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Research Article

Analgesic and Anti-inflammatory Activities of the Residual Aqueous Fraction of *Carissa edulis* Root Bark (Vahl) in Experimental Animals

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Background: *Carissa edulis* is a spiny evergreen shrub that may reach a height of 5 feet and an equal breadth. The bark is grey and smooth with straight woody double-pronged spine often in pairs. The plant is a native of South Africa. It grows in tropical African region and Arabia. It has been used in the traditional treatment of malaria, headache, glandular inflammation, rheumatism and chest complaints among others for many years and their efficacy is widely acclaimed among the Hausa communities of northern Nigeria. Hence, there need for verification of these folkloric claims.

Objectives: The current study aimed at evaluating the analgesic and anti-inflammatory activities of the residual aqueous fraction of the ethanol root bark extract of *C edulis* in mice and rat models.

Methodology: Acetic acid-induced writhing and tail immersion test in mice were used to assess analgesic properties, while anti-inflammatory effect was tested using carrageenan-induced paw oedema in rats.

Results: The fraction (150, 300 and 600 mg/kg) and standard drugs significantly ($p<0.05$) reduced the number of writhes and prolonged the pain reaction time, in acetic acid-induced writhing and tail immersion models respectively. Similarly, the fraction (300 and 600 mg/kg) and ketoprofen (10 mg/kg) exhibited significant ($p<0.05$) decrease in the paw oedema at 1, 2, 3 and 4 hour intervals, while at 150 mg/kg, the decrease was significant at only third hour.

Discussion: The study has shown that the residual aqueous fraction of *C. edulis* possesses analgesic and anti-inflammatory activity, thus, justified the traditional use of the plant in pain and inflammatory conditions.

Keywords: *Carissa edulis,* writhes, inflammation, analgesic, fraction

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1. Introduction

Inflammation is the response of living tissues to injury and it involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Perianayagam et al, 2006), which are aimed at host defense and usually activated in most disease conditions (Iwueke et al, 2006). Non-steroidal anti-inflammatory drugs (NSAIDS) and opioids are used in management of mild to moderate and severe pains respectively. These drugs have serious limitations due to their side effects such as gastrointestinal irritation, tolerance and dependency (Howland and Mycek, 2006).

Medicinal plants have been source of wide variety of biologically active compounds most of which have a potential use in drug discovery and they continued to be the source of lead compounds (Ravalli et al, 2015).

Carissa edulis Vahl is a native of South Africa and belongs to the family Apocynaceae. It is commonly

called *Cizaki* in Hausa; *Kanboro* in Fulfulde; *Emir* in Arabic; *Muyunzo* in Luganda, *Endelkoring-neominoem* in Africana; *Agam* in Amharicand *Mlanoa-mboo* in Swahili (Msonthi, 1986). The plant has been known for its ethnomedical uses which include treatment of fever, sickle cell anemia and hernia, treatment of edema, toothache, cough, ulcer, worm infestation, management of epilepsy, mental illness and cancer (Ya'u et al*,* 2015).

The plant extracts have been previously shown to possess hypoglycemic (El-Fiky et al, 1996), antidiuretic effects (Nedi et al*,* 2004); anticancer (Chandramu et al*,* 2003) effects; analgesic and antimicrobial activity (Ibrahim et al*,* 2005, 2007); antiviral activity (Tolo, 2006); and anticonvulsant, anxiolytic, and sedative activities (Jamilu et al*,* 2007; Ya'u et al*,* 2010; 2011; 2015).

The current study aimed at evaluating the analgesic and anti-inflammatory activities of the residual aqueous fraction of the ethanol root bark extract of *C. edulis* in mice and rats models. To the best of our knowledge such activities were not previously reported on the fraction

2. Materials and Methods

2.1 Plant materials

Root bark of *C. edulis* was collected by a local herbalist at Basawa Village, Zaria, Nigeria. The plant sample was identified and authenticated at the Herbarium Unit, Department of Biological Sciences, Ahmadu Bello University, Zaria. The sample was compared with a deposited specimen in the Herbarium and Voucher number (601) was issued to serve as reference. The plant part was air-dried under shade for two weeks to ensure complete dryness. It was subsequently size-reduced with mortar and pestle. The fine powdered sample (500 g) was cold-macerated with 2 L of 70%$^{v}/_{v}$ ethanol in water with occasional shakings; filtered and the filtrate was concentrated to dryness using water bath regulated at 40^{0}C). The resultant dried extract had characteristic pleasant smell and was brownish in appearance.

2.2 Drugs and Chemicals

Ethanol and Petroleum ether (Sigma, St. Louis U.S.A), Ethyl acetate and n-butanol (BDH Ltd Poole, England), Glacial acetic acid (Searle Essex, England), Ketoprofen (LEK, Slovenia), Pentazocine (Ranbaxy, India).

2.3 Fractionation of the crude extract

Fractionation of the ethanol root bark extract of *C. edulis* to was conducted according to the method of Ciulei (1997), and thus, residual aqueous fraction (RAF) was obtained. Briefly, the ethanol extract was dissolved in the distilled water in separating funnel and defatted with petroleum ether (PE) to get PE fraction. The aqueous portion was partitioned with ethyl acetate (EA) where EA fraction was obtained. Then the aqueous portion was partitioned again with n-butanol (NB) to obtain NB fration. Finally, the left over aqueous portion was referred to as residual aqueous fraction, the fraction of interest.

2.4 Animals

Sixty Swiss albino mice (18–24 g) and thirty Wistar rats (160-200 g) were obtained from Animal House Facility, Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria, Nigeria. They were maintained under good laboratory care and fed with standard animal feeds (Feeds Masters, Ilorin, Nigeria), and were allowed free access to water. The animals were used in compliance with the National Institute of Health Guide for the Care and use of Laboratory Animals (Publication nos. 85-23, revised 1985). The institutional approval number for the protocol was given as DAC/IW-OT/003-10.

2.5 Acetic acid-induced Writhing in Mice

The method of Koster et al*,* (1959) was adopted in this study. Thirty albino mice of both sex were randomly divided into five groups of six mice per group. Groups 1 and 5 served as negative and positive controls and received normal saline (10 ml/kg) and ketoprofen (10 mg/kg), respectively. Groups 2, 3 and 4 were treated with the residual aqueous fraction at doses of 150, 300 and 600 mg/kg respectively, all via intraperitoneal (i.p.) route. Thirty minutes post treatment, 10 ml/kg of 0.6% $^{v}/_{v}$ glacial acetic acid was administered to all the mice via i.p route. The number of abdominal writhes was considered as an index for pain, and were counted for 15 minutes and recorded.

2.6 Tail Immersion Test in Mice

The method described by Uma-Devi et al*,* (1999) was used for this study. Thirty albino mice were grouped into five containing six mice in each group. Group 1 was given normal saline (10 ml/kg); while groups 2, 3 and 4 were treated with the residual aqueous fraction at doses of 150, 300 and 600 mg/kg respectively, all via intraperitoneal (i.p.) route. Mice in group 5 served as positive control and received pentazocine at 10 mg/kg intraperitoneally. Thirty and sixty minutes after, about 5 cm of the tail of each mouse was dipped into a water bath regulated at temperature of 50 ± 1◦C. Time taken for each mouse to flick its tail regarded as an index for pain perception and described as pain reaction time recorded in seconds. The cycle was repeated for all the groups 60 minutes later.

2.7 Carrageenan-induced Paw Oedema in Rats

The experiment was conducted according to the method described by Winter et al*,* (1962). Rats were divided into five groups of six rats per group. Groups 2, 3 and 4 were treated with the residual aqueous fraction at doses of 150, 300 and 600 mg/kg, while groups 1 and 5 served as controls and received normal saline (1 ml/kg) and ketoprofen (10 mg/kg) respectively, all via intraperitoneal (i.p.) route. Thirty minutes post treatment, carrageenan (0.1 ml of 1%) dissolved in normal saline (0.9% $^{w}/_{v}$) was injected to the sub plantar region of right hind paw of the rats. The paw volume was measured at 0. 1, 2, 3 and 4-hour post carrageenan administration, using a Vernier caliper. The percentage inhibition of inflammation by the residual fraction was calculated in comparison with the negative and positive controls.

2.8 Statistical Analysis

Results were presented in tables and expressed as Mean ± SEM and percentages. The level of significance between means was tested where appropriate by one way or repeated measure ANOVA followed by Dunnetts post hoc test and results were regarded as statistically significant from p≤0.05.

3. Results

The residual aqueous fraction significantly (p<0.05) and dose dependently reduced the number of abdominal writhes, at doses of 150, 300 and 600 mg/kg when compared with control group. The reduction was also significant (p<0.05) with ketoprofen (10 mg/kg) treated group as compared to the control (**Table 1**).

Also, the residual fraction (150, 300 and 600 mg/kg) and pentazocine (10 mg/kg), exhibited significant (p<0.05) increase in the latency of pain reaction time; the activity was at 30 and 60 minutes post treatment when each was compared with control group (**Table 2**).

In the carrageenan-induced paw oedema experiment, the fraction (300 and 600 mg/kg) and ketoprofen (10 mg/kg) exhibited significant (p<0.05) decrease in the paw volume at 1, 2, 3 and 4 hour intervals while at 150 the decrease was only significant (p<0.05) at 3 hour; when each was compared with the saline treated group. Percentage inhibition of the paw oedema at the doses of 150, 300 and 600 mg/k for first, second, third and fourth hour were; 22.2%, 38.9%, 45.5% and 18.8%; 70.0%, 73.3%, 70.5% and 42.5%; and 55.6%, 71.1%, 85.5% and 64.4%, each respectively (**Table 3**).

Table 1: Effect of Residual Aqueous Fraction (RAF) of *C. edulis* on Acetic acid-induced Writhes in Mice

Treatment	Dose (mg/kg)	Number of abdominal Writhes	Percentage Inhibition
Control	10 ml/kg	16.3 ± 1.28	-
RAF	150	12.7 ± 3.68	21.8
RAF	300	8.8± 1.11*	46.0
RAF	600	7.6 ± 1.71*	52.8
Ketoprofen	10	5.7 ± 1.76**	65.0

Values are presented as Mean ± SEM, n = 6 per group, RAF = Residual aqueous fraction of ethanol root bark extract of *C. edulis*, Control = Distilled water (10 ml/kg), Significant difference at **p<0.001, *p<0.01 (ANOVA) followed by Dunnetts post hoc test.

Table 2: Effect of Residual Aqueous Fraction (RAF) of *C. edulis* on Tail Immersion Test in Mice

Treatment	Dose (mg/kg)	Mean Pain Reaction Time (s)	
		30 minutes	60 minutes
Control	10 ml/kg	2.07± 0.36	1.69 ± 0.36
RAF	150	4.46 ± 1.88*	4.21 ± 0.86*
RAF	300	3.24 ± 0.14*	4.11 ± 0.99*
RAF	600	7.04 ± 2.25*	7.94 ± 2.54*
Pentazocine	10	4.31 ± 0.60*	5.87 ± 0.32**

Values are presented as Mean ± SEM, n = 6 per group, RAF = Residual aqueous fraction of ethanol root bark extract of *C. edulis*, Control = Distilled water (10 ml/kg), Significant difference at **p<0.001, *p<0.05(ANOVA) followed by post hoc test.

Table 3: Effect of Residual Aqueous Fraction (RAF) of *C. edulis* on Carrageenan-induced Paw Oedema in Rats

Treatment (mg/kg)	Mean Paw Diameter (cm) (Percentage Inhibition %)			
	1 hour	2 hour	3 hour	4 hour
Control	0.09 ± 0.01	0.18 ± 0.02	0.22 ± 0.02	0.16 ± 0.01
RAF (150)	0.07 ± 0.01 (22.2%)	0.11 ± 0.02 (38.9%)	0.12 ± 0.02** (45.5%)	0.13 ± 0.01 (18.8%)
RAF (300)	0.03 ± 0.01** (70.0%)	0.05 ± 0.01* (73.3%)	0.07 ± 0.01* (70.5%)	0.09 ± 0.01** (42.5%)
RAF (600)	0.04 ± 0.01** (55.6%)	0.05 ± 0.01* (71.1%)	0.03 ± 0.01* (85.5%)	0.06 ± 0.01* (64.4%)
Ketoprof (10)	0.04 ± 0.01** (52.2%)	0.08 ± 0.01* (56.7%)	0.07 ± 0.02* (66.8%)	0.08 ± 0.01** (51.9%)

Values are presented as Mean ± SEM, n = 6 per group, RAF = Residual aqueous fraction of ethanol root bark extract of *C. edulis*, DZ = Diazepam, Control = Distilled water (10 ml/kg), Significant difference at *p<0.05 (Repeated Measure ANOVA).

4.0 Discussion

The residual aqueous fraction (RAF) of the ethanol extract of *C. edulis* exhibited significant and dose dependent inhibition of the abdominal writhes thus, showed peripheral and central antinociceptive effects. The intraperitoneal administration of acetic acid produced both peripheral and central nociceptive action which acted through release of endogenous mediators and blocked by nonsteroidal anti-inflammatory drugs (Sudipta et al, 2013). Acetic acid releases prostaglandins and sympathomimetic system mediators like PGE_2 and $PGF_{2\alpha}$ and their levels were increased in the peritoneum fluid of the acetic acid-induced mice (Besra et al, 1996). The observed abdominal constrictions are said to involve local peritoneal receptors (Vongtau et al, 2000), and hence sensitized nociceptive receptors to prostaglandins. This finding corroborated the earlier result obtained using water crude extract of the same plant by Ibrahim et al (2007) where the extract showed analgesic activity for both chemically and mechanically induced pain.

Tail clip, tail flick, and tail immersion models are the well-established methods for measuring the central analgesic effects of drugs through opioid receptors (McCurdy and Scully, 2005). The brain and spinal cord play an important role in central pain mechanism. The dorsal part of the spinal cord is rich with substance P, endogenous opioids, somatostatin, and other inhibitory hormones which are the targets of pain and inflammation (McCurdy and Scully, 2005). The observed analgesic activity of RAF on tail immersion experiment could be via central pain pathways by probably modulating these endogenous substances in addition to opioid receptors. Thus, this finding confirmed earlier studies (Hassan et al, 2010; Ngulde et al, 2013; Gitahi et al, 2015).

The most widely used primary test to screen new anti-inflammatory agents measures the ability of a compound to reduce local oedema induced in the rat paw by injection of an irritant agent (Winter et al, 1962). Carrageenan-induced oedema has been commonly used as an experimental model for acute inflammation and is believed to be biphasic. The early phase (1-2 h) of the carrageenan model is mainly mediated by histamine, serotonin and increased synthesis of prostaglandins in the surrounding damaged tissues. On the other hand, the late phase is sustained by prostaglandins and mediated by bradykinin and leukotrienes, produced by tissue macrophages (Agbaje and Fageyinbo, 2012). Similarly, during the second phase, elevated production of other mediators has been suggested; oxygen-free radicals, inducible cyclooxygenase (COX-2) and the local infiltration and activation of neutrophils (Gepdiremen et al, 2004). Prostaglandins play a major role in the development of the second phase, which usually occurs after 3 hours (Ramirez et al, 2013). Therefore, the anti-inflammatory activity demonstrated by RAF at both phases indicated presence of some biologically active principles capable of modulating some of these pain mediators. Thus, having antihistaminergic and antiserotonergic activity in addition to inhibition of prostaglandins, bradykinins and leukotrienes synthesis. Also, Hassan et al (2010) found similar findings in saponins rich extract of the plant.

Phytochemical screening revealed the presence of flavonoids, saponins, tannins, anthraquinones and steroids in the RAF (Ya'u et al, 2015). These secondary metabolites have been reported to have different extents of analgesic and anti-inflammatory activities. Some flavonoids isolated from medicinal plants have shown analgesic activity mainly by inhibiting the key enzymes involved in prostaglandin biosynthesis (Kumbhare and Sivakumar, 2011). Also, flavonoids, saponins and steroids have been shown to possess analgesic and anti-inflammatory properties (Just et al, 1998). Therefore, the observed analgesic effects may be due some of these bioactive constituents.

5.0 Conclusion

Results of this study indicated that residual aqueous fraction of ethanol root bark extract possesses peripheral and central analgesic effect as well as anti-inflammatory activity. The fraction could therefore, offer potential benefit in the management of pain and inflammation.

Conflict of Interest declaration

The authors declare no conflict of interest.

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