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Pharmacology

Evaluation of hepatoprotective activity of *Cuscuta reflexa* Roxb. stem on experimental animals

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ABSTRACT Aim: To evaluate the hepatoprotective activity of alcoholic extract of *Cuscuta reflexa* Roxb stem against hepatotoxicity induced by paracetamol in albino rats. Materials and methods: Stems of Cuscuta reflexa were collected, air dried and powdered. Methanolic extract was obtained by percolating the dried powder with 99.8% methanol.
Oral toxicity test was done according to OECD guidelines. Hepatoprotective activity was evaluated by the method described by Lahon K and Das S. Alcoholic extracts of *Cuscuta reflexa* (100mg/kg) was used as test drug and Silymarin 100 mg /kg as standard drug. All medicaments were given for 10 days. Hepatotoxicity was induced by a single dose of Paracetamol 2 gm/kg body weight on 8th day of the experiment.
Results: Alcoholic extract of *Cuscuta reflexa* stem showed significant ($p < 0.01$) hepatoprotective activity when compared to the control.

KEYWORDS : Hepatotoxicity, *Cuscuta reflexa*, silymarin, paracetamol.

Introduction

Cuscuta reflexa Roxb. is a leafless yellow twining, parasitic annual belonging to convolvulaceae family. Commonly known as Akashilata, Amarlati and Akashbel, distributed throughout India. Traditionally the whole plant, stem, seeds are used for various medicinal purposes. The herb is used as expectorant, carminative, tonic anthelmintic, purgative, diaphoretic, diuretic, purifies blood and cleanses the body, lessens inflammations, useful in jaundice, pains in the muscles and the joints, heat of the brain, headache, paralysis, diseases of the spleen, vomiting, and lumbago. The seeds are used in the diseases of the liver and the spleen, quartan fever, griping hiccough, purify blood and cleanse the bowels[1].

The plant contains Flavonoids, glycosides, steroids, alkaloids, cuscutalin, cuscutin, and amarvelin[2] odoroside, neritaloside, strospeside, tyramine, ursolic acid, β-sitosterol glucoside, gitoxeginin, methyl cinnamate, dihydroajugapitin, ferulic acid[3].

Pharmacological action: Various studies on Cuscuta reflexa showed that the plant has anticonvulsant[4] antimicrobial[5], antioxidant[6], anti-steroidal[7], anti-inflammatory[8], antitumour[9], diuretic[10], anti-HIV[11], hypotensive[12], hypoglycaemic activity[13].

Materials and Methods

The study was carried out in the department of pharmacology at Assam Medical College. Stems of *Cuscuta reflexa* were collected within Dibrugarh district of Assam. A taxonomist of Dibrugarh University identified and confirmed the stem samples

Stems of *Cuscuta reflexa* were collected and air dried at room temperature. Dried stems of *Cuscuta reflexa* were powdered in electrical grinder. The dried powder then mixed with 99.8% methanol and allowed to stand for few minutes in a tightly covered container. The entire solution transferred to Soxhlet apparatus and sufficient quantity of stem extract was obtained[14].

All the animals used in the study were taken care of under ethical consideration with approval from the institutional ethical committee (Registration no.-634/02/a/CPCSEA), Assam Medical College.

Toxicity studies: Alcoholic extract of *Cuscuta reflexa* stem was subjected to acute oral toxicity (OECD Guidelines, 2001). Mortality in the acute oral toxicity test was not seen in the limit test up to dose 2000 mg/kg[15].

Experimental Design: Twenty four albino rats of either sex weighing 100-200gms were taken for the study. The animals were divided randomly into four groups of six animals per group. The rats were maintained on a standardized diet and water ad libitum. For experimental purpose, the animals were kept fasting overnight, but allowed free access to water. All drugs were administered orally with the help of a feeding tube.

The experiment was performed as follows:

GROUPS	TREATMENT
Group A (Normal control)	Normal saline 10 ml /kg /day orally X 10 days
Group B (Experimental control)	Normal saline10 ml / kg /day orally X 10 days
Group C (Test group)	Alcoholic extract of *Cuscuta reflexa* stem 100mg/ kg /day orally X 10 days.
Group D (Standard drug)	Silymarin 100 mg /kg / day orally X 10 days

The volume of all medicaments were kept constant at 10ml/kg body weight of the animals

Method of inducing hepatic injury by Paracetamol[16]: A single dose of paracetamol 2 gm/kg body weight was given orally to all animals belonging to Groups B, C and D on 8th day of the experiment by using an intragastric tube. It was administered after overnight fasting of the animals, i.e. the diet was restricted 12hrs prior to the administration of paracetamol. Liver damage was seen after 48 hours i.e., on 10th day.

Blood was withdrawn directly from the heart under light (ether) anaesthesia. Serum was separated by centrifugation for biochemical studies.

Technique for estimating liver function[17-19]:

a) AST estimation
b) ALT estimation
c) Serum Protein estimation
d) Serum Albumin : Globulin ratio

Statistical analysis:

The data were subjected to statistical analysis using one way ANOVA followed by Bonferoni's test. *p* values <0.01 were considered significant.

Care of the animal after the experiment: The animal were taken proper care in the central animal house, AMCH. They were provided with proper food and sufficient water and kept in a well ventilated

room. Proper hygienic condition was maintained. They were provided with proper medication after the experiment is over.

Results:

In the present study paracetamol treated group showed a significant ($p< 0.01$) rise in AST, ALT levels and significant ($p< 0.01$) fall in serum protein, albumin globulin ratio when compared to normal control group indicating hepatic damage.

In group C (*Cuscuta* group) and group D (Silymarin group) there was significant ($p < 0.01$) fall in AST, ALT levels and significant ($p< 0.01$) rise in total serum protein, albumin globulin ratio when compared to experimental control group. These results suggests that alcoholic extract of *Cuscuta reflexa* stem has significant ($p <0.01$) hepatoprotective activity.

Table: EFFECT OF AECR ON DIFFERENT BIOCHEMICAL PARAMETERS IN ALBINO RATS

Groups		AST IU/L	ALT IU/L	Serum Protein estimation gm%	Serum albumin: globulin ratio
Group A		99 ± 1.31	42 ± 2.13	6.7 ± 0.09	1.5 ± 0.02
Group B		750 ± 73.36	523 ± 50.17	5.3 ± 0.09	0.1 ± 0.01
Group C		98 ± 1.88	50 ± 7.96	7 ± 0.59	1 ± 0.14
Group D		97 ± 1.18	41 ± 1.18	6.9 ± 0.06	1.3 ± 0.07
One way ANOVA	*f*	72.99	88.53	8.155	64.08
	df	3,20	3, 20	3, 20	3, 20
	p	< 0.01	< 0.01	< 0.01	< 0.01

Values are expressed as mean SEM, n= 6 rats in each group, *p* is < 0.01 compared to paracetamol treated group.

Discussion:

It was observed in the study that administration of paracetamol resulted in a significant rise in serum AST and ALT levels and a significant fall in total proteins and serum albumin globulin ratio.

Compared to the experimental control group (group B), *Cuscuta* group (Group C) and standard drug Silymarin group (group D) showed a significant fall in serum AST and ALT levels. There was a significant rise of serum protein and albumin-globulin ratio in group C and group D.

Extensive hepatocellular damage is produced by larger doses of Paracetamol. A toxic metabolite of paracetamol N-acetyl-P-benzoquinoneimine results hepatotoxicity[20].

Due to this there is exhaustion of reduced glutathione and resulting in cell necrosis and lipid peroxidation[21]. Glutathione depletion potentiates the hepatotoxicity due to paracetamol[20]. In an earlier study done in 2012 by Sharma S *et al*, it was observed that *Cuscuta reflexa* alcoholic extracts neutralized the activities of free radicals and inhibited the peroxidation reactions[6]. Thus the antioxidant activity of *Cuscuta reflexa* may be responsible for its hepatoprotective activity.

The results of the present study suggest that alcoholic extract of *Cuscuta reflexa* stem produces significant hepatoprotective activity at a dose of 100 mg/kg. After observing the results obtained in the study, further and detailed studies on this plant are required to confirm the true potential of this plant, for its hepatoprotective activity, which would make it viable clinically in humans and also have cost effectiveness.

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