

ANTI-INFLAMMATORY ACTIVITY OF ROOT EXTRACT OF *RAPHIOSTYLIS BENINNIENSIS*.

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ABSTRACT

The anti-inflammatory activity of the methanol root extract of *Raphiostylis beninniensis* Dalziel (Icacinaceae) (RbE) was investigated using experimental animal models. The methanol extract (200 and 400 mg/kg) significantly ($p < 0.05$) inhibited (50 and 92.5% respectively) systemic acute rat paw oedema induced by egg albumin after 5h in a dose-dependent manner. The higher dose caused an effect higher than that of 50 mg/kg piroxicam (57%). In chronic inflammation models, RbE (200 and 400mg/kg) showed a significant ($p < 0.05$) non-dose-dependent inhibition of both cotton pellet granuloma formation and formaldehyde-induced arthritis in rats. Evaluation of the acute toxicity and lethality of the extract in mice revealed an oral and i.p LD₅₀ of 3808 mg/kg and 17.55mg/kg respectively. Phytochemical analysis showed the presence of alkaloids, saponins, tannins, steroids, terpenoids, glycosides resins as well as reducing sugars and carbohydrates. The results indicate that RBE has anti-inflammatory effect in both acute and chronic inflammation which is consistent with the folkloric use of the plant in the management of disorders of inflammation.

Keyword: *Raphiostylis beninniensis*, anti-inflammatory, inflammation.

INTRODUCTION

A scientific evaluation of plants and their traditional uses in disease management can permit their incorporation into the official health care systems, especially in the third world countries (Dongmo *et al.*, 2003). In recent years, the belief that plants hold cure for many disease conditions (including inflammatory condition) have led to a reawakening of interest in the use of plants and plant products. (Magaji *et al.*, 2008). Among the African medicinal plants, several species are used as traditional inflammatory remedies (Akah and Nwambie, 1994). One of such plants is *Raphiostylis beninnensis* Dalziel (Icacinaceae).

It is popularly used in south-eastern Nigerian traditional practice in the treatment of tonsillitis and in wound healing. The root is also chewed to neutralise the effect of ingested poison (Muoebonam, personal communication).

Until now, no scientific investigation had been carried out to shed light on the anti-inflammatory property of *Raphiostylis beninniensis*. Thus, this study was designed to validate the tribal use of the plant roots as an anti-inflammatory agent.

MATERIALS AND METHODS

Plant material

Fresh roots of *R. beninniensis* were collected from Akokwa, Imo State, Nigeria in January, 2008. The plant materials were identified and authenticated by Mr. A.O. Ozioko of Biore-sources Development and Conservation Programme (BDCP) Centre, Nsukka, Enugu State, Nigeria where a voucher specimen (2500 AO) is deposited.

The roots were dried in the shade and milled to 5mm² pieces using a hammer mill. About 395g of the root chips was exhaustively extracted with 3 volumes (w/v) methanol in a

soxhlet apparatus. Concentration of the extract by evaporation in a fume chamber afforded (15.54% w/w) of the methanol extract (RbE). The dried extract (RbE) was subsequently subjected to phytochemical analysis for identification of constituents using standard methods (Evans, 1989).

Pharmacological Tests

Animals

Adult Swiss albino rats (120-185g) and mice (24-34g) of either sex were obtained from the laboratory Animals Facility, Department of Pharmacology and Toxicology, University of Nigeria, Nsukka (UNN). The animals were housed in plastic cages under normal room temperature ($25 \pm 2^{\circ}\text{C}$) and natural light cycle and maintained on standard pellets (Vital Feeds, Jos, Nigeria) and water *ad libitum*. Animals handling and use was in compliance with the National Institute of Health Guide for care and use of laboratory Animals (Pub. No.85-23, 1985).

Acute Toxicity Study

The acute toxicity and lethality (LD_{50}) of RbE was studied in mice using the method described by Lorke, 1983. Median lethal dose (LD_{50}) of RbE was determined in mice by oral (p.o) and intraperitoneal (i.p) routes using the method of (Lorke, 1983).

In the initial phase, mice were divided into 3 groups of three and treated with RbE at doses of 10, 100 and 1000mg extract/kg body weight orally (p.o) and were then observed for 24hrs for signs of toxicity including death. In the final phase, mice were divided into 4 groups of one mouse each and treated with the extract at doses of 600, 1000, 1600 and 2900mg/kg body weight p.o. The median lethal dose (LD_{50}) was calculated from the second phase. The same process was repeated intraperitoneally and the median lethal dose was also calculated from the first phase.

Anti-inflammatory Activity Tests

Acute inflammation

Topical acute inflammation of the mouse ear

The effect of ME on topical acute inflamma-

tion was evaluated in the mouse ear using the methods of Tubaro *et al.*, (1985) and Atta and Alkohafi (1998) as modified by Okoli and Akah (2004). Briefly, mice were divided into three groups of five animals ($n = 5$) each. Group I received the RbE (5mg/ear) while group II and III received normal saline (5ml/kg) and indomethacin (5mg/ear) respectively. The extract was applied on the anterior surface of the right ear. Topical oedema was instantly induced on the posterior surface of the same ear by application of xylene (0.05ml). Two hours after induction of inflammation, the mice were killed by overdose of ether anaesthesia and both ears removed. Circular sections (7mm diameter) of both ears were punched out using a cork borer, and weighed. Oedematous response was quantified as the weight difference between the two earplugs. The anti-inflammatory activity was further quantified as oedema reduction/inhibition (%) in the treated animals calculated relative to the control using the relation.

Inhibition of oedema (%) = $100 (R_t - L_t) / (R_c - L_c)$ where R_t = mean weight of right earplug of treated animals; L_t = mean weight of left earplug of treated animals; R_c = mean weight of right earplug of control animals; L_c = weight of left earplug of control animals.

Systemic acute rat paw oedema

The effect of ME on systemic acute oedema was assessed in the rat paw using the method of Winter *et al.*, 1962. Increase in the right hind paw volume (Bani *et al.*, 2000) induced by the sub-plantar injection of fresh egg albumin (Okoli and Akah, 2004) was used as a measure of acute inflammation. Animals were divided into four groups (I-IV) of five rats each. Group I served as normal control and received normal saline (10ml/kg) while animals in group II served as positive control and received piroxicam (50mg/kg). Groups III and IV received 200 and 400 mg/kg of RbE respectively. All the treatments were administered orally by intubation. The animals were deprived of water only during the experiment to ensure uniform hydration and minimize variability in oedematous response (Winter *et al.*, 1963). Thirty minutes after extract administration, fresh undiluted egg albumin (0.1ml)

was injected into the sub-plantar region of the right hind paw of the rats. The paw volume was measured by water displacement in a calibrated measuring cylinder before and at 0.5, 1, 2, 3, 4 and 5.5h after induction of inflammation. Oedema formation was assessed as the difference in the volume of the paw before and the volume at the different time intervals after egg albumin injection.

The extent of inhibition of edema (%) was calculated using the relation (Perez, 1996). Inhibition of oedema (%) = $100 \{1 - [(a-x)/(b-y)]\}$ where a = mean paw volume of treated rats after egg albumin injection; x = mean paw volume of treated rats before egg albumin injection; b = mean paw volume of control rats after egg albumin injection; y = mean paw volume of control rats before egg albumin injection.

Chronic inflammation

Formaldehyde induced inflammation in rats

The anti-arthritic effect of RbE was studied in rats using the formaldehyde-induced arthritis method of Seyle (1949). Adult rats were divided into four groups (I-IV) of five animals each. Groups I and II received RbE (200 and 400mg/kg) respectively. Control groups (III and IV) received piroxicam (50mg/kg) and normal saline respectively. On day 1 of the experiment, one hour after oral administration of extract, arthritis was induced by injection 0.1ml of formaldehyde (2% v/v) solution into the sub-plantar region of the right hind paw of the rats. Stimulus injection was repeated on day 3 of the experiment while treatment was continued once daily until day 10. Paw volume was measured before formaldehyde injection once daily for the ten days. The level of inhibition of oedema (%) was calculated using the relation; Inhibition of oedema (%) = $100 \{1 - [(a-x)/(b-y)]\}$ where a = mean paw volume of treated rats after formaldehyde injection; x = mean paw volume of treated rats before formaldehyde injection; b = mean paw volume of control rats after formaldehyde injection; y = mean paw volume of control rats before formaldehyde injection.

Granuloma formation induced by cotton pellets in rats

The effect of RbE on granuloma tissue formation induced by cotton pellet was evaluated by the method of Winter and Porter (1957). Animals were divided into 4 groups (I-IV; n=5/group). Groups I, II, III and IV received RbE (200 and 400 mg/kg), indomethacin (10mg/kg) and normal saline orally respectively. On day one, one hour after oral administration of extract, two sterilized (by exposure to ultraviolet rays for 24h) cotton pellets (10±1mg) were aseptically implanted one on each axilla under the skin on the previously shaved anaesthetised rats. Extract administration continued orally for seven consecutive days from the day of cotton pellet implantation. On day 8, the animals were sacrificed by cervical dislocation and the cotton pellets were immediately removed surgically and freed from extraneous tissues. The increase in dry weight of the pellets was taken as a measure of granuloma formation and measure of chronic inflammation.

Statistical analysis

The data collected was analysed using One Way ANOVA and subjected to LSD post hoc test for multiple comparisons. Results were expressed as Mean±SEM and differences between means of treated and control groups accepted significantly at $p < 0.05$.

RESULTS

The extraction process yielded 15.54% w/w of the methanol extract (Rbe)

Phytochemical test showed that ME gave positive reactions to alkaloids, saponins, flavonoids, tannins, steroids, terpenoids, glycosides, resins, reducing sugars and carbohydrates.

Acute toxicity studies (LD₅₀)

Acute toxicity tests established an oral and intraperitoneal LD₅₀ of 3808 and 17.55 mg/kg respectively in mice.

Effect of extract on acute topical inflammation

The methanol extract RbE remarkably inhibited topical oedema induced by xylene in the mouse ear. The extract caused an inhibition

greater than that of indomethacin (Table 1).

Table 1: Effect of Extract on Topical Edema of the Mouse Ear

| Treatment | Dose (mg/ear) | Edema (mg) | Inhibition (%) |
|--------------|---------------|------------|----------------|
| Control | 5 | 9.25±2.17 | NI |
| RbE | 5 | 4.50±2.50 | 48.65 |
| Indomethacin | 5 | 0.50±0.71 | 5.41 |

n = 5; RbE = *Raphiostylis beninniensis* extract; NI = No inhibition.

Effect of extract on systemic acute rat paw oedema

The methanol extract significantly (*p*<0.05) suppressed the development of systemic acute oedema of the rat paw induced by egg albumin. The extract provoked a dose-related inhibition with the greatest effect occurring at 5 h with the 400mg/kg dose (Table 2).

Table 2: Effect of Extract on Systemic Edema of the Rat Paw

| Treatment | Dose (mg/kg) | Oedema (ml mean ± SEM) | | | | | |
|-----------|--------------|------------------------|-------------------|-------------------|-------------------|---------------------|---------------------|
| | | 0.5h | 1h | 2h | 3h | 4h | 5.5h |
| Rbe | 200 | 1.17±0.07 (NI) | 0.85±0.09 (03) | 0.70±0.6 (24) | 0.6±0.10 (35) | 0.45±0.05 (42) | 0.35±0.04 (50) |
| Rbe | 400 | 0.81±0.09 (NI) | 0.38±0.10 (57) | 0.25±0.05 (52) | 0.25±0.04 (51) | 0.21±0.03 (66.4) | 0.08±0.02 (92.5) |
| Piroxicam | 50 | (NI) | 0.98±1.01 (11) | 0.78±0.13 (31) | 0.58±1.22 (38) | 0.38±.10 (51) | 0.30±0.15 (57) |

n = 5; **P*<0.05 compared to the control; Rbe – *R. beninniensis* extract, NI=No inhibition. Figures in parenthesis = % inhibition of edema

Effect of extract on arthritis induced by formaldehyde in rats

Chronic oral administration of the methanol extract caused a significant (*P*<0.05), but non dose-dependent inhibition of arthritis induced by formaldehyde in rats (Fig 1).

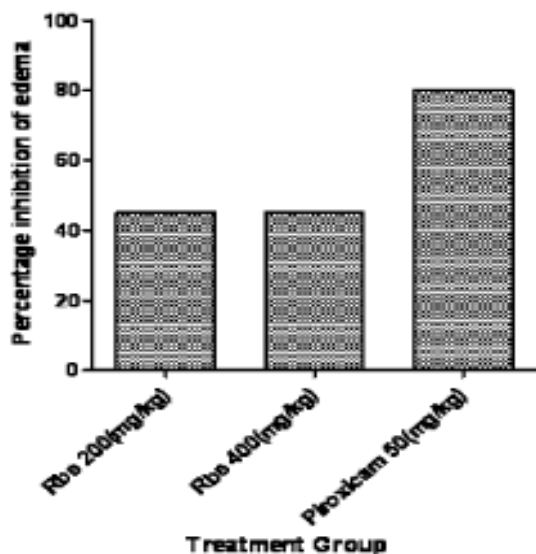


Fig. 1: Effect of extract/piroxicam on formaldehyde induced arthritis in rats

Effect of the extract on cotton pellet-induced granuloma in rats

In granuloma formation induced by cotton pellet, chronic oral administration of the extract caused a non-dose-dependent inhibition of granuloma formation. The effect was however, lower than that of indomethacin (Fig 2).

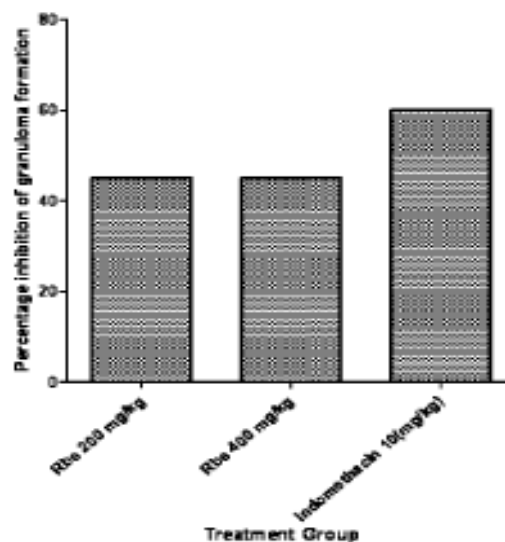


Fig. 2: Effect of extract/indomethacin on cotton pellet-induced granuloma

Table 3: Effect of Extract on Cotton Pellet-induced Granuloma in Rats

| Treatment | Dose (mg/kg) | Edema (mg) | Inhibition (%) |
|--------------|--------------|------------|----------------|
| ME | 200 | 21±0.87 | 45 |
| | 400 | 21±0.87 | 45 |
| Indomethacin | 10 | 18±1.25 | 60 |
| Control | - | 30±1.12 | NI |

DISCUSSION

Studies on the pharmacology of Nigerian medicinal plants in the laboratories and otherwise have indicated that a good number of plants possess therapeutic attributes; both for the management and treatment of a lot of human ailments. The chemical entities that are discovered to have potential medicinal values may serve either as a drug candidate or as a milestone for structural modification.

The low intra-peritoneal median lethal dose (LD₅₀) in mice suggests a possible risk of acute toxicity when administered i.p. Nevertheless the acute intoxication risk seems to be route-dependent; since the p.o. LD₅₀ was relatively high suggesting a good tolerability. This implies that the oral route may reduce the risk of acute toxicity.

The root extract of *R. beninensis* exhibited an anti-inflammatory effect in the topical model of acute inflammation provoking an inhibition greater than that of indomethacin. Ear oedema induced by a various phlogistic substances is mediated by a variety of agents such as leucocytes and prostanoids, which have been shown to mediate croton oil ear oedema (Tubaro *et al.*, 1985). This effect suggests that the extract may have a lipophilic character which enabled it to cross the skin barrier and exert antiphlogistic action (Asuzu *et al.*, 1999; Okoli and Aka, 2004).

Egg albumin induced rat paw oedema which is similar to carrageenan induced hind paw oedema (Okoli and Aka, 2004) is a standard experimental model for acute inflammation (Taesolik *et al.*, 2003; El-Shenawy *et al.*,

2002). Egg albumin is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effect (Chakraborty *et al.*, 2006). Moreover, the model exhibits a high degree of reproducibility (Winter *et al.*, 1962). The probable mechanism of action of carrageenan (egg albumin) induced inflammation is biphasic, the first phase is attributed to the release of histamine, serotonin and kinins in the first hour, while the second phase is attributed to the release of prostaglandins and lysosomal enzymes in the second to the fifth hour (Brooks and Day, 1991).

The ability of the extract to also suppress egg albumin – induced paw oedema at the tested dose levels suggests that it possesses a significant effect against acute inflammation especially in the second phase showing a maximum inhibition of 92.5% at the dose of 400mg/kg after 5h of extract treatment. The extract may have inhibited prostaglandins and or cyclooxygenases (Burke *et al.*, 2006).

Chronic inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents. Chronic inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation. (Dunne, 1990; Gupta *et al.*, 2003). Chronic inflammation occurs by means of the development of proliferative cells. These cells can be either spread or in granuloma form. Efficacy of anti-inflammatory agents in chronic inflammatory states is indicated by their ability to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation (Recio *et al.*, 1995). In formaldehyde induced arthritis in rats, RbE showed a moderate and non-dose dependent inhibitory activity.

Furthermore, the RbE showed an appreciable but non significant anti-inflammatory activity in cotton pellet induced granuloma and thus may be useful in chronic inflammatory conditions.

The presence of flavonoids and tannins might be responsible for the observed anti-inflammatory activities as flavonoids and tan-

nins have been known to inhibit phosphodiesterases (Duke, 2002), which are involved in cell activation, whose effect depend on the biosynthesis of protein cytokines that mediate adhesion of circulating leucocytes to the site of injury. Flavonoids and tannins have also been proven to potently inhibit prostaglandins (Manthey, 2000) and are effective in acute inflammation (Rajnarayana et al., 2001).

In conclusion, the results of our study indicate that RbE was active against both acute and chronic phases at a dose range of 200-400 mg/kg. The overall anti-inflammatory activity of the extract may be due to multiple interactions with several components of different acute and chronic inflammatory reactions.

However, more detailed phytochemical studies are required to identify the active principle (s) and to evaluate its possible mechanism of action.

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