thus *Bacopa monniera* has an efficient antioxidant potential comparable to other powerful natural antioxidants.

In conclusion, *Bacopa monniera* is an effective hepatoprotective and nephroprotective herbal drug as it ameliorates the hepatotoxicity and nephrotoxicity of carbon tetrachloride which might be due to its strong antioxidant activity.

References

1. Williams R. Global challenges in liver disease. Hepatology. 2006;44(3):521-6.
2. Lee WM, Squires RH, Nyberg SL, Doo E, Hoofnagle JH. Acute liver failure: summary of a workshop. Hepatology. 2008;47(4):1401-15.
3. Lee WM. Drug-induced hepatotoxicity. N Engl J Med. 2003;349(5):474-85.
4. Masalkar PD, Abhang SA. Oxidative stress and antioxidant status in patients with alcoholic liver disese. Clin Chim Acta. 2005;355(1):61-5.
5. Choi J, Ou J-HJ. Mechanisms of liver injury. III. Oxidative stress in the pathogenesis of hepatitis C virus. Am J Physiol-Gastrl. 2006;290(5):G847-G51.
6. Jaeschke H, Gores GJ, Cederbaum AI, Hinson JA, Pessayre D, Lemasters JJ. Mechanisms of hepatotoxicity. Toxicol Sci. 2002;65(2):166-76.
7. Eckardt K-U, Coresh J, Devuyst O, Johnson RJ, Köttgen A, Levey AS, et al. Evolving importance of kidney disease: from subspecialty to global health burden. Lancet. 2013;382(9887):158-69.
8. Jha V, Garcia-Garcia G, Iseki K, Li Z, Naicker S, Plattner B, et al. Chronic kidney disease: global dimension and perspectives. Lancet. 2013;382(9888):260-72.
9. Lameire NH, Bagga A, Cruz D, De Maeseneer J, Endre Z, Kellum JA, et al. Acute kidney injury: an increasing global concern. Lancet. 2013;382(9887):170-9.
10. Galle J. Oxidative stress in chronic renal failure. Nephrol Dial Transpl. 2001;16(11):2135-7.
11. Aslam S, Santha T, Leone A, Wilcox C. Effects of amlodipine and valsartan on oxidative stress and plasma methylarginines in end-stage renal disease patients on hemodialysis. Kidney Int. 2006;70(12):2109-15.
12. Lameire N, Vanholder R. Pathophysiologic features and prevention of human and experimental acute tubular necrosis. J Am Soc Nephrol. 2001;12(suppl 17):S20-S32.
13. Russo A, Borrelli F. *Bacopa monniera*, a reputed nootropic plant: an overview. Phytomedicine. 2005;12(4):305-17.
14. Sudharani D. Pharmacological profiles of *Bacopa monnieri*: a review. Int J Pharm. 2011;1(1):15-23.
15. Vollala VR, Upadhya S, Nayak S. Effect of *Bacopa monniera* Linn.(brahmi) extract on learning and memory in rats: A behavioral study. J Vet Behav. 2010;5(2):69-74.
16. Sairam K, Dorababu M, Goel R, Bhattacharya S. Antidepressant activity of standardized extract of *Bacopa monniera* in experimental models of depression in rats. Phytomedicine. 2002;9(3):207-11.
17. Mathew J, Balakrishnan S, Antony S, Abraham PM, Paulose C. Decreased GABA receptor in the cerebral cortex of epileptic rats: effect of *Bacopa monnieri* and Bacoside-A. J Biomed Sci. 2012;19(1):25.
18. Saraf MK, Prabhakar S, Khanduja KL, Anand A. *Bacopa monniera* attenuates scopolamine induced impairment of spatial memory in mice. Evid Based Complement Alternat Med. 2011;2011:1-10.
19. Palanichamy V, Rajasekaran C, Kalaivani T, Sasirekha M, Arunraj D. In vitro evaluation of antibacterial activity of phytochemical extracts from aerial parts of *Bacopa monnieri* (L.) Wettest (Scorphulariaceae). J Pharm Res. 2012;5(3):1636-9.
20. Ghosh T, Maity TK, Sengupta P, Dash DK, Bose A. Antidiabetic and in vivo antioxidant activity of ethanolic extract of *Bacopa monnieri* Linn. aerial parts: a possible mechanism of action. Iran J Pharmacol Ther. 2010;7(1):61-8.
21. Viji V, Helen A. Inhibition of lipoxygenases and cyclooxygenase-2 enzymes by extracts isolated from *Bacopa monniera* (L.) Wettst. J Ethnopharmacol. 2008;118(2):305-11.
22. Kamkaew N, Scholfield CN, Ingkaninan K, Maneesai P, Parkington HC, Tare M, et al. *Bacopa monnieri* and its constituents is hypotensive in anaesthetised rats and vasodilator in various artery types. J Ethnopharmacol. 2011;137:790-5.

23. Janani P, Sivakumari K, Geetha A, Ravisankar B, Parthasarathy C. Chemopreventive effect of bacoside A on N-nitrosodiethylamine-induced hepatocarcinogenesis in rats. J Cancer Res Clin Oncol. 2010;136(5):759-70.
24. Dar A, Channa S. Relaxant effect of ethanol extract of *Bacopa monniera* on trachea, pulmonary artery and aorta from rabbit and guinea pig. Phytother Res. 1997;11(4):323-5.
25. Dar A, Channa S. Calcium antagonistic activity of *Bacopa monniera* on vascular and intestinal smooth muscles of rabbit and guinea-pig. J Ethnopharmacol. 1999;66(2):167-74.
26. Sairam K, Rao CV, Babu MD, Goel R. Prophylactic and curative effects of *Bacopa monniera* in gastric ulcer models. Phytomedicine. 2001;8(6):423-30.
27. Abbas M, Subhan F, Mohani N, Rauf K, Ali G, Khan M. The involvement of opioidergic mechanisms in the activity of *Bacopa monnieri* extract and its toxicological studies. Afr J Pharm Pharmacol. 2011;5(8):1120-4.
28. Chaudhuri P, Srivastava R, Kumar S. Phytotoxic and antimicrobial constituents of *Bacopa monnieri* and *Holmskioldia sanguinea*. Phytother Res. 2004;18(2):114-7.
29. Russo A, Izzo AA, Borrelli F, Renis M, Vanella A. Free radical scavenging capacity and protective effect of *Bacopa monniera* L. on DNA damage. Phytother Res. 2003;17(8):870-5.
30. Sumathi T, Veluchamy G. Inhibitory effect of *Bacopa monniera* on morphine induced pharmacological effects in mice. Nat Prod Sci. 2007;13(1):46-53.
31. Limpeanchob N, Jaipan S, Rattanakaruna S, Phrompittayarat W, Ingkaninan K. Neuroprotective effect of *Bacopa monnieri* on beta-amyloid-induced cell death in primary cortical culture. J Ethnopharmacol. 2008;120(1):112-7.
32. Mohanty IR, Maheshwari U, Joseph D, Deshmukh Y. *Bacopa monniera* protects rat heart against ischaemia–reperfusion injury: role of key apoptotic regulatory proteins and enzymes. J Pharm Pharmacol. 2010;62(9):1175-84.
33. Sumathy T, Subramanian S, Govindasamy S, Balakrishna K, Veluchamy G. Protective role of *Bacopa monniera* on morphine induced hepatotoxicity in rats. Phytother Res. 2001;15(7):643-5.
34. Sumathi T, Devaraj S. Effect of *Bacopa monniera* on liver and kidney toxicity in chronic use of opioids. Phytomedicine. 2009;16(10):897-903.
35. Menon BR, Rathi M, Thirumoorthi L, Gopalakrishnan V. Potential effect of *Bacopa monnieri* on nitrobenzene induced liver damage in rats. Indian J Clin Biochem. 2010;25(4):401-4.
36. Ghosh T, Kumar Maity T, Das M, Bose A, Kumar Dash D. In vitro antioxidant and hepatoprotective activity of ethanolic extract of *Bacopa monnieri* Linn. aerial parts. Iran J Pharmacol Ther. 2007;6(1):77-85.
37. Rauf K, Subhan F, Abbas M, Badshah A, Ullah I, Ullah S. Effect of bacopasides on acquisition and expression of morphine tolerance. Phytomedicine. 2011;18:836-42.
38. Molyneux P. The use of the stable free radical diphenylpicrylhydrazyl (DPPH) for estimating antioxidant activity. Songklanakarin J Sci Technol. 2004;26(2):211-9.
39. Mishra K, Ojha H, Chaudhury NK. Estimation of antiradical properties of antioxidants using DPPH assay: A critical review and results. Food Chem. 2012;130(4):1036-43
40. Janakat S, Al-Merie H. Optimization of the dose and route of injection, and characterisation of the time course of carbon tetrachloride-induced hepatotoxicity in the rat. J Pharmacol Toxicol Methods. 2002;48(1):41-4.
41. Hamed MA, Ali SA, Saba El-Rigal N. Therapeutic potential of ginger against renal injury induced by carbon tetrachloride in rats. Sci World J. 2012;2012(2012):1-12.
42. Basu S. Carbon tetrachloride-induced lipid peroxidation: eicosanoid formation and their regulation by antioxidant nutrients. Toxicology. 2003;189(1):113-27.
43. Manibusan MK, Odin M, Eastmond DA. Postulated carbon tetrachloride mode of action: a review. J Environ Sci Heal C. 2007;25(3):185-209.
44. Shimizu H, Uetsuka K, Nakayama H, Doi K. Carbon tetrachloride-induced acute liver injury in Mini and Wistar rats. Exp Toxicol Pathol. 2001;53(1):11-8.
45. Ozturk F, Ucar M, Ozturk IC, Vardi N, Batcioglu K. Carbon tetrachloride-induced nephrotoxicity and protective effect of betaine in Sprague-Dawley rats. Urology. 2003;62(2):353-6.
46. Khan MR, Rizvi W, Khan GN, Khan RA, Shaheen S. Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of *Digera muricata*. J Ethnopharmacol. 2009;122(1):91-9.
47. Quan J, Li T, Zhao W, Xu H, Qiu D, Yin X. Hepatoprotective effect of polysaccharides from *Boschniakia rossica* on carbon tetrachloride-induced toxicity in mice. J Clin Biochem Nutr. 2013;52(3):244-52.
48. Stickel F, Schuppan D. Herbal medicine in the treatment of liver diseases. Dig Liver Dis. 2007;39(4):293-304.
49. Dhiman RK, Chawla YK. Herbal medicines for liver diseases. Dig Dis Sci. 2005;50(10):1807-12.
50. Ngai HH, Sit W-H, Wan JM. The nephroprotective effects of the herbal medicine preparation, WH30+, on the chemical-induced acute and chronic renal failure in rats. American J Chinese Med. 2005;33(03):491-500.
51. Ghosh T, Dash GK, Nayak SS. Hepatoprotective activity of ethanolic extract of *Bacopa monnieri* Linn. aerial parts against CCl_4-induced hepatotoxicity in rats. Nat Prod Sci. 2007;13(1):61-7.
52. Wong C-C, Li H-B, Cheng K-W, Chen F. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem. 2006;97(4):705-11.
53. Rufino MS, Fernandes FA, Alves RE, De Brito ES. Free radical-scavenging behaviour of some north-east Brazilian fruits in a DPPH system. Food Chem. 2009;114(2):693-5.
54. Loo A, Jain K, Darah I. Antioxidant and radical scavenging activities of the pyroligneous acid from a mangrove plant, *Rhizophora apiculata*. Food Chem. 2007;104(1):300-7.
55. Anand T, Naika M, Swamy M, Khanum F. Antioxidant and DNA damage preventive properties of *Bacopa Monniera* (L) Wettst. Free Radical Antioxid. 2011;1(1):84-90.
56. Puntambekar HM, Upadhye AS, Desai AC, Srivastava P, Raut HN. Stability studies of crude plant material of *Bacopa monnieri* and Its effect on free radical scavenging activity. Journal of Phytology. 2010;2(8):103-9.
57. Sathiyanarayanan L, Paradkar AR, Mahadik KR. In vivo and in vitro antioxidant activity of lipid based extract of *Bacopa monniera* Linn. compared to conventional extract and traditional preparation. Eur J Integr Med. 2010;2(2):93-101.

Concentration (µg/ml)	Percent inhibition (%)		
	Ascorbic acid	BHT	mBME
1	63.03 ± 0.8735	4.393 ± 1.9313	16.89 ± 1.1403
10	91.62 ± 0.4177	55.12 ± 0.7786	37.82 ± 3.5645
30	95.45 ± 0.2281	85.91 ± 0.6804	57.42 ± 3.9432
50	96.11 ± 0.0443	90.63 ± 0.0593	93.37 ± 0.2042
100	96.18 ± 0.1618	91.54 ± 0.0758	91.34 ± 0.7227
200	95.97 ± 0.0356	91.24 ± 0.4984	89.68 ± 1.1838
400	95.99 ± 0.0663	92.09 ± 0.1247	87.94 ± 1.2421
600	95.68 ± 0.0725	91.89 ± 0.2424	83.97 ± 2.5751
800	95.72 ± 0.0544	92.18 ± 0.2191	86.59 ± 0.2987
1000	96.15 ± 0.0675	91.98 ± 0.1944	80.32 ± 2.4823

Table 1: DPPH free radical scavenging activity of mBME or standards (ascorbic acid and BHT). Results are mean ± SEM of three different experiments

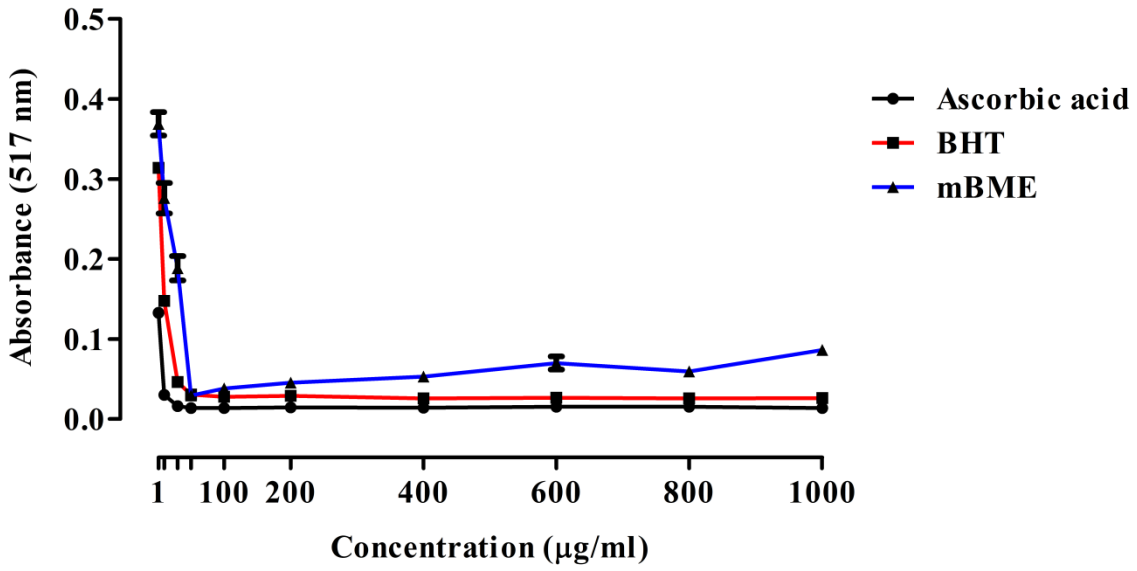

Figure 1: DPPH free radical scavenging activity of mBME or standards (ascorbic acid and BHT). Results are mean ± SD of three different experiments.

Standards/ Extract	EC_{50} (µg/ml)	Antiradical power	Stoichiometry
Ascorbic acid	4.510 ± 0.3596	0.222 ± 0.0177	9.020 ± 0.7192
BHT	8.561 ± 0.3640	0.116 ± 0.0049	17.11 ± 0.7300
mBME	19.20 ± 1.7230	0.052 ± 0.0048	38.40 ± 3.4453

Table 2: The $EC_{50,}$ antiradical power and stoichiometry of mBME or standards (ascorbic acid and BHT). Results are mean ± SD of three different experiments

Organs	Group I (Control)	Group II (CCl_4)	Group III (mBME + CCl_4)	Group IV (mBME)
Liver	8.650 ± 0.104	8.553 ± 0.384	8.367 ± 0.401	8.648 ± 0.195
Right kidney	0.835 ± 0.008	0.646 ± 0.033	0.631 ± 0.002	0.829 ± 0.014
Left kidney	0.853 ± 0.008	0.610 ± 0.031	0.623 ± 0.002	0.803 ± 0.020

Table 3: Effect of mBME or carbon tetrachloride alone or in combination on weight of liver and kidneys. Values are expressed as mean ± SEM; *n* = 5

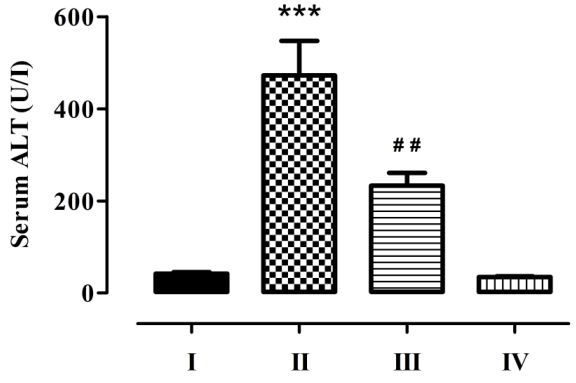

Figure 2: Serum analysis of ALT after treatment with mBME or carbon tetrachloride alone or in combination. Values are expressed as mean ± SEM; *n* = 5. Group I (Control), Group II (CCl_4), Group III (mBME+ CCl_4), Group IV (mBME). $^{***}p < 0.001$ compared to control, $^{\#\#}p < 0.01$ compared to CCl_4.

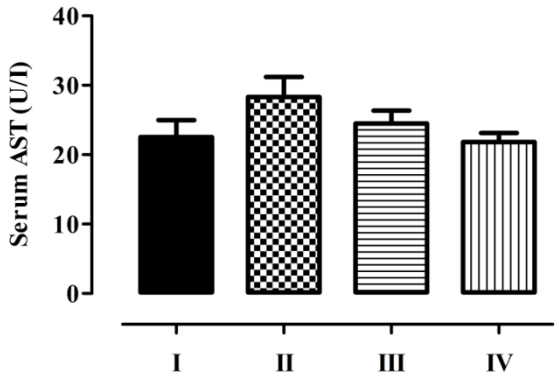

Figure 3: Serum analysis of AST after treatment with mBME or carbon tetrachloride alone or in combination. Values are expressed as mean ± SEM; *n* = 5. Group I (Control), Group II (CCl_4), Group III (mBME+ CCl_4), Group IV (mBME).

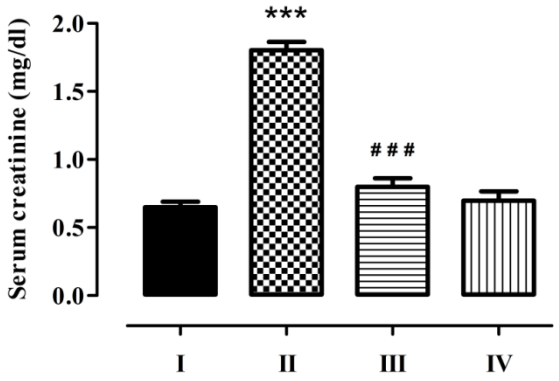

Figure 4: Serum analysis of creatinine after treatment with mBME or carbon tetrachloride alone or in combination. Values are expressed as mean ± SEM; *n* = 5. Group I (Control), Group II (CCl_4), Group III (mBME+ CCl_4), Group IV (mBME). $^{***}p < 0.001$ compared to control, $^{###}p < 0.001$ compared to CCl_4.

Group (Treatment)	Ballooning degeneration	Sinusoidal dilatation	Perivenular necrosis	Steatosis
Group I (Control)	–	–	–	–
Group II (CCl_4)	+++	++	+++	+++
Group III (CCl_4 + mBME)	+	+	+	++
Group IV (mBME)	–	–	–	–

Table 4: Effect of *Bacopa monniera* methanol extract on carbon tetrachloride induced changes on liver morphology; (–) none; (+) mild; (++) moderate; (+++) severe

Group (Treatment)	Tubular cell swelling	Interstitial inflammation	Tubular dilatation	Necrosis of epithelium
Group I (Control)	–	–	–	–
Group II (CCl_4)	++	+++	++	++
Group III (CCl_4 + mBME)	–	–	–	–
Group IV (mBME)	–	–	–	–

Table 5: Effect of *Bacopa monniera* methanol extract on carbon tetrachloride induced changes on renal morphology; (–) none; (+) mild; (++) moderate; (+++) severe

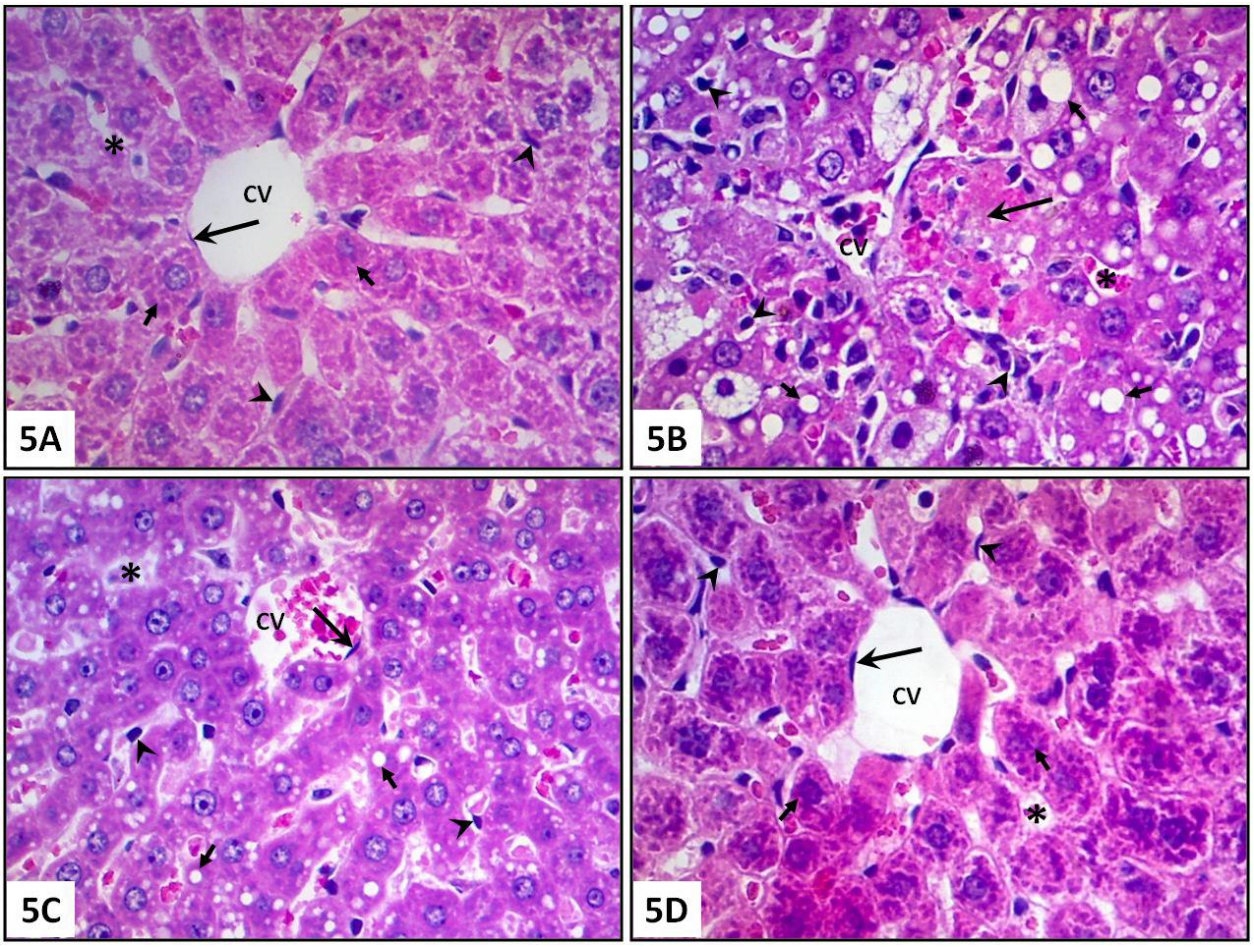

Figure 5: Histological evaluation of carbon tetrachloride induced hepatotoxicity after 14 days of treatment with mBME (H & E; 400x original magnification). **(5A):** Photomicrograph of a section of liver from a rat treated with vehicle showing normal central vein (CV) bounded by an endothelium, plates of hepatocytes (small arrows) and sinusoidal spaces (asterisk) lined by endothelial cells (arrow heads) containing red blood cells. **(5B):** Photomicrograph of a section of liver from a rat treated with carbon tetrachloride showing severe shrinkage of central vein (CV), necrotic hepatocytes (large arrow), ballooning degeneration, microvesicular as well as macrovesicular steatosis (small arrows) and dilatation of sinusoidal spaces (asterisks) infiltrated with red blood cells and lymphocytes (arrow heads). **(5C):** Photomicrograph of a section of liver from a rat treated with mBME and carbon tetrachloride showing congestion of central vein (CV) with red blood cells bounded by an intact endothelium (large arrow), microvesicular steatosis (small arrows) and mild dilatation of sinusoidal spaces (asterisk) infiltrated with lymphocytes (arrow heads). **(5D):** Photomicrograph of a section of liver from a rat treated with mBME alone showing a central vein (CV) bounded by an intact endothelium (large arrow), hepatocytes (small arrows) and sinusoidal spaces (asterisk) lined by endothelial cells (arrow heads).

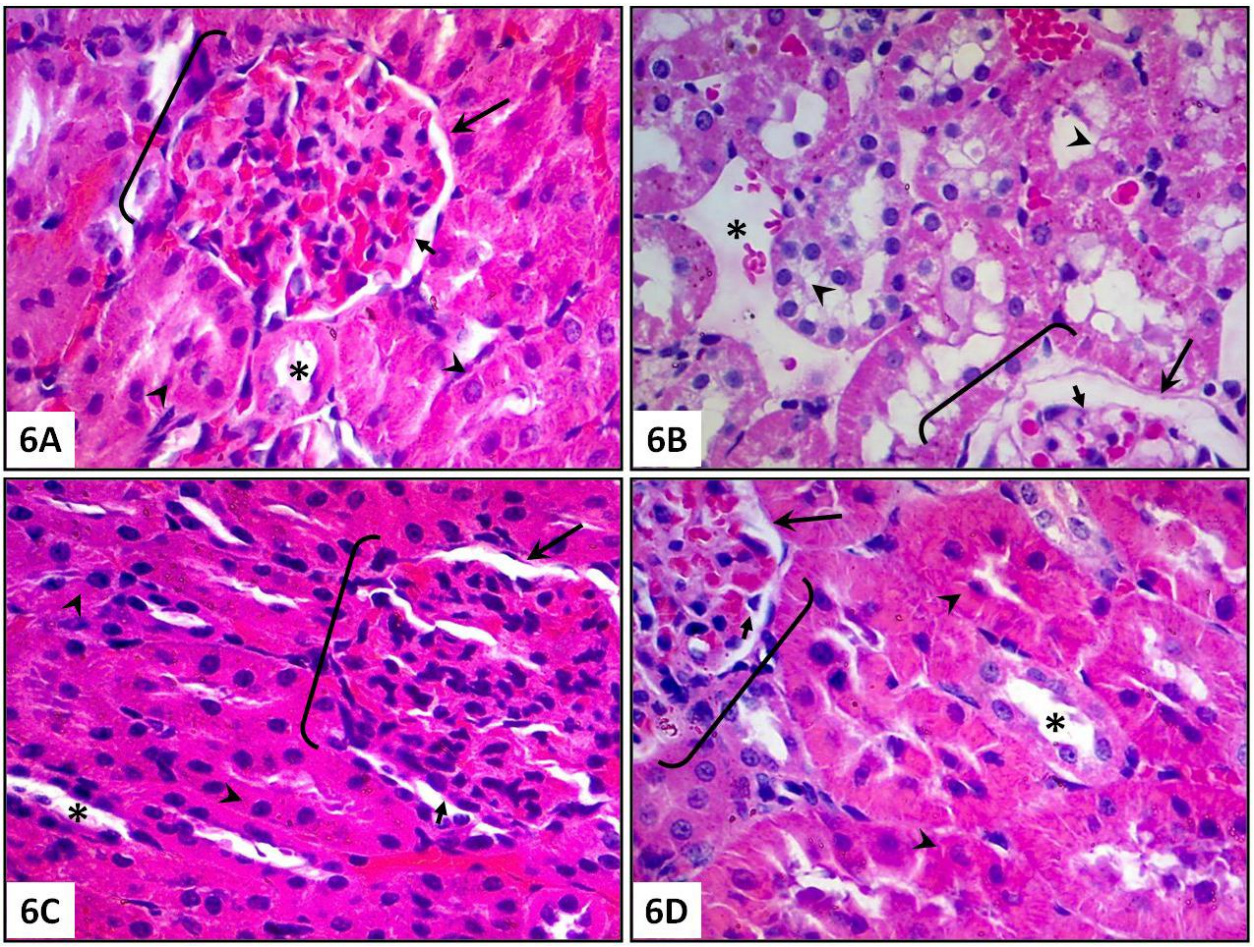

Figure 6: Histological evaluation of carbon tetrachloride induced nephrotoxicity after 14 days of treatment with mBME (H & E; 400x original magnification). **(6A):** Photomicrograph of a section of kidney from a rat treated with vehicle showing a normal renal corpuscle (bar) having a glomerulus bounded by parietal (large arrow) and visceral (small arrow) layers of Bowman's capsule. The numerous proximal convoluted tubules (arrow heads) have a narrow lumen while the distal convoluted tubule (asterisk) has a wider lumen. **(6B):** Photomicrograph of a section of kidney from a rat treated with carbon tetrachloride showing dilatation of renal tubules with destruction of brush border and necrosis of cuboidal epithelial cells (arrow heads), widening of interstitial spaces and increase in the capsular space between parietal (large arrow) and visceral (small arrow) layers of the renal corpuscle (bar). Normal histology of renal corpuscle (bar) bounded by visceral (small arrow) and parietal (large arrow) layers, proximal (arrow heads) and distal (asterisk) convoluted tubules were found in groups of rats treated with **(6C):** mBME and carbon tetrachloride and **(6D):** mBME alone.