



Research Article

ANTIULCER AND ANTI-INFLAMMATORY ACTIVITY OF *CAJANUS CAJAN* LINN.

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ABSTRACT

Cajanus cajan Linn. is commonly used in Indian folk medicine to treat various ulcers and inflammatory conditions. Different extracts of the leaves were prepared with petroleum ether, ethyl acetate, ethanol and water. Each type of Extract of the leaves of *Cajanus cajan* Linn. was investigated for possible anti gastric ulcer properties and anti-inflammatory action. These extracts were effective in protecting rats from developing gastric ulcers induced by three acute models: HCl/ethanol induced ulcer, immersion-restraint stress ulcer and drug induced ulcer. Omeprazole (20 mg/kg p.o.) used as standard in HCl/ethanol, Ranitidine used as standard (50 mg/kg p.o.) in immersion-restraint stress and in drug induced ulcer. There was significant reduction in ulcer index as compared to control. The p value was found to be ($p < 0.0001$). These results suggest that the *Cajanus cajan* Linn. leaves shows the antiulcer activity. Anti-inflammatory effect studied by two models: Cotton pellet granuloma and carrageenan induced paw edema. Indomethacin (20 mg/kg p.o.) used as standard in carrageenan induced paw edema and Diclofenac sodium (4 mg/kg i.p.) used as standard in cotton pellet granuloma. P value was ($P < 0.0001$). The significant reduction in inflammation was calculated as compared to control.

Keywords: *Cajanus cajan* Linn, Ethanolic extract, CMC, Anti-ulcer, Anti-inflammatory, ulcer-index.

INTRODUCTION

Leaves of *Cajanus cajan* Linn. are useful in ulcerogenic and inflammatory conditions, they are also used as diuretic and laxative.¹ Phytosterols such as β -sitosterol, stigmasterol and cholesterol isolated from leaves and are also used in Ayurvedic medicine as poultice over the breast may induce lactation.² Though the plant and its extracts have been used in the folk medicine extensively, but no scientific evidence for such activities is available in established scientific journals of repute. Hence, in the present study, it is planned to investigate various pharmacological activities of this plant's extracts and also it is thought worthwhile to see whether the plant has got any antiulcer activity. The anti-inflammatory and antiulcer activities of the extracts are discussed here; Gastric hyperacidity and ulcer are very common causing human suffering today. It is an imbalance between damaging factors within the lumen and protective mechanisms within the gastro duodenal mucosa. Although prolonged anxiety, emotional stress, hemorrhagic surgical shock, burns and trauma are known to cause severe gastric irritation, the mechanism is still very poorly understood.³ Oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric damage is caused by physical, chemical and psychological factors that leads to gastric ulceration in human and experimental animals.⁴ Most of the available drugs are thought to act on the offensive factors which neutralize acid secretion like antacids, H₂ receptor blockers like Ranitidine, Famotidine, Anticholinergics like Pirenzepin, Telezepine, Proton pump blockers like Omeprazole, Lansoprazole, etc. which interfere with acid secretion. Recently the involvement of neural mechanism in the regulation of stress responsiveness and complex neurotransmitter interactions were reported causing gastric ulceration.⁵ The aim of the present investigation was to evaluate the possible anti-ulcer activity and anti-inflammatory of *Cajanus Cajan* Linn.

MATERIALS AND METHODS

Collection of Plant

The leaves of *Cajanus cajan* Linn. were collected from neelbud area, Bhopal, India and identified by the Botanist Dr. Zea Ul Hasan, Department of Botany, Saifia Science College Barkatulla University, Bhopal (M.P.), India and a voucher specimen of plant (No.226/Bot/Safia/2011) has been deposited in herbarium for further reference. It belongs to the family Fabaceae.

Preparation of Plant Extract

The leaves of the plant were dried in shade, powdered and passed through a 40-mesh sieve. Dried powder was taken and subjected to successive extraction with petroleum ether, ethyl acetate and ethanol and aqueous in soxhlet apparatus. The extracts were concentrated to dry residue by distillation (temperature 40-60°C with vacuum), dried completely in desiccators and weighed, which gave yield of 9.5 % of pet ether, 14.5 % of ethyl acetate, 21.5 % of ethanol and 17.4 % of aqueous extract.

Animal Used

Wistar rats (150-200 g) were used and of approximate same age are used in the present studies. The animals were fed with standard pellet diet (Hindustan lever Ltd. Bangalore, India) and water *ad libitum*. The experimental protocols were approved by Institutional Animal Ethical Committee (IAEC no. - Reg. No. 778/03/C/CPCSEA-03.09.03) and in accordance with CPCSEA guidelines

Acute Toxicity Test

The acute toxicity and LD₅₀ of the extract were determined in rats using the method described by.⁷ Briefly, nine rats randomly divided into three groups (n = 3) received oral administrations of 10, 100, and 1000 mg/kg of the extract respectively and were observed for 24 h for death. Since no

death was recorded, 600,900 and 5000 mg/kg of the extract were administered to a fresh batch of animals and the number of deaths in 24 h recorded. For dosing, the extracts were uniformly suspended in 1 % Carboxymethyl cellulose (CMC) dissolved in water and administered orally (p.o.) depending upon the experimental design.

Anti-ulcer Activity

Ethanol-Induced Gastric Ulcers

Rats were divided into five groups with four to five animals each that were fasted 24 h prior to receiving an oral dose of the vehicle, CMC (1 mL/kg), Omeprazole (20 mg/kg), ethanolic extract (at the doses of 200 and 400 mg/kg body weight). After 60 minutes, all groups were orally treated with 1 mL of ethanol solution for gastric-ulcer induction. Animals were killed 1 h after the administration of ethanol and the stomachs were excised and gastric damage determined as described above. This experiment was done as described by.⁸ The ethanol and aqueous extracts were suspended in Carboxy methyl cellulose (CMC) and administered at the doses of 200 and 400 mg/kg, respectively. Control groups received CMC was given orally, as the reference drug.

Stress- Induced Acute Gastric Lesion

The induction of acute gastric lesions by stress was studied according to the method of Nagura *et al.* modified by Basile *et al.* The animals were deprived of food for 24 h, but not of water. Immediately after oral doses of 1 % CMC solution (1 g/100 ml), ranitidine (50 mg/kg, p.o.), ethanolic (200 and 400 mg/kg, p.o.) and aqueous extract (200 and 400 mg/kg, p.o.) in 1 % CMC solution, each rat was immobilized in a cylindrical cage and immersed vertically to the level of the xyphoid for seventeen hours, at temperature of 21°C, in the presence of intense light. After this time, the animals were sacrificed with ether. Their stomachs were excised and opened along the greater curvature, washed, and stretched on cork plates and the inner surface was examined for the presence of lesions with a magnifier lens, magnification 10X.^{9,10} The number of stress-induced acute lesions was counted for each animal, and the severity was graded as follows: Light (I) = presence of edema, hyperemia, and single submucosal punctiform hemorrhages (petechiae); Moderate (II) = presence of sub mucosal hemorrhagic lesions with small erosions; Severe (III) = presence of a hemorrhagic edge with severe erosions and some invasive lesions.

Drug Induced Ulcer

The ulcers were induced by the method described by Urushidani. The rats were fasted for 24 h but water was allowed. CMC, extract and ranitidine were administered into the 3 groups as before. After 30 minutes, Indomethacin was injected S. C. in the dose of 20 mg/kg as a 1 % suspension in cmc with a trace of Tween 80. After 7 h of indomethacin administration, the animals were scarified by a blow on the head and examined for ulcers as described above.¹¹

Anti-inflammatory Activity

Carrageenan- Induced Paw Oedema

Inflammation was induced by injecting 0.05 ml of 1 % carrageenan sodium salt (Sigma) subcutaneously in the sub-plantar region of the rat right hind paw. The ethanolic extract (200 and 400 mg/kg) was administered orally, 30 minutes before carrageenan injection while control group received CMC (10 ml/kg, p.o.). The hind paw volume was measured

plethysmometrically before and after the carrageenan injection, at hourly intervals for 6 h and then at 24 h.¹²

Cotton Pellet Granuloma

A 50-mg sterilised cotton pellet was implanted subcutaneously on the back of neck in rats under ether anaesthesia. Animals in treated groups received the extract (200 and 400 mg/kg, p.o.) once daily for 14 consecutive days. Animals in the control group received only the vehicle 10 ml/kg, p.o. Diclofenac sodium (4 mg/kg, p.o.) was given as reference drug in a fourth group. On the 14th day, the animals were sacrificed with ether, the pellets' granuloma were removed, freed from extraneous tissue, dried overnight at 55 ± 0.5°C, and weighed.¹³

Statistical Analysis

Mean values ± S.D. were calculated for each parameter. For the determination of significant intergroup differences, each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out.

RESULTS

Phytochemical Screening

The extracts were subjected to phytochemical and pharmacological screening. On preliminary phytochemical study⁶, the extracts showed positive test for the presence of flavonoids, glycosides, traces of alkaloids, tanins and saponins.

Acute Toxicity Studies

As part of this pharmacological study, the ethanolic extract obtained leaves of *Cajanus cajan* Linn. was first investigated for toxicity in mice. In LD₅₀ studies, it was found that the animals were safe up to a maximum dose of 1000 mg/kg body weight. There were no changes in normal behavior pattern and no signs and symptoms of toxicity and mortality were observed. The biological evaluation was carried out at doses of 100 and 200 mg/kg body weight. However, the ethnopharmacological information about the doses of *Cajanus cajan* Linn. leaves traditionally employed were inaccurate. Active principles from medicinal plants are generally present at low concentrations. Active principles at concentrations higher than 1 % are the exception. So, using the example of the antiulcerogenic plant, the maximum dose of 400 mg/kg of crude extract or ethanolic extract presuppose the presence of the active principle in concentrations of 10 % for substances like ranitidine (commonly used at dose of 50 mg/kg) our 2 % for those similar to omeprazole (20 mg/kg).¹⁴

DISCUSSION

In Ethanol induced group of rats, control group found have highest ulcer index which has been reduced in stress induced group while least observed in Drug indomethacin induced group. Which on treatment with different standard drug show approximately similar percentage of inhibition while different extract at 200 mg/kg level p.o. is not significant inhibition statistically. On increasing the dose at level of 400 mg/kg p.o. gives statistically significant p < 0.0001 values.¹⁵ (Table 1). The combination of the anti-inflammatory and gastroprotective effects of *C. cajan* Linn. observed in this study, is extremely favorable when taken to account the serious limitations of a large number of anti-inflammatory agents that show a tendency to produce gastric irritation, bleeding and mucosal cellular damage.

Table 1: Effects of Different Doses of *Cajanus cajan* Linn. Leaves on Gastric Lesions Induced in Rats

Method	Treatment (p.o.)	Dose (mg/kg)	Ulcer index	% Ulcer inhibition
Ethanol	Control (CMC)	1 ml/kg	83.0 ± 1.0	-
	Omeprazole	20 mg/kg	5.0 ± 1.0	93 %
	Ethanol extract	200 mg/kg	33.5 ± 1.5	59 %
		400 mg/kg	28.0 ± 0.5	66 %
Stress	Control (CMC)	1 ml/kg	24.0 ± 1.0	-
	Ranitidine	50 mg/kg	4.5 ± 0.5	81 %
	Ethanol extract	200 mg/kg	10.5 ± 0.5	56 %
		400 mg/kg	7.5 ± 0.5	68 %
Drug (Indomethacin)	Control (CMC)	1 ml/kg	34.0 ± 1.10	-
	Ranitidine	50 mg/kg	5.5 ± 0.64	83 %
	Ethanol extract	200 mg/kg	13.75 ± 0.45	59 %
		400 mg/kg	10.0 ± 0.40	70 %

Results are mean ± S.E.M. Ethanol: ANOVA, Dunnett test $p < 0.0001$; Dunnett test $p < 0.0001$; Dunnett test $p < 0.0001$

Table 2: Effect of Ethanol Extract of *C. cajan* Linn. Leaves on Carrageenan-induced Paw edema in Rats

Treatment	Dose (mg/kg)	% of hind paw edema at time (h) after carrageenan				
		0	1	2	4	24
Control (CMC)	1 ml/kg	0.215 ± 0.005	0.29 ± 0.01	0.55 ± 0.01	0.31 ± 0.01	0.25 ± 0.01
Standard (Indomethacin)	20 mg/kg	0.135 ± 0.005	0.18 ± 0.01	0.23 ± 0.01	0.17 ± 0.005	0.14 ± 0.005
Ethanol extract	200 mg/kg	0.17 ± 0.00	0.235 ± 0.005	0.35 ± 0.01	0.29 ± 0.005	0.19 ± 0.005
	400 mg/kg	0.155 ± 0.005	0.205 ± 0.005	0.31 ± 0.01	0.25 ± 0.01	0.16 ± 0.005

Results are mean ± S.E.M.: ANOVA, Dunnett test $p < 0.0001$

Table 3: Effect of Ethanol Extract of *C. cajan* Linn. Leaves on Cotton Pellet Granuloma in Rats

Treatment	Dose (mg/kg)	% Increase of cotton pellet weight
Control	1 ml/kg, p.o.	133.5 ± 0.5
Standard (Diclofenac sod.)	4 mg/kg, i.p. once	81.5 ± 1.5
Ethanol Extract	200 mg/kg × 14 days	105.5 ± 1.5
	400 mg/kg × 14 days	91.0 ± 1.0

$(100 \times \text{final weight}/\text{initial weight})$ and mean ± S.E; value of $P < 0.0001$

The ethanol extract of *C. cajan* Linn. leaves (200 and 400 mg/kg, p.o.) significantly reduce the increase in hind paw oedema induced by carrageenan, with effects starting 1 hr after carrageenan and lasting for 24 h. In standard group is showing effective as in acute and subacute, where as the ethanolic extract is effective in sub acute (Table 2). The sub-acute oral treatment with *C. cajan* Linn extract, at both the doses used 200 and 400 mg/kg, for 14 consecutive days. Ethanolic extract significantly reduced the increase in weights of the cotton pellet, compared with the control group (Table 3), which shows that ethanolic extract is more effective as Anti inflammatory property than control. Pre-treatment with a *C. cajan* Linn. extract (200 and 400 mg/kg, p.o.) produced significant and dose-dependent decrease in the intensity of gastric mucosal damages induced by ulcerogenic drug (Indomethacin). The results of the present study show that the ethanolic extract of *C. cajan* Linn leaves possesses anti-inflammatory properties, demonstrated following single intraperitoneal administration (carrageenan-induced hind paw edema) and repeated oral administration (cotton pellet granuloma). Moreover, the tested extract has shown significant anti-ulcer activity. Further studies are needed to elucidate the mode of action and better evaluate the potential therapeutic value in the treatment of *C. cajan* Linn leaves.

CONCLUSION

The results of present study show that the ethanolic extract of *C. cajan* Linn. Leaves extract possesses gastroprotective effect and improve ulcer-healing activity. Ethanolic extract also show possible free-radical scavenging property on endogenous PGs. Significant antiinflammatory effect was

shown by the ethanolic extract on carrageenan-induced edema and cotton pellet granuloma in rats.

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