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EXPERIMENTAL PAPER

Preliminary assessment of the anti-inflammatory and analgesic effects of methanol leaf extract of *Cussonia barteri* (*Araliaceae*) in rodents

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Summary

Introduction: Potato (*Solanum tuberosum* L.) is an important vegetable crop in Syria. Potato tuber moth *Cussonia barteri* is a small tree that grows in the sub-Saharan part of Africa. Various parts of the plant are used for the treatment of a variety of ailments in ethno-medicine.

Objective: To evaluate the anti-inflammatory and analgesic effect of the methanol leaf extract of *Cussonia barteri*.

Material and methods: The leaves were air-dried, powdered and repeatedly extracted with methanol using a Soxhlet apparatus. The resulting methanol extract (100, 200 and 400 mg/kg) was evaluated for anti-inflammatory activity using carrageenan-induced paw oedema, xylene-induced ear oedema and formalin-induced arthritis tests. Analgesic effect was evaluated using acetic acid-induced mouse writhing, hot plate and tail flick tests.

Results: All doses of the extract significantly ($p<0.05$) reduced carrageenan-induced paw oedema, however the 400 mg/kg dose gave a sustained effect. The extract significantly inhibited xylene induced ear oedema at all doses. There were no significant ($p>0.05$) reductions in paw swellings due to formalin. In the acetic acid induced writhing test, the extract significantly ($p<0.05$) decreased writhing at 400 mg/kg only. Reaction times were not significantly different from the control in the hot plate and tail flick tests.

Conclusion: This study has shown that the methanol extract possesses acute anti-inflammatory and peripherally mediated analgesic effects.

Key words: ***Cussonia barteri, methanol leaf extract, anti-inflammatory activity, analgesic activity***

Słowa kluczowe: ***Cussonia barteri, metanolowy wyciąg z liści, działanie przeciwzapalne, działanie przeciwbólowe***

INTRODUCTION

Inflammation is a complex, adaptive response that is usually activated by harmful stimuli, such as infection, irritants and tissue malfunction or injury [1]. Inflammation is characterized by fundamental signs which includes: redness (rubor), swelling (tumour), heat (calor), pain (dolor) and loss of function (*functio laesa*) [2]. Inflammation could be acute or chronic and involves cellular and molecular events. It is now understood that virtually all pathological conditions have an inflammatory component [3]. Algesia or pain is a component of the inflammatory process and is a result of the effects of inflammatory mediators on sensory nerves (nociceptors) [2]. Consequently, anti-inflammatory studies are usually carried out concurrently with analgesic studies [4, 5]. The currently available anti-inflammatory agents have side effects that limit their usage, thus leading to increased use of alternative therapies, especially herbal medicines in developing societies [3]. These inadequacies associated with anti-inflammatory drugs underscore the need to search for newer agents and medicinal plants have been cardinal to this search [6]. Several medicinal plants and its isolated compounds have been reported to have anti-inflammatory and analgesic effects [7].

Cussonia barteri Seem (*Araliaceae*) is a medium-sized broadleaf tree, which grows up to about 10 m. The tree grows in the savannah regions of Sub-Saharan Africa, Yemen, and has a thick trunk and hard bark [8]. The plant has greenish-white, fingerlike leaves [9]. The fruits are fleshy and turn purple to white when ripe [10]. The plant undergoes complete defoliation in the dry season. The defoliated tree has the shape of a 'cut off limb' and is thus named 'stump of an amputated limb' in Mali and Volta, 'leper's hand' in Northern Nigeria [11]. The plant is called 'Ako-sigo' in yoruba, 'Tuwon giwa' in hausa, 'Bolo Koro' in Senegal and 'Kokobidua' in Ghana [12], while the seeds are called 'jansa' seeds (Cameroun), 'Ugbaokwe' (Igbo), 'Takandagiwa' (Hausa) and 'Shigo' (Yoruba) in Nigeria [13]. *C. barteri* is utilized for various purposes in ethnomedicine. The whole plant and its stem bark are used as a purgative, an aphrodisiac and as external lotion in Mali [14]. The seeds are used in cooking soup in some societies [13]. Decoctions of the root and stem bark are used in menstrual pain, rheumatism, emetic, epilepsy and mental disorders [15]. The leaves, stem, root and seeds have been documented to contain fatty acids, sugars, flavonoids, saponins, steroids, phenols, tannins, glycosides and alkaloids [16, 17]. Isolated compounds from the leaves and stem bark have been elucidated [16]. The presence of these phytochemical constituents and documented ethnomedicinal use of *C. barteri* stimulated the need for the assessment of the leaf for anti-inflammatory and analgesic effects.

MATERIAL AND METHODS

Plant material

Parts of the *Cussonia barteri* plant comprising leaves were obtained from Basawa area of Sabongari, Kaduna state, Nigeria in July 2016. The plant was identified and authenticated at the National Institute for Pharmaceutical Research and Development (NIPRID) and a herbarium sample (NIPRD/H/6995) was deposited for future reference.

Extraction

The leaves were sundried over a period of one week after which it was reduced to powder form using a mechanical mill. Repetitive Soxhlet extractions were carried out with the powdered plant material (500 g) in 2 l of 100% methanol. The mixture was filtered and concentrated using a rotary evaporator to give a percentage yield of 54.9%. The resulting methanol extract was suspended in 5% gum acacia before use.

Experimental animals

Swiss albino mice (20–30 g) and Wistar rats (150–180 g) of either sexes were purchased at the Department of Pharmacology Animal House, University of Benin. They were housed in polypropylene cages, had free access to clean drinking water and pelletized feed.

The animals were maintained under standardized environmental conditions. The handling procedures were conducted in accordance with recommended procedures of the Faculty of Pharmacy Ethical Committee on Experimental Animals (EC/FP/019/01) and followed the internationally accepted laboratory animal use and care guidelines and rules of IAEC.

Drugs and chemicals

λ-carrageenan and indomethacin (Sigma Chemical, USA), xylene (BDH Chemicals, UK), 40% formaldehyde (Pyrex IG, Nigeria), dexamethasone (Alpha Pharma, Nigeria), acetic acid (BDH Chemicals, England), acetylsalicylic acid powder (Pyrex-IG Company, Nigeria), morphine hydrochloride (Alpha Pharma, Abuja) were used in the experiment.

Acute toxicity study

Swiss albino mice were divided into five groups of five animals each. The control group was orally administered with distilled water (8 ml/kg), while groups 2–5 were orally administered with 0.5, 1, 2.5 and 5 g/kg of extract suspended in 5% gum acacia, respectively [18]. The animals were observed for signs of toxicity and mortality within 24 hours of administration. They were further observed for another two weeks for any signs of delayed toxicity.

Anti-inflammatory study

Carrageenan-induced paw oedema assay

Wistar rats (150–200 g) were divided into five groups of five animals each. Groups 1, 2 and 3 were orally administered with 100, 200 and 400 mg/kg of the extract. Group 4 received indomethacin (10 mg/kg) suspended in 5% gum acacia (p.o.), while the control group received 5% gum acacia (3 ml/kg) p.o. The basal paw thickness of the right hind paw was measured with a vernier caliper. After 1 h of pretreatment, 100 μl of 0.1% *w/v* carrageenan suspension in normal saline was injected into the sub plantar tissue of the right hind paw. Paw thickness was measured using a Vernier caliper at hourly intervals for 5 h [19, 20].

Formalin-induced arthritis test

The modified method of Hosseinzadeh and Younes [21] was applied for this study. Wistar rats (150-200 g) were allotted into five groups of five animals each. There groups were treated as previously described above. One hour after pretreatment, inflammation was induced in all groups by subaponeurotic injection of 100 μl of 2% *w/v* formalin in the right hind paw of the animals; inflammation was also induced on the third day. The inflamed right paw thickness was measured daily for 5 days using the vernier caliper.

Xylene-induced ear oedema assay

The method of Akindele and Adeyemi [22] was used with slight modifications. Five groups of 5 mice each were treated with the extract (100, 200 and 400 mg/kg *p.o.*), dexamethasone (1 mg/kg *i.p.*) and 5% gum acacia (10 ml/kg *p.o.*). Thirty minutes after pretreatment, oedema was induced by applying 30 μl of xylene into the inner surface of the right ear. The left ear was considered as control. After fifteen minutes, the animals were sacrificed under chloroform anaesthesia and both ears were removed and weighed [6]. The mean of the difference between the right and left ears was calculated using the following formula:

$$\% \text{ inhibition} = 100 \left(Vc - \frac{Vt}{Vc}\right)$$

where V_c represents difference in weight of ear in control and V_t difference in weight of ear in group treated with standard/extract.

Analgesic study

Acetic acid-induced writhing in mice

The method of Koster *et al.* [23] was used for this test. The mice were randomly assigned into five groups of five animals each. The extract (100, 200 and 400 mg/kg, *p.o.*), and acetylsalicylic acid (100 mg/kg *p.o.*) suspended in 5% gum acacia and 5% gum acacia (control group) (10 ml/kg *p.o.*) were administered to groups 1 to 5, respectively. One hour after pre-treatments, 10 ml/kg of 0.6% *v/v* acetic acid *i.p.* was administered to all groups. The amount of writhes was immediately recorded every 5 minutes for a period of 30 minutes [4]. Percentage inhibition of writhes in comparison to control group was calculated as:

$$\% \text{ inhibition} = \frac{\text{mean number of writhes (control) - mean number of writhes (treatment) x 100}}{\text{mean number of writhes (control)}}$$

Hot plate test

The modified method of Eddy and Leimbach [24] was used to evaluate the reaction times to pain. Animals were allotted into five groups of five mice. The extract (100, 200 and 400 mg/kg *p.o.*) suspended in 5% gum acacia, 5% gum acacia (control group) (10 ml/kg *p.o.*) and morphine (2 mg/kg, *s.c.*), were administered to groups 1 to 5, respectively. The animals were acclimatized for 30 minutes on the hot plate apparatus; thereafter, the temperature of the hot plate was set at 55±1°C. The latency, defined as the reaction time of each mouse (licking of the forepaws or jumping response) was recorded. Pretreatment reaction for each mouse was determined and 30 seconds was set as the post-treatment cut off time. Reaction time was recorded at time intervals of 0, 30, 60, 90, and 120 minutes post treatment.

Tail flick test

In this model [25], a radiant heat automatic tail flick analgesiometer (Ugo Basile) was used. Five groups of 5 rats each were treated with the extract (100, 200 and 400 mg/kg p.o.) suspended in 5% gum acacia, morphine (2 mg/kg s.c.) and 5% gum acacia (control group) (10 ml/kg p.o.). One hour after pretreatments, the tail tips (1–2 cm) of the animals were placed over the radiant heat source and the tail withdrawal from the heat was taken as the end point. The reaction time (sec) of each rat in each group was determined at 0, 30, 60, 90 and 120 minutes. A cutoff time of 30 seconds was set to avoid tail trauma.

Statistical analysis

All data were expressed as mean ±SEM. Data were analyzed by one way analysis of variance (ANOVA) followed by Dunnet post hoc test (Graphpad Prism® 6, San Diego, USA). Results were considered statistically significant at $p<0.05$.

RESULTS

Acute toxicity of *C. barteri*

There were no toxic or adverse effects at all doses (0.5, 1, 2.5 and 5 g/kg) after 24 hrs. No signs of delayed toxicity and mortality were recorded at all doses after two weeks after treatment (tab. 1).

Anti-inflammatory effects of *C. barteri*

Carrageenan-induced paw oedema assay

In this model (fig. 1), the extract (200 and 400 mg/kg) significantly ($p<0.01$) inhibited paw edema for three hours (1–3 h), however 100 mg/kg dose of the extract only significantly ($p<0.01$) inhibited paw edema in the first hour, compared with the negative control.

Xylene-induced ear oedema

The extract (100, 200 and 400 mg/kg) inhibited xylene-induced edema significantly with percentage values of 47.71%, 67.61% and 65.63% respectively (tab. 2). The inhibitory effect at doses of 200 and 400 mg/kg was higher than that of dexamethasone, with an inhibition of 59.15%.

Formalin-induced arthritis

Table 3 shows the effect of the extract on formalin-induced arthritis. The extract did not significantly inhibit formalin-induced arthritis.

Table 1

Acute oral toxicity of *C. barteri* in mice

Group	Log Dose	Diarrhea	Sedation	Tremor	Writhing	Mortality
Control	–	0/5	0/5	0/5	0/5	0/5
CB 500 mg/kg	2.699	0/5	0/5	0/5	0/5	0/5
CB 1000 mg/kg	3.000	0/5	0/5	0/5	0/5	0/5
CB 2500 mg/kg	3.398	0/5	0/5	0/5	0/5	0/5
CB 5000 mg/kg	3.699	0/5	1/5	0/5	0/5	0/5

Control: 5% gum acacia; CB: *Cussonia barteri*

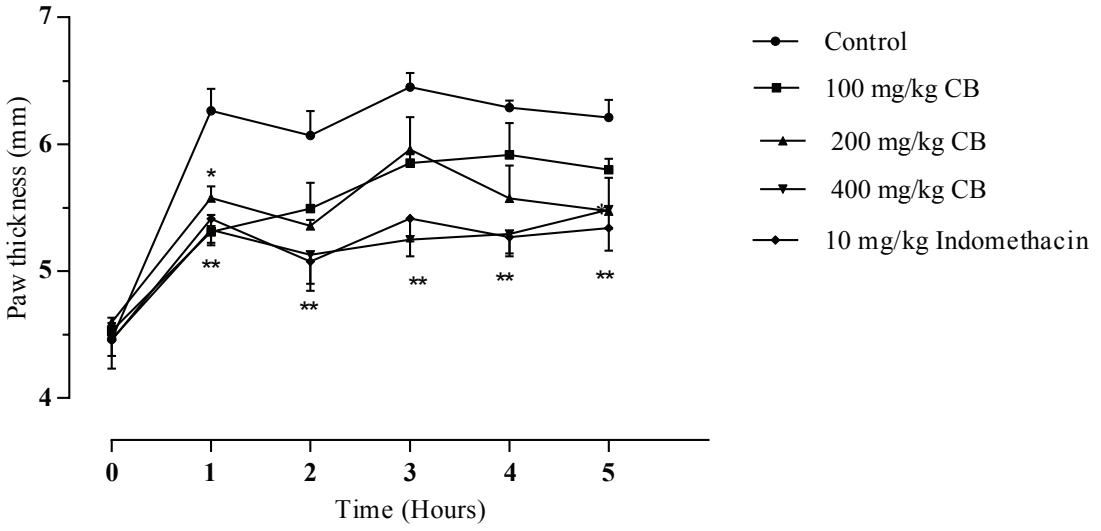

Figure 1

Effect of methanol extract of *C. barteri* on carrageenan-induced paw edema in rats. CB – *Cussonia barteri*. *p<0.05, *p<0.01, when compared to the control (5% gum acacia, 3 ml/kg).

Table 2

Effect of methanol leaf extract of *Cussonia barteri* on xylene-induced ear edema

Treatment	Dose [mg/kg]	Weight of right ear [mg]	Weight of left ear [mg]	Difference [mg]	% Inhibition
Control	3 ml/kg	55.60±6.62	34.30±2.26	21.30±4.36	–
CB	100	41.23±1.97	30.10±2.45	11.13±0.48*	47.7
CB	200	33.80±0.72	26.90±2.20	6.90±1.48*	67.61
CB	400	48.85±10.45	41.53±7.00	7.32±3.45*	65.63
Dexamethasone	1	35.28±4.77	26.58±2.07	8.7±2.70*	59.15

Values are mean ±S.E.M. *p<0.05, as compared to the control. n=5 for each group. CB – *Cussonia barteri*

Table 3

Effect of methanol leaf extract of *Cussonia barteri* on formalin-induced arthritis

Treatment	Dose [mg/kg]	Paw thickness on day 5 [mm]	% Inhibition
Control	3 ml/kg	7.04±0.23	–
CB	100	6.48±0.07	7.95
CB	200	6.85±0.39	2.70
CB	400	6.79±0.25	3.55
Indomethacin	5	5.93±0.36*	15.76

Values are mean ±S.E.M. *p<0.05, as compared to the control. n=5 for each group. CB – *Cussonia barteri*

Analgesic effect of *C. barteri*

Acetic acid induced writhing

The extract at 400 mg/kg, significantly (p<0.05) decreased the number of writhes compared to the control at the 25th and 30th minute with a 27.36% inhibition (fig. 2).

Hot plate test

In this model, the extract did not increase the reaction times in the animals (tab. 4).

Tail flick test

The extract did not produce any significant (p>0.05) increase in reaction times when compared to the control (tab. 5).

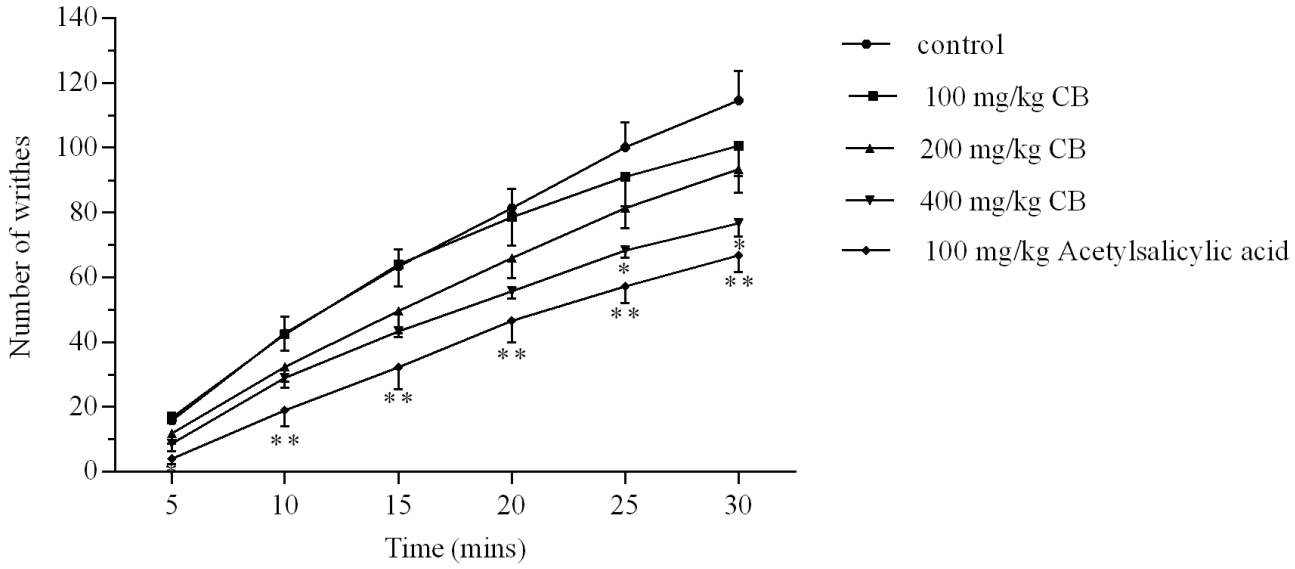

Figure 2

Effect of methanol extract of *C. bateri* on acetic acid induced mouse writhing. *p<0.05, **p<0.01, when compared to control group (5% gum acacia, 10 ml/kg). CB – *Cussonia bateri*

Table 4

Effect of methanol extract of *C. barteri* on hot plate in mice

Groups	Dose [mg/kg]	Reaction times				
		0 min	30 min	60 min	90 min	120 min
Distilled water	–	4.20±0.50	3.58±0.59	4.42±0.39	4.53±0.67	4.24±0.12
CB	100	3.42±0.44	3.40±0.51	4.22±0.59	6.78±0.57	6.02±0.95
CB	200	3.42±1.05	4.93±0.39	5.23±0.78	4.50±1.15	6.52±0.68
CB	400	2.78±0.21	6.42±1.35	5.50±0.58	7.00±0.58	6.80±1.06
Morphine	1	3.43±0.52	11.77±2.17*	11.43±1.37*	10.00±2.29*	8.17±3.60

Values are mean ±S.E.M. *p<0.05, as compared to the control (5% gum acacia, 5 ml/kg). CB – *Cussonia barteri*

Table 5

Effect of methanol extract of *C. barteri* on tail flick in mice

Groups	Dose [mg/kg]	Reaction times				
		0 min	30 min	60 min	90 min	120 min
Distilled water	–	4.90±0.07	5.35±0.89	4.65±0.60	5.78±0.52	4.15±0.52
CB	100	5.28±0.81	5.68±1.24	4.56±0.32	5.56±0.69	5.60±0.87
CB	200	4.22±0.46	4.28±1.15	5.23±1.03	5.90±0.76	4.83±0.11
CB	400	5.28±0.57	4.00±0.61	6.18±0.39	6.56±1.42	4.56±1.04
Morphine	2	4.30±0.43	2.25±4.82***	12.70±3.01**	8.60±1.56	10.43±2.84*

Data are expressed as mean ±SEM. *p<0.05, **p<0.01, ***p<0.001, when compared to control (5% gum acacia 5 ml/kg). CB – *Cussonia barteri*

DISCUSSION

Acute safety evaluation assesses administered doses of a substance for immediate toxic effects [26]. A previous study by Yakubu *et al.* [17], revealed that the ethanol leaf extract of *C. barteri* had an LD_{50} of above 5000 mg/kg at acute oral doses and was reported to be safe on oral administration. This study also demonstrated that the methanol leaf extract was devoid of immediate toxic effects at all administered oral doses.

Phytochemical constituents present in medicinal plants serve as a predictive index for biological

activity [27]. Constituents including saponins, flavonoids, phenols, alkaloids, tannins and oils have been established to possess a plethora of biological effects including analgesic, anti-microbial and antiviral effects [6, 27, 28]. The leaves of *C. barteri* have been demonstrated in previous studies to contain saponins; these saponins have been isolated with documented biological activities [29, 30].

This study assesses the anti-inflammatory and analgesic effect of the methanol leaf extract of *C. barteri*. The carrageenan-induced paw oedema is used to study acute inflammation (<24 h). A subcutaneous injection of 1% carrageenan in rat paw stimulates an oedema that includes an early phase (1–3 h) principally mediated by histamine, serotonin and a late phase (>4 h) mainly caused by prostaglandins, nitric oxide, protease and bradykinins [31-33]. The extract significantly reduced paw sizes at the highest dose in carrageenan-induced inflammation, suggesting that the extract may be effective in acute inflammatory states. This attribute of the extract may indicate the presence of triterpene, saponins and flavonoids that have documented anti-inflammatory effects [30, 34]. The extract's ability to produce a sustained effect in both phases of the carrageenan induced inflammation may suggest the extract interferes with the activity of several mediators (such as serotonin, bradykinin, prostaglandins and histamine) involved. The extract produced similar effects in xylene-induced ear oedema. The local application of xylene into the inner ear surface of the mice stimulates acute inflammatory effects that lead to increases in the ear thickness; the increase in ear thickness is partly due to substance P, which causes plasma extravasations and subsequent oedema [35]. The extract significantly reduced ear weights at all doses, the percentage inhibition of ear oedema by the extract (200 mg/kg) was similar to dexamethasone, which was used as a standard. This further establishes the extracts effect in acute inflammation. In contrast to this observation, the isolated quinic esters, rutin and saponins from the methanol leaf extract did not produce anti-inflammatory effect when tested against 5-lipoxygenase and cyclooxygenase-1 inhibition [29]; we suggest that the extract may possess other biological constituents that may be synergistic to its probable anti-inflammatory effect seen in this study [36].The formalin-induced arthritis test is used to evaluate substances for sub-acute anti-inflammatory effect [37]. The injection of formalin into the hind paw stimulates an intense, painful and proliferative inflammatory reaction partly mediated by products of the cycloxygenase (COX) pathway and may eventually lead to deformity of the hind paw [38, 44]. The extract caused a reduction in paw sizes on the third day, this effect was not sustained as paw size significantly increased on the fourth and fifth days. This is consistent with the acute inflammatory effects of the extract observed in this study from carrageenan and xylene models of inflammation; the involvement of products of the COX pathway may have partially explained the observed acute effects of the extract in the formalin test [44].

The acetic acid-induced writhing assay is one of the most commonly applied tests used to screen substances for peripheral analgesic effect [23, 39]. The intraperitoneal administration of acetic acid irritates resident cells leading to the production of endogenous intermediaries that initiates an inflammatory response which results in the stimulation of peripheral nociceptors [40]. The extract significantly reduced mouse writhing at the highest dose only. The extracts probable effects on prostaglandin activity as reported from anti-inflammatory studies may have also partly resulted in the decrease in the number of writhes as prostaglandins play a role in the acetic acid induced writhing test [42]. Earlier reports by Dubois and his colleagues revealed that the stem bark of *C. barteri* possessed sedative activity and they attributed this sedative effect to the presence of saponins. We suggest that the saponins present in the leaves may have also contributed to the reduction of writhes caused by extract, as substances with sedative and muscle relaxant effects can also reduce the number of observed writhes in this model [41, 43]. The hot plate test employs thermal stimuli and is a simple test for assessing substances for centrally mediated analgesic effect. This test involves the introduction of a mice into a cylindrical metallic plate that is heated by a thermode [24]. Higher spinal mechanisms and brain centres are involved in integrating responses associated with the hot plate test; these responses are attenuated by opioids and substances with centrally mediated analgesic effect [43].The extract did not increase reaction times at all doses significantly, suggesting the extract lacked centrally mediated analgesic effects. However, there was an observed increase in the reaction times (time taken for the mice to lick the paw) at all doses of the extract as the experiment progressed; NSAIDS are known to increase the period taken for the animal to lick its paw in this model and this may be as a result of COX inhibition, a probable effect of the extract [43]. The tail flick test is another test used to screen substances for centrally mediated analgesic effect. All doses of the extract had no significant effect on tail flick reaction times. This further affirms the extracts lack of centrally mediated analgesic effect.

CONCLUSION

The results from this study show that the leaves of *C. barteri* possess dose dependent acute anti-inflammatory and peripherally mediated analgesic effects. Further mechanistic studies may need to be carried out to assess the probable mechanism(s) of the leaf extract.

Conflict of interest: Authors declare no conflict of interest.

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