

Original Research Article

Analgesic, anti-inflammatory and antipyretic activities of ethanol extract from *Ritchiea capparoides* leaf in rodents

ABSTRACT

Background: In Southern Nigeria, the leaf of *Ritchiea capparoides* is used traditionally in the treatment of pains, fever, malaria and snake bites. Therefore, this study evaluated the analgesic, anti-inflammatory and antipyretic activities of the ethanol extract from *Ritchiea capparoides* leaves in rodents.

Methods: The Analgesic effect was carried out using acetic acid and tail immersion models in mice, while anti-inflammatory and antipyretic activities were examined using xylene, egg-albumen, brewer's yeast and dinitrophenol models in mice and rats at doses of 125 mg/kg, 250 mg/kg and 500 mg/kg of the methanol extract. Phytochemical screening and oral acute toxicity tests were also carried out on the leaf extract of the plant.

Results: The methanol leaf extract and the standard drug (Aspirin) significantly decreased the number of writhes caused by acetic acid at $P < 0.05$ and $P < 0.01$. There was significant increase in reaction time in standard and extract groups of the tested agent. The extract produced significant $p < 0.05$ and $p < 0.01$ dose related inhibition of oedema which was comparable to aspirin in egg-albumin induced paw oedema model. *Ritchiea capparoides* leaf extract also demonstrated significant $p < 0.05$ and $p < 0.01$ effect in xylene induced mouse ear oedema test compared to dexamethasone. Statistical significant reduction in rectal temperatures were observed in both Brewer's yeast and dinitrophenol induced pyrexia in rats. The methanol leaf extract contain alkaloids, saponins, tannins, flavonoids, terpenoids, steroids and cardiac glycosides. The oral acute toxicity tests was found to be greater than 5000 mg/kg.

Conclusion: The results obtained showed potential analgesic, anti-inflammatory and antipyretic effects of the methanol leaf extract of *R. capparoides* at doses tested which support the claim for the traditional use of the plant in treatment of these disease conditions.

Keywords: *Ritchiea capparoides*; Leaf extract; Analgesic; anti-inflammatory; antipyretic

1. INTRODUCTION

Herbs have proven to be of high importance for prevention and treatment of diseases, and of high value to immunological provocation against much pathologic conditions [1]. Before synthetic drugs were produced, man was completely dependent on medicinal plants for treatment of different diseases [1]. Extracted substances from variety of these plants are commonly and widely used by traditional healers in Nigeria [2]. Constituents in many plants products have exhibited biological and pharmacological activities, which includes antiplasmodial, antidiabetics' anti-inflammatory, antipyretic, antiviral and many other effects [3]. Therefore, searching for novel pain relievers with analgesic, anti-inflammatory and antipyretic activities is a welcome idea [4].

Ritchiea capparoides, Family (Capparidaceae) is an evergreen climber but when alone it is a self-supporting shrub with compound palmate leaves. The leaves can be collected all – year round as the plant can stand dry season. The roots are tuberous and with strong pungent odour when perceived. Like other capparidaceae, this herb is indigenous to the tropics, found mostly in the lowland area of rain forest, especially beside water body and virgin up -lands. As a shrub it grows to a height of few meter(s) and as climber can grow a considerable length of about 5 meters with several branches. The plant is virtually all over the tropical land of Africa and particularly West Africa [5]. In Igboland it is called “Aka-ato or Nti-ato” [5]. The leaf extract exhibited antinociceptive activities in mice [6]. Ethnomedicinally, the root extract of *R. capparoides* are used as antihelminthic [7]. In the South-Western part of Nigeria, decoction of the leaves and root are widely used for the treatment of microbial and plasmodial infections [8, 9]. Its antifungal activity has also been reported [10]. *R. capparoides* leaf preparations has been used in ophthalmic condition, conjunctivitis, swellings, wounds and in the treatment of Guinea worm [11].

2. METHODS

2.1 Plant collection

Fresh leaves of *Ritchiea capparoides* were collected from the compound of Mazi Obasi Agwu in Ugoni Okposi-Okwu in Ohaozara LGA of Ebonyi State. This plant was identified and authenticated by a Taxonomist, Dr E.I. Aigbokhan of the Department of Botany, University of Benin, Ukwowo Campus, Benin City. The voucher number UBH-R443 was deposited in the Herbarium of the Department for future reference

2.2 Extraction of the leaf

The collected leaves were air-dried for fourteen days at room temperature, and the size was reduced with mortar and pestle. The powdered plant leaf (592 g) was soaked in 1.8 L of methanol for 24 hours. The mixture was sieved using a clean muslin sieve into a conical flask. The filtrate was dried on a water bath at a reduced temperature of 40 °C. The extract yield was stored in a refrigerator before the experiment.

2.3 Experimental animals

Male and female Wistar rats and mice respectively weighing 150-180 g and 20-25 g, were sourced from house of the department of Veterinary Medicine, University of Nigeria, Nsukka. The animals were allowed free access to standard feed and water *ad libitum*. They were kept six each in clean separate cages with saw dust as bedding, which was replaced every two days. The study was conducted according to ethical guidelines on laboratory animal use and care in compliance with Faculty of Basic Clinical Sciences, Nnamdi Azikiwe University Research Policy.

2.4 Phytochemical screening

Phytochemical screening of the extract was carried out using simple chemical tests to detect the presence or absence of secondary constituents as detailed in the literature [12].

2.5 Acute toxicity test

Acute toxicity (LD₅₀) of the extract was determined using Lorke [13] method. This study was in two phases and the mice used were deprived of food overnight before extract administration. In phase 1, three groups of three mice per cage were orally administered the extract in increasing

doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. The mice treated were monitored for the first 4 h and 24 h for signs of toxicity and mortality. With the absence of death after 24 hrs, phase 2 was introduced. Four groups of one mouse were each given the extract orally at doses of 1600 mg/kg, 2900 mg/kg, 5000 mg/kg and 10 mL/kg distilled water. The animals were then observed for signs of toxicity and mortality for 24 h, 48 h and 72 h respectively for late toxicity.

2.6 Analgesic activity

2.6.1 Acetic acid induced writhing in mice

The methanol leaf extract was assessed for analgesic activity using acetic acid-induced writhing method as described by Essien et al., [14]. Albino mice (20-25 g) of both sex randomized into 5 different cages of 6 mice in each cage. Group 1 (drug free) was given normal saline (10 mL/kg), the extract (125 mg/kg, 250 mg/kg and 500 mg/kg) were administered to groups 2-4, accordingly. The positive control received (150 mg/kg) of acetyl salicylic acid (ASA). After thirty minutes, intraperitoneal injection of acetic acid (10 ml/kg, of 0.7%) was administered to each mouse. They were separately placed in a transparent cage for observation. The writhing movements for each mouse was counted for 30 minutes.

2.6.2 Tail immersion test in mice

The method described by Akuodor et al. [15] was used for this study. Thirty randomly selected adult albino mice of both sexes were divided into 5 groups of 6 mice in each cage and were subjected to 24 h fast, but free access to water. Control groups 1 and 5 were treated orally with 10 mL/kg of distilled water and subcutaneously, with 10 mg/kg of morphine, respectively; While groups 2, 3 and 4 received orally, 125, 250 and 500 mg/kg of *R.capparoides* respectively. Thirty minutes after treatment, each mouse was placed in the restrainer cage (Grieve Cooperation, Illinois, U.S.A.), leaving the tail hung out and freely exposed to be dipped in a water bath that was maintained thermo-statistically at $51 \pm 1^\circ\text{C}$. The duration of stay (latency) of the tail in the hot-water bath before the animal withdrew its tail out of the water was recorded. The latency was evaluated at 30, 60, 90 and 120 min.

2.7 Anti-inflammatory activity

2.7.1 Egg-albumin-induced inflammation

Method described by Essien et al [16] was used for this study. Thirty adult Wistar rats of both sex were randomly selected and grouped into 5 with 6 rats per cage. Group 1 received orally, 10 mL/kg of distilled water, while group 2 received 150 mg/kg of aspirin orally. Groups 3, 4 and 5 received orally, 150 mg/kg, 250 mg/kg and 500 mg/kg of *R.capparoides* respectively. After 30 min treatment, the initial volume (size) of each rat's right hind paw was measured and recorded. Thereafter, inflammation was induced in rats by injecting subcutaneously, 0.2 mL of fresh egg-albumin into the sub-plantar of the right hind paw. Assessment of oedema volume using plethysmometer was thereafter measured at 30 min intervals for 120 min.

2.7.2 Xylene induced ear oedema method

This method as described by Akuodor et al., [17] was adopted for the study. Mice recruited were grouped into 5 with 6 animals in each. The animals were orally treated with the leaf extract in graded doses of 125 mg/kg, 250 mg/kg and 500 mg/kg. The negative control group was treated with 10 mL/kg of distilled water, while the positive control was treated with 4 mg/kg of dexamethasone. Oedema was induced in each mouse one hour after treatment with one drop xylene into the inner surface of the right ear. Three hour later, mice were sacrificed and both cut-off to equal size and weighed. The mean difference between the right and left ears were recorded as an indication of inflammation.

2.8 Antipyretic activity

2.8.1 Brewer yeast induced pyrexia

The antipyretic activity was evaluated using Brewer yeast induced pyrexia in rats as described [18]. Hyperthermia was subcutaneously induced by administering 20 mL/kg of 20% aqueous suspension of Brewer yeast in distilled water 24 hours before treatment. Thirty Wistar rats of both sex were divided into five groups. Groups 1 and 2 served as negative control (distilled water 10 mL/kg), and positive control (ASA 150 mg/kg), while groups 3, 4, and 5 received 125 mg/kg, 250 mg/kg, and 500 mg/kg of the extract. All drugs were administered orally. Rectal

temperatures were taken by the use of digital thermometer (Mediklin, China) before yeast injection, 24 h after the injection, and at 1, 2, 3, 4 and 5 hours after drug administration.

2.8.2 D-amphetamine induced pyrexia test

The antipyretic activities of the secondary metabolites of the ethanol leaf extract of *R.capparoides* was screened using D-amphetamine induced pyrexia method [19]. The animals used (wistar rats) of both sex were subjected to 24 hours fasting. The initial temperatures of the selected rats were recorded. The rats were grouped into 5 and 6 rats in each cage. They were each given 5mg/kg of D-amphetamine intraperitoneal in order to induce pyrexia. 24 hours after, the temperatures of the animals were noted for increase and any rat with temperature less than 0.6°C was avoided. Group 1 and 2 were negative (10 mL/kg distilled water) and positive control (ASA 150 mg/kg) respectively. Whereas, group 3, 4 and 4 received orally, 125 mg/kg, 250mg/kg and 500mg/kg of *R.capparoides* methanol leaf extract.

2.9 Statistical analysis

Results are presented as mean±standard error of mean (SEM) and analyzed with statistical package for social sciences (SPSS version 20) using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. Difference in the mean $p < 0.05$ was statistically considered significant.

3. RESULTS

3.1 Phytochemical analysis

The phytochemical screening of methanol leaf extract of *Ritchiea capparoides* revealed the presence of alkaloids, saponins, tannins, flavonoids, terpenoids, steroids, cardiac glycosides, resins and balsam

3.2 Acute toxicity studies

There was no observed changes, mortality or signs of toxicity 72 hours after administration of the methanol leaf extract. The animals were all healthy and active throughout the study. Hence, the median lethal dose (LD50) was found to be greater than 5000 mg/kg.

2.3 Effect of methanol leaf extract of *R.capparoides* on acetic acid induced writhing in mice

The leaf extract of *R.capparoides* significantly and dose dependently decreased the number of acetic-acid induced writhing in mice at $p<0.05$ and $p<0.01$ respectively. The observed effects of the extract at 500 mg/kg was higher than that of 125 mg/kg and 250 mg/kg. This effect was comparable to that of the standard drug (Table 1).

2.4 Effect of methanol leaf extract of *R.capparoides* on Tail immersion test in mice

The methanol leaf extract of *R. capparoides* significantly at ($p<0.05$ and $p<0.01$) reduced the thermal stimuli in mice. In this test, dose dependent reduction was produced by the extract. However, morphine the standard drug showed stronger protection (Table 2).

2.5 Effect of methanol leaf extract of *R. capparoides* on egg-albumen induced paw oedema in rats

The anti-inflammatory activity of *R.capparoides* methanol leaf extract was observed to be dose dependent. There was significant activity at $p<0.05$ with 125 mg/kg and 250 mg/kg doses of the extract, whereas the highest dose of the extract (500 mg/kg) had $p<0.01$ significant activity. The reference drug (ASA) had more activity in this study (Table 3). The anti-inflammatory activity began at 1 hour after administration of drug and lasted for 5 hours before completely vanishing.

2.6 Effect of methanol leaf extract of *R.capparoides* on xylene induced ear oedema in mice

The anti-inflammatory effect of *R.capparoides* methanol leaf extract against xylene induced ear oedema in mice is shown in Table 4. The extract exhibited significant and dose dependent activity reduction of oedema at ($p < 0.01$) with the highest dose of the extract comparable to the standard drug, dexamethasone.

2.7 Effect of methanol leaf extract of *R. capparoides* on Brewer yeast induced pyrexia in rats

Antipyretic studies Table 5 shows the antipyretic effect of the methanol leaf extract of *R. capparoides* determined using Brewer's yeast induced pyrexia in rats. The extract exerted significant and dose dependent antipyretic action at $p < 0.01$, whereas the standard drug showed significant antipyretic activity at $p < 0.01$.

2.8 Effect of the extract on Dinitrophenol – induced pyrexia in rats

The results of the effect of methanol leaf extract of *R.capparoides* against D-amphetamine induced pyrexia is shown in Table 6. There was a progressive dose dependent reduction at $p < 0.05$ in the temperature of rats treated with the methanol extract. The effect of the extract was less than the standard drug, acetylsalicylic acid (ASA)

Table 1: Effect of methanol leaf extract of *R. capparoides* on acetic acid-induced writhing in mice.

Treatment	Dose(mg/kg)	Mean no of writhes	% Inhibition
Distilled water	20 mL/kg	103.67±3.87	-
<i>R. capparoides</i> 125		30.83±2.71	70a
<i>R. capparoides</i> 250		13.33±1.82	87b
<i>R. capparoides</i> 500		5.83±3.80	94b
Aspirin	150	3.50±1.91	97b

Results are mean±SEM; (n=6); ^aP<0.05; ^bP<0.01 compared to control

Table 2: Effect of methanol leaf extract of *R. capparoides* on tail immersion in mice time interval

		Time (min).				
		Pre-treatment		After-treatment		
Treatment	Dose mg/kg	0	30	60	90	120
Distilled water	20 mL/kg	10.17 ± 0.45	10.00 ± 0.58	10.67 ± 0.33	10.55 ± 0.22	10.50 ± 0.20
<i>R. capparoides</i>	125	6.50 ± 0.43	8.50 ± 0.50	10.83 ± 0.83	11.67 ± 0.71	13.17 ± 0.60 ^a
<i>R. capparoides</i>	250	7.50 ± 0.76	9.33 ± 0.76	11.83 ± 0.70	13.50 ± 0.56	16.80 ± 0.68 ^b
<i>R. capparoides</i>	500	8.00 ± 0.52	10.17 ± 0.79	12.50 ± 0.88	14.67 ± 0.56	17.50 ± 0.34 ^b
Morphine	10	7.50 ± 0.62	17.50 ± 0.72	19.17 ± 0.60	21.17 ± 0.87	23.33 ± 0.95 ^b

Results are mean ± SEM; (n=6); ^aP < 0.05; ^bP < 0.01 compared to control

Table 3: Effect of methanol leaf extract of *R. capparoides* on xylene-induced ear oedema in mice.

Treatment	Dose (mg/kg)	Weight of right ear	Weight of left ear	Increase in weight	% Inhibition
Distilled water	20mL/kg	0.042±0.01	0.021±0.03	0.021±0.00	-
<i>R. capparoides</i>	125	0.036±0.05	0.019±0.02	0.017±0.00	55 ^a
<i>R. capparoides</i>	250	0.030±0.04	0.017±0.03	0.017±0.00	60 ^b
<i>R. capparoides</i>	5000.	0.029±0.04	0.016±0.03	0.013±0.00	69 ^a
Dexamethasone	4	0.022±0.03	0.012±0.03	0.010±0.00	76 ^b

Results are mean±SEM; (n=6); ^aP<0.05; ^bP<0.01 compared to control

Table 4: The effect of ethanol leaf extract of *R. capparoides* on Egg-albumin-induced paws oedema in rats.

Time(min)									
Drug	Dose (mg/kg)	0	20	40	60	80	100	120	
Distilled water	10mL/kg		1.24±0.02	1.64±0.01	1.72±0.02	1.79±0.02	1.86±0.02	1.93±0.02	2.05±0.02
<i>R. capparoides</i>	125	1.22±0.04	1.60±0.02	1.53±0.03	1.46±0.02	1.38±0.03	1.31±0.02	1.20±0.03a	
<i>R. capparoides</i>	250	1.24±0.02	1.63±0.01	1.56±0.02	1.48±0.02	1.30±0.02	1.24±0.02	1.16±0.02b	
<i>R. capparoides</i>	500	1.19±0.02	1.57±0.03	1.49±0.03	1.38±0.02	1.30±0.02	1.21±0.03	1.15±0.03b	
Aspirin	150	1.17±0.04	1.61±0.02	1.49±0.02	1.39±0.03	1.30±0.03	1.22±0.03	1.12±0.03b	

Results are mean ±SEM; (n=6) ^aP< 0.05; ^bP< 0.01 compared to control

Table 5: Effect of methanol leaf extract of *R. capparoides* against yeast induced pyrexia in rats.

Treatment	Dos(mg/kg)	Yeast induction (h)					Drug administration (h)								
		0	24	1	23	4	5	0	24	1	23	4	5		
D. water	20 ml/kg	35.37±0.05	37.52±0.04	37.80±0.02	37.63±0.02	37.42±0.02	37.29±0.02	37.30±0.03							
<i>R. capparoides</i>	150	35.26±0.03	37.25±0.03	36.41±0.01	36.20±0.02	35.62±0.03	35.37±0.03	35.24±0.02a							
<i>R. capparoides</i>	250	35.25±0.02	37.27±0.02	36.50±0.03	36.22±0.01	35.61±0.02	35.36±0.03	35.22±0.03a							
<i>R. capparoides</i>	500	35.23±0.02	37.30±0.02	36.47±0.02	36.15±0.01	35.55±0.03	35.30±0.02	35.10±0.03a							
Aspirin	150	35.22±0.00	36.79±0.02	35.69±0.04	35.43±0.02	35.40±0.01	34.60±0.02	34.30±0.03b							

Results are mean±SEM; (N=6) ^aP<0.05; ^bP<0.01 when compared to control.

Table 6: Effect of methanol leaf extract of *R. capparoides* on de-amphetamine induced pyrexia in rats (hours).

	D-amphetamine induction (h)					Drug administration(h)		
Treatment Dose (mg/kg)	0	24	1	2	3	4	5	
D. water 20 ml	35.25±0.04	37.39±0.04	37.61±0.04	37.67±0.02	37.46±0.05	37.26±0.03	37.70±0.03	
<i>R.capparoides</i> 150	35.27±0.04	37.29±0.02	36.43±0.03	36.23±0.03	35.51±0.02	35.30±0.02	35.25±0.02	a
<i>R.capparoides</i> 250	35.20±0.03	37.30±0.02	36.40±0.02	36.23±0.02	35.52±0.02	35.31±0.02	35.23±0.03	a
<i>R.capparoides</i> 500	35.24±0.02	37.30±0.02	36.33±0.02	36.20±0.03	35.48±0.02	35.26±0.02	35.21±0.01	a
Aspirin 150	35.25±0.03	37.31±0.02	36.39±0.03	36.22±0.03	35.49±0.01	35.31±0.01	34.17±0.01	b

Results are mean±SEM; (N=6) ^aP< 0.05; ^bP<0.01 when compared to control

4. DISCUSSION

This study was carried out to establish the potential pharmacological properties of methanol extract of *R.capparoides* based on claims of its use in herbal medicine. The findings of the present study reveal that *R.capparoides* at doses employed exhibited analgesic effects against chemical pains (writhing) induced [20]. Another report revealed the response of mice to acetic acid as a fast and trusted method to test peripheral analgesic effect of herbal agents [14]. In the present work, the extracts of *R. capparoides* and acetylsalicylic acid (aspirin) inhibited acetic acid induced writhing. Results shows the extract has peripheral analgesic properties which suggest the action may be directed via inhibition of local peritoneal receptors [21]. However, irrespective of whether this model evaluates peripheral pain relieving action only or otherwise, the results validates the usefulness of this plant as an analgesic in Nigeria. The injection of acetic acid is reported to induce the release of mediators of pain such as prostaglandins and other cyclokinase [22]. This activity suggests that the extracts might have acted by inhibiting the actions of cyclooxygenase responsible for producing prostaglandins from arachidonic acids [22]. The analgesic effects produced by *R.capparoides* validate their use in traditional medical practice as analgesics being used by local population in Nigeria.

More so, centrally acting model of analgesia (tail immersion) was carried out to confirm the analgesic activity of this leaf extract. This method of assay used to indicate the involvement of central analgesic mechanism is believed to involve spinal reflex [23, 15]. Reported has it that centrally acting agents like morphine, possess this activity in both types of study, whereas peripherally acting agents such as acetylsalicylic acid has been reported to exert analgesic action only in the writhing test [24]. Most importantly, the action of acetylsalicylic acid in writhing assay only could be linked to directly inhibit prostaglandin activity or indirectly inhibit prostaglandin secretion by halting cyclo-oxygenase activity [25]. The essential activity in tail immersion test shows involvement of central analgesic mechanism.

Egg-albumin induced paw oedema has proven to be a suitable way of testing anti-inflammatory agents and has widely been used for screening anti-oedematous action of natural products [26]. This process of testing acute inflammation potency is a highly sensitive tool [27]. The development of oedema which depend on the presence of bradykinin and polymorphonuclear leucocytes with proinflammatory factor like prostaglandins [28]. The leaf extract of *R.capparoides* may not have shown activity on the early phase of inflammation, hence may act by inhibiting the release of prostaglandins. Nonsteroidal anti-inflammatory agents like aspirin, may not inhibit the initial phase of edema induced by egg-albumen whereas the second accelerating phase can be antagonized by the drug [29].

Xylene causes irritation in mouse ear leading to accumulation of fluid and oedema and increase in myeloperoxidase enzymatic activity. Suppression of this response may suggest antiphlogistic activity [15]. The methanol extract of *R.capparoides* leaf exerted significant inhibition of ear oedema in mice. This activity suggests the inhibition of phospholipase A2 which has been involved in the pathophysiology of inflammation resulting from xylene [31]. However, dexamethasone used as the reference drug exhibited significant decrease in the ear weight of positive control rats which indicate an inhibition of PLA2.

Antipyretic agents have been shown to antagonize cyclooxygenase activity through increase in prostaglandin E2 by suppressing high temperature [31]. Increase in temperature may result from damaged tissue, infections, and other factors. This process usually give rise to mediators (interleukins and others), which progresses to prostaglandin E2 formation with increase body temperature [32]. The extract under study reduced rats' anal temperature whose effect was similar to that observed in aspirin. The methanol leaf extract of *R.capparoides* was able to bring fever to a control by getting rid of inflammatory symptoms at both peripheral and nervous system thermoregulator zones. This could bring down pyrogenic secreting cytokines while reducing prostaglandin E2 synthesis from cyclooxygenase possibly through the mechanism reputed for paracetamol [33].

The therapeutic potentials of medicinal plants are mostly attributed to the combination of their secondary metabolites. Flavonoids have been reported to target prostaglandins involved in late phase of acute inflammation and pain and they have therefore been associated with analgesic, anti-inflammatory and antipyretic activities [34, 35]. Therefore, it is not surprising to have seen

these activities in *R.capparoides* leaf extract. There was no lethality observed during the LD50 test which proves the relative safety of the herbal agent.

5. CONCLUSION

The results of present study show that the methanol leaf extract of *R.capparoides* possess analgesic, anti-inflammatory and antipyretic activities, thus confirming the folklore uses of the plant for the treatment of these diseases conditions.

UNDER PEER REVIEW

Ethical approval

The experimental procedures were performed in accordance with Faculty of Basic Clinical Sciences, Nnamdi Azikiwe University, Nnewi Campus Ethics Committee and Guide for Care and Use of Laboratory Animals by The U.S. National Institute of Health (Publication number 85, revised 1996).

UNDER PEER REVIEW

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