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(Research Article)

Hepatoprotective Potency of *Achyranthes aspera*: An In-vivo Study

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ABSTRACT

Achyranthes aspera (Amaranthaceae) is known for many medicinal uses such as purgative, diuretic, used in dropsy, piles, boils, skin eruption and in treating snake bite. Seeds of the plant are used by the ethnic group of Western Ghats for treating jaundice. The objective of the present study was to evaluate ethanolic extract from seeds of Achyranthes aspera for its hepatoprotective potential by carbon tetrachloride (CCl_4) induced liver damage model in rats. Present investigation revealed that the animal group treated with CCl_4 recorded significant rise in serum markers reflecting hepatic damage and pretreatment of rats with ethanolic extract of A. aspera (100mg/kg p.o) inhibited the increase in serum levels of total bilirubin, total protein, serum alanine transaminase, aspartate transaminase and alkaline phosphatase reflecting the liver protection by crude drug.The data obtained was found comparable with silymarin (100mg/kg p.o).

Key Words: *Achyranthes aspera*, Crude drug, Hepatoprotective, CCl_4, Silymarin.

INTRODUCTION

The plant *Achyranthes aspera* (*A.aspera*) is commonly known as Apamarga (Sanskrit) belongs to the family *Amaranthaceae*. The plant is known for many medicinal uses such as purgative, diuretic, used in dropsy, piles, boils, skin eruption and in treating snake bite [1]. The plant is used by tribal groups in treating abdominal disorder, anaemia, anasorea, asthama, cough, diarrhea, dysentery, ear diseases, hydrophobia, insect bite, jaundice, pneumonia, renal dropsy, ulcers, bleeding during delivery, headache, leucoderma, rheumatism, scabies, stomach ache [2] and cancer. Several pharmacological property of *A. aspera* has been tested viz., antifertility [3, 4 and 5], to enhance immunity [6, 7], in inflammation [8], in arthritis, as hypoglycaemic [9], as cardiac stimulant [10] and against leprosy [11]. Though the seeds of the plant are used by the ethnic group of Western Ghats for treating jaundice, no scientific study has been carried out to evaluate its hepatoprotective potency. Hence, the present study was undertaken to evaluate the hepatoprotective potency of the *A. aspera* through CCl_4 induced hepatic damage model.

MATERIALS AND METHODS

Collection of Plant Materials

Seeds of *Achyranthes aspera* were collected from the Shikaripura range forest, Shimoga district, Karnataka State during October 2006 and identified by comparing the herbarium specimen deposited at department of botany, S.R.N.M.N. College and a voucher specimen (MN-1) was also deposited in the departmental herbaria.

Preparation of Extract

Seeds are separated and powdered mechanically (sieve no 10/44) and stored. About 250g of the powdered material was subjected to soxhlation and exhaustively extracted with petroleum ether for 48 hrs. The residual plant material is then shed dried and subjected to soxhlation with 70% ethanol for 48 hrs. The solvent was evaporated at low temperature under reduced pressure using rotary flash evaporator (Buchi, Flawil, Switzerland) till the complete evaporation of the solvent. The yield obtained was 30.8% W/W.

Drug Formulation

Oral suspensions containing 100mg/ml of the aqueous and methanol seed extracts were prepared in 1% W/V gum tragacanth.

Animals

Male wistar albino rats weighing 150-200gms were procured from the National College of Pharmacy, Shimoga. Animals were housed in polypropylene cages and were maintained at 27°±2°C, 60±5% RH, 12:12 hr light/day cycle. They were fed with commercial diet (Hindustan lever ltd., Bangalore) and water *adlibitum* during the experiment.

The study was permitted by the institutional Animal Ethical committee with Reg. No.144/1999/CPCSEA/SMG.

Acute Toxicity Studies

Literature survey revealed that the drug fed orally is non toxic up to 8gm/kg [12]. Hence for the present investigation 100mg/kg b.w. was selected to assess the heptoprotective activity of *A. aspera* seeds.

Evaluation of Hepatoprotective Activity

The animals were divided into 4 groups of 6 rats each. The animals in group I served as control and received the vehicle 1ml/kg/day of 1% W/V gum tragacanth, p.o. for 14 days. All the animals of group II-IV received 0.1 ml/kg per day CCl_4 i.p (Merck, Bangalore, India) for 14 days. Group III animals received the standard drug silymarin 100mg/kg/day p.o (Ranboxy lab, Dewas) for 14 days. Ethanol seed extract 100 mg/kg/day, p.o of *A. aspera* were administered to the animals of group IV for 14 days. The CCl_4, silymarin and extracts were administered concomitantly to the respective groups of animals.The animals of all the groups were sacrificed on 14th day under light ether anesthesia. The blood sample of each animal was collected separately by carotid bleeding into sterilized dry centrifuge tubes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500 rpm for 10 min and was subjected to biochemical investigation viz., total bilirubin [13], total protein [14], serum alanine transaminase (ALT), aspartate transaminase (AST) [15] and alkaline phosphatase (ALP) [16]. Results of biochemical estimations were reported as mean ±SE of 6 animals in each group.

Histopathology

The linear samples were excised from the experimental animals of each group and washed with the normal saline. Initially the materials were fixed in 10% buffered neutral formalin for 48 hours and then with borne solution for 6 hours. They were processed for paraffin embedding. The sections were taken at 5µm thickness using microtome, processed in alcohol xylene series and were stained with alum haematoxylin and eosin [17]. The sections were examined microscopically for the evaluation of histopathological changes.

RESULTS

In the present study, the data in the table-1 reveals that, the animals of toxic control groups (CCl_4 0.1 ml/Kg/day. i.p.) recorded high level of serum markers alanine transaminase (1394.4±1.59), aspartate transaminase (2245.6±7.759) and alkaline phosphatase (403.61±1.496), total bilirubin (2.440±0.109) and drastic decrease in total protein (5.931±0.052) indicating severe hepatic damage due to toxic effects of CCl_4, the serum markers level in the standard drug treated group showed almost normal values indicating the hepatoprotective effect of silymarin. Administration of ethanolic seed extracts of *A. aspera* showed recovery against the toxic effects of CCl_4 which is reflected in terms of serum markers concentration in the blood serum i.e., decrease in the levels of serum total bilirubin (0.576±0.0002), alanine transaminase (84.3±0.202), aspartate transaminase (187.55±0.10) and alkaline phosphatase activities (220.3±0.45) increase in the total protein level (8.022±0.0032). The results are in comparison with the serum analysis of standard drug sylimarin treated group serum total bilirubin (0.507±0.001), alanine transaminase (70.5±0.205), aspartate transaminase (162.02±0.26) and alkaline phosphatase activities (194.78±0.18) and the total protein (8.890±0.0002). Histopathological evidences indicate restoring the damage of liver cells by the therapeutic effect of the plant extract. Histological profile of control animal showed normal hepatocytes (Figure 1), the section of liver of the group II animals exhibited severe intense centrilobular necrosis (N), vacuolization (Figure 2). The liver tissue sections of silymarin treated animals showed normal hepatic architecture (Figure 3). The liver tissue sections of the animals treated with ethanol (Figure 4) at the dose of 100 mg/kg p.o. and exhibited significant liver protection against CCl_4 induced liver damage as evident by the presence of normal hepatic cords, absence of necrosis and fatty infiltration.

DISCUSSION

In the CCl_4 induced hepatic damage model the toxic metabolite CCl_3 radical produced by microsomal oxidase system, binds covalently to the macromolecule and causes peroxidative degradation of lipid membranes of the adipose tissues. The extent of hepatic damage is assessed by the elevated levels of serum markers which are attributed to the generation of trichloromethyl free radicals which in turn causes lipid peroxidation [18]. Hepatocellular necrosis leads to high level of serum markers in the blood, among these, alanine transaminase alone represents 90% of total enzyme and high level of alanine transaminase in the blood is the better index of liver injury, the decreased level of these serum markers indicates the stabilization of plasma membrane and protection of hepatocytes against CCl_4 damage [19]. Alkaline phosphatase concentration is related to the functioning of hepatocytes, high level of ALP in the blood serum is related to the increased synthesis of it due to the increased biliary pressure [20]. Ethanolic seed extracts of *A. aspera* exhibited recovery against the toxic effects of CCl_4 and the results were comparable with that of standard drug silymarin. Histopathological examinations also supported the above results showing normal arrangement of hepatocytes around the central vein, portal artery, hepatic duct, absence of necrosis and moderate fatty vacuoles.

CONCLUSION

As intended, the present study provides scientific evidence to the ethno medicinal use of *A. aspera* in treating jaundice. Further studies on isolation of active constituents, evaluation of their hepatoprotective potency and synergism will be beneficial in designing the ideal drug candidate for the treatment of Jaundice.

Table 1: Effect of ethanolic seed extract of *Achyranthes aspera* on CCl_4 induced hepatotoxicity in rats.

Group (N)	Total Bilirubin (mg/dl)	Total Protein (gm %)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)
Control (1% gum tragacanth, p.o)	0.502±0.020	9.242±0.120	146.0±0.760	57.62±0.278	178.05±3.208
CCl_4 (0.1ml/kg/ Day. i.p)	2.440±0.109	5.931±0.052	2245.6±7.759	1394.4±1.59	403.61±1.496
CCl_4 + Silymarin (0.1ml/kg/ Day. i.p + 100mg/kg/ day .p.o)	0.507±0.001	8.890±0.0002	162.02±0.26	70.5±0.205	194.78±0.18
CCl_4 + Ethanolic seed extract (0.1ml/kg/ Day. i.p + 100mg/kg/ Day. po)	0.576±0.0002	8.022±0.0032	187.55±0.10	84.3±0.202	220.3±0.45

Note: N=six animals in each group, All the values are expressed as Mean± SE(Standard error)

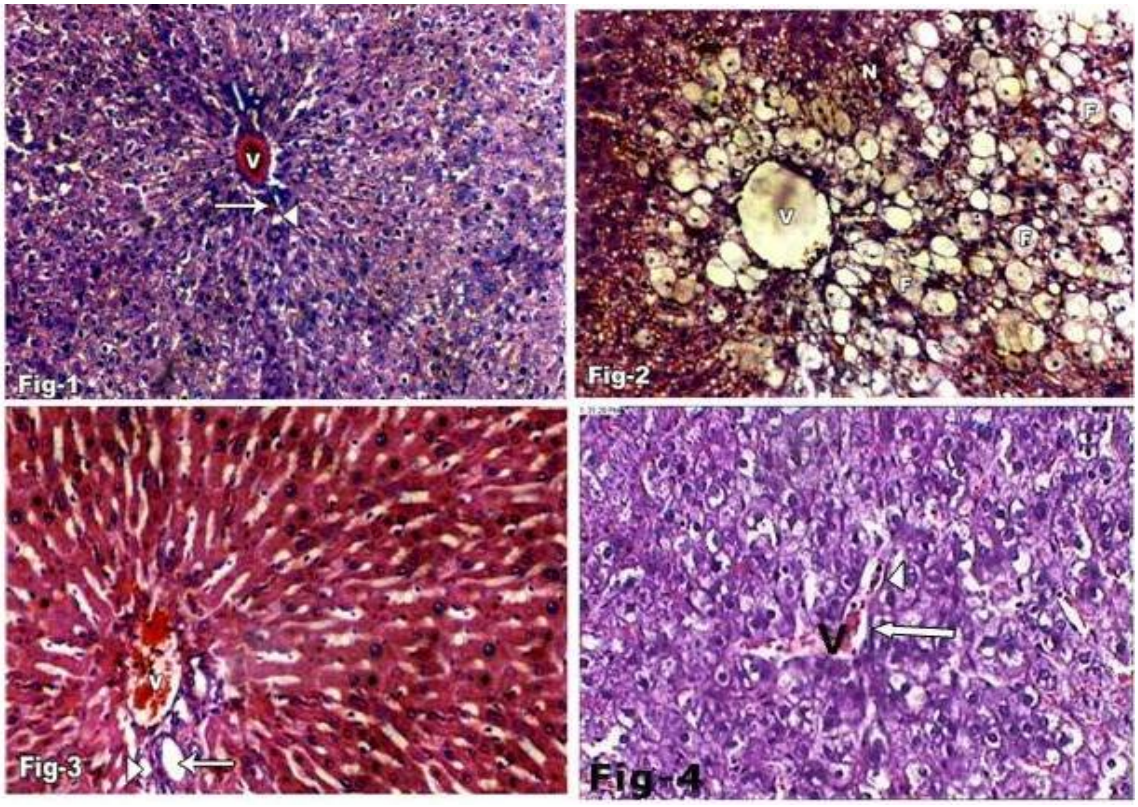

Fig. 1: Section of the liver tissue of control animal showing normal histology, portal triad showing portal vein (V), portal artery (Arrow), hepatic duct (Arrow head). (H and E, 100X).
Fig. 2: Section of the liver tissue of animal treated with CCl_4 showing necrosis (N) and fatty vacuole (F) and portal vein (V). (H and E, 100X).
Fig. 3: Section of the liver tissue of silymarin treated animals showing normal hepatocytes, portal vein (V), portal artery (Arrow), hepatic duct (Arrow head). (H and E, 100X).
Fig. 4: Section of the liver tissue of ethanol seed extract (100mg/kg.b.w.) treated animals showing normal arrangement of hepatocytes around the central vein (V), portal artery (Arrow), hepatic duct (Arrow head), absence of necrosis and moderate fatty vacuoles. (H and E, 100X).

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