



International Journal of Pharmacology

ISSN 1811-7775

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Anti-Nociceptive and Anti-Phlogistic Actions of a Polyherbal Decoction

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Abstract: A polyherbal decoction prepared with *Gongronema latifolia*, *Ocimum gratissimum* and *Vernonia amygdalina* leaves was evaluated for analgesic and anti-inflammatory activities using rats and mice. Three doses of the decoction (2.0, 4.0 and 8.0 g kg⁻¹) were administered to the test animals to evaluate analgesic and anti-inflammatory activities using the test models of formalin-induced pain, mouse writhing, hot plate, carrageenan and xylene induced oedema. Acute toxicity studies showed that the extract may be considered non-toxic as no mortality was observed even at the high doses of 16 g kg⁻¹ p.o. and 2.5 g kg⁻¹ i.p. In the hot plate assay, the decoction caused a significant pain inhibition of 138.5% (12.4±1.9) which is comparable with 180.8% produced by morphine. The decoction, at 8.0 g kg⁻¹ b.wt., significantly inhibited writhing by 138.5% (12.4±1.9) when compared to that of control. A dose-dependent decrease in licking time and frequency in rats injected with 1% formalin was observed. The highest dose of the decoction exhibited a significant inhibition of 46% (42±5.4) while acetylsalicylic acid also produced a significant inhibition of 49% (39.6±2.9) for the formalin induced pain assay. The decoction also significantly (p<0.05) reduced carrageenan and xylene induced oedema. These results suggest that the polyherbal decoction which is used as a tonic in folk medicine has the potential to reduce pain and inflammation and is also, non-toxic.

Key words: *Gongronema latifolia*, *Vernonia amygdalina*, *Ocimum gratissimum*, anti-inflammatory, analgesic

INTRODUCTION

Ocimum gratissimum is a shrub with aromatic smell in the crushed form. The essential oil obtained from this shrub has been used in wound dressing, as mosquito repellent or applied topically for skin infections (Sofowora, 2006). The essential oil obtained from this shrub has been reported to possess antibacterial properties (Orafidiya *et al.*, 2001).

It is used as a preservative in pharmaceuticals (Trevisan *et al.*, 2006). Nakamura *et al.* (2004) had reported its antibacterial properties.

Vernonia amygdalina is also a shrub found mostly in tropical regions. The root and stem are often used as chewing stick in rural areas due to the acclaimed medicinal properties of the plant. The plant has been used to replace hops in beer making due to its bitter principle. Reports have also shown that extracts from *Vernonia amygdalina* have cell growth inhibitory effects in prostate cancer cell line (PC-3) and no effect on normal human Peripheral Blood Mononuclear Cells (PBMC) (Izevbigie *et al.*, 2004).

Gongronema latifolium is a globorous perennial climber which is popularly used to prepare soups for women especially after childbirth because it is claimed to possess medicinal properties that soothes the womb. It

has a bitter taste which has been exploited in beer production. The hopping potentials of blends of *Vernonia amygdalina*, *Gongronema latifolium* and *Garcinia kola* has been reported as potential replacement for hops in sorghum-based lager beer brewing (Eleyinmi *et al.*, 2004). *Gongronema latifolium* was further shown to have an effect on renal oxidative stress and lipid peroxidation in non-diabetic and streptozotocin-induced diabetic rats (Ugochukwu and Cobourne, 2003).

Additionally, these three plants are used individually for food making in a variety of ways in most parts of Nigeria. They have also been ascribed with strong medicinal properties occasioning their use in folk medicine since ancient times. They are still used in most local populations lacking adequate healthcare system. Okpuzor *et al.* (2008) reported that active principles inherent in medicinal plants have been extracted and used in different forms such as infusions, syrups, decoctions, infused oils, essential oils, ointments and creams.

Chronic inflammatory diseases have been implicated as major health problems (Li *et al.*, 2003). Inflammation is the response of living tissues to injury which has been reported to involve a complex array of enzyme activation, mediator release and extravasations of fluid, cell migration, tissue breakdown and repair (Perianayagam *et al.*, 2006).

It has become the focus of global scientific research because of its implication in virtually all human and animal diseases.

The use of Non-Steroidal Anti-Inflammatory Drugs (NSAID) as anti-inflammatory and analgesic agents have not always been successful (Dharmasiri *et al.*, 2003; Park *et al.*, 2004) because of adverse effects such as gastric lesions, tolerance and dependence induced by opiates. Therefore, new anti-inflammatory and analgesic drugs devoid of these side effects are being researched as alternatives to NSAID and opiates (Vane and Botting, 1987; Brooks and Day, 1991).

Plant-based drugs used in traditional medicine practice have become the focus of current research because they are cheap, have little side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies (Dharmasiri *et al.*, 2003).

The use of medicinal plants is becoming increasingly popular across all social classes in developing societies. The high cost of orthodox treatment has ensured that the route to good health is the use of medicinal plants which are cheap, abundant and readily available. This study aims to investigate the claim that a decoction made from a mixture of *Gongronema latifolia*, *Ocimum gratissimum* and *Vernonia amygdalina* leaves may be effective therapy for fever, pains and many types of inflammatory diseases.

MATERIALS AND METHODS

Plant materials: The leaves of *G. latifolia*, *V. amygdalina* and *O. gratissimum* were sourced from Oyinbo market in Lagos metropolis, Nigeria, in December 2008. The botanical authentication was by Mr. T.A. Adeleke of the Department of Pharmacognosy, Faculty of Pharmacy, University of Lagos, Lagos, Nigeria. Herbarium deposit numbers were *G. latifolia* PCGH 444, *V. amygdalina* PCGH 432 and *O. gratissimum* PCGH 443.

Extraction: Five hundred grams each of the fresh leaves of *Gongronema Latifolia*, *Vernonia amygdalina* and *Ocimum gratissimum* were macerated in 5 L of distilled water. The liquid was decanted and filtered to yield filtrate which was evaporated to dryness in an oven set at 40°C. The dried decoction was weighed and dissolved in distilled water to a concentration of 100 mg mL⁻¹.

Animals: Wistar rats weighing between 180-200 g and Swiss mice (25-30 g) of either sex were procured from the Nigerian Institute of Medical Research, Yaba Lagos, Nigeria. Approval to use the animals for experiment was

obtained from the University of Lagos ethical committee on the use of animals. They were housed under standard environmental conditions and allowed free access to clean drinking water and standard diet (Ladokun Feeds PLC, Ibadan, Nigeria) *ad libitum*. The animals were fasted 18 h prior to commencement of experiment but water was allowed *ad libitum*.

Acute toxicity: This was performed according to the Organization of Economic Co-operation and Development (OECD) guidelines for testing Chemical, TG420 (OECD, 2001). Sixty mice were randomly divided into 12 groups of 5 animals each. Doses of the decoction, 1.0, 2.0, 4.0, 8.0 and 16.0 g kg⁻¹, were administered orally to groups 1-5 by gastric intubations while mice groups 7-11 were administered intraperitoneally with doses of 0.5, 1.0, 1.50, 2.0 and 2.5 g kg⁻¹. Group 6 and 12 which served as the control animals received only distilled water (10 mL kg⁻¹). Signs of toxicity and mortality were monitored and recorded for each group 24 h after extract administration.

Evaluation of analgesic activity

Hot plate test: The hot plate method described by Woolfe and MacDonald (1944) was used to evaluate the analgesic properties of the polyherbal preparation. Swiss albino mice were subjected to initial screening and those that did not respond within 60 sec were excluded from the experiment. Twenty five mice were divided into 5 groups of 5 animals each. Groups 1, 2 and 3 were administered doses of 2.0, 4.0 and 8.0 g kg⁻¹ of the extract p.o., while groups 4 and 5 received morphine (2 mg kg⁻¹ s.c.) and normal saline (10 mL kg⁻¹ p.o.), respectively, 30 min before the hot plate test. Thereafter, the animals were dropped on a hot plate maintained at a temperature of 56±1°C. The interval between the time the animal was dropped on the hot plate and the moment it either licked its forepaws or jumped up or out of the hot plate was recorded as the reaction time. Calculation was done as described by Vogel (2002).

Mouse writhing assay: Twenty five Swiss albino mice of either sex were selected and divided into five groups of five animals each. Groups 1-3 were administered with the decoction doses of 2.0, 4.0 and 8.0 g kg⁻¹ p.o., group 4 received acetylsalicylic acid 100 mg kg⁻¹ s.c., while group 5 received normal saline 10 mL kg⁻¹ p.o., 30 min before intraperitoneal injection of 0.6% v/v acetic acid solution. The mice were then gently dropped inside a transparent glass cage and the number of writhes or constrictions (a syndrome characterized by a wave of contraction of the abdominal musculature followed by the extension of the hind limbs) were counted for 15 min. A significant reduction in the number of writhes by treated

animals as compared to the untreated was considered a positive analgesic response. The percentage inhibition of writhes was calculated as the reduction in the number of writhes compared to the control using the formula of Vongtau *et al.* (2000):

$$\text{Inhibition (\%)} = \frac{\text{Mean No. of writhes (control)} - \text{Mean No. of writhes (test)}}{\text{Mean No. of writhes (control)}} \times 100$$

Formalin test: The formalin test method of Turner (1965) was adopted. The test animals were administered with 2.0, 4.0 and 8.0 g kg⁻¹ p.o., doses of the decoction, 100 mg kg⁻¹ s.c. of acetylsalicylic acid and normal saline 10 mL kg⁻¹ p.o., thirty min before injection with 20 µL of 1% formalin subcutaneously into the right hind paw. The time spent in licking and or biting the injected paw was recorded as an indicator of pain response. The responses were measured for 5 min after formalin injection.

Evaluation of anti-inflammatory activity

Xylene induced ear oedema: Twenty five animals were divided into 5 groups of 5 animals each. Groups 1, 2 and 3 received 2.0, 4.0 and 8.0 g kg⁻¹ p.o., of the crude extract, group 4 was treated with Dexamethasone 4 mg kg⁻¹ p.o., while normal saline 10 mL kg⁻¹ was administered to group 5 thirty min before inflammation was induced with topical application of xylene. Two drops of xylene were applied on the inner surface of the right ear and allowed to act for 15 min. They were anaesthetized with ether and the left and right ears were cut off. The difference between the weights of the two ears was recorded as the result of the oedema induced by the xylene (Tjølsen *et al.*, 1992).

Carrageenan induced rat paw oedema: Five groups of Wistar rats populated with 5 rats each were used. Group 1, 2 and 3 were administered with decoction doses of 2.0, 4.0 and 8.0 g kg⁻¹ p.o., group 4 received indomethacin 10 mg kg⁻¹ p.o., while group 5 which served as control animals received normal saline 10 mL kg⁻¹ p.o. Rat paw oedema was induced with carrageenan by injecting 0.1 mL of freshly prepared 1% carrageenan diluted in normal saline (1% w/v in 0.9% normal saline) into the sub plantar region of the right hind paw one hour after the administration of the decoction, indomethacin and normal saline.

The paw size was measured before and immediately after the administration of carrageenan using the cotton thread method. Paw sizes were measured at time intervals of 1, 2, 3, 4, 5 and 6 h. Increases in the linear diameter of the right hind paws were taken as an indication of paw oedema. Oedema was assessed in terms of the difference

in the zero time linear diameter of the injected hind paw and its linear diameter at time t (i.e., 1, 2, 3, 4, 5 and 6 h) following carrageenan administration. Any significant reduction in the volume of the injected hind paw of the test group(s) compared to that of the control group was considered as anti-inflammatory response. The mean increase in paw swelling was measured and the percentage inhibition calculated. The anti-inflammatory effect of the decoction was calculated with the following equation:

$$\text{Anti-inflammatory activity (\%)} = (1 - D/C) \times 100$$

where, D represents the percentage difference in paw volume after the decoction was administered to the rats and C represents the percentage difference of paw volume in the control groups. The percentage inhibition of the inflammation was calculated using the Newbould formula as shown below:

$$\text{Inhibition (\%)} = D_0 - D_t / D_0 \times 100$$

where, D₀ was the average inflammation (hind paw oedema) of the control group at a given time 0. D_t is the average inflammation of the drug treated (i.e., decoction or reference indomethacin) rats at time t (Moody *et al.*, 2006).

Statistical analysis: The observations were expressed as Mean±SEM. Statistical analysis of the data was done using Anova and Least Significant Difference (LSD) test.

RESULTS

Acute toxicity studies showed that the polyherbal decoction did not produce any mortality or any significant change in the general behaviour of the animals at 16.0 g kg⁻¹ (p.o.) and 2.5 g kg⁻¹ (i.p.) doses. The results of anti-nociceptive and anti-inflammatory activities of the polyherbal decoction are indicated in Table 1-4. In the hot plate assay, the decoction caused a significant pain inhibition of 138.5% (12.4±1.9) while 180.8% was produced by morphine (Table 1). The abdominal constrictions

Table 1: Effect of the polyherbal decoction on hot plate test

Groups	Dose (g kg ⁻¹)	Reaction time (sec) (Mean±SEM)	Inhibition (%)
Control (mL kg ⁻¹)	10.0	5.2±0.63	0.0
Polyherbal decoction	2.0	6.6±0.32	26.9*
	4.0	7.8±0.48	50.0*
	8.0	12.4±1.89	138.5*
Morphine (mg kg ⁻¹)	2.0	14.6±1.20	180.8*

Results are expressed as Mean±SEM. *p<0.05 compared to control. n = 5

Table 2: Effect of the polyherbal decoction on acetic acid induced writhing in mice

Groups	Dose (g kg ⁻¹)	No. of writhes (Mean±SEM)	Inhibition (%)
Control (mL kg ⁻¹)	10.0	91.0±1.2	0.0
Polyherbal decoction	2.0	34.6±3.2	61.9*
	4.0	31.2±1.7	65.7*
	8.0	30.4±2.0	66.6*
Acetyl salicylic acid	0.1	28.4±3.4	68.9*

Results are expressed as Mean±SEM. *p<0.05 compared to control. n = 5

Table 3: Effect of the polyherbal decoction on formalin induced pain

Groups	Dose (g kg ⁻¹)	0-5 min (Mean±SEM)	Inhibition (%)
Control (mL kg ⁻¹)	10.0	84.1±5.20	0.0
Poly herbal decoction	2.0	55.4±5.88	28.7
	4.0	48.8±8.18	37.2*
	8.0	42.0±5.40	46.0*
Acetyl salicylic acid	0.1	39.6±2.88	49.0*

Results are expressed as Mean±SEM. *p<0.05 compared to control. n = 5

Table 4: Effect of the polyherbal decoction on xylene induced ear oedema in mice

Groups	Dose (g kg ⁻¹)	Ear weight increase (g)			Percentage (%)	Inhibition (%)
		Left ear weight	Right ear weight	Ear weight increase		
Control (mL kg ⁻¹)	10.0	0.26±0.01	0.61±0.01	0.35±0.01	(57)	0
Polyherbal Decoction	2.0	0.40±0.01	0.62±0.01	0.22±0.01	(36)	37*
	4.0	0.38±0.01	0.54±0.01	0.16±0.01	(30)	54*
	8.0	0.37±0.01	0.50±0.01	0.12±0.01	(24)	66*
Demet (mg kg ⁻¹)	4.0	0.36±0.00	0.49±0.00	0.11±0.00	(22)	69*

Results are expressed as Mean±SEM. *p<0.05 compared to control. n = 5; values in parenthesis indicate (%) increase in ear weight

resulting from treatment with 100 mg kg⁻¹ of acetylsalicylic acid presented 28.4±3.4 writhes in 15 min which is 68.9% inhibition while treatment with the decoction at doses of 2.0-8.0 g kg⁻¹ significantly (p<0.05) and dose dependently reduced the number of writhes from 34.6±3.2 to 30.4±2.0 in 15 min (Table 2). However, the number of abdominal constrictions among the control mice was 91±12.00.

Table 3, showed that in the formalin induced pain assay, the highest dose of the decoction exhibited a significant (p<0.05) inhibition of 46% (42±5.4) while acetylsalicylic acid also produced a significant pain inhibition of 49% (39.6±2.9). In the xylene-induced ear oedema test, the polyherbal decoction significantly and dose dependently exhibited strong anti-inflammatory effect when compared to the control. At 8.0 g kg⁻¹, there was a percentage inhibition of 66% by the extract while dexamethasone presented 69% (Table 4).

The anti-inflammatory studies revealed a dose dependent relationship in the activity of the polyherbal decoction which was also comparable to Indomethacin. It showed significant (p<0.05) inhibition at doses of 4.0 and 8.0 g kg⁻¹, which was 114.71 and 132.35% inhibition, respectively post 6 h induction of inflammation (Fig. 1). No inhibition was observed in the control group.

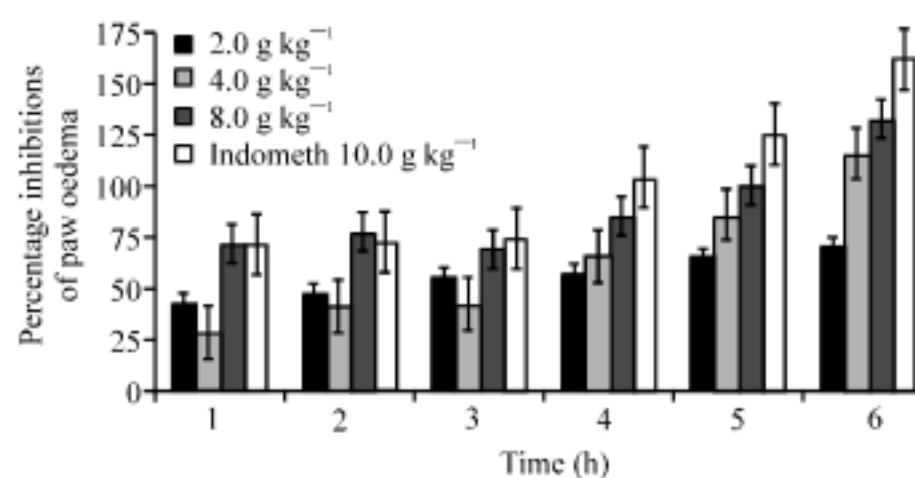


Fig. 1: Percentage inhibitions of rat paw oedema

DISCUSSION

The administration of the polyherbal decoction did not present any mortality or abnormal behavior in the test animals even at its highest concentration of 16.0 g kg⁻¹. Adedapo *et al.* (2008) reported in their acute toxicity studies of aqueous extract of *C. paniculata* that all the animals that were treated with 4.0 to 3.2 g kg⁻¹ dose of their preparation died. Their 2.0 g kg⁻¹ dose however, did not kill the animals. We suggest that an overdose of the extract of *C. paniculata* may have been administered or that it contains certain compounds which are non-toxic at lower doses but become toxic when a certain threshold is reached or, that the products of metabolism of *C. paniculata* becomes toxic at higher doses. This difference in result indicates that this polyherbal decoction is a safe and non-toxic herbal health product.

The expression of significant antinociceptive and antiphlogistic activities observed in the test animals shown to be comparable to the standard drugs at its highest concentration, may be indicative of the potential usefulness of the decoction for managing pain and inflammation. In all the three pain models used for antinociceptive studies, the polyherbal decoction demonstrated effective and significant (p<0.05) pain inhibition in a dose dependent relationship. Guyton and Hall (2006) reported that pain is mainly a body response mechanism which occurs whenever tissues are being damaged and causes reaction to remove the pain stimulus. Consequently, the reaction of the test animals supports our results that the decoction affects peripheral tissues by inhibiting nociception induced by acetic acid. Abdominal constriction test is used for rapid evaluation of peripheral type of analgesic action in drugs and it is related to the sensitization of nociceptive receptors to prostaglandins. Acetylsalicylic acid (Aspirin) offers relief from inflammatory pain by suppressing the formation of pain substances in the peripheral tissues. It has been suggested that endogenous substances such as serotonin (5HT), histamine, prostaglandins (Pgs) and

bradykinins play important roles in the pain process (Paschapuri *et al.*, 2009). Aspirin interferes with synthesis of prostaglandins and thromboxanes by irreversibly inhibiting COX-1 and modifies the enzymatic activity of COX-2 (Wu, 2003). COX-2 modified by aspirin produces lipoxins, most of which are anti-inflammatory. The polyherbal decoction exhibited 138% inhibition in the hot plate assay and this is comparable to the action of morphine which is believed to act directly on the Central Nervous System (CNS) to relieve pain. We, therefore suggest that the polyherbal decoction is likely acting on both the peripheral and central tissues having induced significant antinociceptive activities in all the experimental models. Kanodia and Das (2008) also found that both central and peripheral tissues were involved in their anti-inflammatory studies.

Carrageenan induced rat paw oedema is a multi mediated phenomenon that liberates diversity of mediators and was taken as a prototype of exudative phase of inflammation. It is a biphasic event, in which the first phase (1 h) involves the release of serotonin and histamine while the second phase (after 1 h) is caused by the release of bradykinin, protease, prostaglandins and lysosomes (Perianayagam *et al.*, 2006). The polyherbal decoction possessed significant anti-oedematogenic effect that might be interfering with the prostaglandin pathways.

Xylene-induced mouse ear oedema reflects the oedematization during the early stages of acute inflammation, which was probably related with the release of phospholipase A and inhibition of the inflammation factors.

The inhibitory effect of the decoction on carrageenan-induced inflammation and xylene induced ear oedema is likely due to the inhibition of histamine, 5HT, kinnins, prostaglandins and phospholipase A and is in agreement with the anti inflammatory activities of Hippocratea Africana (Okokon *et al.*, 2008). Preliminary laboratory research on this decoction showed the presence of tannins and flavonoids alkaloids, glycoside anthraquinone, phlobatannin and saponins. Therefore, some of these secondary metabolites may have contributed to its anti-inflammatory and antiphlogistic actions. Silva *et al.* (2005) had reported that plant flavonoids are implicated in anti inflammatory activities.

The results from this study demonstrate significant presence of anti-inflammatory and analgesic properties in the decoction when compared to the standard drugs. This suggests a justification of its use in ethno medicine even though it has not been scientifically screened. Further detailed phytochemical studies are in progress to identify the active principle(s) in the decoction and their specific mode of actions.

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