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Evaluation of Anti-ulcer and Anti-secretary properties of the *Calotropis procera* root extract

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ABSTRACT

The present study was undertaken to determine the anti-ulcer properties of the Methanol extract of *Calotropis procera* root. Antiulcer activity of methanolic extracts of the root *C. Procera* was tested in rats, in which gastric ulcerations were experimentally induced by aspirin, alcohol, stress and pylorus ligation. The extract was administered in the dose of 200 mg/kg (Group-III) and 400mg/kg (Group-IV) orally to the test group of animals for 3 consecutive days and on fourth day the rats were subjected for ulcer index and gastric acid evaluation. The reduction in the ulcer index as well as gastric output in group-III animals was found to be significant with respect to group-IV. The study revealed ulcer protection activity of the extract in dose dependent manner. Lansaprazole was used as standard drug for ulcer protection.

Keywords: *Calotropis procera,* anti-ulcer, Anti-secretary.

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INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrhythmia's, impotence, funaecomastia and haematopoeitic changes) of modern medicine [1]. indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer. There is evidence concerning the participation of reactive oxygen species in the etiology and pathophysiology of human diseases, such as neurodegenerative disorders, inflammation, viral infections, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammations and gastric ulcer [2]. In the anti-oxidant status following ulceration, indicating that free radicals seem to be associated with the pylorus ligation [3] and ethanol induced [4] ulceration in rats. Reactive oxygen species have also been implicated in drug induced gastric hyperacidity and stress induced gastric ulceration [5].

According to traditional system of medicine one of such plant, possessing anti-ulcer activity is *Calotropis procera* (Ait) R.Br. is a plant which is found abundantly in Asia and Africa. Different parts of the plant have been advocated for use in a variety of disease conditions. The plant has been used as a purgative, anthelmintics, antileprotic and antiulcer.The latex of the plant has been claimed to be useful as an abortifacient and leaves have been claimed to cure abdominal pain. The plant has been reported to possess diverse biological activities. Different parts of the plant have been found to possess proteolytic [6]. antimicrobial, larvicidal [7] and anticancer [8] activities. On preliminary pharmacological studies in our laboratory, it was revealed that the methanol fraction of *C.procera* root extract possessed significant anti-inflammatory and analgesic activity [9]. The root extract was also found to possess significant hepatoprotective activity against CCl_4 induced hepatotoxicity [10].

Since it is well established that the nonsteroidal anti-inflammatory agents are potentially ulcerogenic, because of the fact that these drugs inhibit the synthesis of cytoprotective prostaglandins PGE_2 and PGI_2 and might also cause overproduction of leukotrenes (as NSAIDs block the cyclooxygenase pathway, arachidonic acid metabolism is shifted to the lipooxygenase pathway) which in turn might be damaging to the gastric mucosa. Thus it is essential to evaluate the role of *C. procera* on different models of gastro duodenal ulceration in rats.

MATERIALS AND METHODS

Plant Material

C. procera root was collected from the rural area of north Karnataka. The plant was identified and authenticated by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli, Karnataka, India. A voucher specimen (01/2008) has been deposited at the museum of our college. The root was collected in the month of May 2010 and shade dried at room temperature.

Preparation of extract

C. procera root was powdered in electrical grinders. This powder was packed into Soxhlet column and extracted successively with methanol. The dried extracts were stored in air tight container in refrigerator below 10° C. The Methanol extract was dissolved in water before administration to the animals.

Experimental Animals

Albino rats (150-200 g) were procured from National Institute of Mental Health and Neuro Sciences, Bangalore India. After procuring the animals were acclimatized for 10 day's under standard husbandry conditions, room temperature (27 ± 3° C), relative humidity (65 ± 10 %) and 12 hours light / dark cycle. They were allowed free access to standard dry pellet diet (Gold mohr, Lipton India Ltd., Bangalore, India) and water *ad libitum* under strict hygienic conditions. All the described procedure were reviewed and approved by the Institutional Animal Ethical Committee (Reg No:361 /00/CPCSEA).

Aspirin-induced Gastric Ulcer

The male rats were randomly divided into four groups and fasted for 24 h. To the first group it was given of vehicle (normal saline, 1ml/100 g, p.o.), Group I was treated with Lansaprazole (8 mg/kg) orally and the Group II and III received orally 200 and 400 mg/kg of Methanol extract of the *C. procera*. An hour later aspirin (250 mg/kg) was administered orally to all the animals and 6 h later the animals were sacrificed by cervical dislocation[11]. The stomachs were removed, opened along the greater curvature and examined under microscope. Scoring of ulcer was done by the following method: 1= erosions 1 mm or less, 2 = 1-2 mm, 3 = < 2 mm. The overall score was divided by a factor of 10 which was designated as the ulcer index (Main and, Whittle 1975). The percentage of ulcer inhibition was calculated as follows;

Percentage of ulcer inhibition = Mean ulcer index of control - Mean ulcer index of test / Mean ulcer index of control x 100.

Alcohol-Induced Gastric Ulcer

The male rats were randomly divided into four groups and fasted for 24 h with free access to water. Animals were given vehicle or Methanol extract of the *C. procera* @ 200 and 400mg/kg and control were administered with Lansaprazole (8 mg/kg) orally. One hour later, 1 ml of 80% ethanol was administered orally to each animal [12]. One hour after ethanol administration animals were sacrificed by cervical dislocation, stomachs were isolated and cut open along the greater curvature and pinned on a soft board. The length of each gastric lesion was measured and the lesion index was expressed as sum of the length of the entire lesion in mm.

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Stress induced gastric ulcer

Albino wistar rats of either sex weighing between (150-200gms) were divided into four groups of six animals in group. Group–I Positive Control, Group–II Standard (Lansoprazole 8mg / Kg p.o), Group-III Methanolic extract (200mg /kg p.o) and Group-IV methanolic extract (400mg /kg P.o.).Cold restraint stress (CRS) –induced Ulcers to 18hr. fasted rats, cold restraint stress was given by strapping the rats on a wooden plank and keeping them for 2hr at $4^0 – 6^0$ C. The animals were then sacrificed by cervical dislocation and ulcers were scored on the dissected stomachs.

Pylorus- Ligation Induced Gastric Ulcer

In this method male albino rats were fasted in individual cages for 24 h. The vehicle or Methanol extract of the *C. procera* (200 and 400 mg/kg) or reference drug (Lansaprazole, 8 mg/kg) was administered 30 min prior to pyloric legation orally. Under light ether anesthesia, the abdomen was opened and the pylorus was legated. The abdomen was then sutured. At the end of 4 h after legation, the animals were sacrificed with excess of anesthetic ether and the stomach was dissected out. Gastric juice was collected and its volume, pH, free acidity and total acidity were determined. The glandular portion was then exposed and examined for ulceration. Ulcer index was determined [13].

Determination of total acid and free acid were estimated from gastric juice collected from the 4 hr pyloric ligated rats. Total acid output of the gastric juice was estimated by titration of 0.1 ml of gastric juice with 0.01N sodium hydroxide using phenolphthalein as indicator. Total acid output was expressed as mEq/L per 100 gm of body weight [14].

Statistical Analysis

Values are expressed as mean $\pm$ S.E.M. The data were statistically evaluated by analysis of variance (ANOVA) coupled with student't' test. $P<0.05$ were consider statistically significant.

RESULTS

Preliminary phytochemical screening revealed the presence of flavonoids, triterpenes, saponins, carotinoids, alkaloids, glycosides and carbohydrates. Acute toxicity studies of the methanolic extract of the *C. procera* did not exhibit any signs of toxicity up to 2 g/kg body weight. Since there was no mortality of the animals found at high dose. Hence 200 and 400 mg/kg dose of the extract selected for evaluation of anti-ulcer activity.

Aspirin Induced Ulcer

The methanolic extract was found to possess remarkable ulcer-protective properties at 400 mg/kg dose. The maximum effect of ulcer protection (91.01%) was produced at 400 mg/kg

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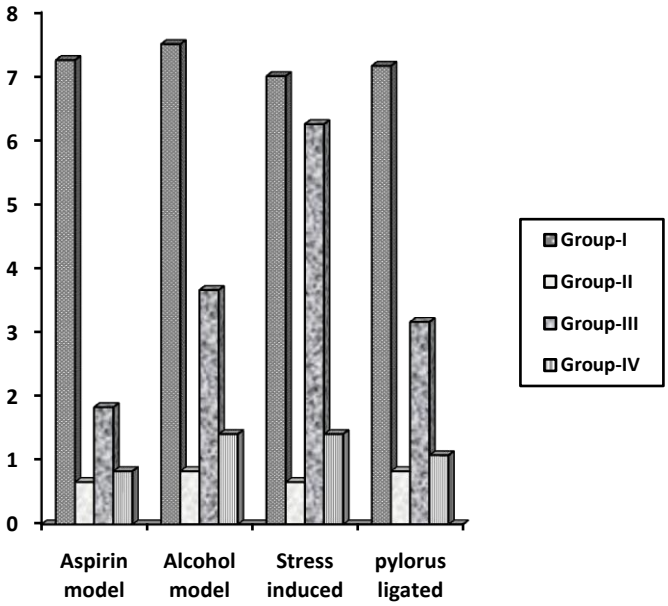
and 79.31% protection @ 200mg/kg of extract dose. The standard drug (Lansoprozole) gave 92.79% of ulcer protection.

Table 1: Effect of methanolic extract of the *C.procera* root against different models of gastric ulcers in rats

Treatment (dose)	Aspirin Mean±S.E	Alcohol Mean±S.E	Stress Mean±S.E	Pylorus ligated Mean±S.E
Control	7.25±0.512^{a}	7.5±0.5^{a}	7.0±0.5^{a}	7.167±0.833^{a}
LANSAPRAZOLE (8mg/kg)	0.667±0.167^{b}	0.833±0.211^{b}	0.667±0.105^{b}	0.833±0.211^{b}
Methanolic extract 1 (200mg/kg)	1.833±0.401^{c}	3.667±0.726^{c}	6.25±0.911^{a}	3.167±0.422^{c}
Methanolic extract 2 (400mg/kg)	0.833±0.0.211^{bc}	1.417±0.490^{b}	1.417±0.490^{b}	1.083±0.271^{b}

P< 0.05, Values with different superscripts differ significantly

Alcohol Induced Ulcer



Effect of methanolic extract of the *C.procera* root against different models of gastric ulcers in rats (values are mean±SE;P<0.05).

Table 2: Range of Protection levels (%) given by different drugs for various gastric ulcer conditions

Treatment(dose)	Protection level	Aspirin	Alcohol	Stress	Pylorus ligated
Control		0	0	0	0
LANSAPRAZOLE (8mg/kg)	Max %	92.791	92.221	92.511	92.221
	Min %	85.877	85.081	88.128	83.423
Methanolic extract 1 (200mg/kg)	Max %	79.312	63.242	28.813	65.692
	Min %	62.123	37.248	18.24	43.037

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Methanolic extract 2 (400mg/kg)	Max %	91.014	88.417	78.036	89.846
	Min %	82.299	72.762	61.241	78.503

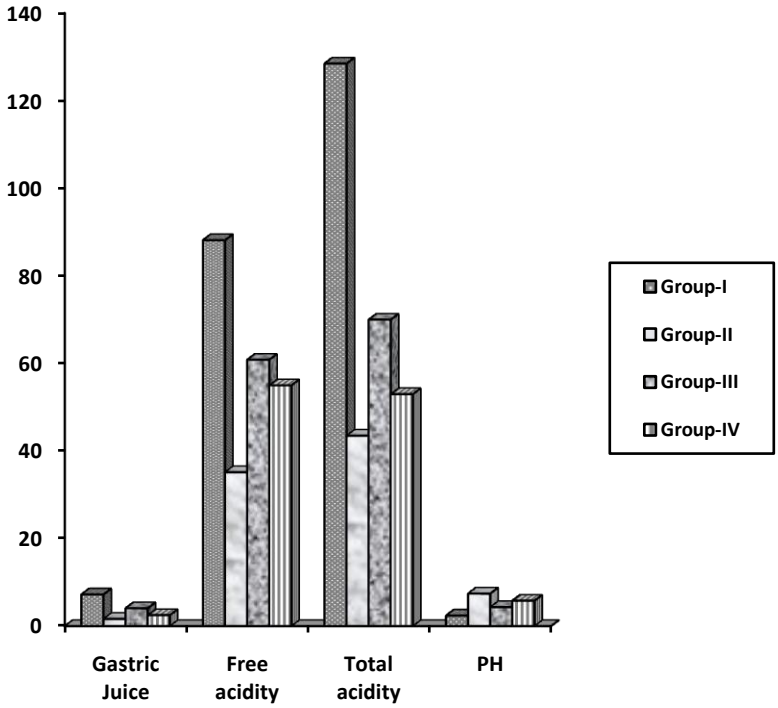
Pretreatment of rats with *C. procera* extract produced a dose dependent protection in the ethanol induced ulceration model as compared to control group. The maximum effect of ulcer protection (88.417%) was produced at 400 mg/kg and 63.242% protection @ 200mg/kg of extract dose. The standard drug (Lansoprozole) gave 92.21% of ulcer protection.

Stress Induced Ulcer

Pretreatment of rats with *C. procera* extract produced a dose dependent protection in the ethanol induced ulceration model as compared to control group. The maximum effect of ulcer protection (78.036%) was produced at 400 mg/kg and 28.813% protection @ 200mg/kg of extract dose. The standard drug (Lansoprozole) gave 92.511% of ulcer protection

Pylorus Ligation Induced Ulcer

The methanolic extract of the *C. procera* in the doses of 200 and 400 mg/kg produced a reduction in the ulcer index, gastric volume, free acidity, total acidity and raised gastric pH significantly in comparison with control group. Lansoprazole, reference drug produced significant reduction gastric ulcer and total acid output as compared to control group.



Comparison of influence of drugs on various acidity parameters in Pylorus ligated ulcer model in rats (values are mean±SE;P<0.05).

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Table 3: Comparison of influence of drugs on various acidity parameters in Pylorus ligated ulcer model in rats

Treatment(dose)	Gastic juce volume (ml/4h)	Free Acidity (mEq/L/ 100 g)	Total Acidity (mEq/L/ 100 g)	P^H
Control	7.233±0.543[a]	88.167±1.922[a]	128.500±4.631[a]	2.40±0.159[a]
LANSAPRAZOLE (8mg/kg)	1.667±0.105[b]	35.167±1.493[b]	43.5±0.992[b]	7.433±0.049[b]
Methanolic extract 1 (200mg/kg)	4.083±0.436[c]	60.833±1.138[c]	70.0±0.577[c]	4.35±0.189[c]
Methanolic extract 2 (400mg/kg)	2.500±0.2111[b]	55.000±1.000[d]	53.0±9.048[b]	5.80±0246[b]

P< 0.05, Values with different superscripts differ significantly

DISCUSSION

The anti-ulcer activity of the root of *C.procera* was evaluated by employing aspirin, alcohol, stress induced and pylorus ligation ulcer models. These models represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study involving, depletion of gastric wall, mucin mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production. NSAID's like aspirin causes gastric mucosal damage by decreasing cytoprotective prostaglandin levels through inhibition of PG synthesis and also results in back diffusion of H^+ ions in to the gastric mucosa [15]. It is evident from the result obtained in our study that the methanol extract of *C. procera* root extract (400mg/kg) significantly inhibit different models of gastric ulceration in rats.

Methanolic extract of the root of *C.procera* was significantly effective in protecting gastric mucosa against aspirin induced ulcers at all the dose level studied. Ethanol induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane [16]. The extract of the root of *C.procera* has significantly protected the gastric mucosa against ethanol challenge as shown by reduced values of lesion index as compared to control group suggesting its potent cytoprotective effect [17]. Showed that stress increases histidine decarboxylase activity in the gastric mucosa,and that the degree of increase correlated positively with the number and severity of lesions. The extract did show a cytoprotective effect against the gastric lesions induced by necrotizing agents,which suggests a direct,protective effect on the gastric mucosa. It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration [18]. In our study, aspirin was administered to pyloric ligated rats, thus aspirin further aggravated the acidity and peptic activity and the resistance of the gastric mucosa was decreased, thereby, causing extensive damage to the glandular region of the stomach. The anti-ulcer activity of *C.procera* extract in pylorus ligation model is evident from its significant reduction in gastric volume, total acidity, free acidity and increase in pH of gastric juice. It is suggested that

C.procera root extract can suppress gastric damage induced by aggressive factors. The ulceration in pyloric-ligated rats is generally caused by increased acid and peptic activity [19].

Thus from our present study the antiulcer effect of *C.procera* is evident and the ability of *C. procera* to inhibit gastric mucosal damage in various ulcer models indicates that the methanol fraction of *C.procera* may be causing an increase in gastric mucosal resistance.

CONCLUSION

On the basis of the present results and available reports, it can be concluded that the anti-ulcer activity elucidated by *C.procera* root could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition.

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