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Research Article

Antiulcer Activity of Chloroform Extract of *Cyperus rotundus*

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ABSTRACT
Cyperous rotundus Linn. belonging to family Graminae, commonly known as "Nut Grass", contains number of chemical constituents, mainly cyperenone, cyperol, cyperolone, cyperone camphene, limonene, oleanolic acid and sitosterol etc.. Chloroform extract of *Cyperous rotundus* two doses, viz., 200 and 400 mg /kg were evaluated by ethanol induced method using Ranitidine (50mg/kg) as standards. The standard drugs and the test drugs were administered orally for 14 days in ethanol induced method. The results of the present study showed that the chloroform extract of *Cyperous rotundus* possessed gastro protective activity as evidenced by its significant inhibition of mean ulcer score and ulcer index. The present experimental findings suggest that *Cyperous rotundus* may be useful for treating peptic ulcers. Preliminary phytochemical screening of extracts revealed the presence of the bioactive compounds, such as alkaloids, anthroquinones, flavonoids, phenolic compounds, saponins, steroids and tannins. The study revealed specific identities for *Cyperous rotundus* linn.

Keywords: *Cyperous rotundus*, Ethanol induced method, Ranitidine, Cyperenone and Ulcers.

INTRODUCTION

Cyperous rotundus Linn. belonging to family Graminae, is commonly known as " Nut Grass" is a perennial herb with long rhizomes[1], leaves are linear, broadly grooved on the surface and dark green in colour. Flowers are small inflorescence with 2-4 bracts. The inflorescence consists of a few slender branches with the longest usually not more than about 7.5cm spikes. The nut is oblong, ovate, nearly half as long as the glume, strongly 3- angled, yellow or black when ripe[2].

MATERIAL AND METHODS

Plant material and Preparation of the Extracts

The fresh *Cyperous rotundus* were collected from locally from the Rohilkhand region and dried in shade and identified correctly by Dr. Alok Kumar Khare, Department of Botany, Bareilly College, Bareilly 243001, (UP) India. (ref-Bareilly College Herbarium, BHRK-1603).

The collected plant material were chopped into small pieces, shade dried and coarsely powdered with suitable pulverizer. The coarse powder was subjected to successive extraction with warm organic solvents like petroleum ether, Benzene, chloroform, acetone and ethanol by soxhlet method[3].

Preliminary Phytochemical Screening[4]

Test for alkaloids

To the test solution in 10 ml menthol, add 1 % (w/v) HCl and any of Mayors reagents, Wagner's reagent or Dragendroff reagent (6 drops). A creamish or brownish red or orange precipitate indicated the presence of alkaloids.

Test for Anthraquinones

To the test solution add a benzene drop and ammonia drop, a pink colour indicates the presence of anthraquinons.

Test for Flavonoids

5ml of dilute ammonia solution was added to a portion of the aqueous filtrate of each plant extract followed by addition of concentrated H_2SO_4. A yellow colour in each extract indicated the presence of flavonoids. The yellow colour disappeared on standing. Few drops of 1% ammonia solution were added to portion of each filtrate. A yellow colour indicates the presence of flavonoids.

A portion of the powdered plant sample was in each case heated with 10 ml of ethyl acetate over a steam bath for 3 min. The mixture was filtered and 4 ml of the filtrate was shaken with 1 ml of dilute ammonia solution. A yellow colour indicates opposite test for flavonoids.

Test for Coumarins

To the test solution add a drop of sodium sulphate developing yellow colour. Indicates the presence of coumarines.

Test for Phenols

To the test solution add a drop of ferric chloride. Developing of intense colour develops.

Test for Saponins

About 2 g of the powdered sample was boiled in 20 ml of distilled water in a water bath and filtered. 10 ml of the filtrates was mixed with 5ml of distilled water and shaken vigorously for a stable persistent froth, which indicates the presence of saponins.

Test for Steroids

2 ml of acetic anhydride was added to the solution along with 2 ml of conc. H_2SO_4. The colour changed from violet

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Table 1: Effect of chloroform extract of *Cyperous rotundus* on ethanol induced ulcers in rat.

Treatment	Dose	Ulcer index %	Ulcer inhibition
Control	10ml/kg	0.0 ± 0.0	-
EtOH	5ml/kg	21.0 ± 4.7	-
Chloroform Extract	200mg/kg	8.6 ± 4.0	51.60
	400mg/kg	7.7 ± 2.1*	48.11
Ranitidine	50mg/kg	8.9 ± 2.3*	51.51

Value are expressed as (Mean± S.E.M.), n=6, *p with compared with controlled group.

to blue or green in some samples. This indicates the steroids.

Test for Terpenoids

5 ml of each extract mixed with 2ml of chloroform, and 3 ml concentrated H_2SO_4 was carefully added to form a layer reddish brown colour of the interface was formed to show positive results for the presence of terpenoids.

Test for Tannins

About 0.5 g of the leaves was dried and powdered samples was boiled in 20 ml of water in a test tube and then filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration.

Test for Amino acids and Proteins

Take 2-3 ml of sample solution in a test tube. Add 3-4 drops of ninhydrine solution and heat. Appearance of purple or violet indicates the presence of proteins.

Test for Carbohydrates

Add 1ml of Benedicts reagents to test tube and heat the mixture to boiling in a water bath for 2mins .the formation of an orange red colour precipitate due to formation of a copper (I) oxide indicates the presence of reducing sugars

Antiulcer Activity

Animals

Adult male albino rats weighing about 200-220g were used for study. The animal room was well ventilated with a 12h light/ dark cycle throughout the experimental period. They were maintained in clean, polypropylene cages and fed with Mona Laboratory animal feeds for rats/mice (Manufactured by Raman Dairy Vikash Udyog and Marketed by Pashu Aahar Kendra, Varanasi, UP, India) and water ad libitum[5]. Institutional Animals Ethics Committee (IAEC) approved the experimental protocol and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare and Government of India[6]. Drugs and Chemicals All the drugs and chemicals were of analytical grade, Ranitidine (Osaka), Ethanol (Research Lab) were used. Acute toxicity study (LD_{50}) Acute toxicity studies were carried out on wistar rats by the oral route at dose levels upto 2000mg/kg ethanol extract of *Cyperous rotundus* as per organization for economic cooperation and development (OECD) guidelines No 423. Animals were divided in groups (n=3). The animals were fasted for 4h with free access to water only. The ethanol extracts of *Cyperous rotundus* were administered orally in doses of 200mg/kg and 400mg/kg to different groups of rat and observed over 14days for mortality and physical/behavioral changes[7]. Estimation of anti-ulcer activity through Ethanol (EtOH) induced ulcer in rats Intragastric application of absolute ethanol is reproducible method to produce gastric lesions in experimental animals. These lesions least partially inhibited by various drugs, such as some prostaglandins. The protective effect against various irritants has been called cytoprotective activity. In this four groups each of six albino rats were taken randomly and designated as Group I to Group III. Group I: Rats were given 0.025% CMC suspension (10ml/Kg) orally for 14 consecutive days. Group II: Rats were given the suspension of chloroform extract of *Cyperous rotundus* (200 and 400mg/kg) orally for 14 consecutive days. Group III: Rats were given the suspension of Ranitidine (50mg/Kg) as a Standard. Rats were deprived from food (but not from water) on day 4 of the experiment. On the last day of experiment (day15) rats were given absolute ethanol (90%) (1ml/200g) by gastric intubation 1hr before sacrificing, except for rats in group IV which consisted of six rats that were fasted for 24 hrs, administered orally with Ranitidine and was given 90% ethanol as above 8 hrs thereafter. Rats were sacrificed after one hrs of ethanol administration. Stomach was removed and incised along the greater curvature and ulceration will be scored.

RESULTS

Acute Oral Toxicity Study Acute oral toxicity was carried out by up-down regulation method. It is found that chloroform extract of *Cyperous rotundus* were safe at limit dose 2000mg/kg and 4000mg/kg with no mortality in studied subjects.1/10th of these doses i.e.200mg/kg and 400mg/kg were used in the subsequent study respectively. Ethanol Induced Gastric Ulceration Ethanol at dose of 5ml/kg showed superficial, deep ulcers and perforations in the control animals (Table 1). However, animals treated

with chloroform extract of *Cyperous rotundus* at 200 and 400mg/kg doses showed significant ($P<0.05$), reduction in the number of ulcer and ulcer index (Table 1). It showed 51.60, 48.11% ulceration inhibition at the dose of 200 and 400mg/kg respectively where as ranitidine showed 51.51%ulceration inhibition. Anti-ulcerogenic effect of *Cyperous rotundus* in ethanol induced ulcers was comparable to that of ranitidine 50mg/kg.

DISCUSSION

The preliminary phytochemical analysis of *Cyperous rotundus* extract shows the presence of alkaloids, flavonoids, terpenoids and glycosides. the significant increase in the antiulcer activity of *Cyperous rotundus* extract could be attributed to the presence of flavonoids, terpenoids alkaloids and saponin glycoside. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. It is suggested that, these active compounds would be able to stimulate mucus, bicarbonate and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants inn gastrointestinal lumen. So the antiulcer activity of *Cyperous rotundus* extract may be attributed to its flavonoids content. The results of the present study suggest that the chloroform extract of *Cyperous rotundus* may be beneficial in the treatment of gastric lesions.

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