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Full Length Research Paper

Investigation of the anti-inflammatory and anti-nociceptive activities of *Elephantorrhiza elephantina* (Burch.) Skeels root extract in male rats

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The anti-inflammatory and anti-nociceptive activities of the root extract of *Elephantorrhiza elephantina* (Burch.) Skeels (Fabaceae) were investigated using wistar rats. The extract was administered intraperitoneally (i.p) to rats at graded doses of 50, 100 and 200 mg/kg BWt. Carrageenan and Histamine were injected into rat paws sub-plantar to induce paw oedema, while acetic acid and formalin were injected i.p to induce pain. Indomethacin (10 mg/kg) was used as reference drug, whereas the vehicle [0.9% normal saline in 3% tween 80 (2 ml/kg)] was used as negative control. Acute toxicity was tested in rats at doses of 200, 400, 800 and 1600 mg/kg BWt. Compared to control, the aqueous extract of *E. elephantina* at all doses investigated significantly ($P < 0.05$) reduced the formation of oedema induced by Carrageenan and Histamine. The extract also caused a significant ($P < 0.05$) reduction in writhings in the acetic acid test and licking time in the formalin test. The rats did not show any signs of acute toxicity. The study revealed the anti-inflammatory and anti-nociceptive activities of the aqueous extract of *E. elephantina,* which may be due to the presence of phytochemical constituents such as tannins and flavonoids. The acute toxicity test showed that the plant is relatively safe to use.

Key words: Inflammation, pain, aqueous extract, acute toxicity.

INTRODUCTION

Chronic inflammatory diseases remain one of the world's major health problems (Adedapo et al., 2008); as a result, it has become the focus of global scientific research. Inflammation is the response of living tissue to injury, involving a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Perianayagam et al., 2006). As a result of adverse effects such as gastric lesions caused by non-steroidal anti-inflammatory drugs (NSAID), tolerance and dependence induced by opiates, use of these drugs as anti-inflammatory and anti-nociceptive agents have not been successful in all cases (Adedapo et al., 2008). New anti-inflammatory and analgesic drugs without these side effects are therefore being researched as alternatives to NSAID and opiates. Attention is focused on investigating efficacy of plant-based drugs used in traditional medicine because they are cheap, have little side effects and according to WHO, about 80% of the world population still rely mainly on herbal remedies (Adedapo et al., 2008).

*Elephantorrhiza elephantina (*Burch.) Skeels. (Fabaceae) is a traditional remedy for a wide range of ailments both in humans and livestock. Among Zulus, an infusion of the inner parts of the roots is administered as an enema for dysentery and diarrhoea and root decoctions are also taken for diarrhoea (Hutchings et al., 1996). In livestock, the root is given to cows for mange (Dold and

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Cocks, 2001); heartwater, blackquarter, (Luseba and Van der Merwe 2006); diarrhoea, coughing, pneumonia, as well as tick-borne diseases (McGaw and Ellof, 2008). Goats are given root decoction to treat helminthosis, (Maphosa and Masika, unpublished data).

Phytochemical screenings have shown the presence of catechins in rhizhomes, thus indicating presence of condensed tannins, as well as flavanoids and other phenolic substances (Naidoo, 2005). Studies have shown that tannins are useful in treating dermatitis and are known to form a protective layer on the skin and mucosa, thereby enhancing tissue regeneration (Van Wyk et al., 1997). The purpose of this study was therefore, to assess the anti-inflammatory and anti-nociceptive activities of *E. elephantina*, so as to validate its ethno-veterinary use.

MATERIALS AND METHODS

Plant material collection and extract preparation

Roots were collected in February 2007 in the Matatiele area, Umzimvubu municipality of the eastern Cape province of South Africa. The area falls within the latitudes 30º25´ - 33º45´S and longitudes 23º15´-30º10´E. The plant was identified in its vernacular name by farmers and authenticated at Albany Museum herbarium in Grahamstown. A voucher specimen (No VMAP18) was deposited in the Griffen herbarium, university of Fort Hare.

The roots were then air dried to constant weights at room temperature and ground into powder. The powder, (100 g) was boiled in water (1000 ml) under atmospheric pressure and the solution filtered using a Buckner funnel and Whatman No 1 filter paper. The filtrate was later lyophilized using a freeze drying system, yielding 12.3% extract.

Animals

Male Wistar rats, (200-240 g) were used for anti-inflammatory and toxicity studies and (90-110 g) for analgesic studies. Animals were maintained in the experimental animal house at the university of Fort Hare, South Africa, under room temperature (25ºC) and normal day light. They were kept in rat cages, fed on commercial pellets (EPOL Feeds, South Africa Ltd.) *ad libitum*, and allowed free access to clean water in bottles. All experimental protocols were in compliance with university of Fort Hare ethics committee on research in animals, as well as internationally accepted principles for laboratory animal use and care.

Chemicals and drugs

Carrageenan, histamine, acetic acid, tween- 80 and indomethacin, all of analytical grade, were obtained from sigma-Aldrich Chemie Gmbh, Steinheim, Denmark.

Acute toxicity test

Using method of Hilaly et al., (2004), sixteen rats were randomly grouped into 4 and fasted for 16 h. Graded doses of the extract (200, 400, 800 and 1600 mg/kg p.o.) were administered to rats using a bulbed steel needle. All animals were later allowed free access to food and water and observed over 48 h, for physiological and behavioural changes and deaths.

Anti-inflammatory activities: Carrageenan-induced rat paw oedema

5 groups each consisting of 4 rats received either plant extract (50, 100, 200 mg/kg p.o), indomethacin (10 mg/kg p.o), or vehicle control [0.9% normal saline in 3% Tween 80 (2 ml/kg). 1 h later, acute inflammation was produced by the sub-plantar administration of 0.1 ml of 1% Carrageenan (in normal saline containing Tween-80) in the right hind paw of the rats. Paw volume was measured at 0, 1, 2 and 3 h after Carrageenan injection using a micrometer screw gauge. Increases in the linear diameter of the right hind paws of rats were taken as an indication of paw oedema. The percentage inhibition of the inflammation was calculated from the expression:

$$\% \text{ inhibition} = [(D0-Dt)/D0] \times 100$$

where *D0* is the average inflammation (hind paw oedema) of rats in the control group at a given time and *Dt* is the average inflammation of the drug treated (that is, extracts or indomethacin) rats at the same time (Gunakunru et al., 2004).

Histamine-induced rat paw oedema

Using the method of Parmar and Ghosh (1978), 5 groups of 4 rats each received either plant extract (50, 100, 200 mg/kg i.p), or indomethacin (10 mg/kg i.p) or vehicle control [0.9% normal saline in Tween 80 (2 ml/kg] i.p. Paw oedema was produced by sub-plantar injection of 0.1% Histamine into the right hind paw of rats 1 h after injection of drugs. Paw volume was recorded before histamine injection (time 0) and 1, 2 and 3h after injection. The percentage inhibition of the oedema was calculated using the same formula as in Carrageenanan -induced paw oedema.

Anti-nociceptive activity: acetic acid-induced writhing test

The test was determined according to the method of Zakaria et al. (2001). 5 groups with 4 rats each, received either plant extract (50, 100, 200 mg/kg), indomethacin (10 mg/kg) or normal saline solution (2 ml/kg) (all i.p). 30 min later, 10 ml/kg of acetic acid (0.7%) was injected i.p on rats to induce writhing. 10 min later, rats were placed in transparent boxes and writhes counted for a period of 10 min. Writhing was accepted as contraction of the abdominal muscles accompanied by stretching of hind limbs in response to pain. Anti-nociceptive effect was expressed as reduction of number of writhes between control and treated groups, using the formula: $[(C-D)/C] \times 100$ where C is the average number of writhings for control group of rats and *D* is the average writhings of the drug/extract treated rats (Gupta et al., 2005).

Formalin test

The method described by Hunskaar et al. (1985) was used. 5 groups of 4 rats were pretreated with either distilled water vehicle (2 ml/kg); plant extract (50, 100, 200 mg/kg) or indomethacin (10 mg/kg) i.p. Thirty minutes later, 2.5% formalin (0.05 ml) was injected sub-planter into the dorsal surface of the right hind paw. Animals were then placed in transparent boxes and observed immediately after injection. Time (seconds) spent licking and biting the injected paw was taken as an indicator of response to pain,

Table 1. Anti-inflammatory activities of aqueous extract of *E. elephantina* root and indomethacin on carrageenan-induced oedema in the right limb of rats.

Group	Dose (mg/kg)	Paw oedema volume (ml)		
		1h	2h	3h
Control	-	1.91 ± 0.14	1.87 ± 0.94	2.05 ± 0.28
Indomethacin	10	0.74 ± 0.32* (61.3)	0.59 ± 0.34* (68.4)	0.52 ± 0.26* (74.6)
Extract	50	0.70 ± 0.37* (63.3)	0.32 ± 0.23* (82.9)	0.13 ± 0.05* (93.7)
Extract	100	0.58 ± 0.29* (69.6)	0.40 ± 0.27* (78.6)	0.98 ± 0.54* (52.0)
Extract	200	0.55 ± 0.18* (71.2)	0.38 ± 0.30* (79.7)	0.24 ± 0.10* (88.3)

Data in mean ± SD., n = 4.
Percentage inhibitions of the carrageenan-induced inflammation (oedema) are indicated a (%).
*Significantly different from control (P < 0.05).

Table 2. Anti-inflammatory activities of aqueous extract of *E. elephantina* root and indomethacin on histamine-induced oedema in the right limb of rats.

Group	Dose (mg/kg)	Paw oedema volume (ml)		
		1h	2h	3h
Control	-	1.99±0.34	2.9±0.32	2.57±0.98
Indomethacin	10	1.72± 0.44 (13.6)	2.22±0.39 (23.4)	1.43±0.29* (44.4)
Extract	50	1.62±0.51 (18.6)	1.67±0.42* (42.4)	1.18±0.37* (54.1
Extract	100	1.35±0.82 (32.2)	1.04±0.46* (64.1)	0.65±0.37* (74.7)
Extract	200	1.68±0.82 (15.6)	1.34±0.74* (53.8)	0.84±0.45* (67.3)

Data in mean ± SD., n = 4.
Percentage inhibitions of the histamine-induced inflammation (oedema) are indicated a (%).
*Significantly different from control (P < 0.05).

which were measured for 5 min (first phase) and 15-30 min (second phase) after formalin injection.

Statistical analyses

The data were expressed as means ± S.D. Differences between control and treatment groups were analyzed using one way ANOVA followed by Turkey post hoc multiple comparisons tests using SPSS version 11.5 software. P < 0.05 was considered significant.

RESULTS

Carrageenan-induced paw oedema

There was a significant (P < 0.05) inhibition of the paw oedema in all doses of the plant extract and with indomethacin. Doses of 50 mg/kg (63.3-93.7% inhibition) 100 mg/kg (69.6-78.6% inhibition) and 200 mg/kg (71.2-88.3% inhibition) were more active than that of indomethacin (61.3-74.6% inhibition) (Table 1).

Histamine-induced paw oedema

The plant extract produced significant (P < 0.05) reduction of paw oedema with all doses after 2 and 3h of histamine injection (Table 2). Indomethacin produced its significant (P < 0.05) anti-inflammatory activity (44.4% inhibition) after 3 h of histamine injection. At all test levels, the extract was more active than indomethacin.

Acetic acid-induced writhing test

The aqueous extract of *E. elephantina* at 100 and 200 mg/kg doses significantly (P < 0.05) decreased the number of writhes when compared to the control. The extract at 50 mg/kg exhibited the lowest analgesic effect (46.2%), followed by indomethacin (53.8%) (Table 3).

Formalin test

At doses of 100 and 200 mg/kg, *E. elephantina* caused a significant (P < 0.05) reduction in licking time that was greater than indomethacin (10 mg/kg) both in the early and late phases (Table 4). The extract also exhibited higher (88.6%) inhibition at 200 mg/kg dose in the early phase and (61.0, 87.0%) in the late phase at 100 and 200

Table 3. Analgesic effect of aqueous extract of *E. elephantina* root and Indomethacin on acetic acid induced writhing test.

Group	Dose (mg/kg)	Number of writhing per 20 min	Inhibition (%)
Control	2	6.5 ± 4.4	0
Indomethacin	10	3.5 ± 1.3*	46.2
Extract	50	3.0 ± 1.6*	53.8
Extract	100	1.8 ± 0.5*	72.3
Extract	200	1.5 ± 0.9*	76.9

Data in mean ± SD., n = 4.
*Data are significantly different from control (P<0.05)

Table 4. Analgesic effect of the aqueous extract of *E. elephantina* and indomethacin on formalin induced pain test.

Group	Dose (mg/kg)	Early phase (5 min)	Inhibition (%)	Late phase (15 - 30 min)	Inhibition (%)
Control	-	8.75 ± 2.8	0	19.25 ± 0.9	0
Indomethacin	10	4.25 ± 1.6	51.4	12.5 ± 5.2	35.1
Extract	50	5.25 ± 2.1	40.0	14.0 ± 4.8	27.3
Extract	100	4.25 ± 1.2	51.4	7.5 ± 2.9*	61.0
Extract	200	1.0 ± 0.2*	88.6	2.5 ± 0.3*	87.0

Data in mean ± SD., n = 4.
*Data are significantly different from control (P < 0.05).

mg/kg respectively than indomethacin (51.4%) in early phase, (35.1%) in the late phase.

Acute toxicity test

No physiological and behavioural changes were observed and no mortalities were recorded.

DISCUSSION

The anti-inflammatory and analgesic activities of the aqueous extract of *E. elephantina* were established by this study. The plant is employed locally in treatment of helminthosis and this could probably be attributable to its anti-inflammatory and anti-nociceptive activity. The extract reduced oedema and pain even better than Indomethacin, a potent inhibitor of prostaglandins (PG) synthesis (Barros et al., 2006), showing that the plant has strong anti-inflammatory and anti-nociceptive activities.

The suppression of Carrageenan-induced paw oedema at the early phase of inflammation can be attributed to inhibition of PG synthesis and antihistamine activities by the plant extract.

The suppression of oedema produced by histamine in the second and third hour by all doses of the extract suggests that the extract do possess some anti-inflammatory activity. Histamine is one of the important mediators of inflammation, a potent vasodilator substance and increases vascular permeability (Vesudevan et al., 2007). Results indicate that the inhibitory effect of *E. elephantina* root aqueous extract on Carrageenan oedema is probably due to histamine reduction since the extract was able to inhibit the oedema induced by this mediator. It is, therefore, speculated that part of the anti-oedematous effect by all doses of the extract is due to inhibition of prostaglandin liberation, since the plant extract effect on Carrageenan oedema was more pronounced than that of histamine.

A dose-dependant decrease in writhings by rats injected with acetic acid signifies the analgesic effect of the plant extract. In the acetic acid-induced abdominal writhing, which is the visceral pain model, the processor releases arachidonic acid via cyclooxygenase and prostaglandin biosynthesis plays a role in the nociceptive mechanism (Gupta et al., 2005). The anti-nociceptive effect of the extract may be due to inhibition of synthesis of the arachidonic acid metabolite, mediated by cyclooxygenase inhibition. It is known that the abdominal constriction response is very sensitive and able to detect anti-nociceptive effect of extracts or compounds that may

appear inactive in other methods like the tail-flick test (Spereni et al., 2005).

The pain in the early phase of formalin test may be due to direct stimulation of sensory nerve fibres by formalin, while pain in the late phase is due to inflammatory mediators like histamine, PGs, serotonin and bradykinin. This test is believed to be a more valid analgesic model which is better correlated with clinical pain (Tjolsen et al., 1992). The decrease in licking time and frequency by rats injected with formalin therefore signifies the analgesic effect of the plant.

Phytochemically, catechins in rhizhomes have been reported, indicating that the plant may contain condensed tannins, as well as flavonoids and other phenolic sustances (Naidoo, 2005). The mechanism of anti-inflammatory activity may be related to anti-phlogistic action of tannins (Adedapo et al., 2008). In another study, Ahmadiani et al. (1998) also reported anti-inflammatory activity of flavornoids from *Sambucus ebulus* rhizome extract. From this study, it can, therefore, be concluded that the root extract of *E. elephantina* possess anti-inflammatory and analgesic activities, thus giving a scientific basis to its ethnoveterinary use and acute toxicity results showed that it is relatively safe to use.

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REFERENCES

Adedapo AA, Sofidiya MO, Maphosa V, Moyo B, Masika P, Afolayan AJ (2008). Anti-inflammatory and analgesic activities of the aqueous extract of *Cussonia paniculata* stem Bark. A C G publications, 2: 46-53.

Ahmadiani A, Fereidoni M, Semnanian S, Kamalinejadand M, Saremi S, (1998) Antinociceptive and anti-inflammatory effects of *Sambucus ebulus* rhizome extract in rats. J. Ethnopharmacol. 61: 229-235.

Barros IMC, Lopes LDG, Borges MOR, Borges ACR, Ribeiro MNS, Freire SMF (2006). Anti-inflammatory and anti-nociceptive activities of *Pluchea quitic* (D.C) ethanolic extract. J. Ethnopharmacol. 106: 317-320.

Dold AP, Cocks ML (2001).Traditional veterinary medicine in the Alice district of the Eastern Cape Province South Africa. S. Afr. J. Sci. 97: 1-5.

Gunakunru A, Padmanaban K, Thirumal P, Vengatesan N, Gnansekar N, Raja S, Rajarajan AT, Vijay Kumar SG, Periyanayagan JB (2004). Chemical investigations and anti-inflammatory activity of fixed oil of *Butea monosperma* seeds. Nat. Prod. Sci. 10: 55-58.

Gupta M, Mazunder UK, Sambath Kumbar R, Gomath P, Rajeshwar Y, Kakoti BB, Tamil SV (2005). Anti-inflammatory, analgesic and antipyretic effects of methanol extract from *Bauhina racemosa* stem bark in animal models. J. Ethnopharmacol. 98: 267-273.

Hilaly JE, Israili ZH, Lyoussi (2004). B. Acute and chronic toxicological studies of *Ajuga iva* in experimental animals. J. Ethnopharmacol. 91: 43-30.

Hunskaar S, Famer OB, Hole K (1985). Formalin test in mice: a useful technique for evaluating wild analgesics. J. Neuroscie Meth. 14: 69-76.

Hutchings A, Scott AH, Lewis G (1996). Zulu medicinal plants*:* An inventory, Natal, South Africa: University of Natal Press. pp. 266-267.

Luseba D, Van der Merwe D (2006). Ethnoveterinary medicine practices among Tsonga speaking people of South Africa. Onderstepoort J. Vet. Res. 73: 115-122.

McGaw LJ, Eloff JN (2008). Ethnoveterinary use of southern African plants and scientific evaluation of their medicinal properties. J. Ethnopharmacol. 10: p. 1016.

Naidoo V (2005). Screening of four plants commonly used in ethnoveterinary medicine for antimicrobial, anti-protozoal and anti-oxidant activity. MSc dissertation. University of Pretoria. pp. 1-130.

Parmar NS, Ghosh MMN (1978). Current trends in flavonoid research. Indian J. Pharm. 12: 213-228.

Perianayagam JB, Sharma SK, Pillai KK (2006). Anti-inflammatory activity of *Trichodesma indicum* root extract in experimental animals. J. Ethnopharmacol. 104: 410-414.

Spereni E, Cervellati R, Innocenti G, Costa S, Guerra MC, Dall' Acqua S, Govoni P (2005). Anti-inflammatory, anti-nociceptive and antioxidant activities of *Balanites aegyptica* (L) Delile. J. Ethnopharmacol. 98: 117-125.

Tjolsen A, Berge O, Hunskaar S, Roseland JK, Hole K (1992). The formalin test: an evaluation of the method. Pain, 51: 5-17.

Van Wyk BE, Van Oudtshoorn B, Gericke N (1997). Medicinal Plants of South Africa. Pretoria: Brizza Publications, p. 304.

Vesudevan M, Gunman KK, Parle M (2007). Antinociceptive and anti-inflammatory effects of *Thespesia populnea* bark extract. J. Ethnopharmacol. 109: 264-270.

Zakaria MNM, Zathakrishnan R, Chen HB, Kamil M, Al-gisri AN, Chan K, Al-attas A (2001). Antinociceptive and anti-inflammatory properties of *Carraluma arabica*. J. Ethnopharmacol. 76: 155-158.