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Analgesic and anti-inflammatory activities of Saponin fraction of aqueous extract of *Caralluma dalzielii* N. E. Brown in Wistar rats

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Abstract

Background: *Caralluma dalzielii* N. E. Brown (*Asclepiadaceae*) is a cactus-like plant found mainly in the North-western Nigeria. Scientific research has revealed its anti-inflammatory and analgesic properties making it a potential lead for newer analgesic medication. The increasing demand for natural products and numerous biological activities of saponins have made it a commercially significant compound with more applications in pharmaceutical industries. This study was conducted to evaluate the anti-inflammatory and analgesic properties of the saponin-rich fraction of the aqueous extract of *Caralluma dalzielii* in rats.

Materials and methods: Aqueous extract of *Caralluma dalzielii* was prepared by cold maceration. Saponin fraction was obtained following standard procedures. Oral acute toxicity study was carried out on the fraction using the up and down method of acute toxicity testing at a limit dose of 2000 mg/kg. The analgesic activity was tested using tail-flick model and the anti-inflammatory activity was evaluated using formalin-induced paw oedema in rats.

Results: At 2000 mg/kg the fraction produced no mortality or obvious toxicity signs in the rats. The inhibition of oedema in the formalin test was significantly ($p<0.05$) increased with increase in dose of the fraction. The difference became more significant within the first two hours with the exception of 100 mg/kg dose in which the inhibition was not consistent. The saponin-rich fraction of the extract showed significant analgesic effect by markedly prolonging tail-flick reaction time.

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Conclusion: This study has shown that the saponin-rich fraction of *Caralluma dalzielii* with LD_{50} greater than 2000 mg/kg, possesses potent analgesic and anti-inflammatory activities.

Keywords: *Caralluma dalzielii*, Saponins, Tail-flick, Anti-inflammatory, Analgesic

Introduction

Several medicinal plants have been used for the treatment of diseases including pain and inflammatory conditions. They have been identified and used throughout human history for curative and preventive medical therapy for human beings and animals (1). About 80% of the world's population, especially the developing countries, depends on traditional medicine and their products for primary healthcare needs (2). These medicinal plants synthesise and accumulate secondary metabolites, like saponins, flavonoids, alkaloids, tannins among others. These secondary metabolites possess important therapeutic properties that are beneficial to humans and animals (3). Scientific validation of the folkloric claims of these medicinal plants will provide basis for the preservation of tropical medicinal resources, the deployment of the beneficial ones as phytomedicine and the development of potential bioactive constituents as novel lead compounds/precursors in drug design (4). One of such phytochemical constituents is saponins.

Saponins are a heterogeneous group of naturally occurring surface active glycosides that are produced mostly by plants, lower marine animals and some bacteria (5). They are called 'saponins' because like soap they possess foaming properties when mixed with water (6). Saponins are made up of triterpenoid/steroid aglycone moiety and complex oligosaccharide substituent. They are characterised based on the number of sugar moiety that they contain (3). The increasing demand for natural products and several biological activities of saponins have made it a commercially significant compound with more applications in food, cosmetics and pharmaceutical industries (7, 8). Information on the biological activities of saponins from variety of sources provide lead for the development and design of new drugs. Many saponins that have been tested have shown analgesic, anti-inflammatory and antipyretic activities (9).

Inflammation is one of the complex biological responses of vascular tissues to harmful stimuli (8). It is characterized by pain of all kind, redness, swellings and loss of joint function. Regarding

visceral pain, it is considered the most common pain produced by diseases and one of the most common reasons for medical consultation (10). Current treatment for pain and Inflammation include the use of NSAIDs amongst others. Unfortunately, these medications cause side effects which include increased risk of blood clot resulting in heart attacks and strokes. Hence, the development of potent analgesic and anti-inflammatory drugs from the natural products is of paramount importance.

Caralluma dalzielii N.E. Br. (family Asclepiadaceae) is a cactus-like shape plant that grows in the Sahel region of Africa. It has a 5-merous flower type a characteristic which serve to easily identify this species (11). It is commonly called the mosque reed and has 'Karan masallanci' as its Hausa traditional name. Previous studies have reported the antinociceptive and anti-I nflammatory activity of the plant (12). Phytochemical analysis of the plant has identified several phytochemicals present in the plant of which saponins is one of them (13). Due to the numerous biological activities associated with saponins attention has been channelled to their characterization and investigation of their biological and pharmacological activities (3, 14). This study was therefore to evaluate the analgesic and anti-inflammatory activities of the saponin-rich fraction of *Caralluma dalzielii* aqueous extract.

Materials and Methods

Plant collection, identification and preparation

The plant was collected from Sokoto North local government area of Sokoto State in the month of June. The plant was identified at the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University, Sokoto, and authenticated by Dr. Halilu Mshelia. A voucher specimen (PCG/ UDUS/BURS/0001) was deposited at the same Department. The plant was air dried to constant weight for a period of 6 weeks and pulverised using mortar and pestle. Two hundred grams (200 g) of the powdered plant material was macerated in 2 L of distilled water at room temperature for 24 h. The mixture was filtered through a Whatman filter paper No 1 and concentrated to dryness in a hot air oven set at 45°C to obtain the extract of *Caralluma dalzielii.* The percentage yield was calculated as follows:

% yield = W_2/W_1 x 100
W_2 = weight of the extract in grams
W_1 = weight of plant material in grams

To obtain the saponin-rich fraction for the study, the extract was dissolved in distilled water at room temperature and then defatted by addition of n-hexane and allowed to stand for 24 h (15). The two different layers formed were collected separately. After that, hydroalcoholic solution (70:30 methanol to water) was added to the water residue. Polar compounds were further removed by dissolving the hydroalcoholic extract in diethyl ether solution and allowed to stand for another 24 h, the two distinct layers formed were also collected separately. Butanol was added to the water residue and the mixture shaken vigorously and allowed to stand for 24 h. The two distinct layers formed were then separated; the butanol fraction contained saponins. The presence of saponins was confirmed by adding water to a little portion of the butanol fraction containing the solutions and agitating for 15 s. The development of persistent foam which lasts more than 15 min indicated the presence of the saponins (16).

Experimental animals

Wistar rats of both sex (150-180 g) were used for the experiment. The animals were obtained from the animal house of Faculty of Pharmaceutical Sciences Usmanu Danfodiyo University Sokoto. Animals were fed with standard feeds and had access to drinking water *ad libitum*. Housing conditions were maintained at 25 ± 2 °C. The care and handling of the animals were according to the established public health guidelines in guide for care and use of laboratory animals (17).

Acute toxicity test

The oral acute toxicity testing of the saponin-rich fraction was carried out using the 'Up-and- Down' method of testing in rats at single dose of 2000 mg/kg in accordance with the Organization for Economic Development (OECD) 425 guidelines (18). Five female rats were used in the study. An animal was picked at a time, weighed and dosed orally with the equivalent volume of extract dissolved in distilled water using gastric feeding tube. Each animal was observed after dosing for the first 5 min for signs of regurgitation and kept in a metallic cage. Each was then observed every 15 min in the first 4 h after dosing, every 30 min for 6 h and daily for 48 h for behavioural signs of toxicity. The animals were afterwards monitored for 14 days for possible Jlong-term lethal outcome.

Anti-inflammatory studies

The formalin (2.5%) induced inflammation model was used in this study (19). Animals were divided into 5 groups of 5 rats each. Thirty minutes before the injection of formalin, the first group received distilled water (1 ml/kg), groups 2-4 received 25, 50 and 100 mg/kg intraperitoneally (i.p) of the extract respectively. The fifth group received 10 mg/kg i.p of piroxicam. The increase in paw diameter was measured using vernier calliper. The difference in the weight of the right hind paw and the left hind paw indicates inflammation. Measurement was done immediately before and after at hourly intervals for 5 hours following formalin injection.

Tail-flick test

In the tail-flick test, before treatment, about 2 cm of each rat's tail was immersed in a beaker of hot water maintained at 55± 1° C using a thermo-regulated hot plate. Tail-flick latency was determined by recording the time between the onset of stimulation and tail withdrawal. Twenty-five rats that showed response within 0–4 s were selected and allotted into five groups of 5 animals each for the study. Group 1 received normal saline

(1 ml/kg i.p.), groups 2–4 received 25, 50 and 100 mg/kg i.p. of the saponin-rich fraction respectively. Group 5 received 10 mg/kg i.p. of piroxicam. The reaction was recorded at 30 (early phase) and 60 (late phase) minutes (12). Cut-off time was placed at 20 s to reduce tissue damage.

Statistical analysis

The results were presented as the mean ± S.E.M. The results were analysed using GraphPad Prism version 6 software. Comparison in all the groups was made using one-way analysis of variance (ANOVA) followed by Student's T-tests. Differences were considered to be significant at $p<0.05$

Results

Extract yield

The yield (w/w) of the aqueous extract of *Caralluma dalzielii* was calculated to be 15.5%.

Acute toxicity screening

A single oral administration of the saponin-rich fraction at 2000 mg/kg did not produce any sign of toxicity and no mortality occurred within 24 h after administration and after 14 days of observation.

Effect of saponin-rich fraction of *Caralluma dalzielii* on inflammatory activity.

The anti-inflammatory activities of the saponin-rich fraction of *Caralluma dalzielii* at 50 and 100 mg/kg were significantly ($p<0.05$) increased when compared to the control (Table 1). At 50 mg/kg the anti-inflammatory activity of the fraction was also significantly increased at the fifth hour similar to that of piroxicam.

Table 1: Anti-inflammatory activity of Saponin-rich fraction in formalin-induced paw oedema model

Treatment	1h	2h	3h	4h	5h
Distilled water	2.05±0.31	1.72±0.23	1.21±0.27	1.16±0.25	0.95±0.23
25mg/kg	1.57±0.34	1.1±0.42	1.3±0.36	0.91±0.23	0.64±0.18
50mg/kg	1.23±0.34*	0.85±0.22*	0.91±0.27	0.74±0.11	0.46±0.06*
100mg/kg	0.77±0.18*	0.95±0.11*	1.09±0.29	1.04±0.28	0.81±0.25
Piroxicam	1.23±0.24*	0.92±0.35*	0.10±0.22	0.67±0.15	0.59±0.15*

Data presented as mean ± SEM, n=5 for all group. *Significantly different from the control at $p<0.05$

Effect of Saponins-rich fraction of *Caralluma dalzielii* on tail-flick test

The saponin-rich fraction of *Caralluma dalzielii* at 25 mg/kg produced a significant ($p<0.05$) increase in the tail-flick reaction time in both the early (Figure 1) and the late phase (Figure 2) of the reaction time. The highest dose did not produce any significant ($p<0.05$) effect in the tail-flick time on both phases when compared to the control (Figure 1 and Figure 2).

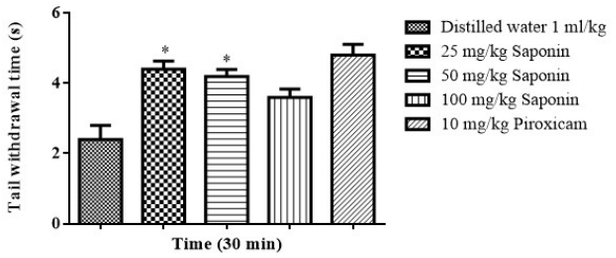

Figure 1. Effect of Saponins-rich fraction of *Caralluma dalzielii* on early phase of tail-flick test. Data presented as mean ± SEM, n=5 for all group. *Significantly different from the control at $p<0.05$

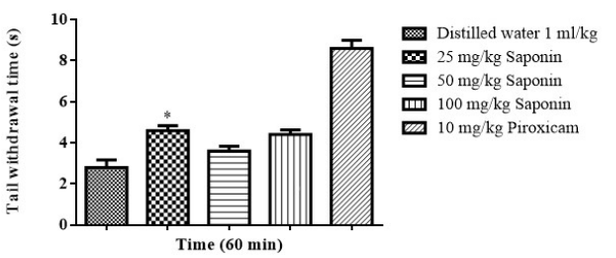

Figure 2. Effect of Saponins-rich fraction of *Caralluma dalzielii* on late phase of tail-flick test. Data presented as mean ± SEM, n=5 for all group. *Significantly different from the control at $p<0.05$

Discussion

The aqueous extract of *Caralluma dalzielii* has been used traditionally for the treatment of pain conditions of different forms. In this study, we investigated the anti-inflammatory and analgesic properties of the saponin-rich fraction of the plant. First, the anti-inflammatory effect of the saponin-rich fraction was determined. The Formalin-induced oedema model was used in the anti-inflammatory studies to confirm peripheral anti-inflammatory activity of the fraction and to evaluate its acute anti-inflammatory activity (20). Generally, the development of oedema in the hind paw following the injection of formalin is known to be biphasic (21). The first phase (0 to 2 h after the injection) is assumed to be due to the release of pro-inflammatory substances such as the bradykinin, histamine and 5-hydroxytryptamine from damaged neighbouring tissues (22). The second is due to enhanced production of prostaglandins. The saponin rich fraction of the plant exerted a dose dependent inhibition of the oedema, at a dose of 25 mg/kg and 50 mg/kg, from the first hour to the fifth, in the hind paw of the rats, while at the dose of 100 mg/kg the effect was not consistent. This could imply that 50 mg/kg was the optimum dose for the anti-inflammatory effect is observed. This also suggests that the underlying mechanism of the anti-inflammatory activity of this extract may involve inhibition of the release of both the pro-inflammatory and the inflammatory mediators. Further studies

are however needed to confirm that. The result however is in contrast with the result from the crude aqueous extract where activity was seen even at 100 mg/kg (12). This could be because of the presence of other secondary metabolites in the crude that may be synergistic in activity.

Next, we investigated the analgesic properties of the saponin-rich fraction of *Caralluma dalzielii*. A thermal nociception model such as tail-flick test was used to elucidate the analgesic activity of this fraction. Piroxicam a non-steroidal anti-inflammatory drug at 10 mg/kg exhibited more inhibitory effects in both the early and the late phases which is typical of cyclooxygenase inhibitor (23) than the fraction. It is a well-established fact that NSAIDs exert their analgesic and anti-inflammatory activity by the inhibition of COX (24).Our fraction at the lower dose acted in a similar manner suggesting cyclooxygenase inhibitor mechanism. The activity at the lower dose could mean that the fraction had reached its optimal dose where any further increase in dose may result in lower activity. Lower doses may be utilise in further studies to confirm this.

The result of the acute toxicity showed that the LD_{50} was greater than 2000 mg/kg. This according to OECD criteria under its Globally Harmonised Classification System (GHS) for chemical substances and mixtures, states that substances with LD_{50} > 2000 mg/kg may be safe (18). This suggests that the saponin-rich fraction of *Caralluma dalzielii* may be safe.

Conclusion

This study has shown that the saponin-rich fraction of *Caralluma dalzielii* possesses potent anti-inflammatory and analgesic activities. These results confirm the potential interest in *Caralluma dalzielii* as a medicinal plant and open the way for future research focusing on the active fractions. It also has LD_{50} greater than 2000 mg/kg.

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Conflicts of interest

The authors declare no conflicts of interest.

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