Advances in Biological Research 9 (4): 242-246, 2015
ISSN 1992-0067

DOI: 10.5829/idosi.abr.2015.9.94109

Hepatoprotective Activity of *Adhatoda vasica* and *Vitex negundo* Leaf Extracts Against Carbon Tetrachloride Induced Hepatotoxicity in Rats

[1]*Manoj Kumar,* [2]*Sukumar Dandapat and* [2]*Manoranjan Prasad Sinha*

[1]Department of Zoology, St. Xavier's College, Ranchi-834001, India
[2]Department of Zoology, Ranchi University, Ranchi-834008, India

Abstract: Liver diseases such as jaundice, cirrhosis etc. are very common worldwide and traditional medicine are proving to be useless or less effective against them. Therefore workers are trying to find out alternate medicine or formulations to combat these diseases. In the present study hepatoprotective activity of aqueous extract of *Adhatoda vasica* and *Vitex negundo* on total protein, bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) in CCl_4 intoxicated rats was studied. Administration of CCl_4 showed significant increase ($p < 0.01$) in elevation of liver marker enzymes in serum namely AST (107.2 ± 13.17), ALT (206.2 ± 28.82), ALP (508.22 ± 10.22), total bilirubin (2.61 ± 0.27) and significantly decreased total protein (5.2 ± 0.085) when compared to normal. *Av* and *Vn* at 250 mg/kg and 500 mg/kg body weight showed significant increase in total protein (8.0 ± 0.05, 7.11 ± 0.044; 7.02 ± 0.02, 6.23 ± 0.03) when compared to CCl_4 treated rats. Both *Av* and leaf extracts lowered enzymes levels, which is a designation of hepatoprotective action of the extracts. The serum AST, ALT and ALP levels are reliable markers of liver function. Thus the present study concludes the aqueous leaf extract of *Adhatoda vasica* and *Vitex negundo* to possess hepatoprotective activity.

Key words: Alanine Transaminase · Alkaline Phosphatase · CCl_4 · Hepatoprotective

INTRODUCTION

Liver diseases, such as jaundice, cirrhosis and fatty liver are very common worldwide. In India, numerous medicinal plants such as *Psidium guajava*, *Coccinia indica* etc... are used for treatment of liver disorders. *Adhatoda vasica* and *Vitex negundo* are traditionally used medicinal plants. *Adhatoda vasica* and *Vitex negundo* have been reported to possess antioxidant and reducing power ability [1], they also possess growth inhibitory effect on pathogens causing typhoid [2]. Both *Adhatoda vasica* and *Vitex negundo* possess many trace elements important for the normal growth and function of the body [3]. Some plants have been found, scientifically, to possess hepatoprotective activity and the underlying mechanisms involves their antioxidant property [4]. Free radicals or oxidative injury now appears to be the fundamental mechanisms behind a number of human diseases [5]. Oxidative stress has been implicated in the pathogenesis of acute and chronic liver injury in a variety of pathophysiological conditions such as hepatotoxin exposure, intrahepatic cholestatis, alcoholic liver injury, liver ischemia and viral hepatitis [6-9]. Effects of antioxidants or free radical scavengers have been widely tested for the prevention and treatment of acute and chronic liver injuries. In some of the studies, antioxidants have shown beneficial effects, specifically for prevention and treatment of chronic liver injury [10, 11]. The present study was undertaken to investigate the hepatoprotective activity of sequential extracts of *Adhatoda vasica* and *Vitex negundo* aqueous leaf extracts in CCl_4 induced hepatotoxicity in rats.

MATERIALS AND METHODS

Plant Materials: the fresh tender leaves of *Adhatoda vasica* and *Vitex negundo* were collected. The leaves were washed with deionized water and disinfected with 0.1% $HgCl_2$ solution for 5 min and dried in shade away from light for 15 days and ground to fine powder using electrical grinded and sieved [12].

Corresponding Author: Manoj Kumar, Department of Zoology, St. Xavier's College, Ranchi-834001, India.
Tel: +9708550235.

Preparation of Plant Extracts: The fine powders of *Adhatoda vasica* and *Vitex negundo* were made into thimble for loading in Soxhlet apparatus and extraction was done using H_2O. The extraction was done continuously for 72 hours. The extracts thus obtained were concentrated under vacuum rotary evaporator and extracts were kept in desiccator until used [13-16].

Phytochemical Screening: Preliminary phytochemical screening were conducted on *Adhatoda vasica* and *Vitex negundo* leaf sample with previously published standards [2].

Antioxidant Activity: The antioxidant properties of plant samples were determined by Spectrophotometric quantitation method [17-19]. Various concentrations of samples (5 µg, 50 µg, 100 µg) were taken in a series of test tubes. The 1.9mL of reagent solution (0.6m Sulphuric acid, 28mm Sodium phosphate and 4mm Ammonium molybdate) was added to the test tubes. The tubes were incubated at 95°C for 90 min and allowed to cool down. The absorbance of aqueous solution of each was measured at 695nm against blank. Antioxidant capacities were expressed as equivalents of ascorbic acid. Butylated hydroxyl anisole (BHA) was used as reference standard.

Animals: Albino rats weighing about 175-200 g were used in the study. They were maintained under standard laboratory conditions at ambient temperature of 25 ± 2°C and relative humidity at 50 ± 15 %, with dark-light cycle of 12h. Animals were fed with a commercial pellet diet and water *ad libitum*. The experiment was performed after prior approval of Ethics committee of Ranchi University, Ranchi (Proceeding no. 46, page no. 137).

Acute Toxicity Studies: the acute toxicity studies were carried out as per stair case method [20]. 50 albino mice of either sex weighing 20-25g and 90 days were used to determine LD_{50} of various extracts. The mice were divided into 3 groups of 10 mice each as follows for *Adhatoda vasica* and *Vitex negundo* each.

Group 1: Received 1 ml of distilled water orally.
Group 2: Received 0.1 ml/Kg/day i.p. of CCl_4
Group 2: received 250 mg/Kg body weight of extract orally.
Group 3: Received 500 mg/Kg body weight of extract orally

Mortality was not observed up to 500 mg/Kg body weight of both extracts.

Assessment of Hepatoprotective Activity: All the animals were sacrificed on day 14 under light ether anesthesia. 5 ml of blood was collected from each animal by cardiac puncture using sterile needle and syringe. Part of the blood sample was put into test tubes and allowed to clot for 30 min at 37 °C. The clear serum was separated at 2500 rpm for 10 min and biochemical investigations were carried out to assess liver function *viz.* total bilirubin [21], total protein [21], serum transaminases [22] and serum alkaline phosphatase [23].

RESULTS AND DISCUSSION

The results of phytochemical screening of leaf extract of *Adhatoda vasica* and *Vitex negundo* is presented in figure 1 and 2, respectively. The antioxidant activity of *Adhatoda vasica* and *Vitex negundo* is summarized in figure 3. Phytochemicals-alkaloids, tannins, saponins and flavonoids were detected in *Adhatoda vasica* and *Vitex negundo* (Figure 1 and 2, respectively). Medicinal properties of plants are due to secondary metaites (Phytochemicals) present in different parts of plants (Growth inhibitory impact). The phenols possess redox properties and thus impart antioxidant property to plants in which they are present. They act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators [2, 24].

The effect of aqueous extract of *Adhatoda vasica* leaf extract on total protein, bilirubin, aspartate aminotransferase, alanine transaminase and alkaline phosphatase in toxicity induced rats and data of controlled rat is summarized in Table 1.

Administration of CCl_4 showed significant increase ($p < 0.01$) in elevation of liver marker enzymes in serum namely AST (107.2 ± 13.17), ALT (206.2 ± 28.82), ALP (508.22 ± 10.22) and total bilirubin (5.2 ± 0.085) when compared to normal control respectively. Total protein was significantly reduced following CCl_4 administration (5.69 ± 0.09) when compared to normal control rats. The aqueous leaf extract of *Adhatoda vasica* at a dose of 250 mg/Kg body weight and 500 mg/kg body weight showed significant increase in total protein (Table 1) levels when compared to CCl_4 intoxicated rats.

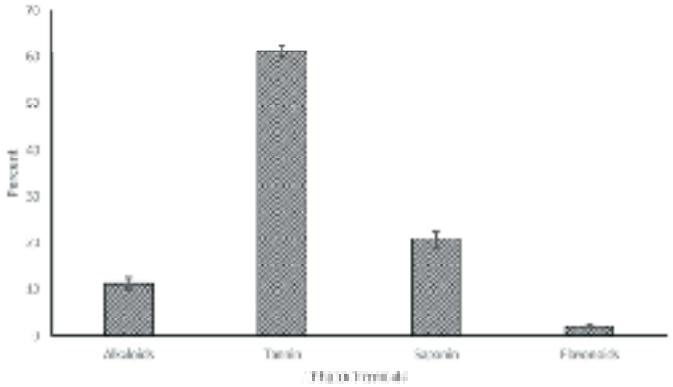

Fig. 1: Phytochemical screening of *Adhatoda vasica*

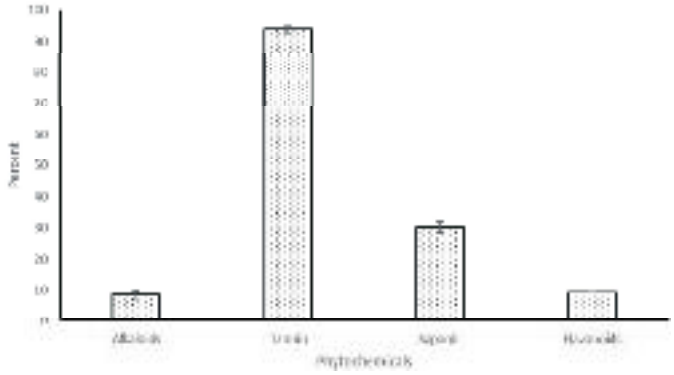

Fig. 2: Phytochemical screening of *Vitex negundo* aqueous leaf extract.

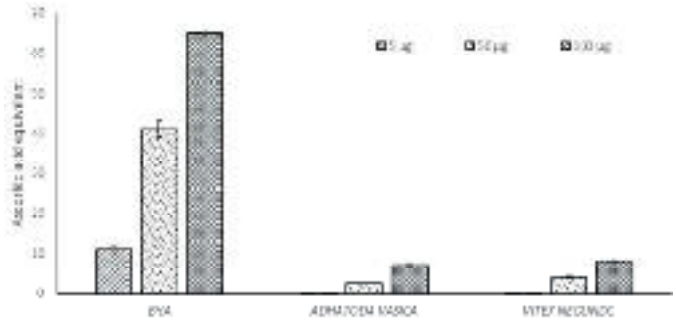

Fig. 3: Antioxidant potency of *Adhatoda vasica* and *Vitex negundo*

Table 1: Effect of *Adhatoda vasica* aqueous leaf extract against CCl_4 induced hepatotoxicity in rats

Groups (n)	Total Protein (g/dl)	Bilirubin (g/dl)	AST (IU/I)	ALT (IU/I)	ALP (IU/I)
Control	9.57 ± 0.17	0.52 ± 0.09	52.30 ± 1.15	146.63 ± 5.79	176.24 ± 5.80
CCl_4 Treated	5.2 ± 0.085	2.61 ± 0.27	107.2 ± 13.17	206.2 ± 28.82	508.2 ± 10.22
Group 1	8.0	0.50	30.05	35.76	120.05
Group 2	7.02	0.90	28.5	46.7	168.0

Table 2: Effect of *Vitex negundo* aqueous leaf extract against CCl_4 induced hepatotoxicity in rats.

Groups (n)	Total Protein (g/dl)	Bilirubin (g/dl)	AST (IU/I)	ALT (IU/I)	ALP (IU/I)
Control	9.57 ± 0.17	0.52 ± 0.09	52.30 ± 1.15	146.63 ± 5.79	176.24 ± 5.80
CCl_4 Treated	5.69 ± 0.09	2.61 ± 0.27	1072.14 ± 13.17	2062.17 ± 28.82	513.04 ± 13.20
Group 1	7.11	0.80	26.8	45.6	101.1
Group 2	6.23	0.70	24.2	38.7	115.02

The effect of aqueous extract of *Vitex negundo* leaf extract on total protein, bilirubin, aspartate aminotransferase, alanine transaminase and alkaline phosphatase in toxicity induced rats and data of controlled rat is summarized in Table 2.

The aqueous leaf extract of *Vitex negundo* at a dose of 250 mg/kg body weight and 500 mg/kg body weight showed consequential increase in total protein (Table 2) levels when compared to CCl_4 intoxicated rats.

The CCl_4 has been utilized as an implement to induce hepatotoxicity in experimental animals [25, 26]. The hepatotoxicity induced by CCl_4 due to metaite CCl_3, a free radical that binds to lipoprotein and leads to peroxidation of lipids of the endoplasmic reticulum [27] this toxic chemical caused peroxidative degradation in adipose tissue resulting in fatty infiltration of hepatocytes. The incrementation in the calibers of serum bilirubin reflected the depth of jaundice and the incrementation in serum transaminases and alkaline phosphatases was the clear designation of cellular damage and loss of functional integrity of the cell membrane [28].

The lowering of enzyme levels is a definite designation of hepatoprotective action of the drug. The serum AST, ALT and ALP levels are reliable marker of liver function [28] in our study, the consequential elevation of marker enzymes following administration of CCl_4 betokened earnest toxicity engendered by the chemical. In CCl_4 induced hepatitis administration of aqueous leaf extract of *Adhatoda vasica* and *Vitex negundo* engendered consequential reduction in AST, ALT and ALP activities and total bilirubin levels while exhibiting a paramount increase in total protein. Thus it could be suggested that, *Adhatoda vasica* and *Vitex negundo* leaf extract possesses hepatoprotective activity in this model.

REFERENCES

1. Cook, N.C. and S. Samman, 1996. Flavonoids: chemistry, metaism, cardioprotective effects and dietary sources. Nutr. Biochem., 7: 66-76.
2. Kumar, M., A. Kumar, S. Dandapat and M.P. Sinha, 2013. Phytochemical screening and antioxidant potency of *Adhatoda vasica* and *Vitex negundo*. The Bioscan., 8(2): 727-730.
3. Kumar, M., S. Dandapat, A. Kumar and M.P. Sinha, 2013. Anti-typhoid activity of *Adhatoda vasica* and *Vitex negundo*. Persian Gulf Crop Protection, 2(3): 64-75.
4. Kumar, M., S. Dandapat, A. Kumar and M.P. Sinha, 2013. Determination of nutritive value and mineral elements of Five-leaf Chaste Tree (*Vitex negundo* L.) and Malabar Nut (*Adhatoda vasica* Nees). Academic Journal of Plant Sciences, 6(3): 103-108.
5. Parvathi, K.M.M., C.K. Ramesh, V. Krishna, Shankarmurthy, I.J. Kuppast and N.M. Shakuntala, 2013. Hepatoprotective activity of *Anogeissus latifolia* leaf and bark extracts against carbon tetrachloride induced hepatotoxicity in rats. The Bioscan, 8(2): 649-653.
6. Atawodi, S.E., 1971. Antioxidant potential of African plants. African Journal of Biotechnology, 9: 91.
7. Laeschke, H., C.V. Smith and J.R. Mitchell, 1988. Reactive oxygen species during ischemica-liver injury in isolated perfused rat liver. J. Clin. Investigation, 81: 1250.
8. McDonough, K.H, 2003. Antioxidant nutrients and alcohol. Toxicology,189: 89.
9. Stephens, W.E., oxidative stress, toxic hepatitis and antioxidants with particular emphasis on Zinc. In Exp. Biology, 31: 316.
10. Kukongviriyapan, V., T. Janyacharoen, U. Kukongviriyapan, P. Laupattarakasaem, S. Kanokmedhkul and P. Chantaranothai, 2003. Hepatotoprotective and antioxidant activities of Tetracera loureiri. Phytother. Research, 17: 717.
11. Kumar, M., S. Dandapat and M.P. Sinha, 2014. Pharmacological Screening of leaf extract of *Adhatoda vasica* for therapeutic efficacy. Global Journal of Pharmacology, 8(4): 494-500.
12. Kumar, M., S. Dandapat and M.P. Sinha, 2014. Plant mediated Synthesis of silver nanoparticles using *Adhatoda vasica* aqueous leaf extract, The Ecoscan, V. (special issue), pp: 29-36.
13. Dandapat, S., M. Kumar, A. Kumar and M.P. Sinha, 2013. Antipathogenic efficacy of methanolic leaf extract of *Cinnamomum tamala* and *Aegle marmelos* with their nutritional potentiality. In The Bioscan; 8(2): 635-341.
14. Dandapat, S., M. Kumar, A. Kumar and M.P. Sinha, 2014. Therapeutic efficacy of Cinnamomum tamala (Buch.-Ham.) and *Aegle marmelos* (L.) leaf. In Balneo Res. J., 5(3): 113-122.
15. Kumar, M., S. Dandapat, M.P. Sinha, Effect of *Vitex negundo* aqueous extract on serum lipid variables and hematological profile of albino rats. The Ecoscan, V. (Special issue), pp: 135-150.

16. Dandapat, S., M. Kumar, A. Kumar and M.P. Sinha, 2014. Effects of *Aegle marmelos* (L.) leaf extract and green nanoparticles on lipid profile. The Ecoscan V. (special issue), pp: 157-167.
17. Prieto, P. and M. Pineda, 1999. Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of Vitamin E1. Analytical Biochemistry, 269: 337-341.
18. Ferreira, I.C.F.R., M. Baptista Vilas-Boas and L. Barros, 2007. Free radical scavenging activity and reducing power of wild edible mushrooms from northeast Portugal: An individual cap and stipe activity. In Food Chem, 100: 1511-1516.
19. Ghosh, M.N., 1984. Fundamentals of experimental pharmacology, 2nd ed. Calcutta. Scientific Book Agency, pp:156.
20. Mallory, H.T. and E.A. Evelyn, 1956. The determination of bilirubin with photoelectric colorimeter. J. Biol. Chem.., 119: 481.
21. Kingsley, S.R. and S.J. Frankel, 1939. The determination of serum total protein albumin and globulin by the biuret reaction. J. Biol. Chem., pp: 128-131.
22. Reitman, S. and S.A. Frankel, 1957. Colorimetric method for the determination of serum goutamisc oxolacetic and pyruvic transaminase, Am. J. Clin. Pathol., pp: 28p56.
23. Bessey, O.A., D.H. Lowery and M.J. Brock, 1964. A method for the rapid determination of alkaline phosphatase with five cubic meters of serum. J. Biol. Chem., 164: 321.
24. Kumar, M., S. Dandapat, A. Kumar and M.P. Sinha, 2013. Growth inhibitory impact of *Adhatoda vasica* and *Vitex negundo* on some human pathogens, The Bioscan, Special issue (IV): 241-246.
25. Recnagel, R.O., 1983. Carbon tetrachloride hepatotoxicity status quo and future prospects. Trends Pharmacol. 4: 129-131.
26. Okuno, H., H. Hazama, T. Muzare and S. Shiozaki, 1986. Drug metaizing activity in rats with chronic liver injury induced by carbon tetrachloride relationship with hydroxyproline content in liver. Japan Journal of Pharmacology, 41: 363.
27. Recknagacl, R., 1967. Carbon tetrachloride hepatotoxicity. Pharmacological reviews, 19: 145.
28. Sarwath, B., P.K. Visen, G.K. Patnaik and B.N. Dhawan, 1993. Anticholestics effect of picrolive active hepatoprotective principle of *Picrorhiza kur*rooa, against carbon tetrachloride induced cholestatis. In Indian J. Exp. Biol., 31: 316.