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Archives of Applied Science Research, 2010, 2 (1) 152-158
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Protective effect of aerial parts of *Amaranthus spinosus* Linn in Paracetamol induced hepatotoxicity in rats

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Abstract

The ethyl acetate soluble and insoluble fraction of the methanolic extract of the aerial parts of *Amaranthus spinosus* Linn (Family: Amaranthaceae) were evaluated for hepatoprotective activity in Paracetamol induced liver damaged rats. Both the fractions, at a dose of 200mg/kg/p.o were administered through oral route for 7 days. The ethyl acetate soluble fraction exhibited a significant hepatoprotective effect by lowering serum levels of glutamic oxaloacetic transaminase (SGOT), glutamic pyruvic transaminase, (SGPT), alkaline phosphatase (ALP), total bilirubin (TBL) and increase in serum total protein levels (TPL). These biochemical observations were also evidenced by histopathological examination of liver sections. The activity was compared with standard drug Silymarin.

Key words: *Amaranthus spinosus* Linn, Amaranthaceae, Hepatoprotective.

Introduction

Liver disease is still a worldwide health problem. Herbs play an important role in the management of various liver disorders. Numerous medicinal plants and their formulations are used for liver disorders in ethnomedical practices and in traditional system of medicine in India. However satisfactory remedy for serious liver disease is not prevalent; most of the herbal drugs speed up the natural healing process of liver. So the search for an effective hepatoprotective drug from herbal origin continues [1-2]. Paracetamol, a well known antipyretic and analgesic agent used in day to day medical practice. But it produces hepatic necrosis at higher doses. The toxicity

is due to the formation of toxic metabolites including N-acetyl-p-benzo quinoleimine, which leads to the depletion of glutathione and causes oxidative stress [3].
Amaranthus spinosus Linn. belonging to the family Amaranthaceae is an erect, glabrous herb found abundantly in the wastelands of India and Srilanka. It is commonly known as Mullukeerai in Tamil [4]. The important constituents of the aerial parts of the plant are sterols (α-spinosterol, stigmosterol, campesterol), fatty acids (stearic acid, gentisic acid), alkanes (hentriacontane), proteins, aminoacids, alkaloids, flavonols and terpenoids [5]. The entire plant has been reported to possess several ethnomedical properties such as antiulcer, antigout, antitumour, diuretic and purgative. The rhizome of the herb was reported to possess antihepatotoxic activity [6]. But still there is no scientific methodological report has been found in literature regarding the action of *Amaranthus spinosus* Linn. on liver. Therefore, the present study was undertaken to evaluate the hepatoprotective activity of aerial parts of *Amaranthus spinosus* Linn. on the biochemical parameters against Paracetamol induced liver damage in rats.

Materials and Methods

Collection of plant material

The aerial parts of *Amaranthus spinosus* Linn. (Amaranthaceae) was collected in the evening hours from the roadsides of Vandalur, Chennai, Tamil Nadu, India and was authenticated by Prof. P. Jayaraman, Taxonomist, Plant Anatomical Research Institute, Chennai, India. A voucher specimen (ASL/SRM/01/09) was deposited in the Department of Pharmacognosy, SRM College of Pharmacy, Kattankulathur, India for future references. The aerial parts were shade dried, pulverized into coarse (sieve: 60) using a cutter mill and stored in an air tight, light resistant container till further use.

Preparation of extract

The pulverized plant material was defatted with hexane (60°-70°C). The defatted plant material was extracted with methanol and then concentrated under vacuum. To the dried methanolic extract ethyl acetate was added and stirred well. The soluble and insoluble fractions of ethyl acetate were separated. On evaporation of these two portions in vacuum, greenish colored residues were obtained with the yield of 2%w/w and 14%w/w respectively and they were stored in a desiccator. For pharmacological experimentation, a weighed amount of dried extract was freshly suspended in 2% v/v Tween-80 solution.

Preliminary phytochemical screening

The dried extracts were subjected to various chemical tests to detect the presence of different phytoconstituents like alkaloids, glycosides, terpenoids, flavonoids etc., according to standard procedure [7].

Animals

Wister albino rats of either sex weighing 180-250g were obtained from the central animal house, Government veterinary hospital and research centre, Madhavaram, Chennai, India. They were maintained under controlled conditions of light and temperature (25°±2°C) and were provided with standard laboratory pellet diet and distilled water *ad libitum*. The animals were randomly divided into five groups of six rats each, the groups being balanced for sex and body weight. The animals were fasted for 24 hrs before experimentation but allowed free access to distilled water

throughout. The experiment was performed after the experimental protocols have been approved by the institutional animal ethical committee, SRM College of Pharmacy (IAEC/SRMCP/17/09).

Induction of hepatotoxicity and drug treatment

For inducing acute hepatic damage, Paracetamol suspension was administered for 3 days at a dose of 2g/kg/p.o except control group rats [8-9]. Following Paracetamol-induced hepatotoxicity, the standard drug Silymarin and the two fractions were administered for 7days (200mg/kg/p.o) individually. The treatment schedules are as follows,

Group 1 was fed with 2%v/v Tween-80 (10ml/kg/p.o.) and considered as vehicle control.
Group 2 was left untreated and considered as negative control.
Group 3 was fed with ethyl acetate soluble fractions of methanolic extract of A.spinosus (200mg/kg/p.o.) and considered as test group-I.
Group 4 was fed with ethyl acetate insoluble fractions of methanolic extract of A.spinosus (200mg/kg/p.o.) and considered as test group-II.
Group 5 was fed with Silymarin (25mg/kg/p.o.) and considered as standard.

Assay of hepatic marker enzymes

Twenty four hours after the final administration of the drug/extract, rats of each group were anaesthetized. Blood samples were withdrawn from the carotid artery and centrifuged at 2000 rpm at 4°C for 10 min to separate the serum. The serum thus obtained was used for the estimation of hepatic marker enzyme levels namely SGOT, SGPT [10], ALP [11] and TBL [12]. The sera were also subjected to estimate the levels of total proteins [13-14].

Histopathological studies

Each rat was laparotomized to excise the liver immediately after collection of blood. Small fragments of the livers were washed in ice-cold saline and fixed in 10% formalin solution. They are dehydrated with ethanol (90%), embedded in paraffin wax and cut into 5 µm thick sections using a rotary microtome for histopathological examinations. The sections were stained with eosin-haemotoxylin dye for photomicroscopic observation of necrosis, steatosis and fatty changes of hepatic cells [15].

Statistical analysis

The mean values ± SEM were calculated for each parameter. For determining the significant inter group difference, each parameter was analyzed separately and one way analysis of variance (ANOVA) was carried out. Then the individual comparisons of the group mean values were done using Dunnet's multiple comparison tests [16].

Results and Discussion

Preliminary phytochemical screening

In the preliminary phytochemical investigation of ethyl acetate soluble portion reveals the presence of alkaloids, sterols, proteins, flavonoids and phenolics while the ethyl acetate insoluble portion showed the presence of carbohydrates, tannins and oils.

Effect on serum enzymatic activity

Paracetamol (acetaminophen), a widely used over-the-counter analgesic and antipyretic produces hepatic necrosis when ingested in very large doses or frequent administration. It is metabolized in the liver primarily to glucuronide and sulphate conjugates. Paracetamol toxicity is due to the formation of toxic metabolites by cytochrome P_{450}[17]. Induction of cytochrome P_{450} or depletion of hepatic glutathione is a prerequisite for paracetamol-induced hepatotoxicity. Therefore the hepatoprotective activity of the drug may be due to inhibition of cytochrome $P_{450,}$ promotion of glucuronidation, stimulation of hepatic regeneration, activation of the functions of the reticuloendothelial systems or inhibition of protein biosynthesis [18].

Acute administration of Paracetamol for 3 days caused a marked hepatocellular injury which was clearly evidenced from the significant elevation in the levels of SGOT, SGPT, ALP, TBL and decrease in TPL (group 2), which are reliable markers of hepatotoxicity. The ethyl acetate soluble fraction of the methanolic extract of *A.spinosus* at a dose of 200mg/kg/p.o. reduced the elevated levels significantly (group 3) and stabilized the deficient protein levels and was found to be statistically significant ($P<0.001$) on comparison with the normal control group. The ethyl acetate insoluble fraction of the methanolic extract of *A.spinosus* was found to be less active (group 4) and was statistically insignificant. The activity exhibited by the ethyl acetate soluble portion was comparable with the standard drug Silymarin (group 5). Silymarin provided a better inhibition of the elevated SGOT, SGPT, ALP and TBL induced by paracetamol and also exhibited protein levels similar to the normal control group.

Table 1. Effect of *A.spinosus* on serum biochemical parameters in Paracetamol induced liver damage in rats

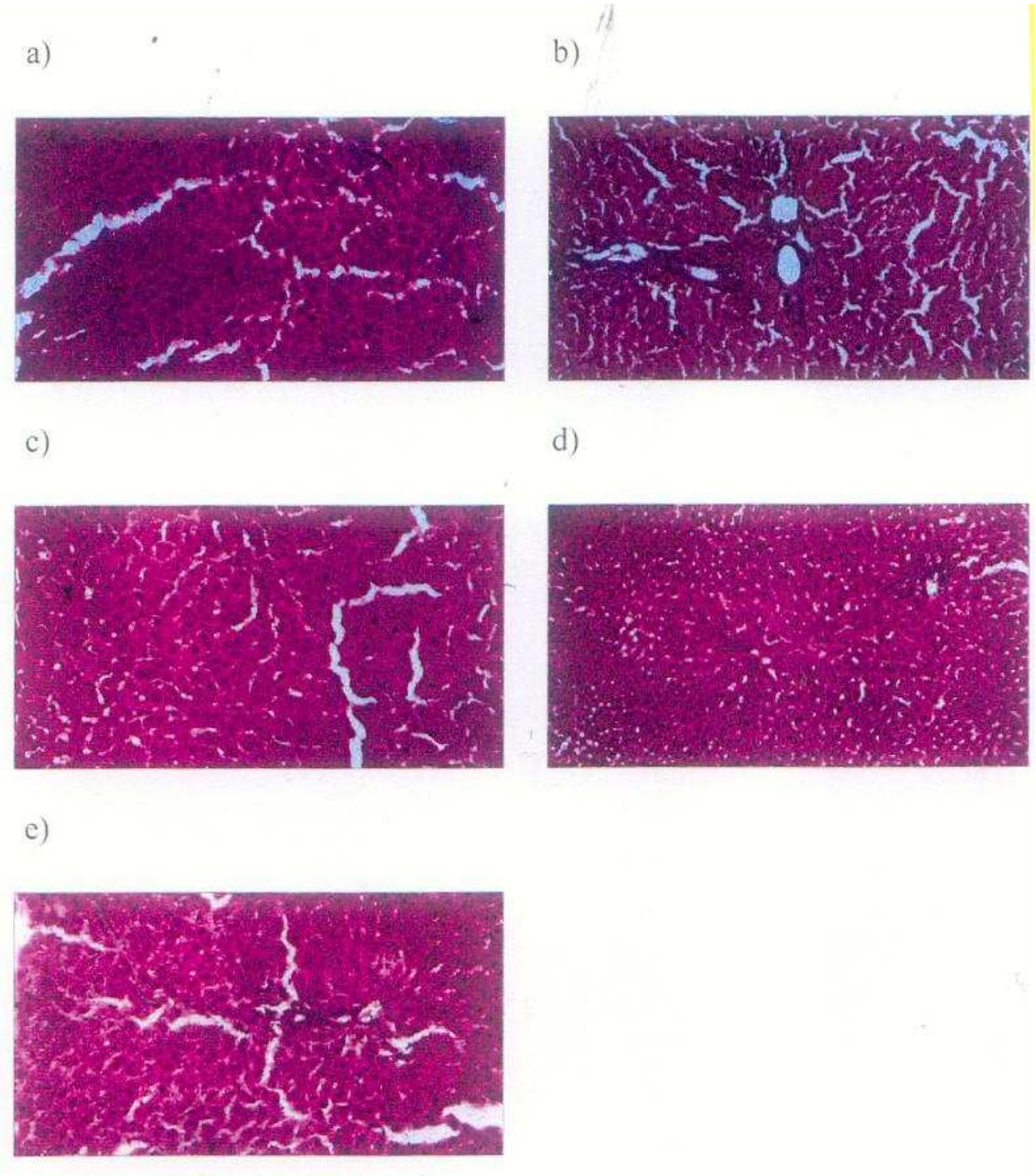
Group	SGOT (IU/L)	SGPT (IU/L)	ALP (IU/L)	TBL (mg/dl)	TPL (g/dl)
Normal control	49.82±1.57	40.5±2.79	109.36±9.12	2.9±0.5	10.7±0.1
Negative control	134.12±12.65	102.50±12.10	451.22±21.20	3.5±0.8	10.1±0.5
Test group-1	80.54±7.57*	69.52±6.11*	191.61±27.62*	2.0±0.4*	11.6±0.3*
Test group-2	110.05±2.22	90.25±3.23	255.39±7.43	3.1±0.04	10.9±0.2
Standard	57.64±2.79*	52.805±6.65*	215.05±3.26*	2.6±0.3*	11.7±0.5*

N=6, values are mean ± SEM. The data were analyzed by one way ANOVA followed by Dunnett's multiple comparison tests. *p<0.001 compared to control group.

Histopathological observations

The histopathological profile of the rat of normal control in group 1 showed normal cellular architecture with distinct hepatic cells, sinusoidal spaces and central vein (Fig a). In the liver section of the rats intoxicated with Paracetamol in group 2, there is a disarrangement and degeneration of normal hepatic cells with intense centrilobular necrosis, sinusoidal haemorrhages and dilatations. There was chronic inflammatory cell infiltrate in portal tracts (Fig b). The liver section of the rats treated with ethyl acetate soluble fractions in group 3 and Silymarin in group 5 shows less vacuole formation reduced sinusoidal dilations, less disarrangement and degenerations of hepatocytes indicating marked regenerative activity (Fig c, e). The intensity of centrilobular necrosis was also less. But in case of rat liver treated with ethyl acetate insoluble

fractions in group 4 only mild changes in regeneration of hepatocytes indicates there is no significant activity when compared to group 3. (Fig d)



Figure

a) Liver section of a normal rat showing normal hepatic cell architecture

b) Liver section of a rat with paracetamol induced hepatotoxicity showing severe focal necrosis

c) Liver section of a rat treated with paracetamol + ethyl acetate soluble portion of methanolic extract showing almost normal cell architecture
d) Liver section of a rat treated with paracetamol + ethyl acetate insoluble portion of methanolic extract showing mild focal necrosis
e) Liver section of a rat treated with paracetamol + standard drug Silymarin showing almost normal hepatic cell architecture

Conclusion

The results obtained from the levels of the hepatic marker enzymes showed significant hepatoprotective activity of the ethyl acetate soluble fractions of the methanolic extract of *A.spinosus* when compared to Silymarin. However, further studies are required such as detailed phytochemical examination of the active constituents will provide the principle(s) responsible for the activity and to elucidate their mechanism of action.

Acknowledgement

The Authors are grateful to Dr. R. Shivakumar, Pro-Vice-chancellor, SRM University and Dr. K. S. Lakshmi, Dean, College of Pharmacy, SRM University, Kattankulathur, for providing necessary facilities to carry out this work.

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