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Antinociceptive and antiinflammatory activities of the hydroethanolic extract of *Clerodendrum volubile* leaf

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Abstract

The leaf of *Clerodendrum volubile* is used in the Traditional African Medicine (TAM) in the treatment of various diseases including painful and inflammatory conditions. This study was carried out to investigate the antinociceptive and antiinflammatory properties of the hydroethanolic leaf extract of *Clerodendrum volubile* (HeCV) in rodents. The antinociceptive activity was evaluated using the formalin, capsaicin and acetic acid-induced writhing assays in mice, while the carrageenan-induced paw edema and cotton pellet-induced granuloma formation tests in rats were used to investigate the antiinflammatory action. In the second phase of formalin induced nociception, the greatest effect (79.84% inhibition) was produced at the lowest dose of 100 mg/kg and had significant ($p < 0.05$) inhibition of nociception reaction. The effect was less and comparable to diclofenac 50 mg/kg (79.51%) but not significantly different ($p < 0.05$) from that of the effective dose of HeCV at 400 mg/kg. In respect of antiinflammatory activity, *Clerodendrum volubile* caused no significant ($p < 0.05$), but dose-dependent inhibition of edema development in carrageenan-induced inflammation and cotton pellet-induced granuloma formation in rat. Pretreatment of mice with sulpiride prevented HeCV induced antinociceptiion in mice writhing test. Findings from this work indicates that the hydroethanolic leaf extract of *Clerodendrum volubile* possesses antinociceptive and anti-inflammatory activities possibly mediated *via* peripheral and central mechanisms involving the activation of dopaminergic receptor. This justifies the use of the plant extract in TAM for the treatment of painful and inflammatory conditions.

Keywords: *Clerodendrum volubile*, Antiinflammatory, Antinociceptive, Pretreatment

1. Introduction

Man's existence on earth has been made possible only because of the vital role played by the plants in sustaining life. In recent years, there has been an increased and immense interest in the revival of herbal and homeopathic system of medicine, both of which are obviously based on the plants. Large number of plants are constantly being screened for their pharmacological value, particularly analgesic, antiinflammatory, hypotensive, hypoglycemic, antifertility and cytotoxic activities. Analgesics are primary need of patients to get rid of any kind of pain [1]. The pain is one of the basic symptoms of almost all human ailments which is a sensorial modality and primary protection. Analgesics only relieve pain in a particular complaint affecting its cause [2]. The most eminent analgesics, including opiates and NSAIDs, are not helpful in all cases due to their adverse effects [3, 4]. Therefore, new compounds with potent pain killer action with no side effects are required to be investigated.

The inflammatory process involves a series of events that can be elicited by numerous stimuli such as infectious agents, ischaemia, antigen-antibody interactions and thermal or other physical injury. The response usually is accompanied by the familiar clinical signs of erythema, edema, tenderness and pain [5, 6]. At present, the natural analgesics and antiinflammatory compounds of plant origin are appreciated due to possible toxicity of synthetic drugs.

Clerodendrum volubile (Lamiaceae) is a widely distributed vegetable in the warm temperate and tropical region of the world. The plant popularly known as 'marugbo' or 'eweta' amongst the Ikale, Ilaje and Apoi people in southern-senatorial district of Ondo State, South-West, Nigeria. It is also known as 'Obnettete' amongst the Itsekiri and Urhobo tribes in Niger–Delta, Nigeria. It is a green climbing shrub reported to have a height of 3 m and possesses numerous flowers. The plant had been used in the treatment of inflammation and pain by traditional medical practitioners but no scientific evidences are yet available.

In view of these facts, this study was conducted to investigate the antinociceptive and antiinflammtory activities of the hydroethanolic leaf extract of *C. volubile.* No report of such study was found in the course of literature study.

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2. Materials and methods

The plant material was collected from a traditional medicinal practitioner in Mushin, Lagos State, Nigeria. Botanical identification and authentication was done by Mr O.O. Oyebanji, a forestry expert of the Department of Botany herbarium in the Faculty of Science, University of Lagos, Akoka, Nigeria. A voucher specimen with identification number LUH 7118 was deposited in the herbarium.

2.1. Preparation of plant material

The dried leaves were mulled into coarse powder and 774.9 g of the material was loaded into a percolator and extraction was done with 1.2 L of absolute ethanol for 72 hr. The filtrate obtained was concentrated in a rotary evaporator (40 °C) under vacuum and the percentage yield was 7.65% w/w. The brownish solid extract obtained was always reconstituted in distilled water to appropriate concentration before administration to experimental animals.

2.2. Experimental animals

Albino mice (20 – 25 g) and rats (80 – 200 g) of either sex obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria were used for this experiment. The animals were fed with rodents chow and had access to drinking water *ad libitum*. All experiments were performed in compliance with institutional and international policies governing the humane and ethical treatment of experimental animals as contained in United States National Institutes for Health Guidelines [7].

2.3. Acute toxicity test

Groups of mice of both sexes (5 per group) were fasted for 12 h prior to the test and were given hydroethanolic extract of *Clerodendrum volubile* (H_eCV) at doses of 2500 and 5000 mg/kg. Animals in each group were observed for any immediate signs of toxicity and mortality within 24 h. The LD_{50} was estimated by the Logdose-probit analysis [8, 9, 10].

2.4. Anagelsic activity

2.4.1. Mouse writhing test

Mice fasted overnight were divided into five groups of five animals each. The animals were then treated with distilled water (10 mL/kg, p.o.); H_eCV (100, 200, 400 mg/kg, p.o.); and diclofenac 50 mg/kg, p.o.). Sixty minutes later, mice were administered with acetic acid (0.6% v/v in saline, 10 mL/kg, i.p.). The number of writhes (characterized by contraction of the abdominal musculature and extension of the hind limbs) was then counted for 30 min at 5 min interval [11, 12].

$$\% \text{ Inhibition} = \frac{\text{Number of writhes (control)} - \text{Number of writhes (treatment)}}{\text{Number of writhes (control)}} \text{ X } 100$$

2.4.2. Formalin test

Mice fasted overnight for 12 h were divided into five groups of five animals each. The different groups of animals were treated with either distilled water (10 mL/kg, p.o.) or H_eCV (100, 200 and 400 mg/kg, p.o.) or diclofenac (50 mg/kg, p.o.). Sixty minutes after administration, formalin (20 µL of 10% v/v solution) was injected subcutaneously into the right hind paw of each mouse. The time (in seconds) spent in licking and biting responses of the injected paw, indicative of pain, was recorded for each animal. The responses of the mice were observed for 5 min (first phase) and 15 – 30 min (second phase) post-formalin injection [13, 12].

2.4.3. Capsaicin test

Mice fasted overnight were divided into five groups of five animals each. The different groups of animals were treated either with distilled water (10 mL/kg, p.o.) or H_eCV 100, 200 and 400 mg/kg, p.o) or diclofenac (50 mg/kg, p.o.). Sixty minutes after administration, capsaicin was injected subcutaneously into the right hand paw of each mouse. The time (in seconds) spent in licking and biting responses of the injected paw, indicative of pain, was recorded for each animal. The response of the mice were observed for 5 min (first phase) and 15 -30 min (second phase) post formalin injection [13, 12].

2.4.4. Elucidation of mechanism of antinociception of Clerodendrum volubile in mice

To investigate the mechanism by which H_eCV produces antinociception in acetic-acid induced writhing, animals were pretreated with antagonist of receptors implicated in pain. The choice of doses was based on previous studies. The acid writhing test was chosen based on its sensitivity in the transmission of pain.

2.4.5. Involvement of opioidergic receptors

To investigate the role of opioid system in H_eCV-induced antinociceptive effect, mice were pretreated with naloxone (5 mg/kg, i.p., a non-selective opioid receptor antagonist) or vehicle and after 15 min, H_eCV (400 mg/kg, p.o.) was given. One hour later, acetic acid 0.6% v/v in saline (10 mg/kg, i.p.) was administered.

2.4.6. Involvement of nitric oxide

To investigate the role played by nitric oxide synthase inhibitory pathway in the anticociceptive effects of H_eCV, mice were pretreated with N^G – nitro-L – arginine (10 mg/kg, i.p., a nitric oxide synthase inhibitor). After 15 min, the animals received H_eCV (400 mg/kg, p.o.) or vehicle (10 mg/kg, p.o.). One hour after treatment, acetic acid (10 mg/kg i.p.) was administered.

2.4.7. Involvement of ATP-sensitive potassium channel pathway

The possible contribution of ATP–sensitive potassium channel pathway was determined. Mice were pretreated with glibenclamide (10 mg/kg, i.p.) and 15 min later, they received H_eCV (400 mg/kg, p.o.). The acetic acid writhing test was carried out one hour post-treatment.

2.4.8. Involvement of L-NNA neuronal nitric oxide inhibitor pathway

The role played by nitric oxide inhibitor pathway in the antinociceptive effect of H_eCV was investigated. Mice were pretreated with L-NNA (10 mg/kg, i.p.), and 15 min later, the animals received H_eCV (400 mg/kg, p.o.) and 1 hour after treatment, acetic acid (10 mg/kg, i.p) was administered.

2.4.9. Involvement of dopaminergic pathway

The possible participation of non-selective dopaminergic pathway, particularly the D_2 and D_3 in the antinociceptive effect of H_eCV was evaluated. Mice were pretreated with sulpiride (50 mg/kg, i.p., a dopamine D_2-receptor antagonist). After 15 min, the animals received H_eCV (400 mg/kg, p.o.). One hour after treatment, acetic acid (10 mg/kg, i.p) was administered.

2.5. Antiinflammatory activity

2.5.1. Carrageenan-induced paw oedema

The rats used in this experiment were divided into five groups of five animals each. The respective groups were treated with distilled water (10 mL/kg, p.o.), H_eCV (100, 200, 400 mg/kg, p.o.) and diclofenac (10 mg/kg, p.o.). One hour later, oedema was induced by injection of carrageenan (0.1 mL, 1% w/v in saline) into the sub-plantar tissue of the right hind paw [14]. The linear paw circumference was then measured using a Vienier Calliper. Measurement of paw circumference was done immediately before injection of the phlogistic agent and at 30 min interval for 3 h.

$$\text{Inhibition \%} = \frac{\text{Increase in paw oedema (control)} - \text{Increase in paw oedema (treated)}}{\text{Increase in paw oedema (control)}} \text{ X } 100$$

2.5.2. Cotton pellet-induced granuloma formation in rats

The pellets of adsorbent cotton wool (20 mg) were sterilized in a hot air oven (model 600, Memmert, Germany) at 120 °C for 2 hr. Two pellets were implanted subcutaneously, one on each side of the abdomen under light ether anesthesia and sterile technique. Then H_eCV at 100, 200 and 400 mg/kg, and celecoxib (30 mg/kg) and distilled water were orally administered to 5 groups of 5 rats (180 – 200g) each, respectively for 7 days. On the 8th day, rats were sacrificed, the cotton wool was carefully removed from the surrounding tissue and weighed immediately, then wrapped inside a foil paper which was dried inside the oven at 40 °C for 24 hr after which the dried weight were determined [15].

2.6. Drugs and chemicals

The chemicals used were: acetic acid (May and Baker Ltd, Dagenham, England), formaldehyde (Griffin and George, Leics, England); diclofenac (Total Healthcare, Parwanoo, India); carrageenan (Sigma Chemical Company USA), capsaicin, celecoxib (Sigma Aldrich, St. Louis, MO, USA).

2.7. Statistical analysis

Results obtained from the study were expressed as mean ± standard error of the mean (S.E.M). Statistical comparisons between the groups were analysed using the ordinary one-way analysis of variance (ANOVA).

3. Results

3.1. Acute toxicity test in mice

Oral administration of HeCV at doses 2500 and 5000 mg/kg produced no mortality and other behavioral changes.

Table 1. Acute toxicity determination of HeCV administered orally in mice

Treatment (mg/kg)	Log dose	Mortality	% mortality	Probit
CV 2500	3.398	0/5	0	0
CV 5000	3.699	0/5	0	0

Therefore, the doses 2500 mg/kg and 5000 mg/kg are safe.

3.2. Acetic acid-induced writhing test

As shown in Table 2, intraperitoneal injection of acetic acid-elicited writhing syndrome in control mice with 121 ± 11.18 writhes counted in 30 min. HeCV produced a significant ($p < 0.05$) reduction in the number of writhes with peak effect (62.31%) produced at the highest dose of 400 mg/kg. This effect was less but not significantly different ($p > 0.05$) from that produced by diclofenac (50 mg/kg; 81.49%).

Table 2. Effect of HeCV on acetic-acid induced writhing in mice

Treatment	Dose (mg/kg)	Total number of writhes	Inhibition
Vehicle	0	121 ± 11.18	
HeCV	100	58.8 ± 9.36**	51.40%
HeCV	200	54.6 ± 7.72**	54.88%
HeCV	400	45.6 ± 13.75***	62.31%
Diclofenac	50	22.4 ± 10.99****	81.49%

Values are expressed as mean ± SEM (n=5); **p < 0.01, ***p < 0.001, ****p < 0.0001 versus vehicle treated control; p < 0.05 versus HeCV 100 mg/kg

3.3. Formalin-induced nociceptive test

As shown in table 3, in the first phase, injection of formalin into the sub-plantar tissue of the right hind-paw of control mice produced nociceptive response of biting and licking of treated paw with a total duration of 38.00 ± 7.04 s. HeCV was most effective at 200 mg/kg but had no significant (p > 0.05) inhibition of nociception reaction. This effect was less and there was no significant difference (p > 0.05) when compared to diclofenac 50 mg/kg (65.79%).

In the second phase, the total duration of nociceptive reaction in the control group was 123.00 ± 36.00 s. The greatest effect (79.84% of inhibition) was produced at the lowest dose of 100 mg/kg and had significant (p < 0.05) inhibition of nociception reaction. The effect was less and comparable to diclofenac 50 mg/kg (79.51%) but not significantly different (p > 0.05) from that of the effective dose (CV at 400 mg/kg).

3.4. Carrageenan-induced paw edema

Injection of carrageenean into the sub plantar tissue of the right hand paw of rats in the control group caused edema development with increase (3.07 ± 0.41 cm) in paw circumference at 180 min post-phlogistic agent injection. The effect of HeCV was dose-dependent with peak effect (48.05%) produced by HeCV at dose 400 mg/kg at 180 min. The effect produced by diclofenac 50 mg/kg (30.09 %), was also less and not significantly different (p > 0.05) from the effective dose of HeCV 400 mg/kg (Table 4).

3.5. Effect of dried transudative cotton pellet in HeCV

Figure 1 shows that the HeCV exhibited a significant and dose-related inhibition of cotton pellet granuloma. The inhibitory values for 100, 200 and 400 mg/kg of the extract were 52.10%, 54.37%, and 72.17% respectively. The effect was dose-dependent and peak effect was produced at the highest dose of 400 mg/kg. The effect of celecoxib (59.22%) was less.

Table 3. Effect of HeCV on formalin induced pain in mice

		0-5 min		15-30 min	
Treatment	**Dose (mg/kg)**	**Response duration (s)**	**Inhibition (%)**	**Response duration (s)**	**Inhibition**
Vehicle	10	38.00± 7.04		123.00± 36.00	
HeCV	100	26.6± 5.11	30	24.80± 9.57**	79.84
HeCV	200	21.00± 3.69	44.74	31.20± 7.85*	74.63
HeCV	400	26.60 ± 4.90	30	34.40± 4.46*	72.03
Diclofenac	50	13.00 ± 4.49*	65.79	25.20± 5.05**	79.51

Values are mean ± SEM (n=5); *p < 0.05, ** p < 0.01 versus vehicle treated control group; p < 0.05 versus 100 mg/kg.

Table 4. Effect of HeCV on carrageenan-induced paw oedema

Treatment	Dose (mg/kg)	30 min	60 min	90 min	120 min	150 min	180 min
Vehicle	10	1.12 ± 0.25	1.64 ± 0.16	1.79 ± 0.43	2.92 ± 0.51	2.53 ± 0.35	3.07 ± 0.41
HeCV	100	0.84 ± 0.17	0.56 ± 0.19*	0.77 ± 0.29*	1.36 ± 0.26***	1.69 ± 0.30	2.52 ± 0.25
% Inhibition		25.27%	66.18%	56.77%	53.43%	33.23%	18.05%
HeCV	200	0.58 ± 0.16	0.82 ± 0.13	0.43 ± 0.08**	1.41 ± 0.25***	1.58 ± 0.31	2.50 ± 0.27
% Inhibition		48.22%	50.12%	75.81%	51.58%	37.34%	18.44%
HeCV	400	0.69 ± 0.10	0.68 ± 0.11	0.54 ± 0.29*	1.44 ± 0.29**	1.53 ± 0.29	1.60 ± 0.24*
% Inhibition		39.08%	38.64%	69.90%	50.62%	39.08%	48.05%
Diclofenac	50	0.77 ± 0.15	0.42 ± 0.12	0.32 ± 0.11**	1.50 ± 0.35**	1.32 ± 0.11	2.14 ± 0.28
% Inhibition		31.67%	74.21%	81.41%	48.49%	47.63%	30.09%

3.6. Capsaicin-induced nociceptive test

Table 5 shows that in the first phase injection of capsaicin into the sub plantar tissue of the right hind paw of the control mice, produced nociceptive response of biting and licking of the treated paw with total duration of 112.80 ± 26.00s. HeCV at 200 mg/kg produced peak effect (87.41%) and had significant ($p < 0.001$) inhibition of nociception. The effect was less and comparable to diclofenac 50 mg/kg (87.06%), but was not significantly ($p > 0.05$) different. In the second phase, the effect was dose-dependent and maximum effect (68.71%) was observed at 400 mg/kg with significant ($p < 0.01$) inhibition of nociception reaction. Diclofenac had more effect (77.19%) and was not comparable to HeCV 400 mg/kg, but had no significant ($p > 0.05$) difference.

Table 5. Effect of HeCV on capsaicin-induced pain in mice

		0-5 min		15-30 min	
Treatment	**Dose (mg/kg)**	**Response duration (s)**	**% Inhibition**	**Response duration (s)**	**% Inhibition**
Vehicle	10	112.80 ± 26.00		68.4 ± 11.91	
HeCV	100	22.80 ± 7.65**	89.79	44.2 ± 7.04	33.38
HeCV	200	14.20 ± 6.38***	87.41	34.8 ± 10.24	49.12
HeCV	400	36.80 ± 9.95**	67.38	21.4 ± 3.97**	68.71
Diclofenac	50	14.60 ± 5.06***	87.06	15.64 ± 5.06**	77.19

Values are mean ± SEM (n=5); **$p < 0.01$, ***$p < 0.001$ versus vehicle treated control; $p < 0.05$ versus 100 mg/kg.

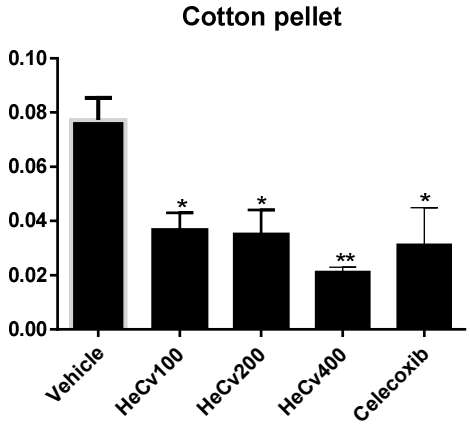

Fig 1. Effect of dried transudative cotton pellet in HeCV. Values are mean ± SEM (n=5); *$p < 0.05$; **$p < 0.01$; versus vehicle treated control. Values are mean ± SEM (n=5); *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ versus vehicle treated control

3.7. Elucidation of mechanism of antinociceptive effect of HeCV

3.7.1. Involvement of opioid system

The result (Fig. 2) showed that the pretreatment of mice with naloxone (5 mg/kg, i.p., a non-selective opioid receptor antagonist) did not prevent the antinociceptive effect elicited by HeCV (400 mg/kg) in writhing test. One way ANOVA revealed no significant ($p < 0.05$) difference.

3.7.2. Involvement of dopaminergic system

The result showed that the pretreatment of mice with sulpiride (10 mg/kg, a dopamine D_2 receptor antagonist i.p.), prevented the antinociceptive effect elicited by HeCV in the mouse writhing assay. One way ANOVA revealed a significant ($p < 0.05$) difference of treatment.

3.7.3. Involvement of ATP-sensitive K^+ channels

The pretreatment of mice with glibenclamide (10 mg/kg, an ATP-sensitive K^+ channel blocker, i.p.), did not prevent the antinociceptive effect elicited by HeCV in the mouse writhing assay. One way ANOVA revealed no significant difference of treatments with HeCV.

3.7.4. Involvement nitrergic system

The pretreatment of mice with N^G–nitro-L-arginine (10 mg/kg, i.p., a nitric oxide synthase inhibitor i.p.), did not prevent the antinociceptive effect elicited by HeCV in the

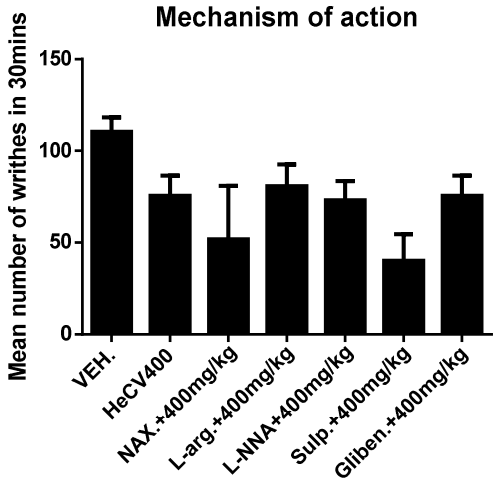

Fig 2. Mechanism of action of HeCV. Values are mean ± SEM (n=5).

mouse writhing test. One way ANOVA revealed no significant difference of treatments with HeCV.

3.7.5. Involvement of L-NNA neuronal nitric oxide inhibitor pathway

Pretreatment of mice with L-NNA (10 mg/kg, i.p., a neuronal nitric oxide inhibitor pathway), did not prevent the antinociceptive effect produced by HeCV in the mouse writhing test. One-way ANOVA revealed no significant difference of HeCV treatments ($p > 0.05$).

4. Discussion

In this study, HeCV was investigated for analgesic activity using acetic acid, formalin and capsaicin tests. In acetic acid-induced writhing test, the results indicated that HeCV given orally at doses 100, 200 and 400 mg/kg significantly decreased the number of writhes in mice, induced by 0.6% acetic acid. The intraperitoneal injection of acetic acid elicited writhing. The writhing test is simple, reliable and affords rapid evaluation of analgesic activity [11]. The induction of writhing by chemical substances injected intraperitoneally results from sensitization of nociceptors by prostaglandins [19] and the test is useful for mild analgesic and anti-inflammatory drugs [20]. Since HeCV at doses 100, 200 and 400 mg/kg significantly decreased the number of writhing response by 0.6% acetic acid, it could be assumed that HeCV decreased visceral pain induced by acetic acid and the mechanism of action for analgesic activity would be mediated by both peripheral and central mechanisms. The results suggested that the effect was dose-dependent and that diclofenac had a higher value than those observed with doses of HeCV. Diclofenac and other NSAIDSs can inhibit cyclooxygenase in peripheral tissues, thus, interfering with the mechanism of transduction in primary afferent nociceptors via inhibiting the synthesis of prostaglandin [20]. The mechanism of analgesic action could probably be due to interference with the synthesis and release of endogenous substances, blockade of the effect of desensitization of nerve fibers that excite pain nerve endings similar to aspirin and other NSAIDS.

The formalin test is another pain model which assesses the way an animal responds to moderate, continuous pain generated by injured tissues [21]. The formalin test was selected because of several advantages including the ability to mimic clinical pain in different conditions, production of tonic stimulus and sensitivity to NSAIDs. This test possesses two distinctive phases, possibly different types of pain. The early phase (0 - 5 min) was mediated by central effect *via* a direct stimulation of the nociceptor and releasing substance P or bradykinin. The late phase (15 - 30 min) was mediated by peripheral effect *via* the release of some chemical transmitters such as histamine, serotonin, prostaglandin and bradykinin [13]. In the present study, the results showed that HeCV inhibited both phases confirming a peripheral and central mechanisms of analgesic effect. The fact that the effect of HeCV in the second phase was greater than that produced in the first phase suggest greater involvement of peripheral mechanism in its anti-nociceptive action. Interestingly, the anti-nociceptive effect was more evident at 200 mg/kg.

In the capsaicin test, HeCV inhibited both phases confirming both peripheral and central effects. It was observed that HeCV at 400 mg/kg was the most effective although this effect was less when compared to diclofenac.

The anti-inflammatory activity of HeCV was evaluated using the carrageenan-induced paw oedema and cotton wool implantation tests. The carrageenean-induced paw inflammation has been accepted as a useful phlogistic tool for investigation of systemic anti-inflammatory agent. The test is sensitive to most clinically effective anti-inflammatory drugs, and it is commonly used as an experimental model for acute inflammation [22]. Edema formation due to carrageenan injected in the rat paw is a biphasic event, namely the initial and second phase. In the first phase, during the first 2 hr after carrageenan injection, chemical mediators such as histamine and serotonin play a role whereas in the second phase which approximates 3-5 hr after carrageenan injection, kinins and prostaglandins are involved [23]. One of the most important features of inflammation is edema, which is caused by the actions of some inflammatory autacoids like kinins, prostaglandin resulting in vasodilation, enhancement of capillary permeability, plasma exudation and these mediators also cause pain and fever [23].

In the present study, HeCV showed significant inhibitory effect on rat paw edema development in the early phase suggesting inhibition of chemical mediators such as histamine, and serotonin.

The response to subcutaneously implanted cotton pellet in rats is an experiment to investigate the ability of an agent to inhibit the proliferative component of the sub chronic and chronic inflammatory process and has been divided into transudative and proliferative phases respectively. The transudative phase is defined as the increase in the wet weight of the granuloma whereas the proliferative phase is defined as the increase of dry weight of the granuloma. Even the nonsteroidal anti-inflammatory drug, celecoxib is reported to only show a slight effect in this model [15]. In the present investigation, it was observed that the extract at 400 mg/kg exhibited significant inhibitory effect. The migration of leucocytes to the injury site occurs during chronic inflammation. Leucocytes accumulation leads to the release of lysosomal enzymes and oxygen radicals at inflammatory site [24]. In cotton pellet-induced granuloma formation, the activity of lysosomal enzymes is markedly elevated on the 7^{th} day after implantation [25] and can therefore be reliably suggested that HeCV at 400 mg/kg normalized the reaction through the stabilization of lysosomal membrane and inhibition of the migration of the inflammatory cells into the inflammatory sites.

Mechanistically, the local peripheral antinociceptive effect of HeCV in acetic acid test was not prevented by naloxone, L-nitro arginine, and glibenclamide, but by sulpiride. These results suggest that the antinociceptive effects induced by HeCV are mediated by non-selective dopaminergic system involving activation of D_2 receptors.

5. Conclusion

The experimental findings in this study indicate that the ethanolic leaf extract of *Clerodendrum volubile* is not acutely toxic orally, and that it possesses analgesic and anti-inflammatory activities possibly mediated *via* peripheral and central mechanisms involving the activation of dopaminergic receptors. Therefore, there is justification for the use of *Clerodendrum volubile* extract in Traditional African Medicine as an orally safe remedy for the treatment of pain and inflammatory conditions.

Conflict of interest

None.

References

1. Eddy NB, Friebel H, Hahn KJ, Halbach H. Codeine and its alternatives for pain and cough relief. Bull. WHO. 1967;40:639-719.
2. Mate GS, Naikwade NS,.Chowki CSA, Patil SB. Evaluation of antinociceptive activity of *Cissus quadrangularis* on albino mice. Intl J Green Pharm. 2008;2:118-121.
3. Way WE, Costley AO. Respiratory sensitivity of the new born infant to meperidine and fentanyl. Clin Pharmacol Ther. 1965;6:454-459.
4. Suave JB, Dahlstroom SN, Paalaw L, Ranc A. Morphine kinetics in cancer patients. Clin Pharmacol Ther.1981;30:629-635.
5. Gallin JL, Goldstein IM, Synderman R. Inflammation: Basic Principles and Clinical Correlates 2nd Edition. Raven Press, N.Y. 1992; pp. 100-115.
6. Kelley WN, Harris ED, Ruddy S, Sledge CB. Textbook of Rheumatology. 4th Edition. W.B. Saunders, Philadelphia.1993; pp.30-35.
7. National Institute for Health Guidelines .Guide for the use of Laboratory Animals. NIH Publication. 1985;85:23.
8. Litchfield JT, Wilcoxon F. A simplified method of evaluating dose-effect experiment. J. Pharmacol Exp Ther. 1949;96:99-113.
9. Adeyemi OO, Akindele AJ, Nwaubani N. Antiinflammatory activity of *Drymaria cordata* extract. J Nat Rem. 2008;8:93-100.
10. Adeyemi OO, Akindele AJ, Ogunleye EA. Evaluation of the antidiarrhoeal effect of *Sanseviera liberia* Gerome and Labroy (Agavaceae) root extract. J Ethno. 2009;123:459-463.
11. Singh S, Majumdar DK. Analgesic activity of *Ocimum sanctum* and its possible mechanism of action. Intl. J. Pharmacol. 1995;33:188-192.
12. Mbagwu HO, Anene RA, Adeyemi OO. Analgesic, antipyretic and anti-inflammatory properties of *Mezoneuron benthamicum* Baill (Caesalpiniaceae). Nig Quart J Hosp Med. 2007;17:35-41.
13. Shibata N, OhkuboT, Takahashi H, Inoki R. Modified formalin test: Characteristic biphasic pain response. Pain.1989;38:347-352.
14. Winter CA, Risley EA, Nuss GW. Carrageenan induced edema in hind-paw of rats as an assay for anti-inflammatory drugs. Proc Soc Exp Biol.1962;111:533-547.
15. Ashok PB, Kati C, Thippesuwamy AM, Tikane VP, Dababi P. Evaluation of anti-inflammatory activity of *Centratherum anthelminticum*. Ind J Pharmaceutical Sci. 2010;72:697-703.
16. Viturro O, Guyton AC, Hall JE, Sayag M, Keppler DK. Liver as an organ. In Textbook of Medical Physiology 10th Edition. WB. Saunders, Philadelphia. 1999; pp.797-802.
17. Braca A, Tommasi N, Dibar L, Morcell J. Antioxidant principle from *Bauhinia terapotensis*. Ind J Pharmaceutical Sci. 2001;64:892-895.
18. Kumaran R, Karuna-karan K. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. Food Drug Analogy. 2007;10:178-182.
19. Wunez Guillenn ME, Emin JA, Souccar C, Lopa AJ. Analgesic and anti-inflammatory activities of the aqueous extract of *Plantago major* L. Intl J Pharmacog. 1997;35:99-104
20. Berkenkop JW, Weichmann BV. Production of prostacylin in mice following intraperitoneal injection of acetic-acid, phenylbenzoquinone and zymosan: its role in the writhing response. Prostaglandins.1998;36:693-709.
21. Tjolsen A, Berge OG, Rosland SH. The formalin test: An evaluation of the method. Pain. 1992;51:5-17.
22. Panthony A, Kanjana D, Thesofikul T. Antiinflammatory and antipyretic properties of *Clerodendrum petasites*. J Ethno. 2003;85:151-156.
23. Hermandez-Perez M, Gailego RM. Evaluation of the anti-inflammatory and analgesic activity of *Sderitis canariensis varpannosa* in mice. J Ethno. 2002;81:43-47.
24. Salmon JA, Higgs GA. Prostaglandins and leukotrienes as inflammatory mediators. British. Med. Bull. 1987;43:285-296.
25. Nishikaze O, Takita H, Takase T. Activity of newly discovered protease in carrageenan-induced inflammation in rats. IRCS. 1980;8:725.
26. Bhaskar R, Rajeswari V, Sathish KT. *In vitro* antioxidant studies in leaves of *Annona* spp. Ind J Exp Biol. 2007;4:480-485.
27. Chowdhury MH, Jimanshu SM, Pranabesh C. Hepatoprotective activity of *Lawsonia inermis* Linn warm aqueous extract in carbon tetrachloride-induced hepatic injury in wistar rats. Asian J. Pharmaceutical Sci. 2011;17:212-34.