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ANTI-INFLAMMATORY AND ANALGESIC EFFECTS OF AQUEOUS LEAF EXTRACTS OF *Gomphrena celosioides* AND *Momordica charantia*

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ABSTRACT

This study reports the anti-inflammatory and analgesic properties of aqueous leaf extracts of *Gomphrena celosioides* and *Momordica charantia* in rats and mice. Hot plate latency test and acetic acid induced writhing movement method were used as the model for analgesic evaluation, while the carrageenan-induced rat paw oedema was used as the model for anti-inflammatory activity. The result of the study revealed that the leaf extracts of the two plants possess anti-inflammatory property as they were found to significantly ($p<0.05$) inhibit oedema induced by carrageenan in the rat paws. The leaf extract of *Momordica charantia* was also found to significantly ($p<0.05$) increase the reaction time of the mice in hot plate test method, while the number of writhing movement of the mice was also significantly($p<0.05$) reduced in dose-dependent manner. Similar result was observed for mice treated with extract of *Gomphrena celosioides*. The pilot toxicity study revealed the plants to have appreciable safety margin, but high dose of *Momordica charantia* could cause purgation in rats. The results of the study suggest the anti-inflammatory and analgesic effects of the aqueous leaf extracts of the two plants.

Keywords: *Gomphrena celosioides, Momordica charantia*, anti-inflammatory, analgesic property

INTRODUCTION

Gomphrena celosioides Mart (Amaranthaceae) is an annual herb with popular usage in traditional medicine. It can be used alone or in combination with other plants for the treatment of human and animal diseases. The plant in combination with shea butter oil is used topically for the treatment of wound and to prevent infection by indigenous people, who also include the plant in livestock feed to treat gastro intestinal tract problems of animals. *Gomphrena celosioides* was reported to contain benzoic acid derivatives with antimicrobial activity (Pereira *et al*., 2004).

Momordica charantia Linn, also known as bitter melon is a herbaceous plant of Curcubitaceae family. It is a Philippine herb that has recently gained international recognition for its possible benefit in the treatment of diabe-

tes mellitus (Basch *et al*., 2003). Despite its bitter taste, it has also become a popular nutritional drink for a boost of vigor; infact the more bitter, the better, as it is believed that the bitterness is proportionate to its potency (Lotikar and Rajarama, 1996). The juice from the fruit or the leaf extract of *Momordica charantia* has been used by indigenous people to treat diabetes, urethra discharge, dysentery, colitis, wounds, infections, hepatitis, rheumatism, gout and mild purgative for children (Lotikar and Rajarama, 1996; Plattel and Srinivasan, 1997). Phytochemical studies revealed plant to contain lutein and lycopene which are responsible for its antibiotic antitumor activities, charatin, momordicine and other alkaloids, saponins, phenolic constituents, glycosides and 5-hydroxytryptamine (Dhalla *et al*., 1981). Plattel and Srinivasan (1997) reported the hypoglycemic effect of the leaf extract of the plant. Antibacterial, antineoplastic, antiviral and antimutagenic activities of the plant have also been reported (Jilka *et al*., 1983; Guevara *et al*., 1990). Sofowora (1979) reported the purgative effect and the contractions of the guinea pig ileum of the plant extract. Literature survey revealed scanty information on the pharmacologic effect of *Gomphrena celosioides*, while little information was also recorded on the anti-inflammatory and analgesic activities of *Momordica charantia*. This study therefore reports the anti-inflammatory and, analgesic effects of the aqueous leaf extracts of the plants.

MATERIALS AND METHODS

Experimental Animals

Adult Sprague-Dawley rats (180-210g) and Swiss albino mice (18-25g) of both sexes were used. The animals were housed in Faculty of Veterinary Medicine, University of Ibadan, Ibadan animal house under standard environmental conditions; maintained on a natural light and dark cycle and had free access to food and water *ad libitum*. All experiments were carried out between 0900 and 1500 hour.

Preparation of plant extracts

The leaves of *Gomphrena celosioides* and *Momordica charantia* were collected from Ologuneru area of Ibadan in Ido Local Government, Oyo State of Nigeria where they grow as weeds in wasteland. The confirmatory identification of these plants was done in the Department of Botany, University of Ibadan, Ibadan.

Ten percent aqueous leaf extract of the plants were prepared by homogenizing 10g of fresh leaves of each plant in 100ml of distilled water. The homogenized solutions were then filtered with Whatman No. 1 filter paper to get the filtrates which make up the aqueous extracts.

Experimental Procedures:
Anti-inflammatory effects

The anti-inflammatory activity of the extracts was evaluated with carrageenan induced oedema model (Winter *et al*., 1962). Inflammation was induced by injection of 0.1ml carrageenan (1% suspension of carrageenan in normal saline) into the plantar surface of the right hind paw. Control group was injected with saline (0.9%). Immediately after injection, the linear circumferences of the injected paws were measured. These measurements were repeated 3 hours after carrageenan or saline injections. The increase in linear circumference after three hours was taken as an index of increase in paw volume, which is a measure of the oedema (Abatan and Adeagbo, 1986), and the percentage inhibition of oedema was calculated;

% Inhibition of oedema = $v_c - v_t \times 100/v_c$

V_c = increase in paw volume of control group, V_c = increase in paw volume of treated group.

The plant extracts and indomethacin (10mg/kg) were administered one hour before induction of inflammation.

Linear circumference of the paws were measured by a loop of thread (diameter = 2mm) tied round the paw such that it was neither too loose nor too tight, but just tight enough to allow the movement of the thread around the paw. The length of the thread covering-the paw was then straightened out and measured on a ruler to the nearest 1.0mm. Five such measurements were made for each paw, such that no such measurements differ by more than 5% (Abatan and Adeagbo, 1986).

Rats (6 per group) were divided into 5 groups per plant. The first 3 groups received oral doses of 100, 200, and 400 mg/kg of the extracts. The 4th and 5th groups were treated orally with indomethacin (10 mg/kg) as a reference drug and distilled water (3ml/kg) as control, respectively.

Analgesic effects

Two methods employed for evaluation of analgesic effect; were the Hot plate test and Acetic acid induced writhing movement test.

Hot plate test

Mice (6 per group) were divided into 5 groups per plant. The first 3 groups received oral doses of 100, 200, and 400 mg/kg of the extracts. The 4th group received morphine (2 mg/kg) i.p and the 5th group received distilled water (3 mg/kg) orally. The hot plate method involves placing the animal on a hot plate maintained at 55^{0}C until the animal reacted to the heat. The reaction time is the time the animal starts to react to the pain of the heat by either rubbing the palms together, licking the paws or jump-off the hot plate. The extracts and drug were administered 1hour before commencing the procedure (Okolo *et al.,* 1995)

Acetic acid induced writhing movement test

Writhing movements were induced in the mice by intraperitoneal injection of 0.6% of 10ml/kg of acetic acid (Koster *et al.*, 1959; Okiemy-Andissa *et al.*, 2004). The mice were divided into 5 groups. Group 1-3 were given 100, 200 and 400mg/kg of the extracts orally one hour prior to induction of writhing movements. Group 4 was administered with paracetamol® (50mg/kg p.o), while group 5 was the control group administered with 3ml/kg of distilled water orally. The number of writhing movements was determined for 10 minutes starting 10 minutes after the injection of acetic acid (Okiemy-Andissa *et al*., 2004).

Preliminary acute toxicity study

Rats (6 per group) were divided into 7 groups. The first 6 groups were administered with oral doses of 20, 40, 80, 160, 320 and 640 mg/kg of the extracts. The 7th group received distilled water (3ml/kg) orally. The animals were observed for toxic symptoms while mortality was determined after 24 hours post treatment.

Statistical Analysis

Data obtained were expressed as the mean ± standard error of mean (SEM). Statistical analysis was performed using student's 't' test. Probability values less than 0.05 were considered statistically significant.

RESULTS

Preliminary acute toxicity study

The preliminary acute toxicity study showed that the extracts possessed high safety margin in the rats as no death was observed at oral doses of 20-640mg/kg. However, the animals administered with 640mg/kg of *Momordica charantia* showed sign of diarrhoea by passing soft faecal sample.

Effect of the extracts on carrageenan-induced oedema

The oedema induced by carrageenan in rats administered with 200 and 400mg/kg extract of *Gomphrena celosioides* was significantly ($p<0.05$) inhibited in dose-dependent manner with 400mg/kg dose having the greatest inhibition of 56.14% as compared to 85.41% of indomethacin (10mg/kg p.o), while the inhibition of oedema of 100mg/kg dose of *Gomphrena celosioides* was not statistically significant ($p>0.05$). The oedema was also significantly ($p<0.05$) inhibited by the extract of *Momordica charantia* in dose-dependent version. The 400mg/kg dose shows inhibition of 71.07 as compared to 400 mg/kg of *Gomphrena celosioides* which showed inhibition of 56.14%. This shows that *Momordica charantia* extract inhibits induced oedema more than the extract of *Gomphrena celosioides* as shown in Table 1.

Effect of the extracts on hot plate test

The 400mg/kg dose of *Gomphrena celosioides* shows a significant ($p<0.05$) reaction time of 20.64±0.51 compared to that showed by morphine (2 mg/kg i.p). The 200 and 400 mg/kg doses of *Momordica charantia* showed a statistically significant ($p<0.05$) reaction time of 22.04±1.43 and 25.71±0.51, respectively, which is dose-dependent. The effect of 200 and 400 mg/kg doses of *Momordica charantia* can be compared to that of morphine (2mg/kg i.p) which is 31.73±1.23 as shown in Table 2.

Effect of the extracts on acetic acid induced writhing movement

The result of this study showed both the extracts of *Gomphrena celosioides* and *Momordica charantia* to significantly ($p<0.05$) reduce the number of writhing movement induced by acetic acid in a dose-dependent manner. The 400mg/kg dose of *Gomphrena celosioides* showed 28.27±2.11 writhing movement compared to that of distilled water which is 42.17±1.77 and paracetamol® which is 22.50±1.27. The highest inhibition of writhing movement by *Momordica charantia* extract was shown by 400 mg/kg dose as shown in Table 3.

DISCUSSION

The result of this study showed that the leaf extracts from two plants, *Gomphrena celosioides* and *Momordica charantia* possess anti-inflammatory activity as they significantly inhibited oedema induced by carrageenan in rats. They also exhibit analgesic property in hot plate and acetic acid induced writhing movement in dose-dependent manner. However, the soft faecal sample produced by rats administered with high dose of *Momordica charantia* may suggests that the extract has purgative action (Sofowora, 1979) and this may suggest the reason for its abortifacient action reported.

The inflammatory action induced by carrageenan may be as a result of step-wise release of the inflammatory mediators such as histamine, serotonin and bradykinin which are released in the early phase of inflammatory reaction, and prostaglandins which are released late in the acute phase (Dirosa *et al*., 1971; Heller *et al*., 1998). The presence of

chemical substances which are inflammatory process mediators results in increase in the vascular permeability at the site of inflammation, thus promoting accumulation of fluid in tissues and this resulted in oedema (Williams and Morley, 1973; White, 1999).

Pain is the normal physiological response to a noxious chemical, associated with invasive procedures (Carr and Goudas, 1999; FSMBUS, 2004). It is an appropriate response to a stimulus that threatens to produce tissue injury (Woolf 2004; Schaibe and Richter, 2004). The intensity of acute pain is proportional to the intensity of the stimulus and persists as long as the stimulus persists. Intraperitoneal injection of acetic acid produces this kind of pain which resulted into writhing movement exhibited by the mice in this study.

The extract of *Momordica charantia* was reported to demonstrate insigficant membrane stabilizing activity (Umukoro and Ashorobi, 2006) and this may suggests that its anti-inflammatory action may be related to the inhibition of the late phase of inflammatory process which is the release of chemical mediators (Umukoro and Ashorobi, 2006). Their analgesic effect may be due to the inhibition of inflammatory process.

Table 1: The mean ±SEM of the effect of aqueous leaf extract of *Gomphrena celosioides* and *Momordica charantia* on carrageenan-induced oedema in rats

Sample	Dose (mg/kg)	Difference in linear Circumference of the paw (cm)	% Inhibition of oedema
G. celosioides	100	0.38±0.03	27.97
G. celosioides	200	0.32±0.09*	39.62
G. celosioides	400	0.23±0.02*	56.14
M. charantia	100	0.33±0.04*	37.89
M. charantia	200	0.24±0.04*	54.36
M. charantia	400	0.16±0.02*	71.07
Indomethacin	10	0.08±0.01*	85.41
Distilled water (3 ml/kg)	-	0.53±0.07	-

*****value was significant at $p<0.05$ compared to control group (student's 't' test), n = 6 for each group

Table 2: The mean ±SEM of the effect of aqueous leaf extract of *Gomphrena celosioides* and *Momordica charantia* on reaction time of hot plate test in mice.

Sample	Dose (mg/kg)	Reaction time (s)
Gomphrena celosioides	100	18.97±0.47
Gomphrena celosioides	200	19.32±1.14
Gomphrena celosioides	400	20.64±0.51*
Momordica charantia	100	19.75±0.78
Momordica charantia	200	22.04±1.43*
Momordica charantia	400	25.71±0.62*
Morphine	2	31.73±1.23*
Distilled water (3 ml/kg)	-	18.18±0.19

*value was significant at $p<0.05$ compared to control group (student's 't' test) ,n = 6 for each group

Table 3: The mean ± SEM of the effect of aqueous leaf extract of *Gomphrena celosioides* and *Momordica charantia* on acetic acid induced writhing movement in mice

Sample	Dose (mg/kg)	No. of writhing movements
Gomphrena celosioides	100	35.78±3.2*
Gomphrena celosioides	200	30.16±1.67*
Gomphrena celosioides	400	28.27±2.11*
Momordica charantia	100	36.50±1.91*
Momordica charantia	200	31.50±1.65*
Momordica charantia	400	25.89±2.23*
Paracetamol®	50	22.50±1.27*
Distilled water (3ml/kg)	-	42.17±1.7

*value was significant at $p<0.05$ compared to control group (student's 't' test), n = 6 for each group

CONCLUSION AND RECOMMENDATION

In conclusion, it may be suggested that the leaf extract of *Gomphrena celosioides* and *Momordica charantia* offer some beneficial effects in the management of inflammatory conditions and further study of these plants could bring a promising anti-inflammatory agent worthy of therapeutic acceptance.

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