INTERNATIONAL JOURNAL OF PHARMACEUTICS & DRUG ANALYSIS

VOL.6 ISSUE 2, 2018; 225 – 231 ; http://ijpda.com; ISSN: 2348-8948

MEDFOOD'18 [1st February 2018]

National Conference on Phytochemicals in Medicinal Plants and Food

Department of Biochemistry, Bharathidasan University Constituent College for Women, Orathanadu - 614 625, Tamil Nadu, India

Research Article

Evaluation of antihepatotoxic effect of *Avicennia marina* against alcohol-induced liver injury

B.Varalakshmi[1*], T.Karpagam[1], A.Shanmugapriya[1], J.Sugunabai[2], B.Ramya[1], P.Anitha[1], N.Abirami[1], K.Parkavi[1], W. Esther Rani[1], S.Kaviyalakshmi[1],

1. Department of Biochemistry, Shrimati Indira Gandhi College,Tiruchirappalli, Tamil Nadu, India
2. Department of Biochemistry, Seethalakshmi Ramaswamy College, Tiruchirappalli, Tamil Nadu, India

Date Received: 23rd January 2018; Date accepted: 29th January 2018; Date Published: 17th February 2018

Abstract

This study aims to evaluate the antihepatotoxic effect of *Avicennia marina* against ethanol induced hepatotoxic rats. Qualitative phytochemical analysis was carried out in alcoholic extract of leaves of *A.marina*. The hepatoprotective effect *A.marina* was investigated against ethanol - induced hepatotoxic (group-III) rats and the activity of *A.marina* was compared with standard drug (Silymarin) treated (Group-IV) rats. Ethanol was used as hepatotoxic inducer for all experimental rats except for normal control (Group-I) rats and ethanol alone was given for disease control (Group-II) rats. Liver marker enzymes in serum (ALT, ALP, AST, GGT), Bilirubin, Protein and histopathological analysis were carried out. Ethanol treatment elevated levels of liver enzymes, decreased protein and histological damage in hepatocytes. However, treatment with *A.marina* significantly reversed the above changes compared with ethanol-challenged rats and was comparable with silymarin treated rats. The results clearly demonstrate that *A.marina* possesses promising antihepatotoxic effect and hence suggests its use as a potential therapeutic agent for protection from ethanol overdose.

Keywords: Ethanol, antihepatotoxic effect, *A.marina*.

INTRODUCTION

Liver plays major role in the metabolism of carbohydrate, protein, fat, detoxification, secretion of bile and storage of vitamin. It is continuously exposed to environmental toxins, abused by poor drug habits, alcohol consumption which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease. Liver disease rates are steadily increasing over the years(1). Liver diseases are recognized as the second leading cause of mortality amongst all digestive diseases in the world(2).Chronic liver disease occurs throughout the world irrespective of age, sex, region or race. According to WHO, about 46% of global diseases and 59% of the mortality is because of chronic diseases and almost 35 million people in the world die of chronic liver diseases(3).

Excess consumption of alcohol is one of the main cause of liver diseases leads to nutritional distur-

bances and liver cells damage[4].The spectrum of alcoholic liver disease ranges from fatty liver to alcoholic hepatitis, ultimately fibrosis and cirrhosis[5].The World Health Organization (WHO) estimated that about 80 percent of the world's population still relies on plant-based medicines for their primary health care.

Avicennia marina commonly called as White or Grey Mangrove, belongs to the family Acanthaceae[6].It is a pioneering species, occurring in mangrove swamps, along seashores and tidal rivers.The leaves of *A. marina* are effective in dispelling inflammatory swelling of joints in acute inflammation, spleen enlargement, catarrhal and the aromatic vapours are employed in baths for the treatment of febrile.

The objective of the present research is to focus on the antihepatotoxic activity of *A.marina leaves* against ethanol induced liver toxicity in albino rats.

MATERIALS AND METHODS

Collection, Identification and Authentication of selected plant

Fresh, healthy and young leaves of *A. marina* were collected from Muthupet, Thiruvarur district, Tamilnadu, India and authenticated by professionals in Department of Botany, St. Joseph's College, Tiruchirappalli, India. The herbarium number of the plant is KM 001.

Preparation of Plant Extract:

The leaves were cleaned,shade dried for 7 days and ground well to fine powder. About 500g of dry powder was extracted with 80%ethanol at 70°C using soxhlet apparatus. The extraction was continued for 24 hours. The ethanolic extract was then filtered and kept in hot air oven at 40°C for 24 hours to evaporate the ethanol, to get a dark brown residue. The residue was stored in a deep freezer and used for analysis.

Preliminary Phytochemical analysis

Phytochemical analysis of the plant extract was done using standard qualitative methods described by Odebiyi and Sofowora[7]. The plant extract was screened for the presence of phytocompounds such as alkaloids, flavonoids, steroids, cardiac glycosides, proteins, tannins, terpenoids, saponins and anthroquinone.

In vivo Antihepatotoxic Actionof *A.marina*

Healthy, adult male albino wistar rats weighing 170-210g were used for the present study. They were kept in plastic cages at animal house maintained at standard temperature and humidity with 12 hours light and dark cycle. The animals were fed with standard pellet diet and water. The ethical clearance was obtained from institutional animal ethical committee as per the Indian CPCSEA guidelines.

Experimental design

20% ethanol was used to induce hepatotoxicity (7.9 g/kg body weight)[8]for 21 days. From 22ndto42ndday the animals were treated with the extract of *A.marina* (100 mg/Kg bw) and standard drug silymarin (1g/kg bw). Inducer and drugs were given orally using intragastric tube.

The animals were divided into four groups. Each group contained six animals.

Group I : Normal control (the animals were given normal saline only).

Group II: Hepatotoxic control (the animals were given 1 ml of 20% alcohol for 21 days).

Group III : Treatment group (the animals were given 1 ml of 20% alcohol for 21 days and from 22nd to 42nd day the extract of *A.marina* was given to animals (100 mg/Kg bw)

Group IV: Treatment group (the animals were given 1 ml of 20% alcohol for 21 days and from 22nd to 42nd days the animals were given silymarin drug (1g/kg.bw).

At the end of the treatment, all the animals were anaesthetized using chloroform and blood samples were collected from each group of animals from dorsal aorta in a vacutainer. Plasma and serum samples were separated and kept at -20°C for biochemical analysis.The whole liver from each animal was removed after killing the animals, was placed in 10% formalin solution.

Assay of liver function

The SGOT, SGPT and ALP levels were assayed using the method of King [9].Serum GGT level was assayed using the method of Rosalki and Rau [10]. The serum bilirubin was assayed by Malloy and Evlyn [11].Serum protein content was estimated by the method of Lowery *et al*.,[12].

Histological Studies

Histology of the liver tissues was performed by the method of Sujai Suneetha [13].The liver in 10 % formalin solution was processed by the paraffin technique, section of 5 μm thickness were cut and stained by haematoxylin and eosin for histological examination. The photomicrographs of histological studies were taken.

Statistical analysis

The data were statistically analyzed and all values were expressed as mean ± SEM. The data were also analyzed by One Way ANOVA using SPSS Software. $P<0.05$ was considered significant.

Results and Discussion

Preliminary Phytochemical analysis

The qualitative phytochemical analysis of ethanolic extracts of *A.marina* leaves revealed the presence of alkaloids, flavonoids, steroids, cardiac glycosides, anthroquinones, tannins, terpenoids and absence of saponins and protein (Table 1).

Alkaloids have been identified to have hepatoprotective and antioxidant activities. The alkaloid has the ability to induce antioxidant enzymes and prevent liver damage[14]. Antihepatotoxic effect of *A.marina* leaves could be attributed to the presence of alkaloids as revealed in the preliminary qualitative phytochemical analysis. Terpenoids have also been proved to have hepatoprotective activity. The presence of terpenoids in leaf extracts of *A.marina* exhibit hepatoprotective properties could serve as a basis for its traditional use as a medicinal plant. The present study agrees with what was reported by Ghoshal *et al*.[14] that alkaloids, and terpenoids are responsible for hepatoprotective activity.

Davila *et al*., [15] studied the hepatoprotective effect of flavonoids on primary cell cultures of neonatal hepatocytes in the presence of hepatotoxin and reported that Catechin and silybin protected the hepatocytes against cell injury produced by erythromycin estolate, amitriptyline, nortriptyline and tert-butylhydroperoxide.

Morin is a kind of flavonoid found in the plants of Moraceae family which are used as dietary agents in herbal medicine[16] and acts as an inhibitor of acute liver damage by blocking the expressions of inflammatory mediators[17].

Sathaye *et al*.,[18] studied the hepatoprotective activity of tannin fraction isolated from aqueous extract of *Murraya koeniggi* against ethanol-induced hepatotoxicity in experimental animals and reported that tannins exhibited excellent hepatoprotective activity and maintained normal morphology even after ethanolic challenge to the cells as comparable to the protection offered by the standard drug L-ornithine L-aspartate (LOLA).

Hepatoprotective Enzymes

Hepatoprotective effects in terms of activities of hepatic enzymes such as Serum glutamate oxaloacetate transaminase (SGOT), Serum glutamate pyruvate transaminase (SGPT), Alkaline phosphatase (ALP), Gamma glutamyl transpeptidase (GGT), levels of total bilirubin were investigated and found to be increased while the level of total protein was decreased in ethanol induced rats (Group II) when compared to control (Group I). The alcohol extracts of leaves of *A.marina* exhibited significant ($p < 0.05$) antihepatotoxicity against the ethanol induced liver disease by improving liver function which was indicated by reduction in the levels of SGOT, SGPT, ALP, GGT, total bilirubin and increase in the level of total protein (Group III) (Table 2).

Table 1: Qualitative phytochemical analysis of ethanolic extract of *A.marina*

S.No	Tests	Appearance	Results
1.	Alkaloids	Pale precipitate	+
2.	Flavonoids	Dirty brown color	+
3.	Cardiac glycosides	Brown ring formation	+
4.	Steroids	Violet to blue color	+
5.	Terpenoids	Reddish brown color	+
6.	Tannins	Yellow precipitate	+
7.	Anthraquinones	Red color	+
9.	Saponins	Absence of honey comb like froth	-

Table 2: Antihepatotoxic effect of*A.marina* leaves extract on experimental rats

PARAMETERS	EXPERIMENTAL GROUPS			
	Group I (Control)	Group II (Ethanol)	Group III (*A.marina*100 mg/kg)	Group IV (Ethanol + Silymarin)
SGOT (IU/L)	30± 0.30	174± 4.20	98± 3.70*	82± 1.40*
SGPT (IU/L)	25± 0.90	123± 1.20	69± 0.50*	46± 0.60*
ALP (IU/L)	28.9± 1.41	81.3 ± 1.30	38.8 ± 0.43*	34.6 ± 2.42*
GGT (IU/L)	27± 0.10	54± 0.68	12± 0.23*	12± 0.15*
Total Bilirubin (mg)	0.2± 0.11	1.35± 0.08	0.31± 0.01*	0.29± 0.01*
Serum protein (mg)	6.9± 0.33	2.5± 0.10	5.28 ± 0.26*	5.78 ± 0.52*

*Values are mean ± SEM (n = 6) One Way ANOVA, where*represents significant at P < 0.05; All values are compared with toxicant.*

Davila *et al*.,[15] in *in vitro* study reported that leakage of AST and ALT as well as morphological parameters, which were used as indices of hepatotoxicity caused by erythromycin estolate, amitriptyline, nortriptyline and tert-butylhydroperoxide. Hepatotoxins caused significant increase in serum level of AST, and ALT ($P < 0.05$) when compared to untreated control groups. Flavonoids like Catechin and silybin protected the hepatocytes against cell injury in treated rats.

The treatment with Livplus (a polyherbal formulation) (200 and 400 mg/kg,bw), silymarin (100 mg/kg,bw) for 14 days significantly reduced the elevated levels of ALT, AST, ALP, bilirubin (direct and total), GGT, TC, TG, and increased levels of TP compared to CCl_4 control rats[19].

Histopathological Study

In histological studies, liver section of normal (control) rats showed normal hepatocytes with well-preserved cytoplasm. There was no sign of inflammation, fatty changes or necrosis in these animals (Figure 1). Severe inflammations and cell swelling were observed in endothelial liver cells of ethanol treated rats and they also showed vacuoles in the cytoplasm as well as ballooning and degeneration of hepatocytes (Figure 2).

The liver section of *A.marina* (100 mg/kg b.wt) treated rats showed higher recovery of inflammatory cells around portal tract. There were few portal triads with periportal lymphocytic infiltration, central vein and rest of the hepatic parenchyma appeared unremarkable. No centrizonal necrosis was identified (Figure 3). Silymarin treated animals showed normal liver lobule with no sign of necrosis in the centrizonal area and portal triad, only focal periportal inflammation was observed (Figure 4).

Figure 1: Liver section of normal control rats showing normal liver lobular architecture with central vein and prominent nucleus and nucleolus.

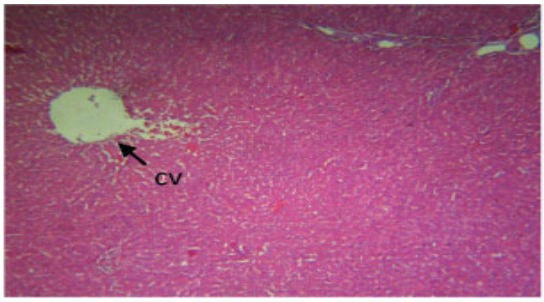


Figure 2: Liver section of alcohol treated rats showing severe toxicity with inflammatory and endothelial cell swelling.

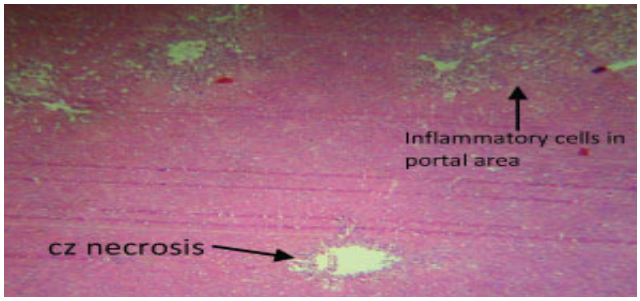


Figure 3: Liver section of rats treated with alcohol and A.marina showing higher recovery of inflammatory cells around portal tract. No centrizonal necrosis was identified.

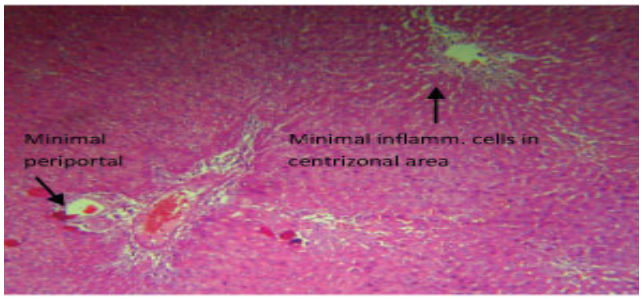
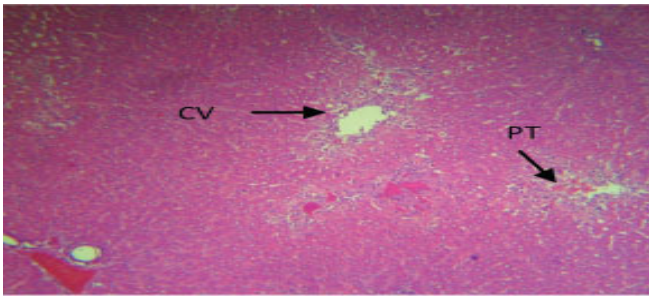


Figure 4: Liver section of rats treated with alcohol and standard drug silymarin showing normal liver lobule with no sign of necrosis in the centrizonal area and portal triad.



Sivakrishnan and Kottaimuthu[20] studied the hepatoprotective activity of ethanolic extract of aerial parts of *Albizia procera* Roxb (Benth.) against paracetamol induced liver toxicity on wistar rats. Liver sections revealed that the normal liver architecture was disturbed by paracetamol treated group of animals, whereas in the liver sections of the rats treated withthe ethanolic extract aerial parts of *Albizia procera* Roxb (benth.) and intoxicated with paracetamol the normal cellular architecture was retained and it is comparable with the standard silymarin treated group.

Therefore, on the basis of our results, the possible mechanism of hepatoprotective activityof *A.marina* leaves extract might be due to the presence of alkaloids, flavonoids and tannins. Histopathological analysis of the liver sections is in agreement with biochemical changes.

Hence, from the results of phytochemical analysis, antihepatotoxic activities and histopathological studies, it is clear that, *A.marina* had more potentiality in treating alcohol induced liver damage in albino rats when compared to standard drug silymarin.The antihepatotoxic effect of alcohol leaves extract of *A.marina* could be due to the presence of bioactive compounds. The results of this experiment indicate that this medicinal plant has the potentiality to treat liver diseases and it could be utilized for the formulation of new drug for ethanol induced liver damage.

Reference

1. UK national statistics, http://www.statistics.gov.uk/
2. Everhart JE, Ruhl CE. Burden of digestive diseases in the United States Part III: Liver, biliary tract, and pancreas. Gastroenterology, 2009; 136: 1134 –1144.
3. Murray CJ, Lopez AD. Evidence-based health policy – lessons from the Burden of Disease Study. Science, 1996; 274: 740-743.
4. Bouoneva I, Abou-Assi S, Heuman DM, Mihas AA. Alcohol liver disease. Hospital Physician, 2003; pp.31-38.
5. Tuma DJ, Sorrell M. Alcohol and alcoholic liver disease. Seminar Liver Disease.2004;24: 215.
6. Duke JA, Ayensu ES. Medicinal Plants of China. Algonac, Michigan: Reference Publication, Inc., 1985;1(4): 352-361.
7. Odebiyi A, Sofowora AE. Phytochemical Screening of Nigerian Medicinal Plants. Part III, Lloydia. 1990; 41: 234-246.
8. Rajakrishnan V, Viswanathan P, Menon VP. Hepatoprotective effect of alcohol on female rats and siblings: Effects of n-acetyl cysteine. HepatologyResearch,1997; 12: 37-50.
9. King J. The dehydrogenase of oxidoreductase-lactate dehydrogenase. In Practical Clinical Enzymology [King, J.C. (ed.)], London: Van D Nostrand Company, 1965, pp.83-93.
10. Rosalki SB, Rau D. Serum gamma glutamyl transpeptidase activity in alcoholism. Clinica Chimica Acta, 1972; 39: 41-47.
11. Malloy HT, Evlyn KA. The determination of bilirubin with the photometric colorimetric. Journal Biological Chemistry,1937; 119: 481-490.
12. Lowry OH, Rose brough NH, Farr AL, Randall RI. Protein measurement with the Folin-phenol reagent. Journal Biological Chemistry,1951; 1932: 265-275.
13. SujaiSuneetha, Histopathological techniques, in handbook of CMAI medical laboratory technology by Robert H carman. Christian Medical Association of India, 1993, 24: 508-541.
14. 14. Ghoshal S, Prasad BNK, Lakshmi V. Anti amoebic activity of *Piper longum* fruits against *Entamoeba histolytica* in vitro and in vivo. J. Ethnopharmacol. 2006; 50: 167-170.
15. Davila JC, Lenherr A, Acosta D. Protective effect of flavonoids on drug-induced hepatotoxicity in vitro. Toxicology.1989, 57(3), 267-286.
16. Sreedharan V, Venkatachalam KK, Namasivayam N. Effect of morin on tissue lipid peroxidation and antioxidant status in 1, 2-dimethylhydrazine induced experimental colon carcinogenesis. Invest New Drugs. 2009;27: 21–30.
17. Lee HS, Jung KH, Hong SW, Park IS, Lee C, Han HK, Lee DH, Hong SS. Morin protects acute liver damage by carbon tetrachloride (CCl_4) in rat. Arch Pharm Res. 2008; 31: 1160–1165.

18. Sathaye S, Bagul Y, Gupta S, Kaur H, Redkar R. Hepatoprotective effects of aqueous leaf extract and crude isolates of Murrayakoenigii against in vitro ethanol-induced hepatotoxicity model. Exp Toxicol Pathol. 2011; 63(6): 587-91.
19. Rajesh Maheshwari, Bhagyashree Pandya, Ramachandran Balaraman, Avinash Kumar Seth, Yogesh Chand Yadav , Vasa Siva Sankar. Hepatoprotective effect of Livplus-A polyherbal formulation. Pharmacognosy Journal, 2015; 7(5): 311-316.
20. Sivakrishnan S, Kottaimuthu A. Hepatoprotective activity of ethanolic extract of aerial parts of Albizia procera Roxb (Benth.) Against paracetamol induced liver toxicity on wistar rats. Int J Pharm and Pharm Sci. 2014; 6 (1): 233-238.