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Membrane Stabilizing Activity: A Possible Mechanism of Action for the Anti-inflammatory and Analgesic Properties of *Russelia equisetiformis*

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Abstract: The Methanolic extract of the whole plant of *Russelia equisetiformis* (10-40 mg kg^{-1}) produced significant ($p<0.05$) anti-inflammatory using carrageenan-induced rat paw oedema method. The extract significantly produced analgesic effect through inhibition of acetic acid-induced writhing and significantly ($p<0.05$) elevated the reaction time to thermal stimulation in mice treated with extract (10-40 mg kg^{-1}). It also significantly ($p<0.05$) stabilized erythrocyte membrane subjected to heat and hypotonic-induced lyses. The anti-inflammatory and analgesic activities of the extract may be due to its membrane stabilizing action.

Key words: *Russelia equisetiformis*, anti-inflammatory, analgesic, membrane stabilization

INTRODUCTION

Russelia equisetiformis (Schlecht and Chan) belongs to the family Scrophulariacae. Phytochemical analysis of the plant extract has been reported to contain triterpenes of lupane type (Burns *et al*., 2001). Triterpenes have been reported by some research workers to have biological activities (Szakiel *et al*., 1995; Olukoga and Donaldson, 2000; Sirtori, 2001). In earlier study on the extract of the plant, we reported that the methanolic extract exhibited strong anti-inflammatory and analgesic activities (Awe *et al*., 2004). Membrane stabilizing action of *Gongronema latifolium* has been reported to be a possible mechanism for its anti-inflammatory property (Morebise *et al*., 2005). The present study was undertaken in an attempt to investigate further the possible mechanism of anti-inflammatory and analgesic activities *R. equisetiformis*.

MATERIALS AND METHODS

The plant material was collected in the month of October from Bodija, Oyo State, Southern Nigeria. The plant was identified in the herbarium, Department of Botany, University of Ibadan, where voucher specimen was deposited with No. 106998.

Extract preparation: The plant sample was air-dried at room temperature. They were then reduced to a powdery form using electric blending machine. A 400 g quantity material was extracted with methanol in a soxhlet extractor. The methanol extract was evaporated to dryness using rotary evaporator under reduced pressure until a solid extract was obtained. The extract weighed 39.2 g (yield 9.8%). This was stored in refrigerator and prepared fresh in 20% Tween 80 for pharmacological studies.

Phytochemical screening of the extract: Preliminary phytochemical screening of the plant extract using the standard method of Trease and Evans (1989) gave positive test for saponins, flavonoids and glycosides.

Animals: Rats (Wistar strains, male, weight (160-220 g) and mice (20-22 g) were housed in the Animal House, College of Medicine, University of Ibadan. They were kept in standard cages, with a maximum of six animals in a cage. The animals were acclimatized in the laboratory for two weeks before experimentation. They were fed on standard diet (Ladokun feeds).

Anti-inflammatory activity: Pedal inflammation in rats was produced according to the method of Winter *et al*. (1962). The animals were divided into groups of six animals each 0.1 mL carrageenan solution was injected into the right hind paw of each rat under the sub- plantar aponeurosis. The test group of rats was treated intraperitoneally i.p., with 10, 20 or 40 mg kg^{-1} of the extract, 30 min before carrageenan injection. At the same time the control group

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received 10 mL kg^{-1} 20% Tween 80 and reference group received 5 mg kg^{-1} indomethacin (MSD, Canada) i.p. Measurement of paw volume was made using a plethysmometer (Ugo Basile Model7140). Measurement was done before and at the third hour following carrageenan injection. Oedema inhibitory values were calculated using the formula

$$\text{Inhibition}(\%) = \frac{\text{Inhibition control}(Vt - Vo) - (Vt - Vo)\text{treated} \times 100}{(Vt - Vo)}$$

Where, Vt is the paw volume 3 h after carrageenan injection and Vo is the paw volume before carrageenan injection.

Analgesic activities: The acetic acid-induced writhing test was carried out using the method of Koster *et al.* (1959). The extract at the doses of 10, 20 or 40 mg kg^{-1} was administered intraperitoneally (i.p.,) to mice divided into groups of six animals each. Thirty minutes after treatment, the mice were injected i.p., with 0.2 mL of 0.6% acetic acid solution to induce characteristic writhing. The number of writhing occurring between 5 and 15 min after acetic acid injection was recorded. The response of the extract and indomethacin (5 mg kg^{-1}) treated groups were compared with those of animals in the control group (20% Tween 80 10 mL kg^{-1}).

Tail flick test: The extract at doses of 10, 20 or 40 mg kg^{-1} was administered i.p., to mice divided into five groups of six animals each. Thirty minutes later, tail reaction response was done with one-third of the tail immersed in a water bath heated to 55°C. The response of the extract and morphine (1 mg kg^{-1}, i.p.,) treated groups were compared with those of the animals in the control group (20% Tween 80, 10 mL kg^{-1}).

Effect of the extract on membrane stabilization: The method used was as described by Olajide *et al.* (2000). Blood was collected from University of Ibadan Teaching Hospital Blood Bank. Blood was centrifuged and the supernatant carefully pipetted with sterile pipettes. The packed cells were resuspended in an equal volume of isotonic saline and centrifuged again. The process was repeated four times until the supernatants were clear. A 10% Human Red Blood Cell (HRBC) suspension was then prepared with normal saline and kept at 4°C undisturbed before use. A reaction mixture (4.5 mL) consisting of 2 mL hypotonic saline (0.25% w/v NaOH and 0.15 M sodium phosphate buffer pH 7.4 and varying concentration of the extract (25, 50 and 100 mg mL^{-1}) in normal saline to make volume 4.0 mL. Then 0.5 mL of 10% HRBC in normal saline was added. Two controls were prepared, one with 1.0 mL of isotonic saline instead of extract, (control 1) and the other one with 1 mL of extract solution without red blood cells (control 2). The mixtures were incubated at 56°C for 30 min. The tubes were cooled under running water for 20 min and mixtures were centrifuged. The absorbance of supernatants was read at 540 nm wave length using spectrophotometer.

The percentage membrane-stabilizing activity was determined using equation of Sadique *et al.* (1989).

$$\text{Stabilizing activity}(\%) = \frac{\text{100-Extract absorbance value -Control absorbance value} \times 100}{\text{Control 1 absorbance value}}$$

Statistical analysis: Values are expressed as mean±SEM. Statistical significance was determined using the student's t-test. Values with $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Results of phytochemical analysis showed that the plant contains Saponins, cardiac glycosides and flavonoids (Table 1). The Methanolic extract of *R. equisetiforms* at 10-40 mg kg^{-1}, i.p., showed significant ($p>0.05$) and dose-dependent anti-inflammatory activity (Table 2). The extract also showed significant ($p<0.05$) and dose-dependent analgesic activities (Table 3 and 4). The extract (25, 50 and 100 mg mL^{-1}) significantly stabilized the erythrocyte membrane subjected to heat and hyposaline induced haemolysis (Table 5).

Table 1: Phytochemical screening of MERE (Trease and Evans, 1989)

Test	Observation
Tanins	Absent
Flavonoids	Present
Phlobatanins	Absent
Cardiac glycosides	Present
Saponins	Present
Triterpenes	Present
Alkaloids	Absent

Table 2: Anti-Inflammatory activity of Methanolic extract of *Russelia equisetiformis* (MERE)

		Carrageenan-induced paw oedema		
Group	Dose (mg kg^{-1})	Paw size[a] (mm) i.p.before 3 h	Paw size[a] (mm) After 3 h	Inhibition (%)
Control (20% Tween 80)	10 mg kg^{-1}	2.76±0.14	2.81±0.14	0.00
Extract:				
R. equisetiformis	10	2.58±0.17*	2.02± 0.18*	21.7*
R. equisetiformis	20	2.68±0.20*	1.92± 0.17*	28.4*
R. equisetiformis	40	2.92±0.22*	1.58± 0.12*	46.5*
Indomethacin	5	2.92±0.22*	1.20± 0.90*	58.90*

[a] Each valve is the mean±SEM of five rats *$p<0.05$ compared with control. Students t-test

Table 3: Effect of MERE on acetic acid-induced writhing

Treatment	Dose (mg kg^{-1} i.p.)	Total No. Writhing[a]	Inhibition (%)
Control 2.0% Tween 80	10 mL kg^{-1}	27.6±2.05	-
R. equisetiformis	10 mg kg^{-1}	22.8±1.70*	17.4±1.29
R. equisetiformis	20 mg kg^{-1}	14.4±1.07*	47.80±3.54
R. equisetiformis	40 mg kg^{-1}	10.0±0.74*	63.71±4.72
Indomethacin	5 mg kg^{-1}	8.20±0.60*	70.3±5.21

[a]Values are mean±SEM (n = 6) *p<0.05 vs control. Students t-test

Table 4: Effect of MERE On tail flick response

Treatment	Dose	Latency (S)[a]	Inhibition (%)
Control (2.0% Tween 80)	10 mL kg^{-1}	2.6±0.24	-
Extract:	10 mg kg^{-1}	3.2±0.30	18.8±1.39
MERE	20 mg kg^{-1}	5.2±0.48	50.0±3.69
MERE	40.0 mg kg^{-1}	6.8±0.63	61.8±4.56
MERE	1 mg kg^{-1}	9.6±0.89	72.9±5.38
Morphine			

[a]Values are mean±SEM (n = 6). [b]Significant difference p<0.05 student t-test compared with contr

Table 5: Effect of *R. Equisetiformis* on human red blood cell (HRBC) stability

Extract	Concentration (mg mL^{-1})	Membrane stability* (%)
R. equisteformis	25	19.7±1.62
R. equisteformis	50	47.6±3.60
R. equisteformis	100	60.3±4.56
Indomethacin	5	62.8±4.75

*Results are: average triplicate experiment

The carrageenan-induced rat paw oedema is the basic model for screening agents with anti-inflammatory activity (Winter *et al*., 1962; Bangbose and Noamesi, 1981). The development of oedema in the paw of the rat after injection of carrageenan has been attributed to the release of mediators of inflammation, such as the prostagladins (Sadique *et al*., 1989; Di-Rosa *et al*., 1971). It is possible that the extract inhibited prostagladins synthesis. Acetic-acid injection is a method of inducing pain which results in the liberation of proinflammatory mediators such as histamine, Kinins and bradykinins (Keel and Armstrong, 1964). This stimulates nerve to produce pain (inflammatory pain). It is also possible that the extract prevents the liberation of these proinflammatory mediators.

A possible explanation for the membrane stabilizing effect could be an increase in the surface area/volume ratio of the cell, which could be brought about by expansion of membrane or shrinkage of the cell and interaction with membrane protein (Abe *et al*., 1991). Moreover, it has also been reported that the deformability and cell volume of erythrocytes are closely related to the intracellular content of calcium (Shinde *et al*., 1999). Hence, it could be speculated that the cytoprotective effect of the extract on the erythrocyte membrane might be due to the ability of the extract to alter the influx of calcium into the erythrocyte (Shinde *et al*., 1999).

Earlier investigations have revealed that various herbal preparations are capable of stabilizing the red blood cell membrane and exert their anti-inflammatory activity (Sadique *et al*., 1989). Since the membrane of red blood cell is similar to that of lysosomal membranes, the effect of drugs or extracts on human red blood cell membrane could be extrapolated to the stabilization of lysosomal membranes (Shinde *et al*., 1999; Oyedapo and Famurewa, 1995)

The present investigation suggests that the lysosomal membrane stabilizing activity of *R. equisetiformis* may be playing a significant role in its anti-inflammatory and analgesic activities, most especially in inflammatory pain produced by acetic acid.

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