PHARMACOLOGICAL ACTIVITY OF THE METHANOLIC EXTRACT OF CASSIA NIGRICANS LEAVES


*Department of Pharmacology and Toxicology, **Medicinal Chemistry and Quality Control, National Institute for Pharmaceutical Research and Development. P. M. B. 21, Garki, Abuja - FCT, Nigeria.

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ABSTRACT

Objective: To evaluate the pharmacological activity of the methanolic extract of C. nigricans leaves on gastrointestinal smooth muscles, pain and inflammation using laboratory animals.

Methods: The investigations were carried out using the combined effects of cold stress and aspirin-induced ulceration, and egg albumin induced hind paw oedema in rats, acetic acid-induced writhing and gastrointestinal transit in mice as well as effects on the isolated rabbit jejunum.

Results: The extract markedly protected rats against cold-stress and aspirin-induced gastric mucosal damage. The extract exhibited significant (p <0.05) anti-inflammatory and anti-nociceptive activities in rats and mice respectively. Furthermore, the extract decreased the amplitude of contraction of the isolated rabbit jejunum and inhibited histamine-induced contractions, but did not affect ACh induced responses. The intraperitoneal LD_{50} values of the extract was 210±4.5 mg/kg in mice.

Conclusion: The extract shows good analgesic, anti-inflammatory effects and protected rats against gastric mucosal damage. The anti-ulcer activity might be via histaminergic receptor inhibition.

KEYWORDS Cassia nigricans ulcer anti-inflammatory analgesia

INTRODUCTION

Cassia nigricans Vahl. (Caesalpinaceae) is a herbaceous plant that grows widely in the savannah grasslands of West Africa. The roots and leaves have been used medicinally in Senegal and Guinea as a substitute for quinine for many years. The root infusion is also used as a vermicide. The pulverized leaves are employed as appetizers and febrifuge, while the leaf decoction is used in treating fevers. The plant parts are widely used ethnomedically in Northern Nigeria for the treatment of various gastrointestinal disorders. A pinch of the grounded leaves is taken with water for the treatment of peptic ulcers. There are few reports on the pharmacological profile of this widely used plant in the literature. The antiulcer properties of the aqueous extract of the leaves were reported recently. The present study was therefore undertaken to evaluate the pharmacological effects of the methanolic extract of C. nigricans in rabbits, mice and rats.

MATERIALS AND METHODS

Plant material: The leaves of C. nigricans were kindly supplied by Mr. S. D. Fumen (ECWA Church Fai-Kwoi, Kaduna State, Nigeria). The plant was identified and authenticated by Mr. A. O. Ohaeri, (Taxonomist), Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD) Abuja. A voucher specimen was deposited at NIPRD herbarium and it was assigned the voucher specimen number 3833.

Preparation of the extract: The clean leaves were sun-dried for 3 days and pulverized into powder. About 60 g of the powder was soxhlet extracted with...
1.0 litre of methanol for 12 h. The solvent was removed under reduced pressure using a rotary evaporator and it gave a yield of 27%.

**Drugs:** Acetycholine chloride, histamine, atropine, cimetidine (all from Sigma Chemical Company, USA), mepyramine (M & B), Acetic acid (BDH) were used. All drugs were prepared fresh to desired concentrations with distilled water just before use. The extract was also prepared fresh using distilled water. Parallel control experiments were always carried out in order to correct possible effects caused by vehicle alone.

**Animals:** Adult wistar rats (150-200 g), Swiss Albino mice (18-22g) and rabbits (1.5 - 2.5kg) of either sex, maintained at the Animal Facility Centre, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja were used for the experiments. The animals were fed with standard feed (Pfizer feed, PLC Lagos) and water ad libitum.

**Phytochemical test:** The freshly prepared extract was subjected to standard phytochemical screening tests for various constituents. The extract was screened for the presence of alkaloid, glycoside, flavonoids, tannins, using conventional protocols.

**Acute toxicity test:** The intraperitoneal (i.p.) acute toxicity (LD$_{50}$) of the extract was evaluated in Swiss albino mice as described by Miller and Tainter. In brief, the method involved the administration of 5 different doses of the extract to 5 groups of mice (6 mice/group). The mortality in each group was recorded within 24 h. The LD$_{50}$ was estimated from the graph of percentage (%) mortality (converted to probit) against log-dose of the extract - probit 5 being 50%.

**Studies on combined cold restraint stress and aspirin induced ulceration:** In this study, cold restraint stress and aspirin (100 mg/kg, p.o.) were utilized simultaneously for the induction of gastric ulceration. The experimental rats were fasted for 18 hours with water ad libitum. The animals were randomly grouped into 5 groups of 5 rats per group. The first group received normal saline (30 ml/kg, i.p.), the second group received cimetidine (100 mg/kg, i.p.). The remaining three groups received doses of the extract (25, 50 and 100 mg/kg, i.p.) respectively. Thirty minutes later, an aqueous suspension of acetylsalicylic acid (ASA) was administered orally to each rat at a dose of 100 mg/kg. The rats were then put in restraining cages and placed in a cold room at a temperature of 4 °C for 2 h. At the end of the 2 h period, the rats were killed and the stomach removed and opened along the greater curvature. The stomach was rinsed under a stream of water and pinned flat on a corkboard. The stomach was coded to avoid observer bias and examined with a hand lens (X10). The scoring of the severity of ulceration was as described.

- < 1 mm (Pin point) = 1
- 1-2mm = 2
- > 2 mm = 3
- > 3 mm = 4

The mean ulcer index was determined by dividing the total ulcer indices in a group by the total number of animals in that group. The percentage severity of ulceration was determined by dividing the scores of ulcers of each group by the total number of scores in the control group and the result multiplied by 100.

**Anti-inflammatory studies:** The method of Winter et al as modified by Akah and Nwamie was used. Rats of either sex were fasted for 12 h and deprived of water only during the experiment. Deprivation of water was to ensure uniform hydration and to minimize variability in oedematous response. Inflammation of the hind paw was induced by injecting 0.1 ml fresh egg albumin (phlogistic agent) into the subplantar surface of the right hind paw. The control group was given normal saline, the second group received ASA (150 mg/kg, p.o.) while the remaining two groups received extract at doses of 50 and 100 mg/kg, i.p. The measurement of foot volume was done by a displacement technique using the plethysmometer (LETICA), immediately before and every 20 min after the injection of egg albumin for 2 hours.

**Studies on acetic acid induced writhing in mice:** The writhing was performed as described by Koster et al. The mice were fasted over night and had water ad libitum. They were randomly divided into 4 groups (12 mice/group). The first group received normal saline (20 ml/kg, i.p.), the second and third groups received the extract at 50 and 100 mg/kg,
i.p., while the fourth group received ASA (150 mg/kg orally). Thirty min later, each mouse was given 0.7% aqueous solution of acetic acid (10 ml/kg, i.p.) and then placed in an observation box for 5 min. The number of writhes is counted for 10 min after acetic acid injection. The number of writhes in each treated group was compared to that of a control saline treated group.

Studies on the rabbit jejunum: The rabbits were killed by a blow on the head, exsanguinated and the abdomen was opened. Segments of the jejunum about 2 - 3 cm long were removed and dissected free of adhering mesentery. The tissue was mounted in a 20 ml organ bath containing Tyrode solution of the following composition (mM): NaCl 136.8, KCl 2.7, CaCl$_2$ 1.3, NaHCO$_3$ 12, MgCl$_2$ 0.5, Na$_2$PO$_4$ 0.14 and glucose 5.5. The preparation was maintained at 37 ± 1°C and aerated. A tension of 1 g was applied. A 60 min equilibration period was allowed during which the physiological solution was changed every 15 min. At the end of the equilibration period, the effects of acetylcholine (5.5 x10$^{-7}$ M), histamine (9.0 x 10$^{-6}$ M) and the extract (0.05 - 0.4 mg/ml) were evaluated. The effect of the extract (0.1 mg/ml) on the contractile response induced by acetylcholine and histamine were also investigated. Determinations were done in quadruplicates.

Studies on gastrointestinal motility test in mice: To test the effect of the extract of C. nigricans on gastrointestinal motility, animals were randomly divided into 5 groups of 5 mice each. The mice were starved for 24 h prior to the experiment but were allowed free access to water. Mice in group I were given 30 ml/kg of normal saline orally, while those in groups II, III and IV received the extract at doses of 100, 200 and 400 mg/kg, i.p. respectively. The fifth group received atropine 0.1 mg/kg, i.p. Five minutes after drug administration, 0.5 ml of a 5% charcoal suspension in a 10% suspension of tragacanth powder was administered p.o. to each mouse. All the mice were killed 30 min later, the abdomen opened and the distance travelled by the charcoal plug from the pylorus to the caecum was determined and expressed as a percentage of the total length of the small intestine.

Statistical analysis: Results were expressed as mean ± SEM. Analysis of Variance was used and the significance of difference between means was determined by the Dunett’s test and results were regarded as significant when P < 0.05.

RESULTS
The preliminary acute toxicity in mice established the i.p. LD$_{50}$ of the extract to be 210 ± 4.5 mg/kg. Phytochemical analysis revealed the presence of tannins, resins and volatile oils.

Effect on combine cold restraint stress and aspirin induced ulceration: The methanolic extract of C. nigricans at doses of 25, 50 and 100 mg/kg, i.p. significantly (P<0.05) attenuated the gastric mucosal damage induced by combined cold stress and aspirin (100 mg/kg). Similarly, cimetidine (100 mg/kg, i.p.) significantly protected the animals from ulceration (Table 1).

Effect on inflammation: The extract demonstrated a significant (p<0.05) anti-inflammatory effect against
Figure 1. Anti-inflammatory effects of the methanolic extract of C. nigricans in rats.
Normal saline ◊. Extract (50 mg/kg) ◐. Extract (100 mg/kg) ○ and ASA (150 mg/kg) △.

*\( p < 0.05 \) when compared to control (normal saline)

Figure 2. Analgesic effect of the methanolic extract of C. nigricans on acetic acid induced writhing in mice.
Normal saline ■. Extract (50 mg/kg) □. Extract (100 mg/kg) △ and ASA (150 mg/kg) ▪.

*\( p < 0.05 \) when compared to control (normal saline)
Figure 3. Concentration dependent relaxant effect of the methanolic extract of *C. nigricans* leaves on the pendular movement of the rabbit jejunum.

![Graph showing concentration dependent relaxant effect](image1)

**C. nigricans (mg/ml)**

0.05 0.1 0.2 0.4 0.8 1.6 3.2

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Figure 4. Inhibitory effect of the methanolic extract of *C. nigricans* (0.2 mg/ml) on the contractile responses of the isolated rabbit jejunum in the presence of ACh (0.1 µg/ml) and histamine (1 µg/ml).

![Graph showing inhibitory effect](image2)

*<p><0.05 when compared to histamine induced contraction*
egg-albumin induced inflammation at doses of 50 and 100 mg/kg as compared to control. ASA (150 mg/kg) also caused a remarkable (P<0.05) anti-inflammatory response (Figure 1).

**Effect on acetic acid-induced writhing in mice:** The methanolic extract of *C. nigricans* significantly (P<0.05) increased the pain threshold in mice at doses of 50 and 100 mg/kg, *i.p.* The maximal analgesic effect was observed 60 min post drug administration (Figure 2).

**Effect on isolated rabbit jejunum:** The extract (0.05 - 0.4 mg/ml) produced a dose dependent reduction in the amplitude of spontaneous contraction of the smooth muscles of the rabbit jejunum (Figure 3). The extract did not affect the contractile effect of acetylcholine (0.1 µg/ml) on the tissue, but it blocked the responses due to histamine (1 µg/ml) (Figure 4).

**Effect on gastrointestinal transit time in mice:** The extract (25, 50 and 100 mg/kg) significantly (p<0.05) reduced the gastrointestinal distance travelled by the charcoal plug in animals. Atropine (a reference drug) also inhibited the gastrointestinal propulsion (Table 2).

**DISCUSSION**

The pharmacological activities of the methanolic extract of *C. nigricans* were evaluated in the study. Cold restraint stress brings about gastric mucosal damage in the glandular part of the stomach that are constant in frequency of occurrence, type and location. Acetyl salicylic acid is also a known ulcerogen. The result of this study revealed that the extract protected rats against the combined effects of cold restraint stress and acetyl salicylic acid, suggesting the protective ability of the extract on gastric mucosal damage. The results were similar to that of cimetidine, which is believed to act via the inhibition of histamine H$_2$ receptors. It was observed that rats treated with *C. nigricans* had their stomachs covered with a relatively thick layer of mucus. These findings agree with earlier studies of Sanyal *et al*., in which they showed that mucous layer of mucin-bicarbonate secretion forms a defensive barrier on the stomach. It is therefore, likely that the extract exerted its effect by forming a cytoprotective barrier, which may be useful against peptic ulcers.

The result obtained on the rabbit jejunum indicated that *C. nigricans* is a potent inhibitor of intestinal motility. The extract did not influence responses to ACh on the rabbit jejunum, but it attenuated responses to histamine, thereby suggesting histaminergic mechanism in the observed effects. The extract also reduced the gastrointestinal transit in mice dose dependently. This activity was revealed by the charcoal meal study, which allows for the *in vivo* evaluation of the effects of drugs on gastrointestinal motility. Therefore, the inhibitory effects of the extract on gastrointestinal motility and the attenuation of histaminergic influence on the gastrointestinal tract may both contribute positively to the beneficial effects of the extract against peptic ulcer.

The extract significantly reduced the number of acetic acid-induced writhes in mice, revealing antinociceptive properties. The abdominal constriction response induced by acetic acid is a very sensitive procedure that enables the detection of antinociceptive activity of compounds in laboratory animals. The abdominal constriction response is thought to involve, in part, local peritoneal receptors, thereby suggesting that the extract of *C. nigricans* could interfere with such peritoneal receptors to bring the observed analgesic effect, which is a desirable property in peptic ulcers. The association of both analgesic and anti-inflammatory effects is well documented for various non-steroidal anti-inflammatory agents. It is therefore interesting that the extract of *C. nigricans* caused a remarkable dose dependent anti-inflammatory activity in the rats; thereby contributing to the overall effects of the extract.

The result of this study support the use of *C. nigricans* in the treatment of gastrointestinal disorders. The purification and isolation of the active principles are being explored.

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REFERENCES


GREATER VIGILANCE NEEDED ON SAFETY OF OVER THE COUNTER DRUGS

It is currently rare for pharmacists to record the details of patients to whom they sell over the counter medicines but an editorial in this week's BMJ argues that it would be in the public health interest for pharmacists to do so.

David Clark of the Department of Pharmacology, University of Otago, New Zealand and Layton and Saad Shakir of the Drug Safety Research Unit at Southampton argue that consumers believe that non-prescription medicines are safe because they are freely available over the counter. However, the trend to self medication and the switch from prescription only to pharmacy only status means that more and more powerful medications are becoming available over the counter.

These products are not always used correctly, so the use of over the counter medicines should be monitored and quantified. The authors argue that spontaneous reporting of adverse reactions is inadequate and a more rigorous system of recording by pharmacists is now indicated.

(Editorial: Monitoring the safety of over the counter drugs) http://bmj.com/cgi/content/full/323/7315/706