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## Evaluation of the Anti-inflammatory and Membrane-Stabilizing Effects of *Eupatorium odoratum*

<sup>1</sup>S. Umukoro and <sup>2</sup>R.B. Ashorobi

<sup>1</sup>Department of Pharmacology and Therapeutics,  
College of Medicine, University of Ibadan, Ibadan, Nigeria

<sup>2</sup>Department of Pharmacology, College of Medicine,  
University of Lagos, Lagos, Nigeria

**Abstract:** The anti-inflammatory and membrane-stabilizing effects of an aqueous leaf extract of *Eupatorium odoratum* was investigated in this study. The effect of the extract on acute inflammation was studied in carrageenin-treated rats. The anti-inflammatory activity of *E. odoratum* was further assessed in rats subjected to sub-chronic inflammatory conditions induced by formaldehyde. The membrane-stabilizing property of the extract was studied using its ability to reduce the levels of haemolysis of rat Red Blood Cells (RBC) exposed to hypotonic solution. The results of the study showed that the extract (100-400 mg kg<sup>-1</sup>, p.o) possess anti-inflammatory property, as it significantly reduced oedema formation induced by the phlogistic agents in rats. At a concentration range of 1.0-2.0 mg kg<sup>-1</sup>, the extract offered significant protection of RBC against the haemolytic effect of hypotonic solution, an indication of membrane-stabilizing activity. It appears that the membrane-stabilizing effect exhibited by *Eupatorium odoratum* might be playing a significant role in its anti-inflammatory activity.

**Key words:** *Eupatorium odoratum*, anti-inflammatory, membrane-stabilizing, property

### INTRODUCTION

In recent times, there is an increase in global utilization of herbal medicines in the treatment of various diseases affecting humans (Vandebroek *et al.*, 2004; Cragg and Newman, 2001). The high safety profile and low cost of herbal medicines have been reported as the major factors responsible for the increased upsurge in herbal medication (Vandebroek *et al.*, 2004).

Inflammatory disorders are the commonest ailments usually managed by traditional medical practitioners in Nigeria (Akah and Nwambie, 1994). A number of plants are utilized by the folk practitioners in the treatment of these disorders. Generally, the plant extracts are taken orally, two to three times daily or applied topically to relieve inflammation by the rural populace (Akah and Nwambie, 1994). In this study, we investigate the anti-inflammatory and membrane-stabilizing effect of one of such notable plants, *Eupatorium odoratum* L. (Asteraceae) in rats.

*Eupatorium odoratum* is an erect shrub of about 3 m high. The leaves are alternate and the fruits are one-seeded. The extract of the leaf have been reported to contained phytochemically active compounds such

as phenols, terpenoids, alkaloids and flavonoids (Richter *et al.*, 2003; Triratana *et al.*, 1991). The aqueous and decoction of the leaves is used for the treatment of soft wounds, burn wounds and skin infections in Vietnam (Phan *et al.*, 1996). The juice of the crushed leaves is applied to cuts to arrest bleeding (Biswal *et al.*, 1997). The macerated leaves is usually applied to swollen portion of the body to relieve inflammation amongst the rural populace in southern part of Nigeria. Although, previous studies have established the anti-microbial, wound healing and haemostatic effects of *Eupatorium odoratum* (Triratana *et al.*, 1991; Biswal *et al.*, 1997), scanty information exist in literature that suggest its usefulness in the treatment of inflammatory diseases. Therefore, we decided to investigate its anti-inflammatory and membrane-stabilizing effect in rats.

### MATERIALS AND METHODS

**Plant material:** The fresh leaves of *Eupatorium odoratum* were purchased from Mushin market, Lagos and authenticated by Prof. J.D. Olowokudejo of the Department of Botany and Microbiology, University of Lagos, Nigeria.

**Laboratory animals:** Sprague-Dawley rats (180-250 g) of either sex obtained from the Laboratory Animals Center, College of Medicine and University of Lagos, Nigeria were used in the study. They had free access to food and water *ad libitum*.

**Preparation of plant extract:** The leaves of *Eupatorium odoratum* were dried in the oven at 40°C. The dried leaves were ground into fine powder and extraction was carried out using Soxhlet apparatus. The powdered material was placed in the Soxhlet apparatus and 600 mL of distilled water was allowed to run continuously through it over a heater for a period of 48 h. The solution obtained was evaporated to dryness in an oven at 40°C. The yield of the extract was 11.7% with reference to the powdered material. A portion of the dried extract was weighed and dissolved in distilled water to make an appropriate concentration for the study.

**Acute toxicity study:** Mice were divided into 6 groups of 6 per group. The first 5 groups received oral doses of the extract (0.10-2.0 g kg<sup>-1</sup>). The other group received saline (10 mL kg<sup>-1</sup>, p.o). Mortality was assessed 24 h after administration. The animals were observed for toxic symptoms and mortality was assessed 24 h after treatment (Miller and Tainter, 1944).

#### Anti-inflammatory test

**Carrageenin-induced acute inflammation:** Carrageenin-induced rat paw oedema was utilized as the model for acute inflammation as previously described (Winter *et al.*, 1962). Rats (6 per group) were treated with the extract (100-400 mg kg<sup>-1</sup>, p.o), acetylsalicylic acid (100 mg kg<sup>-1</sup>, p.o) and saline (10 mL kg<sup>-1</sup>, p.o), respectively. Thirty minutes later, each rat was injected with 0.1 mL of 1% carrageenin into the sub-plantar surface of the right hind paw. The linear circumference of each paw was measured with the aid of cotton thread before and at 3.0 h after induction of inflammation. The mean paw circumference and percentage inhibition of oedema was calculated (Oriowo, 1982).

**Effect on formaldehyde-induced sub-chronic inflammation:** Formaldehyde-induced rat paw oedema was used as a model for sub-chronic inflammation as described by Jain and Khanna (1981). The animals (6 rats per group) were pretreated with the extract (100-400 mg kg<sup>-1</sup>, p.o), acetylsalicylic acid (100 mg kg<sup>-1</sup>, p.o) and saline (10 mL kg<sup>-1</sup>, p.o). Thirty minutes later, each rat was injected with 0.1 mL of 2% formaldehyde into the sub-plantar surface of the right hind paw. The circumference of the paw was measured as described

above before and 24 h after formaldehyde injection. The change in paw circumference and percentage inhibition of oedema, were determined as previously described by Jain and Khanna (1981).

#### Effect on erythrocyte membrane

**Preparation of erythrocyte suspension:** Whole blood was obtained with heparinized syringes from rats through cardiac puncture. The blood was washed three times with isotonic buffered solution (154 mM NaCl) in 10 mM sodium phosphate buffer (pH 7.4). The blood was centrifuged each time for 10 min at 3000 g.

#### Hypotonic solution-induced rat erythrocyte haemolysis:

Membrane stabilizing activity of the extract was assessed using hypotonic solution-induced rat erythrocyte haemolysis (Shinde *et al.*, 1999). The test sample consisted of stock erythrocyte (RBC) suspension (0.50 mL) mixed with 5 mL of hypotonic solution (50 mM NaCl) in 10 mM sodium phosphate buffered saline (pH 7.4) containing the extract (0.5-2.0 mg mL<sup>-1</sup>) or acetylsalicylic acid (0.1 mg mL<sup>-1</sup>). The control sample consisted of 0.5 mL of RBC mixed with hypotonic-buffered saline alone. The mixtures were incubated for 10 min at room temperature and centrifuged for 10 min at 3000 g and the absorbance of the supernatant was measured at 540 nm. The percentage inhibition of haemolysis or membrane stabilization was calculated according to the modified method described by Shinde *et al.* (1999).

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

Where:

OD<sub>1</sub> = Optical density of hypotonic-buffered saline alone  
OD<sub>2</sub> = Optical density of test sample in hypotonic medium

**Data analysis:** Data obtained from this study were expressed as mean±SEM. Statistical analysis was performed using ANOVA. p-values less than 0.05 were considered statistically significant.

## RESULTS

**Acute toxicity test:** The toxicity study showed that the extract has a high safety profile as no death was observed at oral doses of 0.20-2.0 g kg<sup>-1</sup> of the extract. The behavioural changes observed at toxic doses of the extract were sedation, ataxia and hyperventilation.

**Effect on carrageenin-induced acute inflammation:** The effect of the aqueous extract of *E.odoratum* on carrageenin-induced oedema is shown in Table 1. It was

evident that the extract exhibited a significant anti-inflammatory activity against carrageenin-induced oedema in oral doses of 100-400 mg kg<sup>-1</sup>. The dose of 400 mg kg<sup>-1</sup> of the extract produced 50% inhibition of oedema which is comparable that shown by acetylsalicylic acid (100 mg kg<sup>-1</sup>, p.o).

**Effect on formaldehyde-induced oedema:** The values of the inhibitory effect of the extract of *E. odoratum* against formaldehyde-induced oedema are shown in Table 2. The extract was found to significantly reduce the oedema swellings induced by formaldehyde in rats. in a dose dependent manner. At a dose of 400 mg kg<sup>-1</sup>, the inhibitory effect shown by the extract was comparable to that produced by 100 mg kg<sup>-1</sup> of acetylsalicylic acid.

**Effect on erythrocyte membrane stability:** The extract (1.0-2.0 mg mL<sup>-1</sup>) offered a significant (p<0.05) protection of rat RBC against haemolysis induced by hypotonic medium. A similar response was observed in RBC exposed to hypotonic medium containing acetylsalicylic acid (0.10 mg kg<sup>-1</sup>). As shown in Table 3, the extract (0.2 mg kg<sup>-1</sup>) offered 37.50% protection against hypotonic-induced rat red blood cell lysis as compared to 30.21% produced by acetylsalicylic acid (0.10 mg mL<sup>-1</sup>).

Table 1: Effect of aqueous leaf extract of *Eupatorium odoratum* on carrageenin-induced oedema in rats

Sample	Dose (mg kg <sup>-1</sup> )	Paw circumference (cm)	Inhibition of oedema (%)
Saline	-	2.25±0.12	-
<i>E. odoratum</i>	100	2.17±0.17*	46.0
<i>E. odoratum</i>	200	2.11±0.08*	48.0
<i>E. odoratum</i>	400	2.20±0.03*	50.2
Acetylsalicylic acid	100	1.96±0.20*	58.0

Each value represents the mean±SEM for 6 animals in each group. \*p<0.05 when compared to saline-control group (ANOVA)

Table 2: Effect of the aqueous leaf extract of *Eupatorium odoratum* on formaldehyde-induced oedema in rats

Sample	Dose (mg kg <sup>-1</sup> )	Paw circumference (cm)	Inhibition of oedema (%)
Saline	-	2.59±0.05	-
<i>E. odoratum</i>	100	2.27±0.14*	36.50
<i>E. odoratum</i>	200	2.33±0.02*	48.60
<i>E. odoratum</i>	400	2.21±0.07*	58.10
Acetylsalicylic acid	100	2.10±0.13*	60.00

Each value represents the mean±SEM for 6 animals per group. \*p<0.05 when compared with saline-control group (ANOVA)

Table 3: Effect of the aqueous leaf extract of *Eupatorium odoratum* on rat erythrocyte haemolysis induced by hypotonic solution

Sample	Concentration	Optical density (OD)	Inhibition of haemolysis (%)
Hypotonic	50 mM	0.96±0.03	-
<i>E. odoratum</i>	0.50 mg mL <sup>-1</sup>	0.73±0.05	24.00
<i>E. odoratum</i>	1.00 mg mL <sup>-1</sup>	0.69±0.02*	28.13
<i>E. odoratum</i>	2.00 mg mL <sup>-1</sup>	0.60±0.07*	37.50
Acetylsalicylic acid	0.10 mg mL <sup>-1</sup>	0.67±0.01*	30.21

Each value represents the mean±SEM for 6 experiments. \*p<0.05 compared when with hypotonic medium (ANOVA)

## DISCUSSION

The results of the study revealed that the extract possess anti-inflammatory activity, as it significantly reduced oedema induced by carrageenin or formaldehyde in rats. The extract also exhibited membrane stabilizing property, as it offered significant protection of rat RBC membrane against lysis induced by hypotonic medium. The acute toxicity studies showed that the extract has a high safety profile as no death was observed at the tested dose range in mice.

Inflammatory reaction produced by carrageenin has been shown to be due to a step-wise release of vasoactive substances such as histamine, bradykinin and serotonin in the early phase and prostaglandins in the acute phase (Dirosa *et al.*, 1971). Formaldehyde, a potent oedematous agent widely used to induce arthritis in experimental animals, produce inflammation through the release of histamine, bradykinin, serotonin, Substance P, nitric oxide including prostaglandins (Tjolsen *et al.*, 1992). These mediators cause oedema swellings through the induction of vasopermeability that result in the accumulation of fluid in the interstitial tissues (Cotran *et al.*, 1999).

It is well-known that compounds with prostaglandin synthesis inhibitory properties such as acetylsalicylic acid-related drugs reduce paw oedema induced by carrageenin or formaldehyde in rats (Jain and Kahanna, 1981; Nunez-Guillen *et al.*, 1997; Perenz *et al.*, 1996; Shinde *et al.*, 1999). The ability of the extract to reduce oedema size produced by these phlogistic agents, suggests that it contained phytochemically active constituent (s) with anti-inflammatory property.

The probable mode by which the extract exhibits its anti-inflammatory activity was studied on rat red blood cell exposed to hypotonic medium. The vitality of cells depends on the integrity of their membranes. Therefore, heamolysis of RBC on exposure to injurious substances such as hypotonic medium or phenylhydrazine is an indication of injury to its membrane (Ferrali *et al.*, 1992). Since the red blood cell membrane is similar to that of lysosomal membrane, inhibition of RBC haemolysis will therefore, provides good insights into the inflammatory process especially as both events are also consequent of injury. Injury to lysosomal membrane usually triggers the release of phospholipase A<sub>2</sub> that mediates the hydrolysis of phospholipids to produce inflammatory mediators (Aitadafoun *et al.*, 1996).

It is therefore, expected that compounds with membrane-stabilizing properties, should offer significant protection of cell membrane against injurious substances and thereby exhibit anti-inflammatory properties (Shinde *et al.*, 1999; Perenz *et al.*, 1995). This notion is consistent with the observations that stabilization of bio-membranes will interfere with the early step involved in

the inflammatory events, namely the release of phospholipase A<sub>2</sub> that trigger the formation of lipid mediators (Aitadafoun *et al.*, 1996). Studies have also revealed that compounds with membrane-stabilizing properties possess significant anti-inflammatory activities (Perenz *et al.*, 1995; Shinde *et al.*, 1999). In this study, the extract of *E. odoratum* produced significant membrane-stabilizing activity. This effect may have contributed a significant role to its anti-inflammatory activity observed in this study. The presence of phenols, terpenoids and flavonoids in the leaf extract of this plant (Richter *et al.*, 2003; Triratana *et al.*, 1991) may be responsible for the anti-inflammatory property demonstrated by *E. odoratum*, as these phytochemicals are well-known for their ability to inhibit inflammation (Ferrandiz and Alcaraz, 1991; Dewhirst, 1980).

In conclusion, the results of the study provide evidence that may support the ethnomedicinal uses of *Eupatorium odoratum* in the control of inflammation.

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