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Preclinical Evaluation of Protective Effect of *Cynodon dactylon* Pers on Experimentally Induced Gastric Mucosal Damage

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Research Article

ABSTRACT

The present study was undertaken to evaluate the protective effect of *Cynodon dactylon* pers on experimentally induced gastric mucosal damage. In this study two experimental models i.e alcohol model and indomethacin model were used. Ranitidine was taken as standard drug in both the models. Both the models consist of 4 groups with 6 rats in each group (control, standard control, test compound 300mg/kg, tests compound 450mg/kg). The control group received only ulcerogen whereas the standard control group and test compound groups were pretreated with ranitidine and *Cynodon dactylon* respectively before exposure to ulcerogen. 4 hours after exposure to ulcerogen the rats were sacrificed, stomachs were dissected out and opened. The total number of ulcers, size of each ulcer was noted and ulcer index was calculated. Statistical analysis was done by Mann – whitny test. A p value of < 0.05 was considered for statistical significance. In alcohol model the rats pretreated with *Cynodon dactylon* showed significant protection as compared to control and ranitidine pretreated groups. However in indomethacin model the rats pretreated with ranitidine has given better protection.

INTRODUCTION

Peptic ulcer is common disease. Even though mortality associated with this disease is less, morbidity is more. The incidence has been estimated variously as ranging from 3 – 10 %. Pathogenesis of peptic ulcer is far from clear and so is the mechanism of antiulcer drugs to some extent [1]. Peptic ulcer results probably due to an imbalance between the aggressive factors (acid, pepsin, H-pylori) and defensive factors (gastric mucus & bicarbonate secretion) [2]. Because an increase in the gastric secretion was thought to be the main culprit in causation of peptic ulcer, most of the drugs available either neutralize the secreted gastric acid or decrease the acid secretion.

In the past few decades increased knowledge about the pathophysiology of peptic ulcer has paved the path for understanding other possible causes like decreased mucosal defense in presence of normal acid secretion and H.pylori colonization of gastric mucosa and development of new drugs. Therefore the scope for research into the therapy for peptic ulcer has widened considerably. Even though the objectives of therapy remains same viz. relief of pain, promotion of ulcer healing, prevention of recurrences and complications, there will be definite role for new drugs.

There is growing body of experimental data that suggests the generation of oxygen derived free radicals and lipid peroxidation as one of the mechanisms in the pathogenesis of peptic ulcer [3]. Hence there is a need to develop drugs that are directed towards scavenging of these free radicals and produce antiulcerogenic effect. It holds a bright and promising future.

In the past decade studies have shown that many plant extracts do have antioxidant property[4]. In the rural population of Kerala, juice of the plant *Cynodon dactylon* (Bahama grass) is being used for dyspepsia. Europeans use juice of this plant for heart burn[5]. This plant is found to have antioxidant property[6]. Based on these observations this study is of undertaken to evaluate the antiulcer property of juice of *Cynodon dactylon*.

Objectives of study

To study the possible antiulcerogenic property of *Cynodon dactylon* pers and to compare its efficacy with standard drug Ranitidine in albino rats

MATERIALS AND METHODS

48 albino rats of wistar strain of either sex, weighing between 150 – 200g were selected from central animal house of Karnataka Institute of Medical Sciences, Hubli. The animals were kept on standardized diet and allowed food and water ad libitum. The experimental protocol was approved by Institutional animal ethics committee.

Materials

Drugs and dosage

• Ethanol (99.9%) as ulcerogenic agent : a dose of 1 ml
• Indomethacin powder (Micro Labs Ltd, Bangalore) as ulcerogenic agent: 20mg/kg, 2 doses at an interval of 15 hrs.
• Ranitidine (J B Chemicals and Pharmaceuticals Ltd) as standard control : 25mg/kg once daily for 5 days
• *Cynodon dactylon* Juice (Lyophilysed powder): 100mg dissolved in 0.3ml distilled water and given in the dose of 300mg/kg and 450mg/kg

Test compound

Cynodon dactylon (Bahama Grass).

Fresh green Bahama grass was collected from the outskirts of Hubli . Grass was cleaned and slender leaves were separated from the roots and main stalk. With the help of a mixer, juice was collected and measured. 25 ml of juice collected from 100g of processed grass. Lyophylisation was done to get the dry powder. Approximately 100g of grass has yielded 1.23g of dry powder.

Route of administration

All the drugs and *Cynodon dactylon* were administered intragastrically through the infant feeding tube.

Methods

Ethanol Induced Gastric Ulcers

In this method 24 albino rats were divided into 4 groups with 6 rats in each group

Group 1 (Control)   : Received only ulcerogen (ethanol)
Group 2 (Standard control)   : Received ranitidine once daily for 4 days, and 30 prior to ulcerogen on 5th day
Group 3 (Test compound)   : Received *Cynodon dactylon* 300mg/kg, 30 min prior to ulcerogen
Group 4 (Test compound)   : Received *Cynodon dactylon* 450mg/kg, 30 min prior to ulcerogen

Indomethacin Induced Gastric Ulcers

In this method 24 albino rats were divided into 4 groups with 6 rats in each group

Group 5 (Control)   : Received only ulcerogen (Indomethacin)
Group 6 (Standard control) : Received ranitidine once daily for 3 days and 30 prior to ulcerogen on 4th and 5th day
Group 7 (Test compound)   : Received *Cynodon dactylon* 300mg/kg and 30 min prior to ulcerogen
Group 8 (Test compound)   : Received *Cynodon dactylon* 450mg/kg and30 min prior to ulcerogen

In both the groups animals were fasted for 24 hrs prior to the administration of ulcerogen with water ad libitum. On 5th day animals were sacrificed 4 hrs after the administration of ulcerogen by dislocating the cervico – atlanto joint. The anterior abdominal wall was opened and the stomach was dissected out. The number of ulcers, size of each ulcer(mm) and grading of each ulcer was done according to the method described by Laurence and Bacharach[7]

The ulcer Index was calculated by the formula

Ulcer Index = Ulcer number X Ulcer size

Statistical analysis

The results were analyzed by using Mann–Whitney test. A p value of < 0.05 was considered for statistical significance.

RESULTS

Ethanol induced gastric ulcers

Table 1: Effect of pretreatment of Ranitidine and *Cynodon dactylon* on ethanol induced gastric ulcers

	Group 1	Group 2	Group 3	Group 4
Total No of ulcers	53	36	30	22
Mean ulcer No ± SEM	8.8 ± 0.7	a 6.0 ± 0.4	a 5.0 ± 0.9	a , b 3.7 ± 0.5
Mean ulcer size ± SEM (mm)	17.6 ± 0.4	a 9.7 ± 0.4	a , b 5.8 ± 0.4	a , b 5.1 ± 0.3
Severity of ulcer ± SEM	18.3 ± 0.9	a 13.0 ± 1.1	a 10.5 ± 0.8	a , b 7.8 ± 0.7
Ulcer index ± SEM	155.0 ± 10.1	a 58.2 ± 5.5	a , b 29.4 ± 3.6	a , b 18.9 ± 2.8

n = 6. a $p < 0.01$ compared with group 1
 b $p < 0.01$ compared with group 2

Group 1 (Ethanol only): In this group, the mean ulcer number, mean ulcer size and severity of ulcers were 8.8 ± 0.7, 17.6 ± 0.4, and 18.3 ± 0.9 respectively. The ulcer index was 155 ± 10. 1

Group 2 (Ranitidine + Ethanol): In this group, there was reduction in mean ulcer number, mean ulcer size and severity of ulcers as compared to control group. The ulcer index was also reduced

Group 3 (*Cynodon dactylon* 300 mg + Ethanol) : In this group the mean ulcer number, mean ulcer size, severity of ulcers and ulcer index were significantly reduced ($p < 0.01$) as compared to control.

Group 4 (*Cynodon dactylon* 450 mg + Ethanol) : In this group the mean ulcer number, mean ulcer size, severity of ulcers and ulcer index were significantly reduced ($p < 0.01$) as compared to control group

Indomethacin induced gastric ulcers

Table 2: Effect of pretreatment of Ranitidine and *Cynodon dactylon* on indomethacin induced gastric ulcers

	Group 5	Group 6	Group 7	Group 8
Total No of ulcers	36	14	31	28
Mean ulcer No ± SEM	6.0 ± 0.9	a 2.3 ± 0.6	5.2 ± 0.4	b 4.7 ± 0.2
Mean ulcer size ± SEM (mm)	2.93 ± 0.21	a 1.44 ± 0.38	2.92 ± 0.12	2.69 ± 0.13
Severity of ulcer ± SEM	11.3 ± 0.8	a 3.3 ± 0.9	9.5 ± 0.8	b 8.8 ± 0.5
Ulcer index ± SEM	17.7 ± 2.0	a 4.0 ± 1.2	15.0 ± 1.1	b 12.5 ± 0.7

n = 6. a $p < 0.01$ compared with group 5, group 7, group 8
 b $p < 0.05$ compared with group 5

Group 5 (Indomethacin only): In this group, the mean ulcer number, mean ulcer size and severity of ulcers were 6.0 ± 0.9, 2.93 ± 0.21, and 11.3 ± 0.8 respectively. The ulcer index was 17.7 ± 2. 0

Group 6 (Ranitidine + Indomethacin) : In this group, there was reduction in mean ulcer number, mean ulcer size and severity of ulcers as compared to control group. The ulcer index was also significantly reduced (p < 0.01)

 Group 7 (*Cynodon dactylon* 300 mg + Indomethacin) : In this group, even though there was reduction in mean ulcer number, the mean ulcer size, severity of ulcer and ulcer index were not reduced significantly

Group 8 (*Cynodon dactylon* 450 mg + Indomethacin) : In this group, there was reduction in mean ulcer number, severity of ulcer and ulcer index. The p value was significant (p < 0.05). Even though there was reduction in mean ulcer size the p value was not significant. .

DISCUSSION

Present study was to evaluate the protective effect of *Cynodon dactylon* (C.D) against ethanol and indomethacin induced gastric mucosal damage

Ethanol causes gastric mucosal damage by lipid peroxidation. Ethanol causes significant production of free radicals and this is mainly responsible for lipid peroxidation [8] and damage to cell membranes. Xanthine oxidase may be one of the source of these free radicals and neutrophils another source [9, 10].In this study, in alcohol model, the total number of ulcers produced by ethanol in control group (group 1) were 53. The mean ulcer number, mean ulcer size and severity of ulcer were 8.8 ± 0.7 , 17.6 ± 0.4 and 18.3 ± 0.9 respectively. The ulcer index was 155± 10.1.

In our study pretreatment with CD at the dose of 300 mg/kg and 450 mg/kg reduced total ulcer number to 30 and 22 respectively. The mean ulcer number, mean ulcer size, severity of ulcer and ulcer index were significantly reduced when compared to control group (table 1). Chandraprabha et al have reported the role of CD in reducing the lipid peroxidation [6]. As lipid peroxidation is one of the important mechanism of ethanol induced gastric mucosal damage [11], CD probably act by inhibiting the lipid peroxidation. This property of CD can be attributed to one of its important constituents viz. flavonoids [12], which also have the antioxidant property [13].

Ranitidine pretreated animals showed reduction in total number of ulcers(36) as compared to control group, but the ulcer number was more when compared with CD pretreated animals at different doses. The mean ulcer number, mean ulcer size, severity of ulcer and ulcer index in CD pretreated animals (Group 4) were significantly reduced when compared to ranitidine pretreated group (p < 0.01)

Ranitidine is H$_2$ receptor blocker and inhibits gastric acid secretion. Ethanol induces gastric mucosal damage irrespective of the acid content of stomach [14]. In this study the mucosal protection by CD was more significant than ranitidine. In a study conducted by Tarnawski et al, it is shown that H$_2$ receptor blockers could not prevent ethanol induced gastric mucosal damage [15].

One of the most common adverse effect of NSAIDs is gastric mucosal damage. Indomethacin is one of them. NSAIDs cause gastric mucosal damage by inhibiting synthesis of prostaglandins, but normal amount of gastric acid is necessary for development of gastric lesions produced by NSAIDs [14].

In the present study, the total number of ulcers in ranitidine pretreated animals were markedly reduced (14) compared to control group (36) and C.D   pretreated animals at two different doses (31 & 28). Table -2   summarizes the effect of ranitidine. The mean ulcer number, mean ulcer size, severity of ulcer and ulcer index were reduced significantly (p < 0.01) as compared to control group and CD pretreated group at two different doses.

Even though pretreatment with CD in the dose of 300 mg /kg (group7) has reduced mean ulcer number, mean ulcer size, severity of ulcer and ulcer index as compared to control group, it is statistically not significant.

However, CD in the dose of 450 mg/kg (group 8) has significantly reduced mean ulcer number, severity of ulcer and ulcer index as compared to control group (p < 0.05).

Indomethacin induces gastric mucosal damage not only by inhibiting prostaglandin synthesis, but also by causing lipid peroxidation [16]. Probably by inhibiting lipid peroxidation CD exert its gastric mucosal protection against indomethacin induced lesions.

From table 1 and 2 we can say that CD pretreatment has given significant gastric mucosal protection against ethanol induced gastric mucosal damage (better than ranitidine) and indomethacin induced gastric lesions (in the dose of 450 mg/kg). However ranitidine pretreatment has given better protection in indomethacin model.

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