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Preliminary studies of some pharmacological properties of *Croton zambesicus*

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Abstract

The ethanolic leaf extract of *Croton zambesicus* was investigated for pharmacological properties against egg white-induced acute inflammation, acetic acid induced writhing and yeast induced fever in rats. The extract (100-200mg/kg) showed a dose – dependent anti-inflammatory, analgesic, and antipyretic activities. These activities were lower than that of the standard drug (Aspirin, 100mg/kg). The leaf extract possess anti-inflammatory, analgesic and antipyretic properties which can be exploited in health care.

Keywords: *Croton zambesicus*, anti-inflammatory analgesic, antipyretic

Introduction

Croton zambesicus Muell Arg. (Euphorbiaceace) (syn *C. amabilis* Muell. Arg. *C. gratissimus* Buch) is an ornamental tree grown in villages and towns in Nigeria. It is a Guineo-Congolese species widely spread in tropical Africa. The leaf decoction is used in Benin as anti hypertensive and anti-microbial - urinary infections (Adjanohun *et al.*, 1989) and in parts of Nigeria as malarial remedy. Block *et al.*, (2002) reported that ent-trachylobane diterpene, isolated from dichloromethane extract of the leaves has cytotoxic activity on Hela cells. Studies have reported on the antimicrobial properties of the leaf and stem (Abo *et al.*, 1999).The essential oil found in the leaves contain p-cymene, linalool and beta-caryophyllene (Menut *et al.*, 1995). The constituent of the essential oil also found in the flowering tops are pinene, limonene, menthol, carvone, thymol, alpha-humulene and ceisnerolidol (Mekkawi,1985). The ethanolic leaf extract has been reported to possess antiplasmodial activity (Okokon *et al.*, 2005). Although a number of studies have been carried out on this plant, there is no scientific report on the anti-inflammatory, analgesic and antipyretic activity of this plant grown in Nigeria. We therefore investigated the above pharmacological properties of the leaf extract of the plant.

Experimental

Preparation of plant extract. The leaves of *C. zambesicus* Muell. Arg. (Euphorbiaceae) were collected in November 2004 at Uyo area of

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Akwa Ibom State, Nigeria, and authenticated by Dr Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimen number of the plant is FPUU 209. The fresh leaves (2kg) of the plant were dried on a laboratory table for 14 days and reduced to power. The powder 100g was macerated in 95% ethanol (300ml) for 72 hours. The liquid extract obtained was concentrated *in vacuo* at 40°C. The yield was 3. 77%. The extract was stored in a refrigerator at 4°C until used for experiments reported in this study.

Animals: Albino Wistar rats (110 – 180g) and albino Swiss mice (25 – 32g) of either sex were obtained from the University of Uyo animal house. They were maintained on standard animal pellets and water *ad libitum*. Permission and approval for animal studies were obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

Determination of LD_{50}. The LD_{50} of the extract was estimated using Swiss albino mice by intraperitoneal (i.p) route using the method of Lorke (1983).

Anti-inflammatory test. The test was carried out using a phlogistic agent – induced rat hind paw oedema as a model of acute inflammation (Winter *et al.*, 1963). Adult Wistar rats of either sex (180-220g) were used after a 12h fast. Animals were deprived of water only during the experiment. Inflammation of the hind paw was induced by injection of 0.1ml of fresh egg white into the subplantar surface of the right hind paw of the rat. Paw diameters were measured immediately before the administration of the phlogistic agent and at 30 minutes intervals for 3 hours thereafter. For routine drug testing, the increase in paw diameter 3 hours after administration of the phlogistic agent was adopted as the parameter for measuring inflammation (Winter *et al.*, 1962). Thus, (inflammation) was assessed as the difference between zero time paw diameter and that 3 hours after administration of phlogistic agent (Hess and Milong, 1972). The extracts (100, 150 and 200mg/kg) were administered i.p, 1 hour before inducing inflammation. Control rats received equivalent amount of normal saline and the reference group was administered acetyl salicylic acid (ASA) 100 mg/kg. Average oedema (C_t - C_o), percent inflammation and percent inhibition of oedema were calculated for each dose (Oriowo 1982; Akah and Njike 1990). Percent inflammation was calculated as follows:

% Inflammation =
(Average inflammation at time t / Average inflammation of control at the same time) x 100

Analgesic activity. The analgesic activity of ethanolic leaf extract of *Croton zambesicus* was measured against acetic acid induced writhmic movement in rats (Collier *et al.*,1968). The extract at doses of 100, 150 and 200mg/kg, and ASA 100mg/kg and normal saline 5ml/kg were administered intraperitoneally to the respective groups (n = 5) of the 12 hours fasted rats. Thirty minutes later, 0.5 of 2% v/v acetic acid solution was given to each animal intraperitoneally. The animals were then placed in separate plastic cages and closely observed for 10 minutes interval for 50 minutes. The number of writhes for each animal was counted. A writhe was recorded when the contraction of the abdominal muscle followed by the stretching of the hind limbs was noted. Percent inhibition of pain for each group was calculated by comparing the total writhetic number of writhes in the group over the 50 minutes period with the number of writhes in the control group over the same time period. Data were calculated according to following formula

% Inhibition =

{(Mean number of writhes in control group-Mean number of writhes in test group)/ Mean number of writhes in control group} x 100

Antipyretic test. Groups of five rats each deprived of food for 12 hours, were used. At 0 hour, the basal rectal temperature of the rats were taken using the clinical thermometer. Thereafter each animal was administered subcutaneously with 50% v/v aqueous suspension of the yeast in a volume of 10ml/kg (Gural *et al.*, 1955). At suitable interval beginning one hour after yeast injection, rectal temperatures of animals were taken, animals with temperature increase of over 1°C respectively were selected and grouped for the study. The extract understudy was administered intraperitoneally. (i.p) after the pyrogen at the doses of 100, 150, and 200 mg/kg to respective group of rats. The control group received the vehicle 5ml/kg and the reference group administered with 100mg/kg ASA both i.p. The rectal temperatures were taken for the next 5 hours after the treatment.

Statistical analysis. Data were expressed as mean ± SEM for n numbers of experiment. Statistical comparisons and significance levels were analyzed with student's t – test as applicable. A 'p' value less than 0.05 was consider as significant.

Results

Acute toxicity. The extract (100-2000 mg/kg) produced physical signs of toxicity ranging from decreased motor activity, decreased respiratory rate, body and limb tone to death. The intensities of all these effects were proportional to the dose administered. The i.p. LD_{50} of the extract in mice was 1400 ± 48mg/kg.

Anti-inflammatory activity. The study shows that ethanolic leaf extract of *Croton zambesicus* exerts a considerable anti-inflammatory activity. Fresh egg white induced a progressive increase in paw diameter reaching maximum in 90min. The extract exerted a good anti-inflammatory activity against acute inflammation inhibiting the rat paw oedema in a dose related manner. The reduction in oedema was observed 30min after injection of the phlogistic agent and lasted throughout the duration of the study. The mean oedema, percentage inflammation and percentage inhibition of oedema in the extract treated rat are shown in Tables 1, 2, and 3 respectively. Although, all the doses (100-200mg/kg) exhibited significant ($p < 0.05$) anti-inflammatory activity, the effect was highest at the dose of 200mg/kg, but was less effective than that of ASA.

Analgesic activity. The study shows that ethanolic leaf extract of *C. zambesicus* (at doses of 100mg/kg and above i.p) showed significant ($p<0.05$) analgesic activity against acetic acid induced abdominal constriction compared to control statistically (Table 4) in a dose dependent fashion. The antinociceptive activity was less effective than that of ASA.

Antipyretic effect. Table 5 shows the results of antipyretic activities of the leaf extract of *C. zambesicus*. The extract produced a dose dependent reduction in temperature, which though significant ($p < 0.05$) compared to control, was less effective than that produced by ASA.

Table 1: Inflammatory effect of extract on paw

Dose of extract (mg/kg)	Mean diameter (cm) ± SEM (n=5)					
	30 min	60 min	90 min	120 min	150 min	180 min
100	0.3± 0.01	0.32±0.01	0.33±0.01	0.32±0.01	0.31±0.01*	0.30±0.02*
150	0.29±0.02	0.30±0.02	0.32±0.01	0.31±0.01*	0.29±0-.01*	0.28±0.01*
200	0.32±0.02	0.32±0.02	0.34±0.03	0.31±0.01*	0.28±0.01*	0.25±0.01*
Control (5mg/kg)	0.35±0.03	0.36±0.04	0.37±0.03	0.36±0.02	0.35±0.01	0.34±0.01
ASA	0.32±0.03	0.27±0.01	0.28±0.04	0.26±0.04	0.25±0.04	0.24±0.04

*P < 0.05 Significantly different from control (Student's t-test).

Table 2: Percentage inflammation in extract-treated rats

Dose of extract (mg/kg)	30 min	60 min	90 min	120 min	150 min	180 min
100	88.5	88.8	89.1	88.8	88.5	88.2
150	82.8	83.3	86.4	86.1	82.8	82.3
200	91.4	88.8	91.9	91.2	80.0	76.5
ASA	91.4	75.0	75.6	72.2	71.4	70.5

Table 3: Percentage inhibition of oedema by the extract

Dose of extract (mg/kg)	30 min	60 min	90 min	120 min	150 min	180 min
100	11.5	11.2	10.9	11.2	11.5	11.8
150	17.2	16.7	13.6	13.9	17.2	17.7
200	8.2	11.2	8.1	8.8	20.0	23.5
ASA	8.6	25.0	24.4	27.8	28.6	29.5

% Inhibition= 100 - % Inflammation

TABLE 4: Analgesic activity of the ethanolic leaf extract of *Croton zambesicus*

Treatment	Dose	Time (minutes) for onset of writhe movement	No. of writhing (mean ± SEM)	Percent activity against acetic acid
Control	5mL/kg	4.7±0.97	137.6±1.43	-
Extract	100 mg/kg	8.0±0.22	75.0±0.86*	45.5
Extract	150 mg/kg	9.0±0.69	50.0±0.73*	63.7
Extract	200 mg/kg	11.0±0.31	49.8±0.73*	63.8
ASA		13.7±0.81	39.3±0.54*	71.4

TABLE 5: Antipyretic effect of ethanolic leaf extract *Croton zambesicus* on yeast induced pyrexia in rats

Treatment	Dose	Mean temperature after 4 hours
		38.7±0.11
Extract	100 mg/kg	37.6±0.15 *
Extract	150 mg/kg	37.5±0.23*
Extract	200 mg/kg	37.3± 0.17*
ASA	100 mg/kg	36.7±0.09

Results are expressed as mean ± SEM (n = 5), *($p < 0.05$) significantly different from control (Student's t-test)

Discussion

The result of these preliminary pharmacological studies of ethanolic leaf extract of *C. zambesicus* revealed that this plant possesses anti-inflammatory, analgesic and antipyretic activities. The extract inhibited, to some degree, increase in rat paw diameter caused by egg white. Carrageenan and egg white induced oedema has been correlated and reported to have no marked difference (Akah *et al*,1993) and is attributed to the release of histamine, 5-HT, kinins and prostaglandins (Vane and Booting, 1987; Larsen and Henson, 1983).

The anti-inflammatory activity of the ethanolic extract could be due to the presence of a flavonoids (Parmer and Gosh, 1978) reported to be present in this plant (Okokon *et al.*, 2005). The leaf extract was also found to possess significant ($p < 0.05$) analgesic activity against acetic acid induced pain. However, the antinociceptive action of the extract was less than that of aspirin. Pains are known to result from the sensitization of nociceptive afferent nerve by prostaglandins such as PGE_1, or PGE_2 to inflammatory mediators like bradykinin or 5-hydroxytryptamine (Lands, 1985). The extract may be acting in a similar manner like the analgesic drugs by inhibiting archidonate cyclo-oxygenase (COX), enzyme involved in the synthesis of prostaglandins (Ritter *et al.*, 1995), hence reducing pains. The extract demonstrated a significant ($p<0.05$) antipyretic activity and fever is known to be

associated with the production of prostaglandins in the hypothalamus. The extract has been suggested above to act by inhibiting the production of prostaglandins. Its antipyretic action is believed to result from this action. The leaf extract of *C. zambesicus* has been reported to possess antiplasmodial activity (Okokon *et al.*, 2005). The findings of the present study suggest its ability to alleviate malarial symptoms like inflammation, pains and fever.

In conclusion, the result obtained in this preliminary study shows that *Croton zambesicus* possesses anti-inflammatory, analgesic and antipyretic properties which are probably mediated via inhibition of prostaglandin synthesis. Further studies are needed to elucidate the exact mechanism by which *C. zambesicus* inhibits inflammation, pains and fever.

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