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EVALUATION OF ANTI-INFLAMMATORY AND MEMBRANE STABILIZING EFFECTS O F AQUEOUS ROOT EXTRACT OF *Boerhavia diffusa* LINN IN RATS.

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ABSTRACT : *Boerhavia diffusa* is a widely used plant in traditional medicine for various disease problems. In this study, the anti-inflammatory and membrane stabilizing effects of the aqueous extract of its roots was evaluated in rats. Carrageenan-induced rat paw oedema model was used for anti-inflammatory effect while rat red blood cells were used for membrane stabilizing property. The extract in doses of 100-400 mg/kg significantly ($p<0.05$) inhibit carrageenan-induced rat paw oedema in a dose dependent manner. The concentration of 20-80 mg/ml of the extract also showed a dose dependent inhibition of the rat red blood cells haemolysis induced by hypotonic solution. It was concluded that the extract possesses anti-inflammatory as well as membrane stabilizing properties.
Key words: *Boerhavia diffusa*, root, anti-inflammatory, membrane stabilizing, rats.

INTRODUCTION

Boerhavia diffusa or chicken weed is an herbaceous perennial plant of the family Nyctaginaceae. It is widely distributed in the tropics and subtropics (CSIR, 1988). The plant is popular with indigenous people all over the world because of its uses as vegetable and medicinal properties. It was reportedly used in renal ailments as diuretic (Anand, 1995), treatment of stomach ache, anaemia, cough, and cold, and as a diaphoretic, laxative, expectorant, and a potent antidote for snake and rat bites (Chopra *et al.*, 1956). It was also used in the treatment of nephrotic syndrome (Singh and Udupa, 1972), hepatitis, gall bladder abnormalities, and urinary disorders (Cruz, 1995). While the flowers and seeds are used as contraceptive (Chopra *et al.*, 1956), the roots have been reportedly used for treatment of asthma (Ambasta, 1986) and hepatoprotective in action (Rawat *et al*, 1997), it has also been shown to be antispasmodic, anticonvulsant (Borrelli *et al*, 2006) and pain-reliever (Hiruma-Lima *et al*, 2000).

The herb is a diuretic that acts on the glomeruli of the kidney and also protects the kidney from being damaged (Rawat *et al*, 1997). Barthwal *et al*, 1991 reported the antihemorrhagic action, and its antimicrobial effects have also been shown (Agrawal *et al,* 2004). The cytotoxic and anticancer activities have been reported by Mehretra *et al*, (2002), Bharali *et al*, (2003) and Leyon *et al*, (2005). The aqueous leaf extract of *Boerhavia diffusa* significantly reduced thiobarbutric acid reactive substances and hydrogen peroxides with a significant increase in reduced glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione-s-transferase in liver and kidney (Satheesh *et al*, 2004), and its hypoglycemic antidiabetic effects have been demonstrated (Gholap *et al,* 2004; Pari *et al,* 2004). The plant is a good antihypertensive agent (Hensen *et al,* 1995) and it is believed to enhance lactation period and also the amount of milk in cattle (CSIR, 1988). Literature survey of this plant however, revealed very little information about the use of its root as an anti-inflammatory agent and treatment of inflammation related diseases. This study therefore aimed at evaluating the anti-inflammatory and membrane stabilizing properties of the aqueous extracts of the root of the plant, as the root is highly medicinal.

MATERIALS AND METHODS

Laboratory animals: Wistar albino rats (170-200 g) of both sexes were used for the study. The animals were kept in the Faculty of Veterinary medicine animal house in a well ventilated rat cages, with free access to food and water *ad libitum.*

Preparation of the plant material: The roots of *Boerhavia diffusa* used for this study were obtained from a local farm in Ibadan, the plant was authenticated at the Department of Botany, Faculty of Science, University of Ibadan, Ibadan. The roots were cut into smaller pieces and air dry, the dried roots were then ground in a domestic grinding machine to almost fine powder. Two hundred and fifty grams (250 g) of this material was soaked in 500 ml of distilled water for 72 hours with intermittent shaking. The solution was filtered and the filtrate concentrated with vacuum rotary evaporator maintained at 40°C. The percentage yield of the extract was 9.75%. A fresh 10% solution of the extract was prepared with distilled water to make appropriate dosages required for the studies.

Experimental procedures

Acute toxicity study: Five groups of albino rats (6 per group) were used. The first 4 groups received oral doses of 100,200, 400 and 800 mg/kg of the extract. The fifth group received distilled water (10 ml/kg) orally. The animals were observed for toxic symptoms and mortality was determined 24 hours post administration.

Antiinflammatory study: The anti-inflammatory activity of the extracts was evaluated with carrageenan induced oedema model (Winter *et al*., 1962). Rats (6 per group) were divided into five groups. Group 1-3 received oral doses of 100, 200 and 400 mg/kg of the extract. Group 4 was treated orally with indomethacin (10 mg/kg) as standard drug, while group 5 was administered with distilled water (10 ml/kg) as control. After thirty minutes, 0.1 ml of 1.0% carrageenan was injected into the right hind paw of each rat. The linear circumference of the injected paw was measured immediately after injection and also 3 hours after injection and the percentage inhibition of oedema was calculated as described by Jain and Khanna, (1981).

$$\% \text{ Inhibition of oedema} = l_0 - l_1 / l_0$$

Where l_0 = change in paw circumference in control group, l_1 = change in paw circumference in treated groups.

Membrane stabilizing activity: The method use was as described by Shinde *et al.,* (1999). Whole blood was obtained with heparinized syringes from rats through cardiac puncture, centrifuged and supernatant was carefully pipetted. The remaining packed cells were washed four times with equal volume of isotonic buffered solution (154 mM NaCl in 10 mM sodium phosphate buffer (pH 7.4), the packed cells were been centrifuged each time at 1000 rpm. 10% rat erythrocytes suspension was prepared with normal saline and kept in refrigerator at 4^0C as stock erythrocytes.

The test sample consisted of 2 ml of hypotonic solution (50 mM Nacl in 10 mM sodium phosphate buffer, pH 7.4) and varying concentrations of the extract (20, 40 and 80 mg/ml) or indomethacin (0.1 mg/ml) in normal saline to make 4.0 ml, this was then mixed with 0.5 ml of stock erythrocytes to make a total of 4.5 ml. The control sample consisted of 0.5 ml of stock erythrocytes mixed with hypotonic-buffered saline alone. The mixture was incubated for 10 minutes at room temperature, centrifuge for 10 minutes at 1000 rpm and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated according to modified method described by Shinde *et al*, (1999).

$$\% \text{ Inhibition of haemolysis} = 100 \times (OD_1 - OD_2) / OD_1$$

Where OD_1 = optical density of hypotonic buffered-saline solution alone (control) and OD_2 = optical density of test sample in hypotonic solution.

Statistics: The data collected were statistically analysed using one-way Analysis of variance (ANOVA) and Duncan New multiple range post hoc test, mean differences at $p<0.05$ were considered significant.

RESULTS AND DISCUSSION

Acute toxicity study: The extract was safe in rats at the tested doses (40-320 mg/kg), there was no mortality within the studied period. However, there were behavioural changes such as depression, reduced motor activity and ataxia.

Antiinflammatory Effects: The extract significantly ($p<0.05$) inhibited the oedema induced by carrageenan in dose-dependent manner as inhibition of 400 mg/kg is 45.3%, 200 mg/kg is 37.7 while 100 mg/kg is 26.4%. Indomethacin also significantly ($p<0.05$) inhibited oedema of the rat paw (Table 1).

Table 1: Effect of aqueous extract of the root of *Boerhavia diffusa* on carrageenan-induced hind paw oedema in rats.

Sample	Dose (mg/kg)	Difference in paw circumference (cm)	% inhibition of oedema
Root Extract	100	0.39±0.032*	26.4
Root Extract	200	0.33±0.019*	37.7
Root Extract	400	0.29±0.015*	45.3
Indomethacin	10	0.21±0.009*	60.4
Distilled water	10ml/kg	0.53±0.130	-

Each value represents the mean ± SEM from 6 rats in each group. All values are significant at $p<0.05$ compared with control group (distilled water).

Membrane stabilizing activity: The extract at concentration range of 20-80 mg/ml significantly ($p<0.05$) protect the rat blood cells membrane against lysis induced by hypotonic solution. The inhibition is comparable with that of indomethacin as shown in table 2.

Table 2: Effect of aqueous extract of the root of *Boerhavia diffusa* on rat erythrocytes hemolysis

Sample	concentration	optical density (OD)	% Inhibition of haemolysis
Hypotonic soln	50 mM	0.498±0.002	-
Root Extract	20 mg/ml	0.311±0.004*	37.55
Root Extract	40 mg/ml	0.268±0.002*	46.18
Root Extract	80 mg/ml	0.218±0.005*	55.62
Indomethacin	0.1 mg/ml	0.213±0.002*	57.23

Each value represents the mean ± SEM 6 experiments. All values are significant at $p<0.05$ compared with control group (hypotonic solution).

The result of the study showed that the aqueous root extract of *Boerhavia diffusa* possesses anti-inflammatory property, as the extract significantly inhibited carrageenan induced oedema in rat paws. The carrageenan induced rat paw model is the basic model for screening agents with anti-inflammatory effect (Bamgbose and Noamesi, 1981). During inflammation, lysosomal hydrolytic enzymes are released to the sites and these causes damages of the surrounding organelles and tissues with attendance variety of disorders (Sadique *et al*, 1989). The oedema developed after injection of carrageenan into the rat paws has been attributed to the inflammatory mediators released. These mediators include vasoactive substances such as histamine, bradykinin, serotonin and prostaglandins (Sadique *et al*, 1989; Heller *et al*, 1998). The increase in vascular permeability produced by these chemicals promote accumulation of fluid in tissues and thus the oedema (White, 1999). The mode of action of this extract could be due to the prevention of liberation of proinflammatory mediators and inhibition of prostaglandin synthesis which thereby resulted in inhibition of inflammation.

The extract also showed membrane stabilizing effect as it significantly stabilized the rat erythrocytes membrane. A possible explanation could be connected with binding to the erythrocytes membrane with subsequent alteration of the surface charges of the cells which might have prevented physical interaction with aggregating agents or promote dispersal by mutual repulsion of like charges which are involved in the haemolysis of red blood cells (Oyedapo *et al*, 2010).

CONCLUSION

The aqueous extract of the root of *Boerhavia diffusa* possesses anti-inflammatory properties which can be attributed to its cell membrane stabilizing effect which therefore inhibit the lysis and release of the pro-inflammatory mediators.

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