ANTIINFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITIES OF ETHANOLIC ROOT EXTRACT OF *CROTON ZAMBESICUS*

JUDE E OKOKON* AND PAUL A NWAFOR

Department of Pharmacology and Toxicology, University of Uyo, Uyo, Nigeria

ABSTRACT

The ethanolic root extract of *C. zambesicus* (27-81mg/kg) was evaluated for antiiflammatory, analgesic and antipyretic properties in mice. The extract (27-81mg/kg) demonstrated a weak antiinflammatory activity. However, a significant ($P<0.01$-0.001) analgesic and antipyretic activities were observed in all the experimental models tested. The extract may be exerting its effects through central mechanisms. These findings confirms its ethnomedical use in the treatment of malarial-associated symptoms.

Keywords: Antiinflammatory, analgesic, antipyretic, *Croton zambesicus.*

INTRODUCTION

Croton zambesicus Muell Arg. (Euphorbiaceace) (syn *C. amabilis* Muell. Arg. *C. gratissimus* Burch) is an ornamental tree grown in villages and towns in Nigeria. It is a Guineo – Congolese species widely spread in tropical Africa. Ethnobotanically, the leaf decoction is used in Benin as anti hypertensive and anti- microbial (urinary infections) (Adjanohoun *et al,* 1989) and in parts of Nigeria as antidiabetic and malarial remedy (Okokon *et al*., 2005a, 2006). The roots are used as antimalarial, febrifuge and antidiabetic by the Ibibios of Niger Delta region of Nigeria (Okokon and Nwafor, 2009a). The root is also used in Sudan for menstrual pain (El-Hamidi, 1970) and as aperients (Ngadjui *et al*., 1999). Boyom *et al*. (2002) studied the composition of essential oils from the leaves, stem and roots of *Croton zambesicus* and found the three types of oils to be similar in composition, with those from the leaves and stem rich in monoterpenes, while that of the root bark contains sesquiterpenes. The root and stem bark oils were found to be rich in oxygen-containing compounds, with spathulenol and linalool as major components. Okokon and Nwafor (2009a) reported that the root extract whose LD_{50} is 273.86 mg/kg contains alkaloids, saponins, terpenes, tannins, phlobatannins, anthraquinones and cardiac glycosides, while flavonoids were reported to be absent. Block *et al. (*2002) isolated entrachyloban-3β-ol, an ent-trachylobane diterpene from dichloromethane extract of the leaves and reported that the diterpene has a cytotoxic activity on HeLa cells. Also two new trachylobane – and one isopimarane type diterpenoids; ent-18-hydroxy-trachy-loban-3-one, ent-trachyloban-3- one, isopimara-7,15-dien-3β-ol, together with transphytol, β-sitosterol, α-amyrin and stigmaterol have been isolated from the leaves (Block *et al*., 2004). Crotonadiol, a labdane diterpenoid, clerodane, croto-corylifuran and two trachylobanes; 7β-acetoxy-trachyloban - 18 - oic acid, trachyloban - 7β, 18 - diol, lupeol, β-sitosterol and its 3-β-glucopyranosyl derivative were isolated from the stem bark (Ngadjui *et al*, 1999). Ngadjui *et al*., (2002) further isolated three clerodane diterpenoids, crotozambefurans A, B and C from the stem bark. Studies have reported on the antimicrobial properties of the leaf and stem (Abo *et al*, 1999). The ethanolic leaf extract has been reported to possess antiplasmodial (Okokon *et al.*, 2005a), antidiabetic (Okokon *et al*., 2006), anti-inflammatory, analgesic and antipyretic activities (Okokon *et al.,* 2005b), while the root extract has been reported to possess antimalarial (Okokon and Nwafor, 2009a), anticonvulsant and antiulcer activities (Okokon and Nwafor, 2009b).

Information on biological activity of the root are scarce. We therefore investigated the antiiflammatory, analgesic and antipyretic activities of the root extract of the *Croton zambesicus* to ascertain the folkloric claim of its medicinal properties in the treatment of *Plasmodium falciparum* malaria-associated symptoms such as fever and pains.

MATERIALS AND METHODS

Plant materials

The plant part (roots) was identified by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo. The roots were collected from compounds in Uyo metropolis, Akwa Ibom State of Nigeria and were authenticated. A voucher specimen (DPNM. 31c) of the plant was deposited in the herbarium of Department of Pharmacognosy and Natural Medicine, University of Uyo, Uyo, Nigeria

Extraction

The roots were shade dried for 2 weeks. The dried roots were further chopped into small pieces and reduced to powder. The powdered root was macerated in 97% ethanol for 72 hours to give the crude ethanolic extract. The liquid filtrates were concentrated and evaporated to dryness in vacuo 40°C using rotary evaporator. The yield of the extract was calculated. The dry extract was stored

**Corresponding author:* e-mails: judeefiom@yahoo.com

in a refrigerator at 4°C until use for the proposed experiment.

Animals

The animals (mice and rats) both male and female that were used for these experiments were obtained from University of Uyo animal house. The animals were housed in standard cages and were maintained on a standard pelleted Feed (Guinea Feed) and water *ad libitum*. Permission and approval for animal studies were obtained from College of Health Sciences Animal Ethics committee, University of Uyo.

Evaluation of antiinflammatory activity of the extract

Carrageenin – induced oedema in mice

Adult albino mice (22-25g) of either sex were randomised into different groups of 6 mice each.They were used for the experiment after 24 hours fast and deprived of water only during experiment. Inflammation of the hind paw was induced by injection of 0.1ml of freshly prepared carrageenin suspension (1%) in normal saline into the subplanar surface of the hind paw. The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5 hrs after administration of phlogistic agent. For this experiment, the increase in paw circumference 0.5, 1, 2, 3, 4 and 5 hrs after administration of phlogistic agent was adopted as the parameter for measuring inflammation (Winter, *et al*., 1962; Akah and Nwambie, 1994; Ekpendu *et al.,* 1994; Besra *et al.*, 1996; Nwafor and Okwuasaba., 2003). Edema (inflammation) was assessed as difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5 hrs after administration of phlogistic agent (Hess and Milonig, 1992). The extract (27, 54 and 81mg/kg i.p) was administered to various groups of mice, 1 hr before inducing inflammation. Control mice received 10ml/kg of distilled water orally while reference group received acetic salicylic acid, ASA (100mg/kg). The average (mean) oedema was assessed by measuring with vernier calipers.

Egg-albumin- induced inflammation in mice

Adult albino mice (23-28g) of either sex randomised into different groups of 6 mice each were used for the experiment. The root extract (27, 54 and 81mg/kg i.p) and ASA (100mg/kg orally) were administered to mice 1 hr before the induction of inflammation. Control group received 10ml/kg of distilled water orally. Inflammation was induced in mice by the injection of 0.1 ml of fresh egg-albumin into the subplanar tissue of the right hind paw (Akah and Nwambie, 1994). The linear circumference of the injected paw was measured before and 0.5, 1, 2, 3, 4 and 5hrs after the administration of the phlogistic agent. Edema (inflammation) was assessed as the difference in paw circumference between the control and 0.5, 1, 2, 3, 4 and 5hrs after the administration of the phlogistic agent (Hess and Milonig, 1972). All the animals were fasted for 24 hours before the commencement of the experiment. The average (mean) edema was assessed by measuring with vernier calipers.

Xylene – induced ear oedema in mice

Adult albino mice (22-27g) of either sex randomised into different groups of 6 mice each were used for the experiment. *C. zambesicus* root extract (27, 54 and 81mg/kg i.p), dexamethasone (4mg/kg) and distilled water (10ml/kg) were orally administered to various groups of 24 hours fasted mice 30 minutes before the induction of inflammation. Inflammation was induced in mice by topical administration of 2 drops of xylene at the inner surface of the right ear. The xylene was left to act for 15 mins. The animals were sacrificed under light anaesthesia and the both ears were cut off. The difference between the ear weights was taken as the oedema induced by the xylene (Tjolsen *et al*., 1992).

Evaluation of analgesic effect of the extract

Acetic acid induced writhing in mice

Analgesic activity of ethanolic root extract of *C. zambesicus* against acetic acid- induced writhing was carried out according to the procedure of Santos *et al.*, (1994); Correa *et al.*, (1996); Besra (1996); Nwafor and Okwuasaba (2003). Adult albino mice (25-30g) of either sex randomised into five groups of 6 mice each were used for the experiment. The mice were fasted for 24 hours before used but allowed access to water. Group I which served as control were given distilled water (10ml/kg), while groups 2-4 were intraperitoneally pretreated with 27, 54 and 81mg/kg of *C. zambesicus* root extract. Acetyl salicylic acid (ASA), 100mg/kg was given to the reference group. After 30 minutes, 0.2 ml of acetic acid (3%) was administered by the same route. The number of writhing movements (contraction of abdominal muscle together with a stretching of hind limbs) resulting from intraperitoneal (i.p) injection of 0.2ml of acetic acid (3%) was counted for 5 hrs at 30 minutes intervals. Anti-nociception was expressed as the reduction of the number of abdominal constrictions between control animals treated with distilled water (10ml/kg) and mice pretreated with the extract

Formalin-induced paw licking in mice

The method similar to that of Hunskaar and Hole (1987), Gorski *et al.* (1993), Correa and Calixto (1993), Nwafor and Okwuasaba (2003) was used to evaluate the analgesic activity of the extract against formalin-induced paw licking in mice. Adult albino mice (25-30g) of either sex randomised into different groups of 6 mice each were used for the experiment. The animals were fasted for 24 hours and pretreated with *C. zambesicus* root extract (27, 54 and 81 mg/kg i.p) and ASA (100mg/kg) before being challenged with buffered formalin. 20µl of 2.5% formalin solution (0.9% of formaldehyde) made up in phosphate buffer solution (PBS, concentration NaCl – 137mM, KCl

2.7mM and phosphate buffer 10mM) was injected subcutaneously under the surface of the right hind paw of each mice and the responses were observed for 30 minutes. The control animals were given 10ml/kg of distilled water orally. The amount of time spent licking the injected paw was timed and was indicative of pain. The first phase of the analgesic activity normally peaked at 5 minutes after formalin injection and the second phase 15-30 minutes after formalin injection, representing the neurogenic and inflammatory pain responses respectively (Hunskaar and Hole, 1987).

Thermally-induced pain in mice

The effect of the extract on hot plate-induced pain was investigated in adult mice. The hot plate test was used to measure the response latencies according to the method of Vaz *et al.* (1996).. Adult albino mice (23-28g) of either sex randomised into five groups of 6 mice each were used for the experiment. The animals were fasted for 24 hours but allowed water *ad libitum* before used in the experiment. Group I animals served as control and received only normal saline. Groups 2, 3 and 4 were pretreated with 27, 54 and 81 mg/kg of *C. zambesicus* root extract i.p respectively, 30 min prior to the placement on the hot plate, while group 5 animals received 0.1g/kg of acetyl salicylic acid by same i.p route. In these experiments, the hot plate was maintained at 45 ± 1°C. Animals were placed into a glass beaker of 50cm diameter on the heated surface and the time between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30s cut-off was used to prevent tissue damaged.

Evaluation of antipyretic activity of the extract

2, 4 – Dinitrophenol (DNP) induced pyrexia

Adult albino rats (150 – 170g) of both sexes fasted for 24 hours but allowed water *ad libitum* were used for the experiment. They were randomized into groups of 6 rats each. DNP (10mg/kg, i.p) was administered to the rats after obtaining the basal rectal temperatures. Hyperthermia developed within 30 min of DNP administration. Different doses of extract (27, 54 and 81 mg/kg i.p), aspirin (100mg/kg) and distilled water (10ml/kg, orally) were administered respectively to the treatment and control groups of animals. Rectal temperatures of the animals were obtained at an hour interval for 5 hrs (Backhouse *et al*., 1994; Winter *et al*., 1962; Mbagwu *et al*., 2007).

D-amphetamine induced pyrexia

Adult albino rats (150-170g) of both sexes fasted for 24 hours but allowed water ad libitum were used for the experiment. They were randomized into groups of 6 rats each. Amphetamine (5mg/kg, i.p) was administered to the animals after obtaining basal temperatures. Hyperthermia developed 0.5hrs following amphetamine administration. The extract (27, 54 and 81mg/kg, i.p) aspirin (100mg/kg orally) and distilled water (10ml/kg orally) were administered to the animals at peak hyperthermia. Rectal temperatures were obtained at 1hr interval for 5hrs (Blackhouse *et al*., 1994; Bamgbose and Noamesi, 1981; Mbagwu *et al*., 2007).

Yeast-induced pyrexia

Adult albino rats (140-180g) of both sexes fasted for 24 hours but allowed water ad libitum were used for the experiment. They were randomized into groups of 6 rats each. At zero hour, the basal temperature of the rats was taken using digital clinical thermometer. Thereafter, each animal was administered subcutaneously with 20% W/V aqueous suspension of yeast at a volume of 10ml/kg (Gural *et al*., 1955,). At suitable intervals beginning one

Table 1: Effect of *Croton zambesicus* root extract on carrageenin- induced oedema in mice.

Treatment/ dose (mg/kg)	Time Intervals (hr)						
	0	0.5	1	2	3	4	5
Control	0.27 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.34 ± 0.01	0.35 ± 0.01	0.31 ± 0.01	0.30 ± 0.01
Extract 27	0.26 ± 0.01	0.36 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.31 ± 0.01	0.35 ± 0.01	0.34 ± 0.01
54	0.25 ± 0.01	0.36 ± 0.01	0.34 ± 0.01	0.34 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.34 ± 0.01
81	0.23 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.34 ± 0.01	0.32 ± 0.01	0.31 ± 0.01
ASA 100	0.27 ± 0.01	0.35 ± 0.01[a]	0.34 ± 0.01[a]	0.32 ± 0.01[b]	0.30 ± 0.01[b]	0.28 ± 0.01[b]	0.26 ± 0.01[b]

Data are expressed as mean ± SEM. Significant at [a]P < 0.05, [b] P < 0.001 when compared to control. n = 6.

Table 2: Effect of *Croton zambesicus* root extract on egg- albumin induced oedema in mice.

Treatment/ dose (mg/kg)	Time Intervals (hr)						
	0	0.5	1	2	3	4	5
Control	0.25 ± 0.01	0.35 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.32 ± 0.01	0.30 ± 0.01	0.29 ± 0.01
Extract 27	0.26 ± 0.01	0.37 ± 0.01	0.36 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.30 ± 0.01	0.28 ± 0.01
54	0.26 ± 0.01	0.36 ± 0.01	0.36 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.30 ± 0.01
81	0.24 ± 0.01	0.35 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.31 ± 0.01	0.29 ± 0.01	0.28 ± 0.01
ASA 100	0.25 ± 0.01	0.31± 0.01[a]	0.28 ± 0.01[b]	0.28 ± 0.01[b]	0.27 ± 0.01[b]	0.26 ± 0.01[b]	0.25 ± 0.01[b]

Data are expressed as mean ± SEM. Significant at [a]P < 0.01, [b] P < 0.001 when compared to control. n = 6.

Table 3: Effect of *Croton zambesicus* root extract on xylene-induced ear oedema in mice

Treatment/dose (mg/kg)	Weight of right ear (g)	Weight of left ear (g)	Increase in ear weight (g)	% Inhibition
Control (normal saline) 0.2ml	0.078 ± 0.01	0.040 ± 0.00	(48.71) 0.038 ± 0.01	
Extract 27	0.062 ± 0.01	0.041 ± 0.01	(33.87) 0.021 ± 0.01^{NS}	44.78
54	0.058 ± 0.01	0.045 ± 0.01	(22.41) 0.013 ± 0.01^{NS}	53.39
81	0.048 ± 0.01	0.038 ± 0.01	(20.83) 0.010 ± 0.00^{a}	57.23
Dexamethasone 4.0	0.045 ± 0.01	0.036 ± 0.01	(22.22) 0.009 ± 0.00^{a}	54.38

Figures in parenthesis indicate % increase in ear weight. *significant at $^{a}P < 0.01$ when compared with control. n = 6.

Table 4: Effect of *Croton zambesicus* root extract on acetic acid induced writhing in mice.

Treatment/ Dose (mg/kg)	Time Intervals (hr)						
	5	10	15	20	25	30	Total
Control	5.00 ± 1.06	8.83 ± 1.42	19.16 ± 2.13	18.16 ± 1.49	15.66 ± 0.71	12.33 ± 1.20	79.14 ± 3.03
Extract 27	0.00^{c}	9.60 ± 0.88	11.16 ± 2.27^{a}	12.50 ± 1.36^{a}	11.50 ± 2.30	10.66 ± 1.82	55.42 ± 3.26^{c}
54	0.00^{c}	9.16 ± 1.40	11.0 ± 0.96^{a}	11.16 ± 0.94^{a}	8.50 ± 1.23^{a}	6.80 ± 1.37^{a}	46.62 ± 2.23^{c}
81	0.00^{c}	9.50 ± 1.54	11.08 ± 0.67^{a}	9.50 ± 1.28^{b}	7.83 ± 1.53^{a}	3.83 ± 0.87^{b}	41.74 ± 2.33^{c}
ASA 100	0.00^{c}	2.00 ± 0.00^{b}	6.40 ± 1.93^{c}	9.50 ± 1.73^{b}	9.50 ± 1.28^{b}	9.30 ± 1.85^{b}	36.70 ± 2.56^{c}

Data are expressed as mean ± SEM. significant at $^{a}P < 0.05$, $^{b}P < 0.01$, $^{c}P < 0.001$ when compared to control n = 6.

Table 5: Effect of *Croton zambesicus* root extract on formalin-induced hind paw licking in mice

Treatment/ Dose (mg/kg)	Time Intervals (mins)						
	5	10	15	20	25	30	Total
Control	33.3 ± 0.99	14.16 ± 0.60	17.50 ± 0.42	10.66 ± 0.49	6.66 ± 0.33	5.66 ± 0.42	87.94 ± 3.25
Extract 27	29.5 ± 0.88	6.00 ± 0.25^{c}	5.00 ± 0.36^{c}	4.33 ± 0.42^{c}	4.00 ± 0.36^{c}	3.66 ± 0.21^{c}	52.49 ± 2.71^{c}
54	24.16 ± 0.28^{c}	5.33 ± 0.21^{c}	4.83 ± 0.33^{c}	4.16 ± 0.16^{c}	3.33 ± 0.21^{c}	3.00 ± 0.36^{c}	44.76 ± 1.61^{c}
81	18.5 ± 2.74^{c}	0.00^{c}	0.50 ± 0.49^{c}	3.00 ± 0.516^{c}	2.50 ± 0.22^{c}	2.50 ± 0.22^{c}	27.00 ± 4.16^{c}
ASA 100	8.83 ± 0.43^{c}	1.66 ± 0.21^{c}	2.80 ± 0.16^{c}	2.16 ± 0.16^{c}	1.16 ± 0.16^{c}	0.00^{c}	16.61 ± 0.43^{c}

Data are expressed as mean ± SEM. Significant at $^{c}P < 0.001$, when compared to control n = 6.

Table 6: Effect of *C. zambesicus* root extract on hot plate test

Group	Dose Mg/kg	Reaction time (sec) (mean ± SEM)	% inhibition
Control	-	3.08 ± 0.16	-
C. zambesicus	27	3.65 ± 0.37	18.50
	54	4.20 ± 0.20^{a}	36.36
	81	4.61 ± 0.17^{a}	49.67
ASA	100	14.42 ± 0.30^{b}	165.10

Data are expressed as mean ± SEM. Significant at $^{a}P < 0.05$, $^{b}P < 0.01$ when compared to control. n = 6.

hour after yeast injection, rectal temperature of animals were taken, animals with increase of 1°C were selected and grouped for the study. The extract understudy was administered i.p. after the pyrogen at the dose of 27, 54 and 81mg/kg to respective groups of rats. The control group received distilled water (10ml/kg) and the reference group administered with ASA (100mg/kg) both intraperitoneally. The rectal temperature of the groups was taken at 1hr interval for 5hrs.

RESULTS

Antiiflammatory activity

Carragenin-induced oedema in mice

The result of the effect of ethanolic root extract of *C. zambesicus* on carragenin-induced oedema is shown in table 1. The extract exerted a weak antiinflammatory effect at the highest dose which was only significant (P<0.05-0.001) between 1-2 hours post induction.

Table 7: Effect of *Croton zambesicus* root extract on DNP induced pyrexia in rat

Treatment/ Dose (mg/kg)	Time Intervals (hr)						
	0	0.5	1	2	3	4	5
Control	35.01 ± 0.16	36.68 ± 0.13	37.36 ± 0.09	37.03 ± 0.08	36.91 ± 0.21	36.63 ± 0.18	36.30 ± 0.20
Extract 27	34.48 ± 0.10	36.48 ± 0.10	35.86 ± 0.08[b]	35.53 ± 0.07[c]	35.18 ± 0.05[c]	34.86 ± 0.04[c]	34.56 ± 0.06[c]
54	34.31 ± 0.13	36.35 ± 0.19	35.76 ± 0.43[b]	35.45 ± 0.17[c]	35.03 ± 0.16[c]	34.63 ± 0.16[c]	34.26 ± 0.17[c]
81	34.75 ± 0.19	36.75 ± 0.09	36.08 ± 0.12[b]	35.71 ± 0.10[c]	35.26 ± 0.10[c]	34.80 ± 0.13[c]	34.40 ± 0.14[c]
ASA 100	35.08 ± 0.10	38.86 ± 0.23	36.50 ± 0.20	36.28 ± 0.19[b]	35.93 ± 0.20[b]	35.53 ± 0.20[c]	35.25 ± 0.17[c]

Data are expressed as mean ± SEM. significant at [a]P < 0.05, [b]P < 0.01, [c]P < 0.001 when compared to control. n = 6.

Table 8: Effect of *Croton zambesicus* root extract on amphetamine induced pyrexia in rat

Treatment/ Dose (mg/kg)	Time Intervals (hr)						
	0	0.5	1	2	3	4	5
Control	34.60 ± 0.08	36.30 ± 0.12	36.78 ± 0.13	36.63 ± 0.11	36.46 ± 0.10	36.11 ± 0.13	35.80 ± 0.11
Extract 27	34.35 ± 0.16	36.45 ± 0.10	36.03 ± 0.15	35.76 ± 0.15[a]	35.48 ± 0.14[a]	35.18 ± 0.17[a]	34.76 ± 0.17[b]
54	34.58 ± 0.23	36.58 ± 0.18	35.88 ± 0.32[a]	35.65 ± 0.32[a]	35.15 ± 0.30[c]	34.65 ± 0.31[c]	34.56 ± 0.29[b]
81	34.78 ± 0.22	36.68 ± 0.29	35.98 ± 0.18	35.63 ± 0.21[a]	35.18 ± 0.20[b]	34.56 ± 0.17[c]	34.38 ± 0.13[c]
ASA 100	34.50 ± 0.25	36.76 ± 0.17	36.50 ± 0.17[a]	36.16 ± 0.19[a]	35.86 ± 0.20[a]	35.43 ± 0.21[a]	35.00 ± 0.22[a]

Data are expressed as mean ± SEM. significant at [a]P < 0.05, [b]P < 0.01, [c]P < 0.001 when compared to control n = 6.

Table 9: Effect of *Croton zambesicus* root extract on yeast induced pyrexia in rat

Treatment/ Dose (mg/kg)	Time Intervals (hr)						
	0	0.5	1	2	3	4	5
Control	35.01 ± 0.11	36.93 ± 0.15	36.93 ± 0.11	36.86 ± 0.08	36.75 ± 0.07	36.58 ± 0.06	36.55 ± 0.03
Extract 27	34.73 ± 0.15	37.01 ± 0.14	36.86 ± 0.11	36.51 ± 0.20	36.01 ± 0.21[b]	35.61 ± 0.17[c]	35.21 ± 0.16[c]
54	34.78 ± 0.12	36.85 ± 0.14	36.61 ± 0.12	36.30 ± 0.08[a]	36.10 ± 0.09[b]	35.65 ± 0.08[c]	35.23 ± 0.09[c]
81	34.83 ± 0.09	36.85 ± 0.08	36.65 ± 0.06	36.23 ± 0.05[b]	35.86 ± 0.05[c]	35.43 ± 0.08[c]	35.08 ± 0.06[c]
ASA 100	35.03 ± 0.08	36.05 ± 0.11	36.56 ± 0.09	36.20 ± 0.22[b]	35.86 ± 0.06[c]	35.46 ± 0.06[c]	35.21 ± 0.06[c]

Data are expressed as mean ± SEM. Significant at [a]P < 0.05, [b]P < 0.01, [c]P < 0.001 when compared to control n = 6.

However, the standard drug, ASA, caused a significant (P<0.01-0.001) reduction of oedema caused by carragenin.

Egg albumin- induced oedema

Table 2 shows the results of the effect of root extract of *C. zambesicus* on egg albumin-induced oedema in mice. The extract did not exert any considerable antiinflammatory effect at all doses tested against oedema caused by egg albumin. A significant (P<0.01-0.001) anti-inflammatory effect was demonstrated by the standard drug, ASA (100 mg/kg).

Xylene- induced ear edema

Anti-inflammatory effect of root extract of *C. zambesicus* against xylene-induced ear oedema in mice is shown in table 3. The extract exerted a weak anti-inflammatory effect which was only significant (P<0.01) at the highest dose of the extract and comparable to that of the standard drug, dexamethasone (4.0 mg/kg)

Analgesic activity

Acetic acid induced writhing in mice

The extract (27-81mg/kg) dose dependently reduced acetic acid induced abdominal constrictions and stretching of hind limbs. The reduction was significant (P<0.05-0.001) when compared to control (table 4). The effect of the extract was comparable to that of the standard drug, ASA (100mg/kg).

Formalin induced hind paw licking in mice

The result of the effect of the extract against formalin induced hind paw licking in mice is shown in table 5. The extract pretreated animals showed a significant (P<0.05-0.001) dose-related reduction of hind paw licking caused by formalin when compared to control. However, the analgesic effect of the extract was less than that of the standard drug, ASA (100mg/kg).

Hot plate induced pain in mice

The effect of the extract on hot plate induced pain is shown in table 6. Rats pretreated with *C. zambesicus* (27-

81mg/kg, i.p) demonstrated a dose-dependent increase in latency of response in the hot plate test. The increase in the latency of response (analgesic effect) were statistically significant (P<0.05-0.001) and were incomparable to that of the standard drug, ASA (100mg/kg).

Antipyretic test

Dinitrophenol induced pyrexia

The antipyretic effect of the extract on DNP induced pyrexia is shown in table 7. Administration of the root extract of *C. zambesicus* (27, 54 and 81mg/kg) in the presence of the pyrogen caused a significant (P<0.05-0.001) reduction in the temperatures of the extract treated rats when compared with the control. The antipyretic effect though non dose dependent was comparable to that of the standard drug, ASA (100mg/kg).

Amphetamine-induced pyrexia

Table 8 shows the effect of the extract on amphetamine induced pyrexia .The extract exerted a significant (P<0.05-0.001) dose dependent antipyretic effect when compared to control.

The antipyretic effect of the extract was more than that of the standard, ASA (100mg/kg).

Yeast-induced pyrexia

The result of the effect of the extract against yeast-induced pyrexia is shown in table 9. There was a progressive dose dependent reduction in the temperature of rats treated with the extract. The reductions caused by the extract was significant (P<0.005-0.001) when compared to control and more than that of the standard drug, ASA (100mg/kg).

DISCUSSION

In this study pharmacological evaluations of anti-inflammatory, analgesic and antipyretic activities of ethanolic root extract of *Croton zambesicus* were carried out using different experimental models.

In the carragenin induced oedema, the extract (81mg/kg) exerted pronounced effect only at the early stage of inflammation (1-2hr) indicating effect probably on histamine, serotonin and kinnins that are involved in the early stage of carragenin induced oedema (Vane and Booting, 1987). The extract could not reduce later stage of the oedema maybe due to its inability to inhibit prostaglandin which is known to mediate the second phase of carragenin induced inflammation (Vane and Booting, 1987). Moreso, the extract has been reported above to contain sesquiterpenes (Boyom *et al,* 2002) which are likely to cause peroxidation by generating free radical. Peroxidation of lipids components of biomembrane affects primarily function of membranes and pathological changes in them (Stroev and Makarava, 1989). Peroxidation of lipids components of bio-membrane by the extract may in part be responsible for the aggravated oedema observed in this study which was even higher than that of untreated control. This could have been due to distortion of cell membrane intergrity by the extracts' peroxidative activity even if the extract were to inhibit prostaglandin synthesis. However, ASA (100mg/kg) a prototype NSAID, a cyclooxygenase inhibitor whose mechanism of action involves inhibition of prostaglandin, inhibited significantly (P<0.01-0.001) the paw swelling due to carragenin injection.

The extract was ineffective in egg-induced oedema showing that it does not inhibit inflammation by blocking the release of histamine and 5-HT, two mediators that are released by egg albumin. This further point to the fact the inhibition observed above in carragenin induced oedema was not due to due inhibition of histamine and 5-HT, but possibly kinnins or other mediators like eicosanoids which may be involved in this process also. However, ASA, a cyclooxygenase inhibitor reduced significantly oedema produced by egg albumin.

The root extract exerted a significant (P<0.01) inhibition of ear oedema caused by xylene only at the highest dose of the extract (81mg/kg). This suggest the inhibition of phospholipase A_2 which is involve in the pathophysiology of inflammation due to xylene (Lin *et al.*,1992). However, dexamethasone, a steroid antiinflammatory agent produced significant reduction in the mean right ear weight of positive control rats indicating an inhibition of PLA_2. This action of the extract ressembles that of para-amino phenol group of the NSAIDS which possess analgesic and antipyretic activities but lack antiinflammatory effect particularly in the peripheral tissues (Nsihida *et al.,* 1979). The weak antiinflammatory actvity of the extract is likely to be due to the absent of flavonoids in the extract. Flavonoids are reported to be involved in antiinflammatory activity of plants (Parmer and Gosh, 1978).

Experimental evidence obtained in this study indicate that the extract reduced acetic acid induced writhes and formalin induced paw licking in mice. Similarly, it significantly delayed the reaction time of animals to the heat stimulus. Acetic acid causes inflammatory pain by inducing cappillary permeability (Amico-Roxas *et al.,* 1984), formalin exhibits neurogenic and inflammatory pain (Vaz *et al.,* 1996, 1997), while hot plate-induced pain indicates narcotic involvement (Turner, 1995; Besra *et al*., 1996). These results pattern portrays a similarity in mode of action of paracetamol which has been reported to lack or possess a weak antiinflammatory activity but has a strong analgesic and antipyretic activities. Paracetamol has been reported to inhibit centrally the synthesis of prostaglandin in the brain by inhibiting COX-3 (Botting, 2000). However, it does not inhibit peripheral

biosynthesis of prostaglandin and therefore lacks peripheral antiinflammatory effect (Ayoub *et al.,* 2006; Botting, 2000). Paracetamol has also been reported to inhibit acetic acid induced writhing (Ayoub *et al.,* 2006). The extract seems to act centrally just like paracetamol. The central action of the extract is further supported by its ability to inhibit both phases of formalin induced paw licking, which is a characteristic of drugs (such as narcotics) that act centrally (Santos *et al.,* 1994). Furthermore, the extract may have exerted its action through other mechanisms of antinociception thereby leading to the observed analgesic effect. The extract further demonstrated central action by increasing the reaction time to heat. This indicate the involvement of narcotic or opiod receptors.

In this study, the extract was observed to inhibit significantly ($P<0.001$) DNP-, amphetamine and yeast induced pyrexia. As discussed above, the extract acts centrally like paracetamol, the extract is likely to reduce pyrexia by reducing brain concentration of prostaglandin E_2 especially in the hypothallamus through its action on COX-3 (Ayoub *et al.,* 2004; Botting and Ayoub, 2005) or by enhancement of the production of the body's own antipyretic substances like vasopressin and arginine (Chandrasekharan, 2002).

In conclusion, the extract have demonstrated significant analgesic and antipyretic activities though with a weak antiinflammatory activity. These actions are exerted through central activity of the extract in the brain. These findings confirm its traditional use in the treatment of malaria symptoms.

ACKNOWLEDGEMENTS

The authors are grateful to Ms. Sifon Akpan of Department of Pharmacology and Toxicology, Univeristy of Uyo, Uyo for her technical assistance.

REFERENCES

Abo KA, Ogunleye VO and Ashidi JS (1999). Antimicrobial potential of *Spondias mombin, Croton zambesicus* and *Zygotritonia crocea. Phytotherapy Research,* **13**: 494-497.

Adjanohoun EJ, Adjakide V and deSouza S (1989). Contribution to ethnobotanical and floristic studies in Republic of Benin. Vol.1. Agency for Cultural and Technical Cooperation, p.245.

Amico-Roxas M, Caruso A, Trombadore S, Scifo R and Scapagnime U (1984). Gangliosides antinociceptive effects in rodents. *Archieves of international Pharmacodynamics and Therapeutics,* **272**: 103-117.

Ayoub SS, Colville-Nash PR, Willoughby DA and Botting RM (2006). The involvement of a cyclo-oxygenase1 gene-derived protein in the antinociceptive action of paracetamol in mice. *European Journal of Pharmacology,* **538**: 57-65.

Baccelli C, Block S, Van Holle B, Schancke A, Chapon D, Tinant B, Van Meervelt L, Morel N and Quertin-Leclkercq J (2005). Diterpenes isolated from *Croton zambesicus* inhibit KCl-induced contraction. *Planta Medica,* **71**: 1036-1039.

Bamgbose SO and Noamesi BK (1981). Studies on crytolepine II: Inhibition of carrageenan-induced oedema by Crytolepine. *Planta Medica,* **42**: 392-396.

Besra SE, Sharma RM and Gomes A (1996). Anti-inflammatory effect of petroleum ether extract of leaves of *Litchi Chinesis*. Caertn (sapindaceae). *Journal of Ethnopharmacology,* **54**: 1-6.

Blackhouse N, Delporte C, Negrete R, Munoz O and Ruiz R (1994). Anti-inflammatory and antipyretic activities of *Maytenus boaria*. *International Journal of Pharmacognosy,* **32**: 239-244.

Block S, Baccelli C, Tinat B, Meervelt L, Rozenberg R, Jiwan J, Llabres G, De Pauw-Gillet M and Quetin-Leclercq J (2004). Diterpenes from the leaves of *Croton zambesicus*. *Phytochemistry,* **65**: 1165-1171.

Block S, Stevigny C, Llabres G, deHoffman E, Adjakide V, DePauw-Gillet M and Quetin-Leclercq J (2002). Ent-Trachyloban-3β-ol,a new cytotoxic diterpene from *Croton zambesicus*. *Planta Medica,* **68**: 647-648.

Botting RM (2002). Mechanism of acetaminophen: Is there a cycloooxygenase 3? *Clinical Infectious Diseases,* **31**(Suppl.5): S202- S210.

Botting R, Ayoub SS (2005). COX-3 and the mechanism of action of paracetamol/acetaminophen. *Prostaglandins, Leukotrienes and Essential fatty Acids,* **72**: 85-87.

Boyom FF, Keumdjio F, Dongmo PM, Ngadjui BT, Amvam-Zollo PH, Menut C and Bessiere JM (2002). Essential oil from *Croton zambesicus* Muell. Arg. growing in Cameroun. *Flavour and Fragrance Journal* **17**: 215-217.

Chandrasekharan NV (2002). COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs:cloning,structure and expression. *Proceeding of National Academy of Science,* **99**: 13926-13931.

Correa CR and Calixto JB (1993). Evidence of participation of β1 and β 2 Kinin receptors in formation- induced nociceptive response in mouse. *British Journal of Pharmacology,* **110**: 193-198.

Correa CR, Kyle DJ, Chakrararty S and Calixto JB (1996). Antinociceptive profile of the pseudopeptide β2 brady kinin receptor antagonist NPC 18688 in mice. *British Journal of Pharmacology,* **117**: 552-558.

Ekpendu TO, Akah PA, Adesomoju AA and Okogun JI (1994). Antinflammatory and antimicrobial activities of *Mitracarpus scaber* extracts. *International Journal of Pharmacology,* **32**: 191-195.

El-Hamidi A (1970). Drug Plants of the Sudan Republic in native medicine. *Plantae Medica,* **18**: 278-280.

Gorski F, Correa CR, Filhe V, Yunes RA and Calixto JB (1993). Potent antinociceptive activity of a hydroalcoholic extract from *Phyllanthus corcora-densis. Journal of Pharmacy and Pharmacology,* **45**: 1046-1049.

Gural ML, Kohli PP and Saxena PH (1955). Antipyretic activity of some indigenous drugs. *Indian Journal of Medical Research,* **6**: 89-92.

Hayllar J and Bjarnason I (1995). NSAIDS, COX-2 inhibitor and the gut . *Lancet,* 346-522.

Hess SM and Milonig RC (1972). Inflammation. *In*: Lepow LH, Ward PS (Eds). Inflammation, Mechanism and control. Academic Press, New York, USA, pp.1-2.

Homburger F (1989). *In vivo* testing in the study of toxicity and safety evaluation. *In*: A guide to general toxicology. Marquis JK (Ed). 2nd Edn. Karger, New York, p.198.

Hussein G, Miyashiro H, Nakamura N, Hattori H, Kakiuchi N and Shimotohno K (1999). Inhibitory effect of Sudanese medicinal plant extracts on HIV-1 protease. *Phytotherapy Research,* **13**: 31-36.

Hussein G, Miyashiro H, Nakamura N, Hattori H, Kakiuchi N and Shimotohno K (2000). Inhibitory effect of Sudanese medicinal plant extracts on hepatitis C virus (HCV) protease. *Phytotherapy Research,* **14**: 510-516.

Lorke D (1983). A new approach to practical acute toxicity testing. *Achieves of Toxicology,* **54**: 275-286.

Mbagwu HOC, Anene RA and Adeyemi OO (2007). Analgesic, antipyretic and antiinflammatory properties of *Mezoneuron benthamianum* Baill Caesalpiniaceae. *Nigerian Quarterly Journal of Hospital Medicine,* **17**(1): 35-41.

Mekkawi AC (1985). The essential oil of *Croton zambesicus. Fitoterapia,* **56**: 181-183.

Menut C, Lamaty G, Bessere JM, Sulean AM, Fendro L, Maidou E and Denamgania J (1985). Aromatic plants of tropical Central Africa. *Journal of Essential oil Research,* **7**(A): 419-422.

Ngadjui BT, Abeghaz BM, Keumedjio F, Folefoe GN, and Kapche GW (2002). Diterpenoids from the stem bark of *Croton zambesicus*. *Phytochemistry,* 60: 345-349.

Ngadjui BT, Folefoc GG, Keumedjio F, Dango E, Sondegam BL and Conolly JD (1999). Crotondiol, a lablane diterpenoid from the stem bark of *Croton zambesicus*. *Phytochemistry,* **51**: 171-174.

Nwafor PA and Okwuasaba FK (2003). Anti-conceptive and anti-inflammatory effects of methanolic extract fo Asparagus pubescens root in rodents. *Journal of Ethnopharmacology,* **84**: 125-129.

Okokon JE and Nwafor PA (2009a). Antiplasmodial activity of root extract and fractions of *Croton zambesicus. Journal of Ethnopharmacology*, **121**: 74-78.

Okokon JE and Nwafor PA (2009b). Antiulcer and anticonvulsant activities of *Croton zambesicus* root extract. *Pakistan Journal of Pharmaceutical Sciences,* **22**(4): 384-390).

Okokon JE, Nwajuakwu N and Udokpoh A (2005b). Preliminary study of analgesic, anti inflammatory and antipyretic activities of ethanolic leaf extract of *Croton zambesicus*. *Journal of Pharmacy and Bioresources,* **2**: 75-79.

Okokon JE, Bassey AL and Obot J (2006). Antidiabetic activity of ethanolic leaf extract of *Croton zambesicus* on alloxan diabetic rats. *African Journal of Traditional, Commplementary and Alternative Medicine,* **3**: 21-26.

Okokon JE, Ofodum KC, Ajibesin KK, Danladi B, and Gamaniel KS (2005a). Pharmacological screening and antiplasmodial activity of *Croton zambesicus* against *Plasmodium berghei berghei* infection in mice. *Indian Journal of Pharmacology,* **37**: 243-246.

Santos AR, Cechinel Filho V, Niero R, Viana AM, Moreno FN, Campos MM, Yunes RA and Calixto JB (1994). Analgesic effects of callus culture from selected species of *Phyllanthus.* Journal of Pharmacy and Pharmacology, **46**: 755-759.

Sofowora A (1993). Medicinal Plants and Traditional Medicine in Africa. 2nd ed. Spectrum Books Limited, Ibadan, Nigeria.

Stroev EA and Makarava VG (1989). Laboratory Manual in Biochemistry. Translated from Russian by BV Rassadin, Mir Publishers, Moscow, p.245.

Tjolsen A, Berge OG, Hunskaar S, Rosland JH and Hole K (1992). The formalin test: An evaluation of the method. *Pain,* **51**, 5-17.

Trease GE and Evans WC (1989). Pharmacognosy. 13th edn. Bailliere Tindall, London, pp.683-684.

Vane T and Booting R (1987). Inflammation and Mechanism of action of anti-inflammatory drugs. *FASSEB J,* **1**: 89-96.

Vaz ZR, Cechinel V, Yunes RA and Calixto JB (1996). Antinociceptive action of 2-(4bromobenzoyl)-3-methyl-4-6-dimethoxy bezofuran, a novel xanthoxyline derivative of chemical and thermal models of nociception in mice. *Journal of Pharmacy and Experimental Therapeutics,* **278**: 304-312.

Vaz ZR, Risley EA and Calixto JB (1997). Analgesic effect of the herbal medicine catuama in thermal and chemical models of nociception in mice. *Phytotherapy Research,* **11**: 101-106.