Studies on the Analgesic and Anti-inflammatory Properties of Hydro-alcohol Extract of *Caralluma dalzielii* N.E.Br (Asclepiadaceae) in Rats and Mice

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Abstract: The analgesic and anti-inflammatory potentials of the hydro-alcohol extract of *Caralluma dalzielii* N.E.Br, a Nigerian traditional medicinal plant was studied using thermal and chemical-induced pain models and carrageenan-induced acute inflammation. The acute toxicity and the phytochemical constituents of the extract were also determined. The results showed that the extract at a dose of 80 mg/kg significantly ($p<0.05$) prolonged the pain reaction times in the hot-plate pain model at 30 and 60 min and reduced acetic acid-induced writhing in mice and decrease pain score in formalin induced pain in rats at all doses tested. The hydro-alcohol extract of *Caralluma dalzielii* N.E.Br demonstrated significant ($p<0.05$) anti-inflammatory activity against acute inflammation induced by carrageenan. The estimated LD_{50} of the extract was found to be 288.53 mg/kg. Phytochemical analysis revealed the presence of tannins, saponins, anthraquinones, glycosides and flavonoids. These findings indicate that the hydro-alcohol extract of *Caralluma dalzielii* has analgesic and anti-inflammatory properties and could be beneficial in alleviating painful inflammatory conditions

Keywords: Acetic acid-induced abdominal constriction, analgesic, anti-inflammatory, *Caralluma dalzielii*

INTRODUCTION

It has been a well-accepted concept that pain, whether acute or chronic, peripheral or central, originates from inflammation and the inflammatory response (Omoigui, 2007). The release of cyclooxygenase products and sympathomimetic amines which are the final mediators of inflammatory hyperalgesia is preceded by the generation of cytokines by resident macrophages (Ribeiro *et al*., 2000).

Medicinal plants and traditional healthcare practices are important in providing clues to new areas of research and biodiversity conservation. It has been widely observed that medicinal plants are used in most developing countries as a normative basis for the maintenance of good health (Ranjan *et al*., 2010). Screening of natural sources such as plant extracts has led to the discovery of many clinically useful drugs that play important roles in the treatment of human diseases (Kumar *et al*., 2009). Many medicinal plants are used in developing countries for the management of pain and inflammatory conditions. Scientific proof of the folkloric claims of these medicinal plants through research will provide basis for the conservation of tropical medicinal resources. These could provide useful compounds in drug development and testing processes (Musa *et al*., 2009).

Caralluma dalzielii N.E.Br (Family: Asclepiadaceae) is a succulent herb occurring wild on the sahelian region of West Africa, from Senegal to North Western Nigeria and also in the Sahara region. It is used in folk medicine as antispasmodic and analgesic remedy (Marinella *et al*., 2005). *Caralluma dalzielii* is perennial, erect and sparsely-branched to 40 cm high, with green stems, quadrangular branches and scattered dark reddish-purple star-like flowers. The plant is called "Karan Masallaci" by the Hausas and "gubehi" by the Fulanis in Northern Nigeria (Burkill, 1985).

Analgesic and anti-inflammatory drugs are mostly used in many diseases for relief of pain and inflammation (Heidaria *et al*., 2009). NSAIDs usually produce side effects such as stomach irritation, peptic ulcers and kidney toxicity. These make them less appealing for long-term use. Many medicinal plant products are used in Africa for the management of pain and inflammation and their efficacy and potency are traditionally acclaimed. Various studies have demonstrated the analgesic and anti-inflammatory activities of many traditional plant extracts such as *Syzygium aromaticum* flower bud (Tanko *et al*., 2008), *Spathodea campanulata* Linn (Emmanuel and Akah, 2009), *Securinega virosa* (Yerima *et al*., 2009), among others. In this study, the analgesic and anti-inflammatory activities of *Caralluma dalzielii* N.E.Br is investigated.

MATERIALS AND METHODS

Plant material: This study was conducted in the department of Pharmacology and Therapeutics,

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Ahmadu Bello University Zaria, Nigeria between June to September, 2011. Fresh whole plant of *Caralluma dalzielii* N.E.Br. (Asclepiadaceae) was collected around Zaria, Northern Nigeria and was identified by Musa *et al*. (2009) in the herbarium section of the department of biological sciences, ABU Zaria where a voucher specimen no. 1897 has been deposited.

Experimental animals: Adult Wister rats (weight: 110 to 189 g) of both sexes and adult Swiss albino mice (weight: 19 to 26 g) of both sexes were used for the experiments. The animals were obtained from the animal house of department of Pharmacology and Therapeutics, Ahmadu Bello University Zaria, Nigeria and maintained under normal laboratory conditions of humidity, temperature and light for 7 days prior to the experiment and allowed free access to food and water *Ad libitum*.

Preparation of extract: The fresh plant was dried under the shed at room temperature. The fresh dried plant was grounded into powder. Three hundred sixty four grams of the fine powder was cold macerated with 70% ethanol for 72 h. The extract was concentrated to dryness on a water bath at 37°C to give 24 g of a greenish mass of extract. The extract was reconstituted in distilled water at appropriate concentrations for the various experiments. The extract shall be called "Hydro-alcohol Extract of *Caralluma dalzielii* (HECD)".

Phytochemical screening: The HECD was subjected to preliminary phytochemical screening tests for carbohydrates, steroids, flavonoids, alkaloids, saponins, tannins, anthraquinones and glycosides according to the method described by Trease and Evans (1983).

Acute toxicity study (determination of LD_{50}): This was conducted in two phases by using the method of Lorke (1983). In the initial phase, mice were divided into 3 groups of three mice each and treated at doses of 10, 100 and 1000 mg/kg, respectively of HECD, intraperitoneally (*i.p.*). These were then observed for 24 h for signs of toxicity, including death. In the final phase, mice were divided into 4 groups of one mouse each and treated with HECD at doses of 140, 225, 370 and 600 mg/kg, respectively. The LD_{50} was calculated from the results of the final phase as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e., the geometric mean of the consecutive doses with 0 and 100% survival, respectively.

Hot-plate test: The 'hot-plate' (thermal) analgesic test method described by Turner (1965) was used for this study. A 600 mL glass beaker was placed on a hot-plate (with adjustable temperature). The temperature of the hot-plate was regulated to 45°C (±1°C). Thirty mice were divided into 5 groups of 6 mice/group. Group 1

received normal saline (1 mL/kg, *p.o.*) while groups 2, 3 and 4 were treated with HECD (20, 40 and 80 mg/kg *i.p.*, respectively). The 5th group was treated with Pentazocine (20 mg/kg, i.p.). After 30 min, the animals were placed singly on the hot plate. The reaction time (i.e., time taken by the mouse to lick its paw or jump out of the beaker), in seconds, was taken for each animal and repeated 4 times at 1 h intervals. The percentage thermal pain inhibition or protection was calculated using the formula:

$$\% \text{ protection against thermal pain} = \frac{(\text{test mean} - \text{Control mean}) \times 100}{\text{Control mean}}$$

Acetic acid-induced writhing test in mice: The method described by Taber *et al*. (1969) was used. Thirty mice were divided into 5 groups of 6 mice/group. Group 1 received normal saline (1 mL/kg, *p.o.*) while groups 2, 3 and 4 were treated with HECD *i.p.*, (20, 40 and 80 mg/kg, respectively). The 5th group was treated with Piroxicam (20 mg/kg, *i.p.*). After 30 min, 1% acetic acid solution (0.1 mL/kg, *i.p.*) was administered to all groups. The number of writhes (each of which is characterized by a wave of contraction of abdominal musculature followed by extension of the hind limbs) was counted 5 min after acetic acid injection for a period of 10 min.

Formalin test in rats: Thirty rats were divided into 5 groups each containing 5 rats. Rats in group 1 were given normal saline (1 mL/kg). Rats in groups 2, 3 and 4 were treated with HECD i.p., (20, 40 and 80 mg/kg, respectively). Animals in the 5th group were treated with pentazocine (20 mg/kg) i.p., 30 min after this treatment, 50 µL of a freshly prepared 2.5% solution of formalin was injected subcutaneously on the plantar surface of the left hind paw of each rat. The rats were placed individually in an observation chamber and monitored after 5 min and then at the end of 45 min. The severity of pain response was recorded for each rat based on the following scale:

- Rat walk or stand firmly on the injected paw
- The injected paw is favored or partially elevated
- The injected paw is clearly lifted off the floor
- The rat lick, chew or shake the injected paw (Yerima *et al*., 2009)

Carrageenan induced paw edema test in rats: Thirty rats were divided into 5 groups of 6 rats each. Group 1 was given normal saline (1 mL/kg). The HECD was administered to groups 2, 3 and 4 (20, 40 and 80 mg/kg, respectively). The 5th group was treated with Piroxicam (20 mg/kg, *i.p.*). Acute inflammation of the hind paw was induced in each of the rats by injecting a freshly prepared 0.1 mL of 1% w/v suspension of carrageenan in saline solution into the sub-plantar surface of the right hind paw. The diameter of the injected paw was

measured with a vernier caliper after 30, 60, 90 and 120 min of injection of carrageenan, respectively. Increase in the diameter of the right hind paws was taken as indicators of paw edema (Ojewole, 2006).

Statistical analysis: Data obtained from the experiments was pooled and expressed as mean±SEM. The results were analyzed statistically using one-way analysis of variance (ANOVA; 95% confidence interval). Values of $p \leq 0.05$ were taken to imply statistical significance.

RESULTS

Three hundred and sixty four grams of the fine powder of *Caralluma dalzielii* gave 24 g of a greenish mass of extract. The percent yield of the extract was calculated to be 6.59% *w/w*. Phytochemical screening test revealed the presence of Carbohydrates, Glycosides, Anthraquinones, Saponins, Steroids, Flavonoids and Tannins (Table 1).

Acute toxicity test showed that the Hydro-alcohol Extract of *Caralluma dalzielii* N.E.Br (HECD) produces 100% mortality at doses of 370, 600 and 1000 mg/kg, respectively. At these doses, the animals show signs of toxicity including respiratory depression, ataxia, convulsions and death. The minimum lethal dose was 370 mg/kg and the maximum non-lethal dose was found to be 225 mg/kg. The LD_{50} was calculated to be 288.53 mg/kg.

Effect on hot-plate test: The result of the hot plate test experiment shows a significant ($p<0.05$) increase in reaction time by the hydro-alcohol extract of *Caralluma dalzielii* N.E.Br only at dose of 80 mg/kg at 30 and 60 min when compared to the control as shown in Table 2. There was no significant increase in reaction time by the extract on the hot plate test model at doses of 20 and 40 mg/kg at all times and at dose of 80 mg/kg at 90 and 120 min, respectively.

Effect on acetic acid-induced writhing in mice: In the acetic acid-induced writhing test experiment, the hydro-

Table 1: Phytochemical constituents of hydro-alcohol extract of *Caralluma dalzielii*

Constituent	Inference
Carbohydrate	+
Glycosides	+
Antraquinones	+
Cardiac glycosides	+
Saponins	+
Steroids	+
Flavonoids	+
Tannins	+
Alkaloids	-

alcohol extract of *Caralluma dalzielii* shows a significant ($p<0.05$) decrease in number of writhing in the animals at all doses tested when compared to the control (piroxicam) in a dose dependant manner, as shown in Table 3. Percentage inhibition of pain was calculated to be 69.25% at dose of 20 mg/kg, 70.78% at dose of 40 mg/kg and 74.61% at a dose of 80 mg/kg. The percentage inhibition at all doses was found to be higher than the standard drug used (piroxicam) which shows a percentage inhibition of only 50.78%.

Effect on formalin test in rats: The hydro-alcohol extract of *Caralluma dalzielii* shows a significant decrease in the pain scale during the second phase of formalin test at doses of 40 and 80 mg/kg but not at 20 mg/kg. There was no significant decrease in pain scale at all doses tested during the first phase of the experiment (Table 4).

Effect on carrageenan induced paw edema: In the carrageenan induced paw edema test in rats, the hydro-alcohol extract of *Caralluma dalzielii* shows a significant decrease in paw edema after 1 h of carrageenan injection only at dose of 40 mg/kg when compared to the control. There was a statistically significant ($p<0.05$) decrease in paw edema at 2, 3 and 4 h of carrageenan injection at all doses tested when compared to the control, as shown in Table 5. The result shows a dose dependant decrease in paw edema after 2 and 3 h of carrageenan injection.

Table 2: Effect of Hydro-alcohol Extract of *Caralluma dalzielii* (HECD) on hot plate test in mice

Group	Treatment	Dose (mg/kg)	Mean reaction time (sec) ±SEM			
			30 min	60 min	90 min	120 min
I	Control (normal saline)	1 mL/kg	1.41±0.18	0.94±0.13	1.04±0.18	0.84±0.08
II	HECD	20	2.88±0.41	1.66±0.38	1.97±0.19	1.26±0.22
III	HECD	40	2.45±0.46	2.00±0.33	1.24±0.11	2.87±1.20
IV	HECD	80	3.23±0.62*	2.66±0.58*	2.01±0.28	2.73±0.48
V	Pentazocine	20	2.14±0.20	2.46±0.40	2.42±0.82	2.91±0.89

*: The mean difference is significant at $p \leq 0.05$ level

Table 3: Effect of Hydro-alcohol Extract of *Caralluma dalzielii* (HECD) on acetic acid induced writhing in mice

Group	Treatment	Dose (mg/kg)	Mean no. of writhing (±SEM)	Inhibition (%)
I	Control (normal saline)	1 mL/kg	21.66±2.73	-
II	HECD	20	06.66±2.55*	69.25
III	HECD	40	06.33±1.80*	70.78
IV	HECD	80	05.50±1.76*	74.61
V	Piroxicam	10	10.66±2.97*	50.78

*: The mean difference is significant at $p \leq 0.05$ level

Table 4: Effect of Hydro-alcohol Extract of *Caralluma dalzielii* (HECD) on formalin induced pain in rats

Group	Treatment	Dose (mg/kg)	Mean pain score (±SEM)	
			First phase (5 min)	Second phase (45 min)
I	Control (normal saline)	1 mL/kg	1.83±0.17	2.00±0.36
II	HECD	20	1.17±0.17	1.17±0.17
III	HECD	40	1.33±0.21	1.00±0.00*
IV	HECD	80	1.33±0.21	1.00±0.00*
V	Pentazocine	20	1.17±0.17	1.00±0.26*

*: The mean difference is significant at $p \leq 0.05$ level

Table 5: Effect of Hydro-alcohol Extract of *Caralluma dalzielii* (HECD) on carrageenan induced paw edema in rats

Group	Treatment	Dose (mg/kg)	Mean paw diameter (cm) ±SEM				
			0 h	1 h	2 h	3 h	4 h
I	Control (normal saline)	1 mL/kg	0.15±0.00	0.26±0.02	0.33±0.02	0.37±0.04	0.32±0.02
II	HECD	20	0.14±0.00	0.21±0.01	0.25±0.01*	0.25±0.01*	0.25±0.01*
III	HECD	40	0.14±0.01	0.19±0.01*	0.24±0.02*	0.24±0.02*	0.23±0.01*
IV	HECD	80	0.14±0.00	0.21±0.01	0.24±0.01*	0.23±0.01*	0.24±0.01*
V	Piroxicam	10	0.15±0.00	0.21±0.01	0.23±0.01*	0.23±0.01*	0.22±0.01*

*: The mean difference is significant at $p \leq 0.05$ level

DISCUSSION

Natural products have long been recognized as an important source of therapeutically effective compounds. Many traditional medicines have been used in the treatment of pain and inflammation with success (Singh *et al.*, 2008). In the present investigation, the Hydro-alcohol Extract of *Caralluma dalzielii* (HECD) was studied for its analgesic and anti-inflammatory potential in both peripheral (non-narcotic) and central (narcotic) type of pain models and carrageenan induced paw edema. Piroxicam (10 mg/kg, i.p.) and pentazocine (20 mg/kg, i.p.) were used as standard drugs.

The relatively low LD_{50} value (288 mg/kg) obtained in this study for HECD probably indicates that the plant extract is relatively toxic in mice. The toxicity observed may be due to some of the phytochemical constituents of the extract like flavonoids. It has been observed that acute poisoning with rotenone, a biologically active isoflavonoid, is characterized by an initial respiratory stimulation followed by respiratory depression, ataxia, convulsions and death by respiratory arrest (Corbett, 1974). These were also observed in this study.

In the hot plate experiment, the extract only shows significant effect at a dose of 80 mg/kg after 30 and 60 min of administration. The hot plate method is one of the most common tests of nociception that is based on a phasic stimulus of high intensity. Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception (Aiyelero *et al.*, 2009). The ability of the extract to significantly prolong the reaction latency to thermally-induced pain in mice by the hot plate at 30 and 60 min suggests that the extract may have a mild central analgesic activity.

Result of the acetic acid-induced writhing in mice shows that the extract possesses strong peripheral analgesic effect. The extract showed strong inhibition of nociception at all doses, in a dose dependent manner.

Percentage inhibition was highest (74.61%) at the highest dose and relatively lower (69.25%) at the lowest experimental dose when compared with the normal saline control. The result also show that the extract possess stronger anti-nociceptive effect than the standard drug (piroxicam 10 mg/kg) which shows 50.78% nociceptive inhibition compared to the normal saline control. Acetic acid causes pain by liberating endogenous substances that excite pain nerve endings (Khan *et al.*, 2009). The use of abdominal constriction (writhing) model is known to be very sensitive when compared with other models such as tail flick model. It has been postulated that Local peritoneal receptors are partly involved in the abdominal constriction response. There is also increased level of PGE2 and PGF2α in peritoneal fluids (Vyas *et al.*, 2008), as well as lipoxygenase products (Dhara *et al.*, 2000). Evidence also suggests that there is increased Nitric oxide synthesis (Larson *et al.*, 2000). This method affords rapid evaluation of peripheral type of analgesic action (Bhukya *et al.*, 2009). The result therefore suggests that the action of the extract may be linked to cyclooxygenase and/or lipoxygenase pathways. Some of the phytochemical constituents of HECD like flavonoids, saponins and tannins have been demonstrated to have analgesic effects on acetic acid induced writhing test (Calixto *et al.*, 2000).

Edema and pain induced by formaldehyde are mediated by substance P, bradykinin, histamine, serotonin and prostaglandins (Wheeler-Aceto and Cowan, 1991). Acute inflammation induced by formaldehyde results from cell damage, which provokes the production of endogenous mediators such as histamine, serotonin, prostaglandins and Bradykinin. The two distinct phases in formalin test are due to direct effect of formalin on nociceptors and due to inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degree, the sensitization of central nociceptive neurons.

Centrally acting drugs such as opioids inhibit both phases equally but peripherally acting drugs, such as cyclooxygenase inhibitors and corticosteroids only inhibit the late phase (Amanlou *et al*., 2005).

The extract was ineffective in significantly reducing the number of paw lickings in the early phase of the formalin test. However, there was a significant reduction in paw licking by the same mice in the late phase. The mean pain scale in the late phase by animals given only saline was 2.00±0.36 whereas it was 1.00±0.00 in animals given 40 and 80 mg/kg of the extract. The result shows that the extract possesses significant anti-nociceptive effect in formalin test model at doses of 40 and 80 mg/kg. It also showed that the extract possessed significant effect in the second phase (resulting from an inflammatory pain) than in the first phase (caused by the stimulation of nociceptors) of the formalin test. This could be due to some of the phytochemical constituents of the extract like flavonoids, which have been shown to reduce number of paw lickings in formalin test in rats (Calixto *et al*., 2000).

Carrageenan-induced rat paw edema is a suitable test for evaluating anti-inflammatory activities of natural products (Panthong *et al*., 2003). The carrageenan-induced inflammatory reactions have been shown to be due to the release of inflammatory mediators (Ndebia *et al*., 2007). The HECD at all doses show significant reduction in paw edema at 2-4 h after carrageenan injection. Ueno *et al*. (2000) found that the injection of carrageenan into the rat paw induced the liberation of bradykinin and then further induced the biosynthesis of prostaglandin and other autacoids. However, in the carrageenan-induced rat paw edema model, the production of prostanoids has been through the serum expression of COX-2 by a positive feedback mechanism (Lai *et al*., 2009).

Carrageenan edema is a multimediated phenomenon that liberates diversity of mediators (Adedapo *et al*., 2009). The probable mechanism of action of carrageenan-induced inflammation is biphasic; the first phase is attributed to the release of histamine, serotonin and kinins in the 1st h, while the second phase is attributed to the release of prostaglandins and lysosomal enzymes in 2 to 3 h. The extract significantly inhibited the carrageenan-induced inflammation in the 2nd to 4th h. Analgesic and anti-inflammatory effects have been observed in flavonoids as well as tannins (Ahmadiani *et al*., 2000). There are also reports on the analgesics effects of saponins (Choi *et al*., 2005).

The effect on carrageenan induced edema may results from the ability of HECD to inhibit mediators of acute inflammation (Aboyade *et al*., 2010). It is possible that the HECD produce its effect through the inhibition of COX enzymes which are active players in the carrageenan induced paw edema model. Since carrageenan-induced inflammation model is a significant predictive test for anti-inflammatory agents acting by the mediators of acute inflammation (Sawadogo *et al*., 2006), the results of this study are an indication that the HECD can be effective in acute inflammatory disorders.

The hydro-alcohol extract of *Caralluma dalzielii* N.E.Br at the doses tested was shown to possess significant anti-nociceptive activity in all the nociceptive models. The extract show more significant peripheral analgesic and anti-inflammatory properties and less central analgesic effect. The co-existence of both anti-nociceptive and anti-inflammatory effect seen with HECD is well defined for various Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), particularly the salicylates and their derivatives. It is therefore evident that the extract behaved similar to NSAIDs in this study which correlates well with the traditional application of the plant in pain and inflammatory condition. The flavonoids, saponins and tannis might be responsible in part for the observed analgesic and anti-inflammatory effect. Flavonoids are known to inhibit the metabolism of the arachidonic acids whereas the tannins inhibit the synthesis of prostaglandins (Davis *et al*., 1984).

CONCLUSION

The results of this study revealed that the hydro-alcohol extract of *Caralluma dalzielii* possess both peripheral and central analgesic properties. These results suggested that the extract may possess NSAID-like activities mediated through peripheral mechanism. Although the results of the present study are inconclusive, they tend to suggest that the hydro-alcohol extract of *Caralluma dalzielii* used probably produces its anti-inflammatory effect by inhibiting the release, synthesis and/or production of inflammatory mediators, including polypeptide kinins, prostaglandins and so forth, like piroxicam. This research study justified the traditional use of the plant in the treatment of pro-inflammatory disease. Further studies will be carried out on pharmacodynamic pattern to establish the exact mechanism of action of the plant extracts.

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