

Full Length Research Paper

Studies on anti-ulcer, analgesic and antipyretic properties of the ethanolic leaf extract of *Gongronema latifolium* in rodents

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The ethanol extract of *Gongronema latifolium* leaves were evaluated for anti-ulcer, analgesic and antipyretic activities in rats and mice. Ethanol-induced gastric ulceration, acetic acid-induced writhing and formalin-induced nociception were used. Yeast-induced hyperpyrexia was used to investigate the antipyretic activity. The extract produced a significant ulcer protective activity in rats. The extract also decreased pain induced both by acetic acid in mice and early phase of formalin test in rats. A significant reduction in hyperpyrexia was also produced by the extract in rats. This present study provides a strong evidence of anti-ulcer, analgesic and antipyretic activities of *G. latifolium*.

Key words: *Gongronema latifolium*, analgesic, antipyretic, ulcer-protective.

INTRODUCTION

Gongronema latifolium, known as 'utazi' in the south-eastern and 'arokeke' in the south-western part of Nigeria. It is a tropical rainforest plant which belongs to the family of *Aslepiadaceae* (Ugochukwu and Babady, 2002, 2003). It is a climber with tuberous base found in deciduous forest from Guinea Bissau and western Cameroons. Various parts of these plants, particularly the stems and leaves are used as chewing sticks or liquor in places such as Sierra Leone. The liquor, usually obtained after the plant is sliced and boiled with lime juice or infused with

water over three days is usually taken as a purge for colic and stomach pains as well as to treat symptoms connected with worm infections (Okafor, 1975). The plant has also been widely used in folk medicine as a spice and vegetable (Morebise et al., 2002) for maintaining healthy blood glucose levels (Okafor, 1981, 1987, 1989). Antibacterial activity of the leaf extract has also been reported (Nwinyi et al., 2008).

The use of medicinal plants in curing diseases is as old as man (Grabley and Thierickee, 1999; Abinu et al., 2007). The World Health organization (WHO) has long recognized and drawn the attention of many countries to the ever increasing interest of the public in the use of medicinal plants and their products in the treatment of various ailments. These plants which are found in our environment enjoy wide acceptability by the population and serve as cheaper alternatives to orthodox medicine (Sofowora, 1993; Akah and Nwabie., 1994).

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Abbreviations: I.P, Intraperitoneally; ASA, acetylsalicylic acid; S.C, subcutaneously; COX-2, cyclooxygenase- 2; PG, prostaglandin; PGE₂, prostaglandin E₂; LD₅₀, half maximum lethal dose; SEM, standard error of mean.

G. latifolium is one of such medicinal plants whose therapeutic application has a folkloric background. The plant enjoys widespread reputation as a remedy for inflammation, bacteria, ulcer, malaria, diabetes and analgesic. Hence a scientific verification of its uses would be important in establishing a pharmacological basis for some of the claimed ethnomedicinal uses. This study establishes a scientific basis for the folkloric use of *G. latifolium* leaves as antiulcer, pain relief and antipyretic.

MATERIALS AND METHODS

Plant material

Fresh leaves of *G. latifolium* were collected from Ihiagwa, Owerri, Imo State, Nigeria. The plant was identified and authenticated by Mrs. Grace Ugbabe, Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria. A voucher specimen has been deposited in NIPRD herbarium with the voucher number: (NIPRD (H) 6395). The leaves were air-dried and pulverized into fine powder. 300 g of the powdered material was macerated with ethanol for three consecutive days at room temperature with constant shaking. The liquid extract obtained was concentrated to dryness in vacuum at 40°C. The yield was nine grams (9 g).

Phytochemical screening

The ethanolic extract of *G. latifolium* was subjected to qualitative phytochemical screening according to standard methods (Trease and Evans, 1983).

Animals

Adult wistar rats (200-250 g) and Swiss albino mice (20-25 g) of either sex obtained from the Animal Facility Centre of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria were used for the experiments. All animals were kept in metal cages at room temperature and housed under standard conditions of 12:12 h light/dark cycle. They were fed with NIPRD formulated standard feed and allowed free access to tap water *ad libitum*. Studies were carried out in accordance with the principles of good laboratory practice and animal handling (NIH) guidance for the care and use of laboratory animals, Publication No. 85-23, 1985.

Acute toxicity studies

The acute toxicity LD₅₀ was estimated both orally (*p.o*) and intraperitoneally (*i.p*) in both adult Wistar rats and Swiss albino mice following Locke's method (1989). Dose levels used range from 100 to 5000 mg/kg body weight. The LD₅₀ was calculated as the geometric mean of the dose that caused 100% mortality and the dose which caused no mortality at all.

Ethanol induced ulceration

The rats were fasted for 48 h but allowed free access to water *ad libitum*. They were randomly selected and divided into five groups with six animals in each group. Group 1 received normal saline 10 ml/kg body weight while group II received standard drug (Ranitidine 20 mg/kg). Groups III, IV and V received 100, 200 and 400 mg/kg of

the extract, respectively, and all drugs were administered orally. One hour later, ulceration was induced by intragastric instillation of 0.5 ml of 90% ethanol and 1 h after ethanol administration, rats were anaesthetized using ether and the stomachs were removed and opened along the greater curvature to macroscopically examine any ulcerative lesions. The number, length and severity of the ulcers were noted and scored on an arbitrary 0 - 6 point scale (Magistretti et al., 1988). The scores were as below:

- 0 = No lesion
- 1 = 1 - 3 small lesions
- 2 = 1 - 3 large lesions
- 3 = 1 - 3 thick lesions
- 4 = More than 3 small lesions
- 5 = More than 3 large lesions
- 6 = More than 3 thick lesions

Acetic acid induced writhes

This test was conducted using the method described by Koster et al. (1959). Mice were divided into five groups of six animals each and pre-treated as follows: Group 1, II and III received 25, 50 and 100 mg/kg of the extract, respectively, while IV received acetyl salicylic acid (ASA) 150 mg/kg and group V which served as control received normal saline in appropriate volumes, administered *i.p*. 30 min after pre-treatment, each mouse was administered 0.7% of an aqueous solution of acetic acid (10 mL/Kg). Mice were then placed in transparent perspex observation boxes and the number of abdominal constrictions was counted for 10 min after treatment. The percentage inhibition of constrictions for the extract and ASA groups were calculated as:

$$\% \text{ Inhibition} = [(\text{Control mean} - \text{test mean}) / \text{Control mean}] \times 100$$

Formalin induced nociception in rats

The test was carried out as described by Dubuisson and Dennis (1977) and modified by Tjolsen et al. (1992). Adult Wistar rats were divided into five groups of six animals each and pre-treated *i.p* as follows: Group 1 received normal saline, this served as control. Group II, III and IV received 25, 50 and 100 mg/kg extract, respectively, while group V received 150 mg/kg ASA, all drugs administered *i.p*. 30 min after treatment, all groups were administered 50 μ L of a 2.5% solution of formalin, subcutaneously (*s.c*) under the sub plantar surface of the left hind paw (Table 3). They were then placed in an observation chamber and monitored for 60 min, recording severity of nociceptive responses based on the observation scale below:

- (0) rats can bear weight on injected paw with free movement.
- (1) Light resting of the injected paw on the floor.
- (2) Partial elevation of the injected paw.
- (3) Total elevation of the injected paw, licking and /or biting of the injected paw.

These observations were recorded every minute for the first 10 min (early phase) and then every 5 min thereafter up to 60 min (late phase).

Antipyretic activity

The method by Al-Ghamdi (2001) was adopted to evaluate the antipyretic activity of the extract in rats. The rats were injected subcutaneously (*s.c*) with 10 ml/kg of 15% suspension of yeast (Danbaoli) to induce pyrexia. The rectal temperature of each animal

Table 1. The effect of the ethanolic extract of *G. latifolium* on the ethanol induced gastric ulceration in rats.

Drug	Dose (mg/kg)	Ulcer index (U.I.)	% Maximal protection of ulceration
Control (Saline)	10 ml/kg	5.33 ± 0.33	—
Ranitidine	20	0.33 ± 0.21	93.81*
<i>G. latifolium</i>	100	3.17 ± 0.60	40.52*
<i>G. latifolium</i>	200	1.00 ± 0.68	81.24*
<i>G. latifolium</i>	400	0.50 ± 0.50	90.61*

*P < 0.01 as compared with control groups.

Table 2. The effect of the ethanolic extract of *G. latifolium* on acetic acid induced writhes in mice.

Drug	Dose (mg/kg)	Writhes	% Inhibition
Control (saline)	10 ml/kg	31.85 ± 3.06	—
ASA	150	3.33 ± 3.33	89.54 *
<i>G. latifolium</i>	25	14.17 ± 1.87	55.55 *
<i>G. latifolium</i>	50	9.50 ± 5.61	70.15 *
<i>G. latifolium</i>	100	5.33 ± 2.15	83.25 *

*P < 0.01 as compared to control group.

was taken before and 24 h after injection using a clinical thermometer (Geon Corp. U.S.A). Rats that did not show a minimum increase of 0.5°C in temperature 24 h after yeast injection were screened out of the study. Thirty selected rats were grouped into five and treated as follows: Group I received normal saline (10 ml/kg i.p) while group II received 20 mg/kg drugamol (Drugfield Nigeria) to serve as positive control. Group III, IV and V received 25, 50 and 100 mg/kg of the extract, respectively, all administered i.p. The rectal temperature of each rat was again recorded at 30 min intervals for 120 min.

Statistical analysis

Results were expressed as mean ± standard error of the mean (SEM). The data were analyzed using student's t-test and 2 way ANOVA. P < 0.01 was considered significant.

RESULTS

Phytochemical screening

Phytochemical analysis of the extract revealed the presence of alkaloids, saponins, tannins and flavanoids.

Acute toxicity studies

When administered i.p in mice, the ethanolic extract of *G. latifolium* produced 100% lethality at 2000 mg/kg and 0% lethality at 1000 mg/kg. Hence, the LD₅₀ in mice was estimated to be 144.2 mg/kg ip. No lethality was observed in mice upon oral administration even at doses as high as 5000 mg/kg. Thus, the oral LD₅₀ was estimated to be >5000 mg/kg body weight. Apart from

weakness, *G. latifolium* did not produce any major signs of clinical toxicity over the 4 days observation period.

Acute gastric ulcer induced by ethanol

The extract was found to possess remarkable and significant ulcer protective properties at 200 and 400 mg/kg body weight with maximum effect at 81.24 and 90.61% inhibition of ulceration, respectively (P < 0.01). Ranitidine, the standard drug produced 93.81% protection (P < 0.01) (Table 1).

Acetic acid induced writhes

Dose dependent and significant antinociceptive effect was noticed with the ethanolic extract at the doses tested. In the acetic acid induced writhing model, the extract with 100 mg/kg dose exhibited a maximum of 83.25% inhibition of writhing (P < 0.01) and the effect was comparable to standard acetylsalicylic acid (89.54%) (P < 0.01) while 25 and 50 mg/kg doses have shown 55.55 and 70.15% reduction, respectively (P < 0.01) (Table 2).

Formalin induced nociception

The ethanolic extract of *G. latifolium* significantly inhibited both phases of the formalin test dose dependently. In the early phase, the extract produced significant inhibition of up to 51.55% at 100 mg/kg (P < 0.01) when compared with ASA, which produced an inhibition of 58.52% at a

Table 3. The effect of ethanolic extract of *G. latifolium* on early and late phase formalin induced pain in rats.

Drug	Dose (mg/kg)	Early phase Pain score	% Pain Inhibition	Late phase Pain score	% Pain inhibition
Control (saline)	10 ml/kg	2.58 ± 0.07	—	2.9 ± 0.21	—
ASA	150	1.07 ± 0.11	58.52	1.0 ± 0.18	65.5*
<i>G. latifolium</i>	25	2.03 ± 0.24	21.32	2.53 ± 0.11	12.78
<i>G. latifolium</i>	50	1.75 ± 0.25	32.17	2.47 ± 0.10	14.83
<i>G. latifolium</i>	100	1.25 ± 0.20	51.55	2.22 ± 0.23	23.45*

*P < 0.01 as compared to control groups.

The results given are mean ± SEM; number of animal used (n = 6).

Table 4. The effect of ethanolic extract of *G. latifolium* on yeast induced pyrexia in rats.

Drug	Dose - (mg/kg)	Rectal temperature (°C)					
		24 h	0.0 h	0.5 h	1.0 h	1.5 h	2 h
Control (saline)	10 mg/kg	37.15 ± 0.24	38.27 ± 0.20	37.70 ± 0.33	38.35 ± 0.21	38.57 ± 0.29	38.98 ± 0.16
Drugamol	20	36.24 ± 0.27	37.06 ± 0.43	36.50 ± 0.14	35.91 ± 0.14	34.91 ± 0.26	34.54 ± 0.18*
<i>G. latifolium</i>	25	36.81 ± 0.24	36.96 ± 0.35	36.11 ± 0.22	35.76 ± 0.27	35.69 ± 0.35	35.67 ± 0.25*
<i>G. latifolium</i>	50	36.50 ± 0.18	37.76 ± 0.14	36.14 ± 0.26	35.60 ± 0.20	35.64 ± 0.34	35.10 ± 0.24*
<i>G. latifolium</i>	100	36.79 ± 0.20	37.07 ± 0.35	36.15 ± 0.33	35.73 ± 0.38	35.21 ± 0.37	34.71 ± 0.40*

The results given are mean ± SEM; number of animal used (n = 6).

*P < 0.01 as compared to control groups.

dose of 150 mg/kg (P < 0.01). In the late phase of formalin test however, there is a large difference between the levels of inhibition produced by the extract compared with ASA. The extract produced an inhibition of 23.45% at 100 mg/kg whilst ASA produced 65.52% at 150 mg/kg (P < 0.01). The extract produced pain inhibition at the lower doses of 25 and 50 mg/kg.

Yeast induced pyrexia

The subcutaneous injection of yeast suspension markedly elevated the rectal temperature of the rats after 24 h of administration. Treatment with the ethanolic extract of *G. latifolium* at the dose of 25, 50 and 100 mg/kg body weight significantly decreased the rectal temperature of the rat in dose dependent manner (P < 0.01). The antipyretic effect started from the first 30 min and was maintained for 120 min after extract administration. The result obtained from both the standard drug and the extract - treated rats were statistically compared with control group and a significant reduction in temperature was observed (Table 4).

DISCUSSION

The ethanolic extract of *G. latifolium* was evaluated for its anti-ulcer activity in rats. A significant anti-ulcer activity was observed for the extract in the ethanol induced ulcer model. Ethanol induced damage to gastric mucosa is

associated with a significant production of free radicals (Szelenyi and Brune, 1988) leading to increased lipid peroxidation. Moreover, in the stomach, ethanol causes solubilization of mucus constituents and depresses tissue levels of proteins, leading to flow stasis in gastric blood (Szabo et al., 1986). Ethanol provoked gastric mucosal lesions are caused by the direct toxic effect of ethanol through the reduction in mucus production, gastric mucosal blood flow and bicarbonate secretion. Endogenous glutathione and prostaglandin (PG) levels are also lowered by ethanol while the release of histamine, influx of calcium ions and generation of free radicals and production of leukotrienes are all increased (Glavin and Szabo, 1992).

The active constituents of the extract revealed by the phytochemical screening, especially tannins and flavanoids, may also have a contributory role to play in its anti-ulcer activity. Tannins are known to 'tar' the outermost layer of the gastric mucosa rendering it less permeable and more resistant to chemical and mechanical injury or irritant (Asuzu and Onu, 1990). Thus, the result of this study has shown that *G. latifolium* probably antagonizes the aggressive factors like acid, pepsin and *H. pylori*, which play an important role in the pathogenesis of gastric ulcers (Kumar and Clarke, 2002) while augmenting the defensive mucosal factors that protect the gastric mucosa from injury (Germano et al., 1998). This assertion is further buttressed by the fact that the extract has been shown to have a remarkable antimicrobial activity against bacteria (*Escherichia*, *S. aureus*, etc) (Nwinyi et al., 2008).

It is also possible that flavanoids present in *G. latifolium* may play a role in this regard. Flavanoids possess antioxidant properties in addition to strengthening the mucosal defence system through stimulation of gastric mucus secretion (Martin et al., 1994). Further tests however need to be undertaken to elucidate the exact mechanism of action.

Analgesic activity is another biological property significantly exhibited by the extract. The extract inhibited acetic acid induced writhing response in the animals. Acetic acid induced writhes is a sensitive procedure in detecting analgesic effect of medicinal agents (collier et al., 1968). This pain mechanism is believed to involve local peritoneal receptors (Bentley et al., 1983) caused by peritoneal fluid concentration of PG-E₂ and PG-F_{2 α} (Deraedt et al., 1980). This method is not only simple and reliable but also affords rapid evaluation of peripheral type of analgesic action. In this, the animals react with characteristic stretching behavior which is called writhing. The abdominal constriction is related to the sensitization of nociceptive receptors in prostaglandins. It is therefore possible that the analgesic effect may be due to the inhibition of synthesis or action of prostaglandins. The extract was also effective in the formalin test which involves two phases: neurogenic with the release of substance P and inflammation with the release of serotonin, histamine, bradykinin and prostaglandins (Murray et al., 1988; Tjolsen et al., 1992). It was able to block both phases of formalin response, although, higher inhibition was seen in the first phase.

A dose dependent significant reduction in yeast-induced pyrexia was also observed in rats treated with the ethanolic leaf extract of *G. latifolium*. Pyrexia may be as result of infection or one of the sequelae of tissue damage, inflammation, graft rejection or other disease state. Antipyretics are drugs which reduce elevated body temperature, graft rejection or other disease state. Regulation of body temperature requires a delicate balance between production and loss of heat and the hypothalamus regulates the set point at which body temperature is maintained. Most of the antipyretic drugs inhibit COX-2 expression thus inhibiting PGE₂ biosynthesis to reduce elevated body temperature. The present result shows that the ethanolic extract of *G. latifolium* possesses a significant antipyretic effect in yeast induced pyrexia in rats and its effects are comparable to that of drugamol. Based on the results obtained, it can be concluded that the ethanolic leaf extract of *G. latifolium* possesses potential antiulcer, antinociceptive and antipyretic activities.

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