http://dx.doi.org/10.434/jpb.v13i2.11
Vol. 13 no. 2, pp. 155-162 (September 2016)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND BIORESOURCES

Antinociceptive and anti-inflammatory activities of the methanol extract of *Chlorophytum alismifolium* tubers

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Received 29th July 2016; Accepted 30th August 2016

Abstract
The tubers of *Chlorophytum alismifolium* Baker (Family: Liliaceae) are used in Herbal Medicine for the management of various ailments including diabetes mellitus, erectile dysfunction, abdominal pains and inflammatory conditions. Despite its wide usage for management of pain and inflammation, there is no scientific justification to validate this claim. This study was aimed at screening the methanol tuber extract of *Chlorophytum alismifolium* for antinociceptive and anti-inflammatory activities using experimental animal models. The antinociceptive activity was tested using acetic acid-induced writhing response in Swiss albino mice and formalin-induced pain in Wistar rats, while the anti-inflammatory activity was tested using carrageenan-induced paw edema in rats at doses of 200, 400 and 800 mg/kg. The extract significantly ($p< 0.001$) reduced the number of writhes at all tested doses. At 800 mg/kg, it significantly ($p< 0.01$) inhibited the first phase of formalin-induced pain while the second phase was inhibited significantly ($p< 0.05$ and $p< 0.01$) at doses of 400 and 800 mg/kg respectively. The extract (400 and 800 mg/kg) significantly ($p< 0.05$) inhibited the carrageenan-induced inflammation at the third hour. A similar activity was also observed at the fourth hour with 61.61% inhibition of paw oedema at 400 mg/kg. These findings suggest that *Chlorophytum alismifolium* tuber extract possesses antinociceptive and anti-inflammatory activities, thus support the claim for the ethnomedical use of the plant in the management of pain and inflammatory diseases.

Keywords: *Chlorophytum alismifolium*, anti-nociception, anti-inflammation, Carrageenan, Formalin

INTRODUCTION

Pain is a transitory unpleasant sensation resulting from noxious or potentially injurious stimulus, acting as a warning system tissue protection against injuries. It is a complex experience that involves not only the transduction of noxious environmental stimuli, but also cognitive and emotional processing by brain (Julius and Bashaum, 2001; Levine and Alesandri-Haber, 2007). Inflammation is the primary physiologic defense mechanism that helps the body to protect itself against infection, burns, toxic chemicals, allergens or other noxious stimuli. An uncontrolled and persistent inflammation may act as an etiologic factor for many chronic illnesses (Kumar *et al.*, 2004) such as cancer, cardiovascular diseases, arthritis, inflammatory bowel syndrome, atherosclerosis and autoimmune diseases (Ricciotti and FitzGerald 2011; Viljoen *et al.*, 2012).

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ISSN 0189-8442

Drugs currently used for the management of pain are opioids or non-opioids and those for inflammation are non-steroidal anti-inflammatory drugs (NSAIDs) or corticosteroids. However, the use of these drugs are associated with potentially adverse effects. NSAIDs for example cause severe adverse effects like gastric lesions and liver damage (Pilotto *et al.*, 2003; Zulfiker *et al.*, 2010) while opioids cause sedation, constipation, dependence and tolerance (Benyamin *et al*., 2008).

Medicinal herbs have been used as a form of therapy for relieve of pain throughout history (Almeida *et al.*, 2001) with little or no toxicity; and the most important analgesic prototypes (e.g. acetyl salicylic acid and morphine) were originally derived from plant sources. *Chlorophytum alismifolium* (Family: Liliaceae) is a short stem herb with tuberous root stocks and white flowers found around stony sites in forest streams (Burkill, 1995). Common vernacular names include *Rogon makwarwa* (Hausa) and *Cigorodi* (Fulfulde). The tubers are used by the Fulani tribe in Toro Local Government Area of Bauchi State, North Eastern Nigeria for the management of pain and inflammation (personal communication, 2014). There is no information in the literature demonstrating the scientific justification for the analgesic and anti-inflammatory efficacy of *Chlorophytum alismifolium*, hence the reason for this research.

EXPERIMENTAL

Drugs and chemicals. Piroxicam (Pfizer), Morphine (Martindale Pharma®, UK), Acetic acid (BDH), 10 %$^{v}/_{v}$ Formalin, Carrageenan and Methanol (Sigma Aldrich, Germany).

Plant collection and extraction. The tubers of *Chlorophytum alismifolium* were collected in July, 2014 at Tudun Fulani River, Toro Local Government Area of Bauchi State, Nigeria. The botanical identification and authentication was done by Mallam Musa Muhammed of the Herbarium Unit of the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen number (6785) was assigned and a specimen deposited for future reference. The plant material was washed, size-reduced, shade dried until constant weight was obtained and then pulverized into a dry powder with the aid of a mortar and pestle. About one thousand grams (1000 g) of the powdered material was extracted exhaustively with aqueous-methanol (1:9) for 72 hours using Soxhlet extractor. The extract was concentrated over the water bath set at 50°C and the yield calculated. The extract was sealed in an airtight container and stored in a desiccator prior to use. The extract was reconstituted in distilled water at appropriate concentrations for each experiment.

Phytochemical screening. Standard screening test as described by Evans, (2002) was employed in screening the plant extract. The extract was screened for the presence or absence of alkaloids, flavonoids, saponins, cardiac glycosides, tannins, anthraquinones and carbohydrates.

Animals. Adult Wistar rats (160-200g) and Swiss albino mice (25-30g) of either sex were obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. The animals were maintained under standard environmental conditions and fed with standard rodent pellet diet (Vital feed, Jos-Nigeria) and water *ad libitum.* The experiments were carried out in Ahmadu Bello University in accordance with the criteria outlined in the Guide *for the Care and Use of Laboratory Animals* by the National Institutes of Health (Publication No. 80-23, revised 1996).

Acute toxicity studies. The method described by Lorke, (1983) was employed in the determination of the oral median lethal dose (LD_{50}) in mice and rats The test was in two

phases; in phase one, three groups of animals (n=3) were administered widely differing doses of the extract (10, 100 and 1000 mg/kg) and were observed for signs of toxicity and mortality for 24 hours. In the second phase, 3 animals of either species were administered 1200, 2900 and 5000 mg/kg of the extract and then observed for signs of toxicity and mortality for 24 hours. The LD_{50} was calculated as the geometric mean of the lowest lethal dose and highest non-lethal dose.

Analgesic Studies

Acetic acid-induced writhing in mice. The method described by Koster *et al.*, (1959) was employed. A total of 30 mice were divided into five groups of six mice each. Group I was administered distilled water (10 ml/kg), groups II, III and IV received graded doses of the extract (200, 400 and 800 mg/kg respectively) and group V received piroxicam (20 mg/kg), all through the oral route (p.o.). Sixty min. post treatment, all the animals received 10 ml/kg of 0.6 % $^{v}/_{v}$ acetic acid intraperitoneally. Five minutes after acetic acid injection, the mice were placed in individual observation cages and the number of abdominal writing was recorded for each mouse for a period of 10 minutes. A reduction in the number of writhes as compared to the control animals was considered as evidence for the presence of analgesia and expressed as percent inhibition of writhes using the formula:

% Inhibition = {[Mean number of writhes (control) – Mean number of writhes (test)] ÷ Mean number writhes (control)} × 100

Formalin-induced pain in rats. The method described by Dubuisson and Dennis (1977) and modified by Tjolsen *et al.*, (1992) was employed for this test. Rats were divided into five groups of six rats each. Group I was administered distilled water (1 ml/kg), groups II, III and IV received graded doses of the extract (200, 400 and 800 mg/kg respectively) and group V received morphine (10 mg/kg); all through the oral route. Sixty minutes after this treatment, 50 µl of 2.5% $^{v}/_{v}$ freshly prepared solution of formalin was administered subcutaneously under the plantar surface of the left hind paw. The rats were then placed in an observation chamber and monitored for 1 hour, recording severity of pain responses based on the following scale: (0) rats walked or stood firmly on injected paw; (1) the injected paw was favoured or partially elevated; (2) the injected paw was clearly lifted off the floor; (3) the rat licked, chewed or shook the injected paw. The animals were placed in such a way to ensure an un-obstructed view of the injected paw. The anti-nociceptive effect was determined in two phases; the early phase (Phase I) and the late phase (Phase II) which were recorded during the first 5 min and during the last 45 min respectively, with a 10 min lag period between both phases.

Anti-inflammatory studies.

Carrageenan induced rats' paw edema. The carrageenan-induced paw edema method in rats was used as an experimental model for screening the anti-inflammatory activity of *C. alismifolium* according to the method described by Winter *et al.*, (1962). The animals were divided into five groups consisting of six rats each. The control group (I) received 1 ml/kg distilled water. The test groups (II, III and IV) received graded doses of *C. alismifolium* extract (200, 400 and 800 mg/kg, p.o.) respectively. Group V rats received piroxicam (20 mg/kg), a standard anti-inflammatory drug and was used as a positive control. Sixty minutes after the administration of the various agents, edema was induced by injection of carrageenan (100 µl, 0.1% $^{w}/_{v}$) into the sub-planter tissue of the left hind paw. The paw size up to tibio-tarsal articulation was measured at times 0, 1, 2, 3, 4 and 5 hour using a digital Vernier caliper. The increase in paw diameter (edema index) for each rat was calculated as the difference in paw diameter before carrageenan injection

and after carrageenan injection at each time interval, while the percentage inhibition of edema was calculated for each group with respect to its vehicle-treated control group according to the following relationship:

$$\text{Percentage Inhibition} = \{[(C_t - C_0)_{\text{control}} - (C_t - C_0)_{\text{treated}}] \div [(C_t - C_0)_{\text{control}}]\} \times 100$$

where Ct = mean edema index for each group at time t, and C_0 = mean edema index for each group before carrageenan injection

Statistical Analysis. Results obtained were expressed as Mean ± Standard Error of the Mean (S.E.M.). Data obtained for acetic acid-induced writhes was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Data of formalin-induced pain was analyzed by non-parametric Kruskal-Wallis test, while data of edema index was analyzed using one way and repeated measure ANOVA followed by Dunnett and Bonferroni post hoc tests respectively. Values of $p \leq 0.05$ were considered significant.

RESULTS AND DISCUSSION

The percentage yield of methanol extract of *Chlorophytum alismifolium* was 5.16 $\%^{w}/_{w}$. Preliminary phytochemical test revealed the presence of carbohydrates, saponins, flavonoids, glycosides, cardiac glycosides, alkaloids and triterpenes while anthraquinones, steroids and tannins were absent (Table 1). Phytochemicals such as flavonoids, saponins, tannins and alkaloids have been shown to be responsible for analgesic and anti-inflammatory activities of medicinal plants (Anilkumar, 2010; Abdelwahab *et al.,* 2011; Oliveira *et al.*, 2014). The observed analgesic and anti-inflammatory activities of the methanol tuber extract of *Chlorophytum alismifolium* may therefore be due to the presence of one or more of the reported phytochemicals.

The LD_{50} of the crude methanol extract of *C. alismifolium* was estimated to be > 5000 mg/kg in both rats and mice and no adverse symptoms or death was observed in either species. This showed that the methanol tuber extract of *Chlorophytum alismifolium* is relatively safe (Lorke, 1983) when administered orally, as no mortality was recorded at the dose of 5,000 mg/kg.

The methanol tuber extract of *C. alismifolium* at doses of 200, 400 and 800 mg/kg significantly ($p < 0.001$) reduced the number of writhes induced by acetic acid in mice when compared to control group. The reduction was however not dose-dependent. The extract also produced a peak inhibitory effect of 69.01% at the dose of 400 mg/kg. No significant difference was observed in all the doses as against the standard drug, piroxicam (20 mg/kg) (Table 2). The acetic acid-induced writhing test is a common method for measuring peripheral analgesic activity even though it is sensitive to both peripherally acting and centrally acting analgesics (Vogel, 2008; Odoma *et al.,* 2014; Musa *et al*., 2016). The response induced by acetic acid is thought to be mediated by peritoneal mast cells, acid sensing ion channels and the prostaglandin pathway. It has also been associated with increased level of prostaglandin E_2 and prostaglandin $F_{2\alpha}$ in peritoneal fluids as well as lipoxygenase products which enhance inflammatory pain by increasing capillary permeability (Voilley, 2004; Lakshman *et al.,* 2006; Khan *et al.,* 2009). NSAIDs such as piroxicam, reduce writhes induced by acetic acid by inhibiting cyclooxygenase (COX) in peripheral tissues by blocking the release and or synthesis of inflammatory mediators (Odoma *et al.,* 2014). The data obtained therefore suggested that the methanol tuber extract of *C. alismifolium* possess peripheral analgesic activity as evidenced by the significant reduction in the number of writhes. The extract may also be eliciting its analgesic effect through the inhibition of COX in the peripheral tissues by blocking the release and or synthesis of

prostaglandins; or interfering with other mediators responsible for peripheral pain.

C. alismifolium extract inhibited the first phase (neurogenic pain) of formalin induced pain in rats in a dose-dependent manner. The inhibition was significant ($p< 0.01$) at 800 mg/kg when compared to distilled water control group. Similarly, *C. alismifolium* inhibited the second phase (inflammatory pain) of formalin-induced pain significantly ($p< 0.05$ and $p< 0.01$) at doses of 400 and 800 mg/kg respectively. The standard drug, morphine (10 mg/kg) provided a significant ($p< 0.01$) inhibition of only the first phase of formalin induced pain in rats (Table 3). The formalin test is an important animal model in the study of acute long-lasting pain which encompasses inflammatory, neurogenic and central mechanisms of nociception (Ellis *et al.*, 2008; Ma and Zhang, 2011). The nociceptive behavior observed in this model is bi-phasic; the acute or early phase which is due to direct chemical stimulation of nociceptors, and the chronic or late phase which suggests the involvement of peripheral inflammatory processes with the release of histamine, serotonin, bradykinin, and prostaglandins (Hunskaar and Hole, 1987; Tjolsen *et al.*, 1992). Centrally acting analgesics such as morphine inhibit both phases, while peripherally acting analgesics such as NSAIDs and steroids inhibit only the late phase (Vogel, 2008; Couto *et al.*, 2011; Odoma *et al.*, 2014; Maina *et al.*, 2015). The significant suppression of both phases of formalin test as observed with the highest dose of methanol tuber extract of *C. alismifolium* suggests that it may be acting through both peripheral and central mechanism. This further supports the peripheral analgesic effect of the extract as observed with the acetic acid test.

C. alismifolium extract significantly decreased paw edema in rats at different dosages and at different time intervals. At the 3rd hour, which marks the peak of inflammation due to carrageenan, the extract at all the doses tested reduced the edema index.

Table 1: Phytochemical constituents of methanol tuber extract of *Chlorophytum alismifolium*

Constituents	Remark
Alkaloids	+
Anthraquinones	-
Carbohydrates	+
Cardiac glycosides	+
Flavonoids	+
Saponins	+
Steroids	-
Tannins	-
Triterpenes	+

Key: + = Present, - = Absent

Table 2: Effect of methanol tuber extract of *Chlorophytum alismifolium* on acetic acid- induced writhing in mice

Treatment	Dose (mg/kg)	Mean number of writhes	% inhibition
Distilled water	10 ml/kg	40.33 ± 1.56	-
CAL	200	17.67 ± 4.23***	56.19
CAL	400	12.50 ± 3.05***	69.01
CAL	800	14.00 ± 3.03***	65.29
Piroxicam	20	14.50 ± 2.50***	60.05

Values represent Mean ± SEM, *** = $p< 0.001$ compared to distilled water treated group
One-way ANOVA followed by Dunnett's post hoc test; CAL= *Chlorophytum alismifolium*, n=6

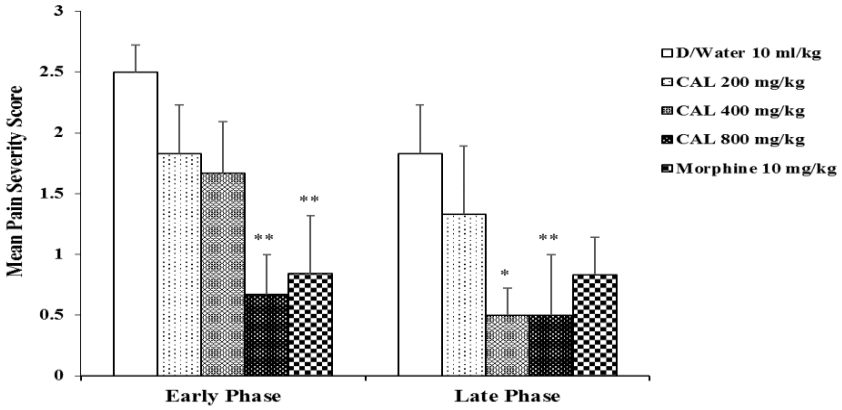

Fig. 1: Effect of methanol tuber extract of *Chlorophytum alismifolium* on formalin-induced pain in rats. Values are presented as Mean ± S.E.M., * = p< 0.05, ** = p< 0.001 compared to distilled water treated group – Nonparametric Kruskal-Wallis test; CAL= *Chlorophytum alismifolium*; n=6

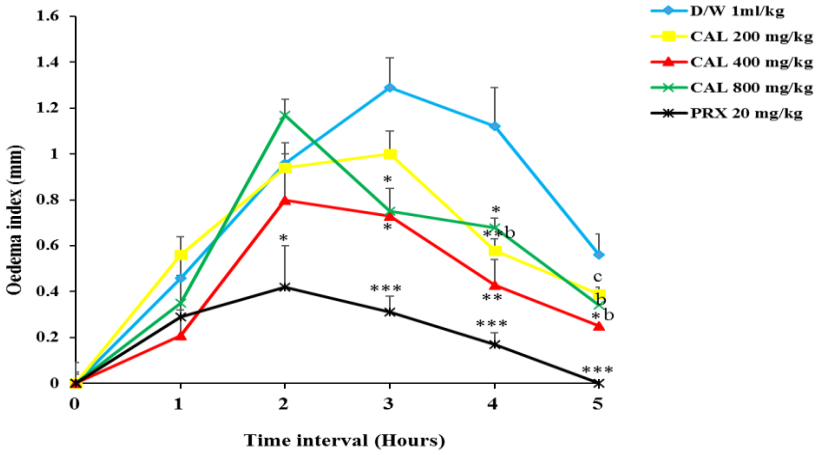

Fig. 2a: Effect of methanol tuber extract of *Chlorophytum alismifolium* on carrageenan-induced rat paw edema. Values represent Mean ± S.E.M., * = p< 0.05, ** = p< 0.01, *** = p< 0.001 compared to distilled water (D/W) treated group; b and c are p< 0.01 and p< 0.001 respectively which denotes significant difference from time 3 h – one way and repeated measure ANOVA followed by Dunnett's and Bonferroni post hoc tests respectively. CAL= *Chlorophytum alismifolium*, PRX = Piroxicam, n=6

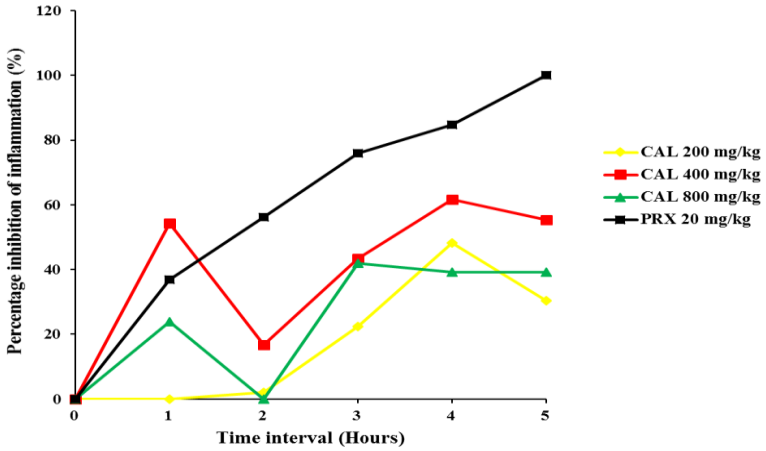

Fig. 2b: Percentage inhibition of inflammation by methanol tuber extract of *Chlorophytum alismifolium* (CAL) on carrageenan-induced rat paw edema. PRX = Piroxicam, n = 6

The reduction was significant ($p< 0.05$) at doses of 400 and 800 mg/kg when compared to distilled water treated group (Fig. 2a). The extract also reduced the edema index at the 4th hour, which was also significant ($p< 0.01$, $p < 0.01$ and $p< 0.05$) at doses of 200, 400 and 800 mg/kg respectively (Fig. 2a). At the same period (4th hour), the extract at 400 mg/kg inhibited inflammation induced by carrageenan by 61.61% (Fig 2 b). The effect of *C. alismifolium* was evaluated over time by comparing the 3rd hour (peak inflammation) to other times. A significant decrease in edema index ($p< 0.01$) was produced by the extract at the fourth and fifth hour across all the doses tested (Fig 1a).

The standard drug, piroxicam (20 mg/kg) significantly ($p< 0.05$ and $p< 0.001$) reduced the edema index from the 2nd to the 5th hour with a 100% inhibition of inflammation at the fifth hour (Fig. 2a and 2b). Carrageenan is a commonly used phlogistic agent for testing anti-inflammatory drugs (Vogel, 2008; Aiyelero *et al.,* 2009, Ismail *et al.,* 2015). The formation of edema induced by carrageenan is said to be in two phases; the first or early phase (the first hour after carrageenan injection) involves the release of serotonin, histamine and bradykinin; while the second or late phase (2–5 hours after carrageenan injection) is associated with increased edema formation that remains up to the fifth hour and involves the release of prostaglandins and lysosomal enzymes (Ratheesh and Helen, 2007; Khan *et al.,* 2009). From the data obtained, *C. alismifolium* extract significantly inhibited edema only at the third, fourth and fifth hour (second phase) which shows that it's activity is more related to inhibition of inflammatory mediators including prostaglandins. The methanol tuber extract of *C. alismifolium* also exerted similar effects to the NSAIDs used in this study and thus correlates with its ethnomedicinal use in the management of pain and inflammatory conditions.

Conclusion. The results obtained suggest that the methanol tuber extract of *Chlorophytum alismifolium* possesses analgesic and anti-inflammatory activities. This supports the ethnomedical claim of the use of the plant in management of pain and inflammatory conditions.

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