



Full length Research Article

Anti-Inflammatory and Anti-Nociceptive Effects of Ethanolic Extract of *Mammea Africana* Stem bark In Rodents

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ABSTRACT: The ethanolic stem bark extract of *M. africana* (30-90 mg/kg) was investigated for pharmacological properties against egg white albumin - induced inflammation, Chemical as well as thermal- induced pain as well as yeast induced pyresis in rats. The extract demonstrated a dose – dependent anti- inflammatory , antinociceptive and antipyretic activities. These activities were comparable to that of ASA (100mg/kg). The stem bark extract possess anti inflammatory , analgesic and antipyretic properties, which can be exploited in health care.

Keywords: *M. africana*, anti inflammatory, analgesic, antipyretic.

INTRODUCTION

Mammea africana sabine (Guttiferae) (syn. *Ochrocarpus africana* Oliv.) is a large forest tree of 50 to 100 feet high with bark often yellow with pale scales and resinous yellow sap (Daziell, 1956). The plant is widely distributed in tropical Africa. The stem bark of the plant is used traditionally by the Ibibios of Niger Delta region of Nigeria in the treatment of malaria related fever, internal heat, and microbial infections. The chloroformic and ether stem bark extract are reported to posses cytotoxic activity on cell culture (Chapuis *et al.*, 1988). Ouahouo *et al.*, (2004) reported cytotoxic coumarins with antimicrobial activity against *Staphylococcus aureus* from the plant stem bark. Methanolic fractions of the stem bark have been

reported to contain compounds that are potent urease inhibitor (Rahman, 2001). Also, Okokon *et al* (2006) reported of the antiplasmodial activity of the stem bark. The stem bark has been reported to contain 5,-7-dihydroxy-8-(12-methyl-butryl) –4-N-Pentyl coumarins (Carpenter *et al.*, 1971; Crichton and Waterman, 1978; Carpenter *et al.*, 1970), Mesuxanthone B (Carpenter *et al.*, 1971). Alkaloids have been reported to be absent in the entire plant parts. (Gartlands *et al.*, 1980). Although reports of scientific studies on *Mammea africana* have been widely published, there is no information regarding the anti inflammatory, antipyretic and analgesic activity of the stem bark extract in rats.

The present study, therefore, was to establish if the stem bark of *M. africana* has any antipyretic and analgesic/anti-inflammatory effect especially because of its ethnomedical uses in the treatment of inflammatory cases

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MATERIALS AND METHODS

Plant materials

Fresh leaves of *M. africana* were collected in November, 2004 at Anwa forest in Uruan, Akwa Ibom State, Nigeria. The plant was identified and authenticated by Dr. Margaret Bassey, a taxonomist in the Department of Botany, University of Uyo, Uyo, Nigeria. Herbarium specimen was deposited at Faculty of Pharmacy Herbarium with voucher no. FPHUU 381. The fresh stem bark (2kg) of the plant were dried on laboratory table for 2 weeks and reduced to powder. The powder 100g was macerated in 95% ethanol (300ml) for 72 hours. The liquid filtrate obtained was concentrated in vacuo at 40°C. The yield was 2.08% w/w. The extract was stored in a refrigerator at 4°C until used for experiment reported in this study.

Animals:

Albino wistar rats (105 – 165g) and albino swiss mice (21-28g) 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.

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) The phlogistic agent employed in this study was fresh egg-albumin (Akah and Nwambie, 1994). Adult wistar rats of either sex (180-230g) 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 mice. Paw diameters were measured immediately before the administration of the phlogistic agent and 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 Milonig, 1972). The extract (30,60 and 90 mg/kg) were administered i.p 1 hour before inducing inflammation. Control rats received equivalent amount of normal saline and the reference group administered Acetic salicylic acid (ASA) 100mg/kg. Average oedema ($C_t - C_o$) and percent inhibition of oedema

were calculated for each dose (Oriowo,1982; Akah and Njike, 1990).

Acetic acid – induced writhing in mice

The analgesic activity of ethanolic stem bark extract of *Mammea africana* was measured against acetic acid induced writhmic movements in mice (Collier, 1968; Santos *et al*, 1994), consisting of the contraction of abdominal muscle together with the stretching of hind limbs. The extract at doses of 30, 60 and 90mg/kg and ASA 100mg/kg and normal saline 5ml/kg were administered intraperitoneally to the respective groups (n=5) of the 18hours fasted mice. Thirty minutes later, 0.5ml 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 at 10 minutes interval for 50minutes. The number of writhes for each animal was counted. 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 the following formula.

$$\% \text{ Inhibition} = \frac{\text{Mean number of writhes in control group} - \text{Mean number of writhes in test group}}{\text{Mean number of writhes in control group}}$$

Thermally – induced pain in mice

The effect of extract on hot plate – induced pain was investigated in adult mice. The hot plate test was used to measure response latencies according to the method of Vaz *et al*, (1996, 1997). The animals were divided into 5 groups of 5 mice per cage. Group 1 mice served as the control and received only saline. Groups 2, 3 and 4 were pre- treated with 30, 60 and 90mg/kg *M. africana* extract i.p respectively, 30 min prior to the placement on the hot plate, while group 5 animals received 100mg/kg of ASA by i.p route. The hot plate was set at $45 \pm 1^\circ\text{C}$ and animals were placed into a glass beaker of 50cm diameter on the heated surface and the time(s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency.

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.

There after each animal was administered subcutaneously with 50% v/v aqueous suspension of the yeast in a volume of 10ml/kg(Gural,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 under study was administered intraperitoneally. (i.p) after the pyrogen at the doses of 30, 60, and 90 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 are expressed as mean \pm SEM for n numbers of experiment. Statistical comparisons and significance levels were analyzed with student's t – test. A 'p' value less than 0.05 was considered as significant.

RESULTS

Fresh Egg Induced Inflammation In Mice

The extract showed significant anti-inflammatory activity against acute inflammation. (Table 1). It suppressed in a dose related manner the increase in the mice paw edema caused by egg albumin. The inhibition by the extract was maximal after 3hours of administration of phlogistic agent.

Acetic Acid – Induced Writhing In Mice

The extract (30 – 90mg/kg) dose – dependently reduced acetic acid induced abdominal constructions and stretching of hind limbs. The reduction was significant (Table 2)

Thermally- Induced Pain In Mice

Administration of *M.africana* extract (30 – 90mg/kg i.p) elicited a dose – dependent increase in the latency response in the hot plate test. These increases in latency responses (analgesic effect) were statistically significant (P<0.05) (Table 3).

Antipyretic Effect

Table 4 shows the results of antipyretic activities of the stem bark extract of *M.africana*. 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

Effect of *M. africana* on fresh egg albumin induced inflammation in rats.

TREATMENT	DOSE Mg/kg	Paw diameter (mean SEM) cm	Inhibition %
Control		0.69 \pm 0.03	-
<i>M. africana</i>	30	0.28 \pm 0.03*	57.97
extract	60	0.27 \pm 0.01*	59.42
	90	0.26 \pm 0.01*	60.86
	100	0.26 \pm 0.01*	62.31

Results are expressed as mean \pm SEM (n=5) *P<0.05 significantly different from control.

Table 2

Analgesic activity of ethanolic leaf extract of *M.africana* on acetic acid induced writhing in mice.

Treatment	Dose mg/kg	No. of Writhing (Mean \pm S. D)	Percent activity against acetic induced pain
Control		237.3 \pm 18.50	
MAE	30	85.6 \pm 18.5*	63.92
	60	79.8 \pm 10.2*	66.37
	90	48.2 \pm 3.9*	79.68
ASA	100	53.0 \pm 2.80*	77.66

MAE= *M. africana* extract

Results are expressed as mean \pm S.D (n=5) *P< 0.05 significantly different from control.

Table 3

Effect of *M. africana* extract on thermally induced pain in rats

Treatment	Dose Mg/Kg	Time (Seconds)
Control		2.50 \pm 0.5
<i>M. africana</i> extract	30 i.p	5.71 \pm 0.05*
	60 i.p	9.40 \pm 0.34*
	60 i.p	13.02 \pm 0.66*
ASA	90 i.p	19.25 \pm 0.19*

Results are expressed as mean \pm SEM (n=5) *P<0.05 significantly different from control.

DISCUSSION

The ethanolic leaf extract of *M.africana* significantly reduced edema of the mouse hind paw induced by fresh egg albumins. This dose -dependent

action; was comparable to that of a acetylsalicylic acid, a cyclo-oxygenase inhibitor (Singh *et al* , 1996). Flavonoids which are some of the constituents of the extract have anti inflammatory property (Trease and Evans, 1989; Parmer and Gosh, 1978), Edema in attributed to the release of histamine, 5-HT, Kinins and prostaglandins (Vane and Booting, 1987, Larsen and Henson, 1983) and the anti inflammatory action of this extract may be due to the inhibition of the release of the above mentioned autocooids.

The stembark extract was also found to possess significant ($P < 0.05$) dose and time - dependent analgesic activity against chemical and thermal - induced pains. Acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Roxas *et al*, 1984) while hot plate-induced pain indicates narcotic involvement (Turner,1965; Besra *et al*, 1966). The ability of the extract to show significant effect in these two types of pain induction suggest that its analgesic effect may in part be related to its anti inflammatory and narcotic properties. Increased body temperature and pain are two major signs of inflammation (Meli *et al*, 2001). A drug with anti inflammatory activity usually exhibit antipyretic and analgesic properties (Perianayagam *et al*, 2004) such as non steroidal anti inflammatory drugs (NSAIDS) which possess all three activities (Buffum and Buffum, 2000). This same situation was observed with this extract. This antipyretic activity is related to the ability of the extract to inhibit the release of prostaglandin which is known to cause elevation of temperature from the hypothalamus.

Table 4.

Antipyretic effect of ethanolic stembark extract *M. africana*

Treatment Dose (mg/kg)	Dose	Mean temperature after 4 hours
control	-	38.7 ± 0.11
Extract	100 i.p	37.6 ± 0.15 *
	150 i.p	37.5 ± 0.23*
	200 i.p	37.3 ± 0.17*
ASA	100	36.7±0.09*

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

Therefore, the result obtained in this study shows that *M. africana* possess anti-inflammatory and

analgesic properties which are probably mediated via inhibition of various autocooids formation and release. Further studies are needed to elucidate the exact mechanism by which *M. africana* inhibits inflammation and pains

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