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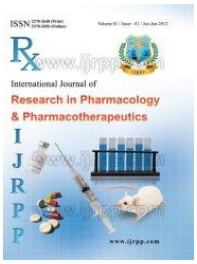

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Evaluation of anti-ulcer activity of ethanolic extract of *Dactyloctenium aegyptium*

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ABSTRACT

The ethanolic extract of *Dactyloctenium aegyptium* was investigated for its anti-ulcer activity against aspirin plus pylorus ligation induced gastric ulcer in rats, HCl -Ethanol induced ulcer in mice and water immersion stress induced ulcer in rats at 300 mg/kg body weight. p. o. A significant ($P<0.01$, $P<0.001$) anti-ulcer activity was observed in all the models. Pylorus ligation showed significant ($P<0.01$) reduction in gastric volume, free acidity and ulcer index as compared to control. It also showed 89.71% ulcer inhibition in HCl- Ethanol induced ulcer and 95.3% ulcer protection index in stress induced ulcer. This present study indicates that *Dactyloctenium aegyptium* extract has potential anti ulcer activity in the three models tested.

Keywords: *Dactyloctenium aegyptium,* Aspirin, Gastric ulcer in rats, Anti Ulcer activity.

INTRODUCTION

The genesis of gastroduodenal ulcer requires acid, peptic activity and a breakdown of mucosal defense mechanism[1]. Recent studies have implicated the production of free radicals and lipid peroxidation in the development of ulcers[2]. Efforts were made to find a suitable agent for the treatment of peptic ulcer in natural products of plants and animal origin. In an Indian traditional system of medicine, *Dactyloctenium aegyptium* is used in the treatment of ulcers, small pox, fever, and as a cooling agent[3].

The aim of the work is the Evaluation of Antiulcer Activity of ethanolic Extracts of *Dactyloctenium aegyptium*. *Dactyloctenium aegyptium* has documented *to possess antimicrobial* activity, but the effect of *Dactyloctenium aegyptium* as an Antiulcer agent is still not reported. Hence it was thought worth while to screen extract of *Dactyloctenium aegyptium* for its Antiulcer activity.

MATERIALS AND METHODS

Wistar rats (175 + 5 g) were provided with standard rat feed and tap water ad libitum. The animals were kept in our animal room with

maintenance of room temperature (22 + 2oC) and light: dark exposure of 12:12 h.

Plant collection and identification

The collected plant materials were washed, sliced, and completely dried in a hot air oven at 60oC. The dried materials were ground and macerated in 95% ethanol for three days and filtered. The marc was remacerated in 95% ethanol for another three days and filtered. The two sets of the filtrate were pooled and evaporated to give a crude extract, which was dissolved in mixed solvents of methanol and water (9:1). The dissolved crude extracts were re-extracted with an equal volume of hexane, dichrolomethane and butanol in succession at least three to four times for each solvent. The extracts obtained from each solvent were combined and concentrated to dryness under reduced pressure[4].

Phytochemical Analysis

The ethanolic extract prepared was analyzed for the presence of alkaloids, saponins, tannins, steroids, flavinoids, anthraquinones, cardiac glycosides and reducing sugars based on the protocols available in the literature [5,6,7,8.]

Tests for Alkaloids

Mayer's Test (Potassium Mercuric Iodide)

A fraction of the extract was treated with Mayer's reagent and observed in the formation of a cream-colored precipitate.

Dragendroff's Test

A fraction of the extract was heated with Dragendroff's reagent and observed in the formation of a reddish orange-colored precipitate.

Wagner's Test

A fraction of the extract was treated with Wagner's reagent and observed in the formation of reddish brown -colored precipitate.

Hager's Test

A fraction of the extract was treated with Hager's reagent and observed in the formation of yellow -colored precipitate.

TESTS FOR CARBOHYDRATES

Molisch's test

Fraction of the extract was treated with a solution of 2-napthol and few drops of sulfuric acid was added through the sides of the test tube and observed in the formation of a violet ring between the junction show the presence of carbohydrates.

Fehling's Test

A fraction of the extract was treated with Fehling's A solution and B and they are heated on a water bath for a few minutes and observed in the formation of a red -colored precipitate.

Barfoed's Test

A fraction of the extract was treated with Barfoed's reagent and observed in the formation of a red -colored precipitate.

Benedict's Test

A fraction of the extract was treated with Benedict's reagent and in boiling water bath for a few minutes and observed in the formation of an orange red -colored precipitate.

TEST FOR GLYCOSIDES

Legal test

To the sample 1 ml of pyridine and a few drops of sodium nitroprussside solution was added and then it was made alkaline with sodium hydroxide solution. Appearance of pink color shows the presence of a glycoside.

Kiddes Test

Cardenolides give blue or violet with firs reagent which fades after 1-2 hours. This reagent is prepared by mixing equal volume of 0.21 solutions of 3, 5 di nitro benzoic acid in 100 ml of 0.5 N KOH solution on 50% methanol.

KELLER KILLIANI TEST

1gm of powdered drug extracted with 10 ml of 70% alcohol for a few minutes and filtered. To 5 ml of filtrate add 10 ml of hydrogen peroxide and 0.5 ml of strong solution of lead acetate was added. Precipitate thus obtained was filtered. The filtrate is shaken with 5 ml of chloroform and the layer is separated and to this 1 m l of mixture of volume of 5%ferric sulfate and 99 volumes of glacial acetic acid was added.

To this mixture 1-2 drops of conc. Sulphuric acid is added. Appearance of blue color confirms the presence of deoxy sugars.

Antimony trichloride test

Solution of the extract is heated with antimony trichloride and tri chloro acetic acid to obtain blue or violet color. Both Cardenolides and bufadienolides give this test.

Borntrager's Test

The extract was treated with chloroform and chloroform layer was separated. To this equal quantity of dilute ammonia solution was added ammonical layer acquires rose pink color shows the presence of a glycoside.

Test for fixed Oils

Small quantity of extract was separately passed between two filter paper. Appearance of stain on the paper indicates the presence of fixed oil. Few drops of 0.5 alcoholic KOH were added a small quantity of extract along with drops of phenolphthalein. Then the mixture was heated on a water bath for 1-2 hours. Formation of soap neutralization of alkali indicates the presence of fixed oil and fats.

Tests for Tannins and Phenolic Compounds

Ferric chloride test

A fraction of the extract was treated with ferric chloride solution and observed in the formation of brownish colorization.

Lead acetate test

To the extract adds 10% lead acetate solution and observed in the formation of white precipitate.

Gelatin solution test

To the extract, add 1% solution gelatin containing sodium chloride solution and observed in the formation of white precipitate.

Test for Saponins

Foam test

The extract was diluted with 20 ml of distilled water and it was agitated on a graduated cylinder for 15 minutes. The formation of 1 cm layer of foam shows the presence of saponins.

Test for Proteins

Millon's Test

To the extract, add little amount of water and millon's reagent. The appearance of red color shows the presence of proteins.

Ninhydrin test

To the extract adds a little amount of Ninhydrin reagent. Appearance of purple color shows the presence of proteins.

Test for Flavoniods

Aqueous NaOH Test

To the extract adds a little amount aqueous sodium hydroxide solution and observed in the formation of color. Blue-violet color (anthocynanine), Yellow color (flavones), Yellow-orange (flavones)

Conc. H_2SO_4 Test

To the extract adds a little amount of conc. Sulfuric acid and observed in the formation of color.Yellow- orange (anthocynanine), Yellow colour (flavones), Orange-crimson (flavononones)

Schinodo's test

For a small amount of extract adds a piece of magnesium followed by conch., Hydrochloric acid and heated slightly, and then observe the color changes. Dark pink color (flavonoids).

Pharmacological Screening

Drug induced gastric ulcer in rats

Animals of the control group received saline (5 ml/kg) and test group received *Dactyloctenium aegyptium* (100 mg/kg and 200 mg/kg) for 6 days. From day 6, the animals received saline or test drug, 2 h prior to the administration of indomethacin (20 mg/kg, orally). Overnight fasted animals were sacrificed by cervical dislocation 3 h after the last dose of ulcerogen. The stomach was incised along the greater curvature and examined for ulcers. The gastric lesions were counted and the mean ulcerative index was calculated as follows: I, presence of edema, hyperemia and single submucosal punctiform hemorrhages; II, presence of submucosal hemorrhagic lesions with small erosions; III, presence of deep ulcer with erosions and invasive lesions [9]. Cold restraint stress induced ulcers: Dactyloctenium aegyptium (25, 50 mg/kg) were introduced for 7 days. On the day 7, the overnight fasted rats were restrained in a metallic restraint chamber 30 min after the administration of test drug and kept for 2 h in a refrigerator at 4-6°C. After the period of immobilization, the rats were sacrificed by cervical dislocation and the stomachs were removed for ulcer scoring[10]. Biochemical estimations: Serum calcium[11] and gastric tissue

lipid peroxidation were estimated in rats that develop ulcers due to indomethacin. The stomach homogenates were prepared in chilled 0.15 M KCl and lipid peroxidation (thiobarbituric acid reacting substances or TBARS) was determined[12]. Protein estimations of tissue homogegenates were made according to Lowry et al [13].

Statistical analysis

Results were analyzed using Student's ' t ' test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Preliminary Phytochemical Screening

The preliminary phytochemical screening of the ethanolic extract showed the presence of plants phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins was carried out on the powdered fruits following standard procedure.

Dactyloctenium aegyptium showed dose dependent reduction of ulcer index in indomethacin treated rats as well as in rats subjected to cold restraint stress, when compared to control (Table 2). It showed a reduction in TBARS content of stomach tissue in indomethacin treated ulcer group (Table 2). No significant difference was noted in serum calcium activity between the groups (Table 2).

It is generally accepted that gastric ulcer results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism[14]. Several studies have indicated that gastroduodenal protection by prostaglandins (PG) include both increase in mucosalresistance as well as decrease in aggressive factors, mainly acid and pepsin[15]. Inhibition of PG synthesis by indomethacin coincides with the earlier stages of damage to the cell membranes of mucosal, parietal and endothelial cells[15]. Similarly, cold restraint stress induced ulcer represents a unique ulcer model in examining the cause, course, consequence and treatment of peptic ulcer[16]. In this study, we observed a dose dependent protection offered by Dactyloctenium aegyptiumin indomethacin and cold restraint B stress induced gastric ulcers. There is extensive experimental evidence that indicates certain substances, through scavenging of free radicals, protect the gastric mucosa[17]. The thiobarbituric acid reactive substance (TBARS) is used as an indicator of lipid peroxidation and free radical activity in biological samples.

Table 1 Phytochemical Evaluation of Ethanolic extract of Dactyloctenium aegyptium

S.NO.	TESTS	ETHANOL
1.	Alkaloids	+Ve
2.	Carbohydrates	+Ve
3.	Glycosides	+Ve
4.	Fixed Oils	+Ve
5.	Tannins	+Ve
6.	Sterols	+Ve
7.	Saponins	+Ve
8.	Proteins	+Ve
9.	Flavinoids	+Ve

+Ve Indicates Present,-Ve Indicates Absent

Table 2 Effect of Dactyloctenium aegyptium on ulcer index, serum calcium and tissue T BARS in rats.

Treatment	Ulcer index		Serum calcium	TBARS
	Indomethacin	Cold restraint		
Control	31.5 ± 0.76	28.7 ± 1.52	12.6 ± 1.36*	14.65 ± 0.52

Dactyloctenium aegyptium (100 mg/kg)	14.6 ± 1.08*	16.9 ± 2.04*	10.76 ± 0.48^{ns}	10.01 ± 0.43*
Dactyloctenium aegyptium (200 mg/kg)	10.1 ± 0.57*	12.6 ± 1.36*	10.93 ± 0.83 ^{ns}	8.76 ± 0.71*

Values are mean + SEM of 6 animals in each group * P< 0.001 when compared to control; ns = statistically not significant**.**

CONCLUSION

In the present study, Dactyloctenium aegyptium exhibits a potent anti-peroxidative effect without altering serum calcium level. Hence, it can be suggested from our study that Dactyloctenium aegyptium provides anti-ulcer activity in rats. It may act as gastric cytoprotective agent by modulating scavenging of free radicals. Further studies like, acids and mucopolysaccharides estimations by pyloric ligated models are required to establish the role of Dactyloctenium aegyptium in protection against gastroduodenal ulcer.

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