



**POTENTIAL GASTROPROTECTIVE EFFECTS OF *CAJANUS CAJAN* LEAF
EXTRACT ON GASTRIC ULCERATION INDUCED BY ETHANOL IN ALBINO RATS**

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ABSTRACT

Humans often use plants to treat a wide range of illnesses, and they are also a major source of new pharmaceuticals. This study investigated the gastro-protective potential of aqueous and methanol extracts of *Cajanus cajan* leaves in ethanol induced gastric ulcer in Albino rats. Twenty-four rats weighed and divided into six groups (A-F) of four rats each were used for this study. Group A and B served as negative and positive controls respectively, all groups received an oral dose of 1mL/kg of 95% ethanol, either with or without a seven-day pre-treatment with the conventional medication (Omeprazole), or leaf extract (methanol and aqueous extract in low and high doses). The rats were euthanized, the stomach tissues removed, and the ulcers graded. Additionally, a histological analysis was done. The rats' stomach tissues were shown to be protected against ethanol-induced ulcers by *Cajanus cajan* leaf extract to varied degrees, as evidenced by the high dose (240 mg/kg) aqueous extract group, which had an ulcer prevention index (UPI) of 69%. The positive control group had a negative UPI signifying that the control drug did not afford any protection to the animals in the group. Results from histology likewise correlated with the calculated ulcer indices; rats that received *C. cajan* leaf extracts had notably fewer stomach epithelial cell lesions than those that did not receive the extract. In conclusion, aqueous leaf extract of *Cajanus cajan* at higher concentrations possesses gastro-protective potentials to prevent stomach ulcers caused by ethanol.

Keywords: Gastro-protective, *Cajanus cajan*, Albino rats, ulceration, histology

INTRODUCTION

Plant extracts are one of the most potential sources of novel medications, with encouraging results in various experimental models for the treatment of stomach ulcers [1]. The study determined the anti-ulcer potentials of *Cajanus cajan* leaf extracts on Albino rats induced with gastric ulcer using ethanol. The *Cajanus cajan* plant, sometimes referred to as the "pigeon pea," is widely cultivated and utilized as a rich source of protein. Flavonoids and alkaloids are also abundant in the leaves of *C. cajan* [2]. In folk medicine, *Cajanus cajan* is used to treat a variety of gastrointestinal disorders since flavonoids, tannins, and carbohydrates have been found in this plant, with flavonoids and tannins showing powerful anti-ulcer activity [3, 4]. These days, gastric hyperacidity and ulcers are among the most prominent causes of discomfort in people [5]. It results from an imbalance between the protective mechanisms found in the gastro-duodenal mucosa and the harmful elements present in the lumen.

Cajanus cajan leaves are an upright, woody, annual, small tree or short-lived perennial shrub that grows widely and is grown in tropical and subtropical regions [2]. The administration of absolute ethanol to experimental animals has long been utilized as a reproducible and reliable means of inducing

stomach lesions [6]. Stomach lesions are accompanied by the development of reactive oxygen species (ROS) and free radicals (FRs). These radicals, specifically seem to play an essential role in variety of erosive and ulcerative gastrointestinal lesions [7, 8].

Various works have been done on this plant as reported in the literature, but the bulk of the work were on the seed even though the leaves have several beneficial health uses just like the seed. In search for a cheaper and effective alternative to conventional medicine for the treatment and management of gastric ulcer, this study was necessitated, to examine the gastro-protective potentials of *Cajanus cajan* leaf extract of both aqueous and methanolic forms against ethanol induced ulcer.

MATERIALS AND METHODS

Experimental Animals

For this study, apparently healthy twenty-four (24) Albino rats weighing 170-200g were employed. The rats were purchased from the Animal house of the physiology Department, University of Nigeria and housed in well ventilated steel cages at the College of Medicine Animal House, University of Nigeria, Enugu Campus. The rats were allowed to acclimatize for two weeks to the laboratory ambient conditions (28 – 30°C temperature, 12 hour light-dark cycle). The

animals were weighed and divided into six groups (A - F) of four rats each. They were fed on regular commercial rat feed (Guinea Feed®, Enugu, Nigeria) and had unlimited access to clean water. To ensure adherence to the regulations for the use of animals in research, animal care personnel from the University of Nigeria's College of Medicine supervised the treatment and experimentation of the animals.

Plant collection and preparation

Fresh leaves of *Cajanus cajan* were collected from a seed-bearing plant in a farm settlement in Nsukka, Enugu state, in south Eastern Nigeria, in the months of May and June 2021. A botanist at the Botany Department, Faculty of Biological Sciences, University of Nigeria, Nsukka, identified and confirmed the leaves with voucher number NO.13/ Botany/ Biological/ 2021. The leaves were properly washed, let to dry in the shade and then processed into powder using a grinding machine that ran on gasoline.

Aqueous extraction:- Two hundred (200) grams of the powdered sample of the plant was extracted by soaking in 1500ml of distilled water and homogenized with a wooden stir intermittently and left to extract for 48 hours in the refrigerator. The homogenate was then passed through a muslin

sieve and the resulting filtrate was refrigerated at 2-8°C until when needed.

Methanol extraction:- Another two hundred (200) grams of the powdered leaves was dissolved in 1500ml of methanol for 72 hours. The mixture was allowed to extract with intermittent stirring while it was refrigerated. Whatman No. 1 filter paper was used to filter the mixture, and the filtrate was allowed to evaporate until it was completely dry. The residual concentrate was dissolved in 3% Tween 80 and kept in the refrigerator until when needed. Both extracts' extractive values were determined.

Experimental design and conduct

Six groups (A – F) of rats grouped according to their body weights were used for this study. Group A and B served as the negative and positive control respectively while Group C – F served as the test groups. Group A received water while Group B received 40mg/kg body weight of Omeprazole via the intramuscular route using hypodermic needle. Group C and D received 120mg/kg and 240mg/kg body weight of aqueous *Cajanus cajan* leave extract orally via oral gavage respectively. Group E - F received 120mg/kg and 240mg/kg body weight of methanol *Cajanus cajan* leave extract respectively for 7 days.

Ethical consideration

The Department of Animal Science of the University of Nigeria Nsukka's Animal Welfare and Ethics Committee granted ethical approval as was requested. The entire investigation was carried out in compliance with Institutional and International recommendations on the humane care of experimental animals [9].

Phytochemical constituents of *Cajanus cajan*

Several studies had identified the phytochemical components of different parts of *Cajanus cajan* including the leaf. According to the work by [10], flavonoids, tannins, saponins, cyanogenic glycoside, glycosides and anthocyanins were all isolated as phytochemical constituents of the leaf of *Cajanus cajan*. Alkaloids and flavonoids were also shown to appear in greatest quantities [10]. Also, the research by [11], showed positive tests for flavonoids and glycosides but conversely, only showed traces of alkaloids and tannins.

Gastric ulcer induction using absolute alcohol

Gastric Ulcer was induced using ninety-five (95) percent ethanol utilizing a modification of the methodology of Robert (1979) as explained by [12]. All the rats in the six groups (A-F) were fasted for 24 hours but had unlimited access to water. After the fasting period was over, the negative control (Group

A) received 1ml/rat of water, the (positive control), rats in group B received a single dose of Omeprazole intramuscularly. Group C and D were given a single dose of 120mg/kg and 240mg/kg body weight of aqueous *Cajanus cajan* leaf extract orally respectively. While group E and F received a single dose of 120mg/kg and 240mg/kg body weight of *Cajanus cajan* leaf methanol extract. The rats were each given 1ml/kg of 95% ethanol orally thirty minutes following the administration of the extract.

Calculation of ulcer index

The rats were sacrificed under chloroform anaesthesia after an hour and their stomach tissues excised, washed in saline and opened along the greater curvature. With the aid of a magnifying lens, the ulcers were viewed and counted. The ulcerative index was calculated thus: - ulcerative lesion > 1mm < 2mm = 2, Ulcerative lesion > 2mm = 3

The ulcer index for every rat was calculated by dividing the total scores by 10.

The effectiveness of the extract and drug was calculated as follows.

$$\text{Ulcer Preventive index (\%)} = \frac{\text{ulcer index of control} - \text{ulcer index of treated}}{\text{ulcer index of control}} \times 100 \text{ [13, 14].}$$

Histological processing and Microscopy

Following a twenty-four (24) hour fixation in 10% formal saline the excised stomach tissues

were processed using standard tissue processing protocol for producing formalin fixed paraffin embedded tissue blocks. The blocks were cut to obtain tissue sections with a thickness of 5 micron which were stained using the haematoxylin and eosin technique. The tissue sections were then viewed under an Olympus™ light microscope. A digital camera; Samsung NX1000 connected to the microscope was used to take pictures of the sections' areas of interest.

Statistical Analysis

The data was analyzed using Statistical Package for Social Sciences software program (SPSS, Chicago, IL, version 26.0). The results of the analysis were presented as mean \pm SEM. A statistical comparison between the treatment and negative control groups was performed using Dunnett's test, with a significance level of $P < 0.05$.

RESULTS

Ulcer index and ulcer preventive index

Varying degrees of mucosal ulcerations were revealed upon gross examination of the excised stomach tissue as shown in **Figure 1**. **Table 1** below shows a summary of the calculated mean ulcer indices (UI) and ulcer preventive indices (UPI) for the test rats. All the animals pre-treated with both aqueous and methanol leave extracts had lower ulcerative indices, however, only animals in Group D had a statistically significant lower ulcer index compared to the negative control. The group of rats treated with 240mg/kg body weight of aqueous leave extract of *Cajanus cajan* had the least UI of (0.88 \pm 0.17) and UPI of 69% showing that aqueous leave extract of *Cajanus cajan* at that dose provided the highest protection against stomach ulcers. Compared to group (B) treated with Omeprazole which is a standard anti ulcer drug which had ulcer index (3.03 \pm 0.34) and UPI of -6.32%, both extracts at 120 and 240mg/kg body weights offered better gastro-protection.

Table 1: Mean ulcer indices and ulcer preventative indices for each treatment group after ethanol-induced gastric ulceration

GROUP	TREATMENT	DOSES (mg/kg)	ULCER INDEX	P VALUE	ULCER PREVENTIVE INDEX (%)
A (Negative control)	Water	1ml/rat	2.85 \pm 1.05	-	
B (Positive control)	Omeprazole	40(mg/kg)	3.03 \pm 0.34	0.9969	- 6.32
C	Aqueous extract of <i>C. cajan</i>	120 (mg/kg) (Low Dose)	1.95 \pm 0.26	0.3601	31.58
D	Aqueous extract of <i>C. cajan</i>	240 (mg/kg) (High Dose)	0.88 \pm 0.17*	0.0097	69.12
E	Methanol extract of <i>C. cajan</i>	120 (mg/kg) (Low Dose)	1.55 \pm 0.15	0.1073	45.61
F	Methanol extract of <i>C. cajan</i>	240 (mg/kg) (High Dose)	1.77 \pm 0.38	0.2520	37.89

Results of ulcer indices are shown as mean \pm SEM (n = 4 rats per group). * Value significantly different from group A (negative control) at $p < 0.05$ using the Dunnett's test (F ratio: 5.952; $P = 0.0097$).

Tissue histopathology

Gross images (Figure 1)

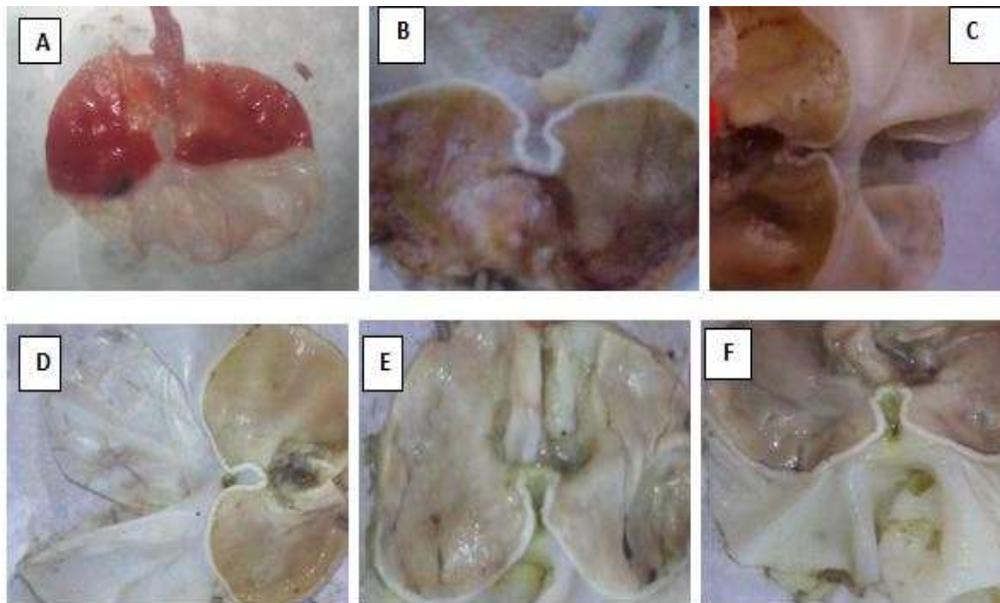


Figure 1; Macrographs revealing the gross appearance of the excised stomach mucosae of rats in various treatment groups. Group A; (1ml/kg absolute ethanol only) showing severe stomach mucosa hemorrhagic ulcer, Group B (40mg/kg Omeprazole) showing moderately severe streaks of haemorrhagic ulceration. Group C, (120mg/kg aqueous extract of *Cajanus cajan*) showing mild streak ulcers. Group D; (240mg/kg aqueous extract of *Cajanus cajan*) revealing very mild spot ulcerations. Group E; (120mg/kg methanolic extract of *Cajanus cajan*) showing very mild streak ulcers, Group F; (240mg/kg methanolic extract of *Cajanus cajan*) showing very mild spot ulcerations.

Histology images (Figure 2)

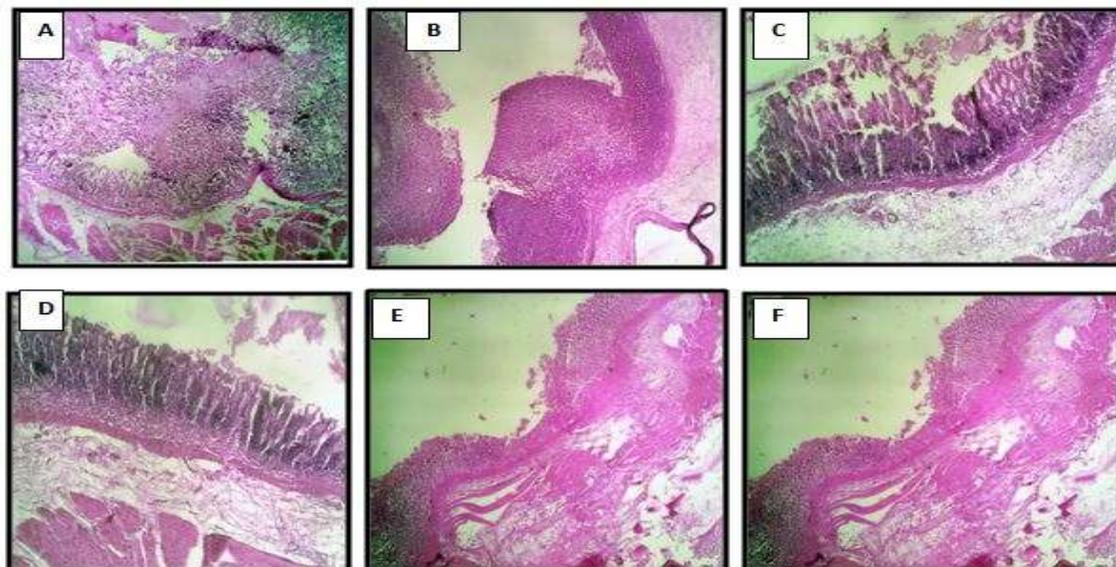


Figure 2; Micrographs of the histological sections of the stomachs of rats in the various treatment groups (A–F). Sections of all treatment groups showed varying degrees of eroded gastric epithelial cells, deposits of mucosal debris and infiltration of mucosae by inflammatory leucocytes.

DISCUSSION

The present study evaluated the gastro-protective properties of crude aqueous and methanol *Cajanus cajan* leaf extract in ethanol induced gastric ulceration in Albino rats. Oral administration of 95% ethanol (1ml/kg) was enough to cause remarkable gastric ulcer in rats. Gastric ulcer is a common ailment which affects a considerable number of people worldwide [15, 16].

In humans and experimental animals, stomach damage is brought about by physical, chemical, and psychological variables, and oxygen-derived free radicals have been linked in the etiology of gastric ulcers [17]. Studies on both humans and animals have revealed that the effectiveness of herbal medicines is comparable with or greater than that of prescription treatments like omeprazole or cimetidine, and that herbal remedies also have fewer adverse effects [18].

In the present study, it was found that extract pre-treatment of the experimental rat models reduced the degree of ethanol-induced injury to the stomach mucosa. In comparison to the negative control, the excised stomach tissues of rats pre-treated with the extract of *Cajanus cajan* leaves showed less mucosal lesions. However, only Group D animals that received 240 mg/kg of *C. cajan's* aqueous leaf extract showed a statistically significant reduced

ulcer index than the Group A negative control ($P < 0.05$).

According to a prior study, pre-treatment with *Vitex doniana* leaf extract (200 mg/kg and 400 mg/kg) showed a significant dose-dependent reduction in the severity of stomach mucosal damages brought on by the ulcerogenic medication Indomethacin [19]. Both the aqueous and methanol leaves extract of *C.cajan* afforded varying degrees of protection to the animals against gastric ulcer caused by ethanol. Group D had the highest ulcer preventive index (UPI) of approximately 69% while Group C had the lowest UPI of approximately 32% among the treated group. The positive control group had a negative UPI signifying that the control drug (Omeprazole) did not offer any protection to the animals in the group. This finding disagrees with the result of a previous study which documented a statistically significant ulcer protective index of rats that received 500 mg/kg of ethanol extract of *Cajanus cajan* and the positive control rats that received the control drugs (famotidine), with *C. cajan* treated rats having UPI closer to that of the control drug [20].

When compared to the negative control, the stomach tissues of the pre-treated rats' showed less mucosal lesions. Further evidence that the extracts provided protection for the stomach

comes from the mean ulcer indices and ulcer protective indices. The aqueous extract seems to offer protection in a dose-dependent manner, with larger doses showing higher percentage ulcer preventative indices. Histological results also agreed with the calculated indices, with treated rats having fewer erosions/lesions of the stomach epithelial cells than untreated rats.

The gastro-protection reported may be due to the phytochemical components of *C. cajan*. Previous studies documented that tannins' have the capacity to precipitate microproteins, induce vasoconstriction and provide gastric protection by creating an additional protective layer on the gastrointestinal mucous membranes, reducing their susceptibility to damage from irritants [21, 22]. Due to their surfactant-like properties, saponins have also been found to have antiulcer capabilities [21]. Additionally, flavonoids are well-known antioxidants that can provide protection for the stomach mucosa due to their capacity to eliminate reactive oxygen species, maintain mucous membrane stability, and increase gastric mucosal prostaglandin [10, 21].

Similarly, alkaloids have been quantitatively identified in great amount in the leaf of *Cajanus cajan*. Also, they have emerged as promising agents for countering stress-induced gastric ulcers due to their anti-

ulcerogenic properties which primarily involve the reduction of gastric acid secretion and the reinforcement of the mucosal barrier [10, 21]. On the other hand, some reports have shown that glycosides possess anti-ulcer potential by adjusting the secretion of gastric acid, promoting mucin production, and through the enhancement of antioxidant defense systems [19, 21]. The mythical use of *C. cajan* for healing stomach disorders may have been supported scientifically by one or more of the processes outlined above that exert the gastro protective effect of the plant's extracts.

CONCLUSION

In conclusion, according to the results and available reports in the present study, it can be suggested that *Cajanus cajan* leave extracts have the potential to prevent gastric ulcer with the high dose aqueous leave extract showing significant gastro-protective properties against ethanol induced gastric ulcer in rats.

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