Asian Journal of Chemistry Vol. 20, No. 1 (2008), 705-710

Evaluation of the Protective Effect of Ethanol Extract of *Acalypha indica* Leaf Against Paracetamol-Induced Liver Dysfunction

MOLLY MATHEW† and BEENA JOY*
Agro Processing & Natural Product Division
Regional Research Laboratory, Thiruvananathapuram-695 019, India
Fax: (91)(471)2491712; (91)(471)2490186; E-mail: bjoy39@yahoo.co.in

A number of medicinal plants, traditionally used for many years, in the treatment of liver ailments, are available in the tropical and subtropical regions. One such plant, *Acalypha indica*, though widely used by tribal people, is not much investigated therapeutically. Hence an effort was made to evaluate the hepatoprotective activity of the ethanol extract of the leaves of *Acalypha indica* in male albino rats of average weight (100-120 g) using paracetamol as hepatotoxicant (3 g/kg). As 100 mg was identified as the optimum dose to exert the said effect, this dose was selected for the programmed study. Silymarin was used as a reference standard. Levels of marker enzymes like aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALKP) total protein (TP), albumin (Alb) and total bilirubin (T.Bil) were estimated in serum. A probe into the mechanism of action was attempted by estimating the thiobarbituric acid reactive substances (TBARS) and glutathione (GSH) levels in liver homogenates in order to evaluate the degree of lipid peroxidation. Histopathological studies were also done to confirm the biochemical changes. *Acalypha indica* extract produced substantial improvement in hepatocellular function as evidenced by reversal of ballooning degeneration and sinusoidal congestion. It also showed reversal of important enzyme functions of liver which were comparable to the effects of silymarin.

Key Words: *Acalypha indica*, Hepatoprotective, Silymarin, Paracetamol.

INTRODUCTION

Liver diseases remain one of the serious health problems. In the absence of reliable liver protective drugs in allopathic medical practices, herbs play an important role in the management of various liver disorders[1]. Many unknown and lesser known plants are used in folk and tribal medical practices in India. The medicinal values of these plants are not known to the scientific world. One such plant is *Acalypha indica* Linn. popularly known as "kuppamani" of the family *Euphorbiaceae* found as a weed in

†Nirmala College of Pharmacy, Medical College, Thiruvananthapuram, India.

gardens, waste places and along the road sides, throughout the plain of India, Srilanka and Pakistan[2-4]. Traditionally this herb is a fovourite remedy in chronic bronchitis, asthma and pneumonia. It is also an expectorant and a substitute for senega[3]. It is an emetic, diuretic and carminative. It is a safe and speedy laxative. 'Kani' tribes of South India use this plant for different types of liver ailments[5]; but so far no scientific study has been carried out. Hence an evaluation of the hepatoprotective activity of this plant against paracetamol (overdose)-induced liver damage had been done in rats. Silymarin was the reference drug used.

EXPERIMENTAL

The leaves of *Acalypha indica* were collected from the suburbs of Thiruvananthapuram and Kanyakumari Districts in the months of July and August (2002) where it is available in plenty.

Preparation and isolation of active principles of the ethanol extract: 1 kg of the shade dried leaf powder was subjected to successive solvent extraction using petroleum ether, hexane, chloroform, acetone, ethanol and water. All the extracts were evaporated to dryness at a low temperature under reduced pressure. The ethanol extract being the most effective after pilot study, was used for studying the hepatoprotective activity after suspending in carboxy methyl cellulose (CMC). Bioactivity directed isolation yielded three compounds from the ethanol extract.

Animal: Adult male Wistar rats weighing about 100-120 g were used. The animals were maintained in the animal house of Medical College, Thiruvananthapuram under standard laboratory conditions with commercial pellet diet and water *ad libitum*.

Experimental procedure[6]

A total of 24 animals were equally divided into 4 groups (n = 6 in each group). Group I which served as control, received 1 % CMC orally (1 mL/100 g). Group II received 1 % CMC for 4 d and a single dose of paracetamol on the 3rd day. Group III received suspension of *Acalypha indica* extract for 4 d (100 mg/kg), orally and a single dose of paracetamol on day 3. Group IV received silymarin suspended in 1 % CMC (100 mg/kg) and paracetamol on the day 3. Animals were sacrificed after 48 h paracetamol intoxication under mild ether anesthesia. Blood and liver samples were collected for biochemical and histopathological studies. The livers were washed with ice cold saline: one part was fixed in 10 % formaline and used for histopathological studies. Using the other part a 50 % homogenate was prepared in 0.05 M sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 700 × g for 10 min at 4 ºC and the supernatant was used for the estimation of malonedialdehyde (MDA), the end product of lipid peroxidation and glutathione (GSH).

Assay of serum enzymes: Aspartate amino transferase (AST)[7], alanine amino transferase (ALT)[8] and alkaline phosphatase (ALKP)[9] were assayed in serum using assay kits from Span Diagnostic Pvt Ltd. The results were expressed as units/L (IU/L). Total bilirubin (T.Bil)[10] was also assayed and expressed as mg %.

Protein estimation[11]: The levels of total protein (TP) and albumin (Alb)[12] were estimated in serum of experimental animals. Standard kits from Ranbaxy laboratories, Delhi were used for this purpose.

Lipid peroxidation: The quantitative measurement of lipid peroxidation was done by measuring the concentration of thiobarbituric acid reactive substances (TBARS) in liver using the method of Yagi[13]. The amount of malondialdehyde (MDA) formed was quantitated by reaction with thiobarbituric acid (TBA) and used as an index of lipid peroxidation. The results were expressed as n mol of MDA/g wet tissue using molar extinction co-efficient of the chromophore (1.56×10^{-5} M^{-1} CM^{-1}).

Glutathione estimation: Glutathione (GSH) was estimated in the liver homogenate using 5,5-dithiobis-2-nitrobenzoic acid (DTNB) by the method of Buetler[14]. The absorbance was read at 412 nm and the results were expressed as mg GSH/g of wet tissue.

Histopathological examination: Animals were sacrificed on the day of withdrawal of blood and liver was removed, sliced and washed in saline. Tissue specimens were preserved in 10 % formaldehyde solution; 3-5 µm thick paraffin sections of formaline-fixed liver samples were stained with haematoxylin and eosin and photographed.

Statistical analysis: The statistical analysis were carried out by One-way analysis of variance (Anova), $p < 0.05$ were considered significant.

RESULTS AND DISCUSSION

Biochemical parameters: There was a significant ($p < 0.05$) increase in the levels of serum marker enzymes and significant ($p < 0.05$) decrease in the total protein and Alb levels after administration of paracetamol. This was reversed with *Acalypha indica* extract (Tables 1 and 2). These effects were comparable to silymarin.

Just to probe the possible mechanism of prevention of hepatic damage due to increased dose of paracetamol, investigation on levels of TBARS and glutathione were carried out. There was elevation in the levels of TBARS after administration of paracetamol and this was significantly ($p < 0.05$) reversed by the drug extract (Table-3). There was a significant rise in the GSH content after treatment with *A. indica* extract. The effect of the extract was comparable to that of the reference drug.

The following observations were made after histopathological examination. Liver sections of control group showed perivenular ballooning degeneration and sinusoidal congestion. But the drug treated group showed normal architecture with mild inflammation. The standard group exhibited same results.

TABLE-1
EFFECT OF *A. indica* EXTRACT ON SERUM ENZYMES IN ALBINO RATS INTOXICATED WITH PARACETAMOL

Group	Treatment	AST (IU/L) Mean ± SEM	ALT (IU/L) Mean ± SEM	ALKP (IU/L) Mean ± SEM
I	Control	165.50 ± 2.78	71.50 ± 1.45	119.33 ± 5.36
II	Paracetamol (3 g/kg)	686.33 ± 19.00[a]	314.66 ± 14.36[a]	218.16 ± 8.00[a]
III	Paracetamol + *A. indica* (100 mg/kg)	297.33 ± 10.38[b]	124.33 ± 7.65[b]	183.66 ± 6.31
IV	Paracetamol + silymarin (100 mg/kg)	301.00 ± 21.16[b]	139.66 ± 6.92[b]	177.66 ± 14.77[c]

AST = Aspartate amino transferase; ALT = Alanine amino transferase; ALKP = alkaline phosphatase; Means in the same column scored by the same alphabet are not significantly different at 1 % level. [a]$p < 0.001$ compared to group I; [b]$p < 0.001$ compared to group II [c]$p < 0.05$ compared to group II; Number of animals per group is six.

TABLE-2
EFFECT OF *A. indica* ON LEVELS OF TOTAL PROTEIN, ALBUMIN AND TOTAL BILIRUBIN IN ALBINO RATS AFTER ACUTE INTOXICATION WITH PARACETAMOL

Group	Treatment	T. Protien (g/100 mL) Mean ± SEM	T. Albumin (g/100 mL) Mean ± SEM	T. Bilirubin (mg/100 mL) Mean ± SEM
I	Control	8.05 ± 0.12	5.93 ± 0.18	0.46 ± 0.03
II	Paracetamol (3 g/kg)	4.22 ± 0.20[a]	2.95 ± 0.04[a]	1.79 ± 0.13[a]
III	Paracetamol + *A. indica* (100 mg/kg)	6.00 ± 0.13[b]	3.12 ± 0.07	0.70 ± 0.08[b]
IV	Paracetamol + silymarin (100 mg/kg)	7.52 ± 0.22[b]	3.23 ± 0.13	0.71 ± 0.07[b]

Means in the same column scored by the same alphabet are not significantly different at 1 % level; [a]$p < 0.001$ compared to group I; [b]$p < 0.001$ compared to group II; Number of animals per group is six.

TABLE-3
ACTIVITY OF *Acalypha indica* ON LIPID PEROXIDE AND GLUTATHIONE CONTENTS AFTER PARACETAMOL INTOXICATION

Group	Treatment	MDA* (n mol/g wet tissue) Mean ± SEM	GSH* (mg/g) Mean ± SEM
I	Control	0.74 ± 0.01	3.93 ± 0.01
II	Paracetamol (3 g/kg)	2.33 ± 0.01[a]	2.91 ± 0.02[a]
III	Paracetamol + *A. indica* (100 mg/kg)	1.19 ± 0.01[b]	3.26 ± 0.01[b]

*MDA = Malondialdehyde; GSH = Glutathione; n = 6;
[a]$p < 0.001$ compared to group I; [b]$p < 0.001$ compared to group II.

Paracetamol or acetaminophen is a commonly used safe analgesic drug, which is known to cause centrilobular hepatic necrosis upon overdose[15]. Its toxicity also accounts for many emergency hospital admissions and continues to be associated with high mortality[15]. The hepatotoxicity has been related to the production of a highly reactive intermediate metabolite, N-acetyl-*p*-benzoquinone-imine (NAPQI), formed by cytochrome P_{450} mediated oxidation[16]. Following an overdose, hepatic glucuronide and sulphate become depleted with a consequent increase in P_{450} catalyzed oxidation. The increased production of NAPQI coupled with a decreased capacity to render the substance non-toxic, results in its intracellular accumulation[16], NAPQI with electrophilic and oxidant characteristics consequently can deplete intracellular glutathione and protein thiol groups, by alkylation and oxidation and lead to the formation of mixed disulfide. These events subsequently give rise to changes in the cellular calcium homeostasis, lipid peroxidation and loss of mitochondrial respiratory function[17-19]. The damaged hepatocytes release factors that both attract and activate hepatic macrophages, causing cell necrosis by release of proteolytic lysosomal enzymes and reactive oxygen species[20]. This is evidenced by elevation in the serum marker enzymes like AST, ALT and ALKP. *A. indica* has significantly reduced these enzyme levels. It also has increased total protein and albumin in the serum, indicating hepetoprotective activity. Stimulation of protein synthesis[21] has been advanced as a contributory hepatoprotective mechanism which accelerates the regeneration process and the production of liver cells.

Since *A. indica* extract increased glutathione content of liver it can also be used in hepatotoxicity induced by other agents also.

In present study, treatment with paracetamol in rats produced increase in MDA level suggesting enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defence mechanisms and production of excessive free radicals. As the test group showed reversal of these changes, it is assumed that the mechanism of hepatoprotection of *A. indica* is due to its antioxidant effect.

Histopathological studies also substantiated the biochemical results. All the results were comparable to that of silymarin, a proven hepatoprotective.

The total results reveal that simultaneous treatment with *A. indica* protects the liver against paracetamol-induced hepatotoxicity probably due to its antioxidant effect. Bioactivity directed fractionation and isolation yielded three well established antioxidant secondary metabolites from ethanol extract.

REFERENCES

1. M. Ali, *Indian J. Pharm. Educ.*, **31**, 13 (1997).
2. The Wealth of India, A Dictionary of Indian Raw Materials and Industrial Products, Publication and Information Directorate, CSIR, New Delhi, India.
3. J.E. Caius, The Medicinal and Poisonous Plants of India, Scientific Publishers, Jodhpur, India, pp. 186-187 (1986).
4. R.P. Bhagwagar and M.K. Patel, *Poona Agricultural College Magazine*, **43**, 46 (1952).
5. K.E. Vaidyar, Role of Kuppameni in Sidha Vaidya, In: Glympses of Indian Ethno Pharmacology, pp. 99-107 (1986).
6. A. Subramoniam, D.A. Evans, S. Rajasekharan and P. Pushpangadan, *Indian J. Expt. Biol.*, **36**, 385 (1998).
7. H.U. Bergmeyer, S. Bowers Jr., M. Horder and D.W. Moss, *Clin. Chem.*, **23**, 887 (1977).
8. H.U. Berg and M. Horder, *J. Chem. Clin. Biochem.*, **18**, 521 (1980).
9. G.N. Bowers Jr. and R.B. McComb, *Clin. Chem.*, **21**, 1988 (1975).
10. J. de la Huerga, G.W. Smetters and J.C. Sherrick, in eds.: F.W. Sunderman and S.W. Sunderman Jr., Serum Proteins and Diproteinemias, Philadalphia, J.B. Lippincott Co. (1964).
11. J. Jayaraman, Protein, In: Laboratory Manual in Biochemistry, New Age International Publishers, New Delhi, p. 78 (1981).
12. G.H. Lathe and C.R.J. Ruthven, in ed.: H. Varley, Julab Vazirani for Arnold Heiemann Publisher New Delhi, Practical Clinical Biochemistry, edn. 4, pp. 278-79 (1988).
13. K. Yagi and R. Rastogi, *Ann. Biochem.*, **95**, 351 (1979).
14. E. Buetler, O. Duron and B.M. Kelly, *J. Lab. Clin. Med.*, **61**, 882 (1963); K. Yagi and R. Rastogi, *Ann. Biochem.*, **95**, 351 (1979).
15. Y. Kiso, M. Tohkin, H. Hikino, M. Hattori, T. Sakamoto and T. Namba, *Planta Med.*, **50**, 298 (1984).
16. M.W. Robin, W.J. Reez and T.F. McElligot, *Am. J. Pathol.*, **13**, 321 (1983).
17. R.W. Hong, J.D. Rounds, W.S. Helton, M.K. Robinson and D.W. Wilmore, *Ann. Surg*, **215**, 114 (1992).
18. E.L.Y. Miki, F.Y. Ichi, H. Simizu and K. Karia, *Toxicology*, **67**, 37 (1991).
19. M.F. Dixon, J. Nimmo and L.F. Prescott, *J. Pathol.*, **103**, 225 (1971).
20. B. Weishen, L.M. Kamendulis, D.R. Sidharthan and G.B. Corcoran, *Toxicol. Appl. Pharmacol.*, **112**, 32 (1992).
21. D. Awang, *Milk Thistle. Can. Pharm. J.*, **23**, 749 (1993).

(*Received*: 22 December 2006; *Accepted*: 18 September 2007) AJC-5878