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Anti-diarrhoeal and anti-inflammatory effects of methanolic extract of *Guiera senegalensis* leaves in rodents

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Abstract

Objective: To investigate if the methanolic extract of *Guiera senegalensis* leaves actually possesses any anti-diarrhoeal and anti-inflammatory effects in rodents. Methods: The anti-diarrhoeal effect of extract (250 –750 mg/kg; p.o.), on intestinal propulsion in unanaesthetized rats was tested using charcoal meal method, castor oil-induced diarrhoea and fluid accumulation respectively. Its anti-inflammatory property was also investigated in carrageenin - induced rat hindpaw edema, acetic acid-induced writhing in mice as well as indomethacin-induced gastric ulceration in rats. The extract effects were compared with standard drugs (atropine, 1.0 mg/kg; yohimbine, 1.0 mg/kg; diphenoxylate, 5.0 mg/kg and cimetidine, 100.0 mg/kg respectively). Results: The extract (250 –750 mg/kg) dose-dependently, reduced significantly ($p < 0.01 – 0.001$) the intestinal propulsive movement, castor oil-induced diarrhoea and intestinal fluid accumulation. Similarly, it also reduced indomethacin-induced ulceration, carrageenin-induced inflammation and acetic acid- induced writhing in mice. These effects were statistically significant. Conclusion: The results indicate that the observed anti-diarrhoeal and anti- inflammatory effects might in part be due to α_2 – adrenoceptor stimulation, possession of anticholinergic properties, inhibition of prostaglandin synthesis as well as improvement of microcirculation.

Keywords: *Guiera senegalensis*, antidiarrhoeal, anti-inflammatory, methanolic extract, rodents.

1. Introduction

Guiera senegalensis (Combretaceae) is a herb of a wide range of geographical distribution in Africa, starting from rain forest region of Nigeria to the arid zone areas of Mali [1, 2]. It is called *Sabara* in Hausa and *Shafa pitu* in Marghi. It grows luxuriantly in the North Eastern Nigeria between the months of June – September, where it is being used for gastrointestinal disorders and treatment of rheumatoid pains (Asabe Magomya,

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Department of Biochemistry, University of Maiduguri, personal Communication). It is also used in Senegal for similar purpose [1, 3]. The present study was to establish if the leaves actually possess any true anti-diarrhoeal and anti-inflammatory properties.

2. Material and methods

2.1 Preparation of extract

The plant material used in this study was collected from University of Maiduguri, Borno State between the months of May – June, 1999.   The plant was identified and authenticated by Dr. S. S. Sanusi, Department of Botany, University of Maiduguri. Specimen vouchers (CMS 016) were made and deposited at the herbarium of Department of Pharmacology, College of Medical Sciences, University of Maiduguri. The leaves were dried at room temperature and pulverized by grinding using pestle and mortar. Then, 100 g of the ground leaves were subjected to exhaustive soxhlet extraction in methanol (250 ml) for 72 h at 60°C. The solvent was removed by distillation. This gave a mean yield of 14.5 ± 0.24 g w/w of extract. The extract was stored at – 40°C from   where it was used when required.

2.2 Animal stock

Adult albino mice and rats (weighing 25-30 g and 165-200 g) respectively were used in the study. All the animals were housed in a cross ventilated room (temperature 22 ± 2.5°C, 12 h light/12 h dark cycle) and were fed with standard mash (ECWA feed Nig;) Jos, Nigeria and water *ad-libitum*.

2.3 Small intestinal propulsion

The effect of extract on intestinal propulsion in unanaesthetized rats was tested using the charcoal method of Capasso *et al.* [4]. The animals were fasted for 24 h but allowed free access to water. They were randomized and placed in five cages of six animals per cage. Group 1 was administered with normal saline (p.o.) using orogastric cannula. Groups 2-4 were pretreated with G. senegalensis extract 250-750 mg/kg (p.o.), respectively. Group 5 was pretreated with 100 μg/kg atropine (p.o.). After 1h, each rat was administered with 1 ml charcoal meal (5% activated charcoal suspended in 10% aqueous tragacanth), orally. The rats were killed 30 min later by cervical dislocation and bled, and the small intestine was rapidly dissected out and placed on a clean surface. The small intestine was carefully inspected and the distance traversed by the charcoal meal from the pylorus was measured. The length of the whole small intestine was also measured. The distance traversed by the charcoal meal from the pylorus was expressed as a percentage of the distance from the pylorus to the ileocaecal junction.

$$\text{Intestinal propulsion} = \frac{\text{Distance moved by the suspended charcoal head}}{\text{Whole length of small intestine}} \times 100$$

2.4 Castor oil-induced diarrhoea

A modification of the method of Awouters *et al.* [5] by Nwodo and Alumanah was adopted [6]. The rats were fasted for 24h but allowed free access to water. They were randomized and placed in cages of five rats per cage. Group 1 was administered with normal saline. Group 2-4 were given 250-750 mg/kg of extract (p.o.) respectively. Groups 5 & 6 were administered with diphenoxylate (5.0 mg/kg, p.o.) and yohimbine (1 mg/kg) respectively, and after 10min, 500 mg/kg of extract was given orally to both groups. After 1h, each rat received 2 ml castor oil (p.o.) and was observed for consistency of faecal matter and the frequency of defaecation for 3 h.
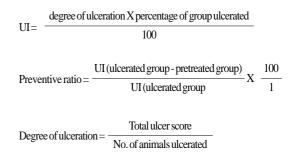
2.5 Castor oil-induced fluid accumulation

This was determined according to the method of Robert *et. al.* [7] modified by Dicarlo *et. al.* [8]. The rats were fasted for 24 h but allowed free access to water. They were randomized and placed into six cages of six rats each. All drugs were orally given except yohimbine which was administered subcutaneously. Group 1 was administered with castor oil. Groups 2-4 received 250-750 mg/kg (p.o.) of extract respectively. Groups 5 & 6 were administered with diphenoxylate (5.0 mg/kg p.o.) and yohimbine (1.0 mg/kg, sc) respectively. After 30min, the rats were killed by cervical dislocation and exsanguinated, the small intestine was ligated at both pyloric sphincter and at the ileocaceal junctions. The entire small intestine was dissected out, its contents were expelled into a graduated measuring cylinder and the volume of the contents was recorded.

2.6 Indomethacin-induced gastric ulceration

Pilot tests aimed at determining the effective dose of indomethacin required to produced reliable acute gastric ulceration in rats were done. This was achieved by administering varying doses of indomethacin (40, 60 and 100 mg/kg) (Studer Arcola, India) to rats. In this way, the least effective dose (p.o.) of indomethacin that produced 100% gastric ulceration was obtained. The dose was repeated to verify if the degree of ulceration will be reproducible. From these tests, 100 mg/kg produced gastric ulceration in all rats in 4 h.

The rats were randomized and divided into five groups of six rats each. Food was withdrawn 24 h and water 2 h before the commencement of experiment (9). Group 1 was administered with 100 mg/kg indomethacin (p.o.). Groups 2-4 were pretreated with 250-750 mg/kg of extract while group 5 was pretreated with 100 mg/kg of cimetidine, (Lek, India) 1h prior to administration of 100 mg/kg of indomethacin. The drugs were administered intragastrically via the aid of an orogastric cannula. Four hours later, the animals were killed by cervical dislocation. The stomachs were removed and opened along the greater curvature. The tissues were fixed with 10% formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of lesions using Alphin and Ward method [9] modified by Evbuonwu and Bolarinwa [10]. Ulcer index (UI) and preventive ratio of reach of the groups pretreated with extract were calculated using the standard methods [11, 12].

$$UI = \frac{\text{degree of ulceration X percentage of group ulcerated}}{100}$$

$$\text{Preventive ratio} = \frac{\text{UI (ulcerated group - pretreated group)}}{\text{UI (ulcerated group}} \times \frac{100}{1}$$

$$\text{Degree of ulceration} = \frac{\text{Total ulcer score}}{\text{No. of animals ulcerated}}$$

2.7 Carrageenin-induced rat hind paw edema

Increase in the rat hind paw linear circumference induced by subplantar injection of a phlogistic agent was used as the measure of acute inflammation [13]. Adult albino rats of either sex were used after 24 h fast and deprived of water only during experiment. Inflammation of the hind paw was induced by injecting 0.1 ml of freshly prepared carrageenin 1% suspension in normal saline into the subplantar surface of the hind paw. The linear circumference of the injected paw was measured before and 0.5 h, 1 h, 2 h, 4 h and 5 h after administration of phlogistic agent. For routine drug testing, the increase in paw circumference 0.5 h, 1 h, 2 h, 4 h and 5 h after administration of phlogistic agent was adopted as the parameter for measuring

Table 1. Effect of *Guiera senegalensis* extract on intestinal propulsion in rats

Dose (mg/kg)	Mean intestinal Length (cm)	Mean distance moved by charcoal	% Inhibition
1ml (saline)	72.00 ± 4.91	71.50 ± 2.28	0.69
250	68.80 ± 3.34	46.20 ± 2.02*	32.85
500	67.40 ± 2.98	32.70 ± 1.89*	51.48
750	66.60 ± 5.98	21.71 ± 1.73*	67.40
0.1 (Atr.)	68.30 ± 2.01	10.15 ± 1.50*	85.14

Values are mean ± S.E.M; (n=6) ; Atr.=atropine; [a]p<0.001 relative to control.

Table 2. Effect of *Guiera senegalensis* extract on castor oil-induced diarrhoea in rats

Dose (mg/kg)	Total number of Faecal matter	% Reduction (inhibition)
1ml	78	0.00 ± 0.00
250	15	80.77 ± 0.24[a]
500	4	94.87 ± 0.54[a]
750	3	96.15 ± 0.32[a]
1.0 (yoh) + 500	18	76.92 ± 0.01[a]
5.0 (diph) + 500	0	100.00 ± 0.00[a]

Values are mean ± S.E.M; (n=6); yoh = yohimbine; diph = diphenoxylate; [a]p<0.001 relative to control.

Table 3. Effect of extract on castor oil-induced intestinal fluid accumulation in rats.

Extract Dose (mg/kg)	Mean volume of intestinal fluid ± S.E.M (ml)	Inhibition (%)
Control (2ml castor oil)	3.21 ± 0.05	—
250	2.35 ± 0.20[a]	24.67
500	2.20 ± 0.08[a]	29.49
750	1.88 ± 0.04[a]	39.74
5.0 (diph)	1.25 ± 0.09[a]	59.94
1.0 (yoh)	2.98 ± 0.01	4.49

Values are mean ± S.E.M; (n = 6); yoh = yohimbine; diph = diphenoxylate ; [a]p < 0.001 relative to control.

inflammation [13-16]. Edema (inflammation) was assessed as the difference in paw circumference between the control and 0.5 h, 1 h, 2 h, 4 h, 5 h after administration of the phlogistic agent [17]. The extract (250-750 mg/kg) was administered intraperitoneally to groups 2-4, 1h before inducing inflammation. Control rats received carrageenin while group 5 rats received 100 mg/kg acetyl salicyclic acid intrapentoneally. The average (mean) edema was assessed by measuring with Vernier calipers.

2.8 Acetic acid-induced writhing in mice

The abdominal constrictions resulting from intraperitoneal (i.p) injection of (0.1 ml) acetic acid (3%) consisting of the contraction of abdominal muscle together with a stretching of hind limbs, were carried out according to standard procedures [16, 18, 19]. The animals were divided into five groups of six mice per group. Group 1 served as control while groups 2-4 were pretreated with 250-750 mg/kg of *Guiera senegalensis* extract intraperitoneally. Group 5 was treated with acetyl salicylic acid (100 mg/kg, i.p). After 30 minutes, acetic acid (0.1 ml) was administered (i.p). The numbers of writhing movements were counted for 30 minutes. Antinociception was expressed as the reduction of the number of abdominal constrictions between control animals (saline treated mice) and mice pretreated with the extract.

2.9 Statistical analysis

Multiple comparisons of mean ± S.E.M were carried out by one way analysis of variance (ANOVA), followed by Tukey-Krammer multiple comparisons tests. A probability level of less than 5% was considered significant.

Table 4. Effect of extract on indomethacin-induced ulceration in rats

Dose (mg/kg)	Ulcer index	Preventive ratio
100 Indo. (control)	19.23 ± 1.05	-
250	16.00 ± 0.08a	16.80
500	8.08 ± 1.32a	58.00
750	0.50 ± 0.69a	97.40
100 (cimet)	4.58 ± 1.39a	76.18

Values are mean ± S.E.M; (n = 6); cimet = Cimetidine; ap < 0.001 relative to control

3. Results

3.1 Small intestinal propulsion

In control animals (saline treated rats), the charcoal meal traversed 71.50 % of the total length of the small intestine. All the tested doses of the extract inhibited dose-dependently the intestinal propulsion from 32.85 to 67.40 % (Table 1). These inhibitions were significant. Atropine, an anticholinergic drug, caused 85.14% of intestinal propulsive inhibition.

3.2 Castor oil-induced diarrhoea

G. senegalensis leaf extract (250-500 mg/kg, p.o) decreased the castor oil-induced diarrhoea in rats by 80.77-96.15%. This effect was enhanced in the presence of diphenoxylate (5 mg/kg, p.o), an anticholinergic drug. However, yohimbine (1 mg/kg, sc) an α_2 – blocker, inhibited this effect (Table 2).

Table 5. Effect of *Guiera senegalensis* on carrageenin-induced inflammation in rats

Dose (mg/kg)	Tim (h)						
	0	0.5	1.0	2.0	3.0	4.0	5.0
Control (0.1ml)	0.37 ± 0.01	0.49 ± 0.01	0.58 ± 0.00	0.69 ± 0.01	0.77 ± 0.02	0.75 ± 0.02	0.69 ± 0.02
250	0.38 ± 0.01	0.45 ± 0.01	0.51 ± 0.01a	0.61 ± 0.04	0.61 ± 0.04b	0.54 ± 0.03b	0.51 ± 0.04b
500	0.35 ± 0.39	0.51 ± 0.02	0.45 ± 0.01b	0.43 ± 0.01b	0.48 ± 0.00b	0.45 ± 0.02b	0.42 ± 0.01b
750	0.34 ± 0.00	0.41 ± 0.01a	0.41 ± 0.02b	0.36 ± 0.01b	0.36 ± 0.01b	0.34 ± 0.01b	0.33 ± 0.01b
100 ASA	0.37 ± 0.01	0.43 ± 0.02	0.45 ± 0.01b	0.47 ± 0.01b	0.46 ± 0.02b	0.46 ± 0.00b	0.43 ± 0.01 b

Values are mean ± SEM; (n = 6); ASA = Acetyl salicylic acid; ap< 0.01; bp< 0.001 relative to control.

Table 6.   Effect of *Guiera senegalensis* on acetic acid-induced writhing in mice

Dose (mg/kg)	5min	10min	15min	20min	25min	30min	Total mean +SEM
Control (0.1ml AA)	6.50 ± 0.66	17.16 ± 1.83	19.66 ± 1.72	17.16 ± 1.01	13.66 ± 0.49	11.50 ± 0.93	85.64 ± 6.64
250	4.00 ± 0.26a	11.33 ± 0.35a	12.50 ± 0.69a	9.83 ± 0.10a	7.50 ± 0.43a	5.66 ± 0.60a	50.82 ± 1.83a
500	2.50 ± 0.08a	7.00 ± 0.95a	8.33 ± 0.02a	6.66 ± 0.20a	7.16 ± 0.50a	6.00 ± 0.80a	37.65 ± 1.75a
750	1.16 ± 0.16a	0.0 ± 0.00a	1.50 ± 0.33a	2.00 ± 0.04a	1.50 ± 0.71a	1.83 ± 1.04a	7.99 ± 1.64a
100 ASA	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a	0.00 ± 0.00a

Values are mean ± SEM; (n = 6); AA = Acetic acid; ASA = Acetyl salicylic acid; ap < 0.001 relative to control.

3.3 Castor oil-induced intestinal fluid accumulation

G. senegalensis extract (250-750 mg/kg, p.o) dose-dependently reduced the intestinal fluid accumulation by 24.67 – 39.74% relative to control. Yohimbine (1 mg/kg, sc) antagonized the fluid reducing effect of the extract by 4.49%. However, diphenoxylate (5 mg/kg, p.o) enhanced the fluid reducing effect of the extract by 59.94 % (Table 3).

3.4 Indomethacin-induced gastric ulceration

The oral treatment with the extract dose-dependently inhibited the ulcerogenic effect of indomethacin, the ulcer index being reduced from 19.23 (control) to 0.50 with the highest dose used (750 mg/kg, p.o) which was equivalent to preventive ratio of 97.40. Cimetidine, a H_2-blocker, reduced ulcer index to 4.58 equivalent to 76.18 of its preventive ratio (Table 4).

3.5 Carrageenin-induced inflammation

The extract showed good anti-inflammatory activity against acute inflammation.It suppressed in a dose-related manner the increase in the rat paw oedema caused by carrageenin. The inhibition was significant ($p < 0.01$ - 0.001). The inhibition by the extract was maximal after 2 h of administration of phlogistic agent. The effect of acetyl salicyclic acid was comparable to that of extract (750 mg/kg, p.o) after 1h (Table 5).

3.6 Acetic acid-induced writhing

The extract (250-750 mg/kg, i.p) dose-dependently, reduced acetic acid-induced abdominal constrictions and stretching of hindlimbs. The reduction was significant ($p < 0.001$; Table 6).

Discussion

Methanolic extract of *G. senegalensis* leaves inhibited dose-dependently the small intestinal propulsive movement (IPM) in rat. The data suggests that this effect on IPM is mediated by α_2-adrennoceptor stimulation because α_2-adrenoceptor antagonist, yohimbine, significantly reduced the extract-induced transit delay in rats. The result further supports the idea that activation of α_2-adrenoceptor induce delay in IPM [20, 21].

The extract also showed a dose-related decrease in castor oil-induced diarrhoea. It has been shown that drugs affecting motility, frequency and consistency of diarrhoea also affect secretion [8]. The intraluminal fluid accumulation induced by castor oil was blocked by the extract in a dose-related fashion. The involvement of α-adrenoceptor effect was further confirmed by the antagonistic action of yohimbine, an α-adrenoceptor antagonist. The inhibitory effect of the extract on the gastrointestinal tract was also further enhanced by anticholinergic drugs atropine and diphenoxylate. Anticholinergics, are known to slow both the motility and the secretion of gastrointestinal tract [22]. All the results therefore suggest that the extract produced an inhibitory action on gastrointestinal functions, motility and secretion, and this effect is mediated in part through the activation of α_2-adrenoceptor and anticholinergic receptor systems.

The extract reduced indomethacin-induced ulceration in rats in dose-dependent manner. Indomethacin is an established ulcerogen especially in an empty stomach [23]. The incidence of indomethacin-induced ulceration is mostly on the glandular (mucosal) part of stomach [12, 24, 25]. Although the mechanisms underlying the ulcerogeoncity of indomethacin is not completely understood, it has been known that inhibition of prostaglandin synthesis may be important [26]. The view is supported by the fact that prostaglandins normally serve protective function in stomach by maintaining gastric microcirculation [26, 27] and causes gastric secretion of bicarbonate [28] and mucus [29].

It has been proposed that mucosal protection induced by nonprostanoid compounds may be mediated through the mobilization of endogenous prostaglandins [30, 31]. It is possible that one of the mechanisms of antiulcerogenic effects of the extract may be due to its ability to mobilize prostaglandins in gastric mucosa by increasing its microcirculation or through an unknown mechanism.

The extract dose-dependently inhibited carrageenin-induced inflammation in rats. Carrageenin is said to mediate its action through the mobilization of prostaglandin synthesis [32]. The phytochemical analysis of *Guiera senegalensis* revealed that it contained flavonoid [33]. Flavonoids inhibit both inflammatory and allergic reactions as well as offer some protection in ulcer development by increasing capillary resistance and improving microcirculation which renders the cells less injurious to precipitating factors [34, 35].

The extract also inhibited acetic acid-induced writhing in mice. Acetic acid causes irritation, pain and inflammation [36]. Besra *et. al.* [16]

and Turner [37] have shown that hot plate-induced pain indicates narcotic involvement. Therefore the inhibition of acetic acid induced-writhing may in part be due to its anti-inflammatory/analgesic properties.

In conclusion, the exact mechanism of antidiarrhoeal and anti-inflammatory effects may not be fully elucidated. They may in part be due to activation of α_2-adrenoceptor, possession of anticholinergic properties, inhibition of prostaglandin synthesis, improvement of microcirculation as well as its narcotic involvement/direct mechanism. Though the investigation is not exhaustive, it however lends credence to the local usage of *G. senegalensis* leaves.

Acknowledgement

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