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Full Length Research Paper

Analgesic, anti-inflammatory, antipyretic and anti-plasmodial effects of the methanolic extract of *Crossopteryx febrifuga*

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Preparations of *Crossopteryx febrifuga* have been used in traditional medicine for treatment of pain and malaria for many years and their efficacies are widely acclaimed among the Hausa communities of northern Nigeria. The methanolic extract of *C. febrifuga* was evaluated for analgesic, anti-inflammatory, antipyretic and anti-plasmodial activities of in rodents. The extract significantly diminished acetic acid-induced writhes in mice and increased the pain threshold in rats dose-dependently. It also demonstrated significant anti-plasmodial, antipyretic and anti-inflammatory activities in mice and rats in a dose-related manner. The results suggest that *C. febrifuga* contains biologically-active substances with potential values in the treatment of malaria, fever, pain and inflammation. These provide scientific evidence to support the isolation and development of biologically active components as anti-malarials, analgesic, antipyretic and anti-inflammatory agents.

Key words: *Crossopteryx febrifuga*, pain, inflammation, pyrexia, anti-malarial effects.

INTRODUCTION

African indigenous herbal medicines are widely used throughout the African continent, despite an apparent lack of scientific evidence for their quality, safety and efficacy (Amos et al., 2002). Recent pharmaco-chemical exploration of African medicinal plants in our laboratories and elsewhere has shown that many of the Nigerian medicinal plants possess therapeutic attributes (Audu, 1989). One of such therapeutically useful Nigerian medicinal plant is *Crossopteryx febrifuga* Benth, (Family Rubiaceae). *C. febrifuga* is a twisted tree with conspicuous tubular flowers, which is widely distributed throughout the Savannah region of Central, East and West Africa.

Preparations of the tree is used in traditional medicine for symptomatic relieve of dry cough and for treatment of septic wounds, respiratory infections, fever, dysentery and pain (Kodio, 1986; Lathan, 2001; de Boer and Kool, 2004). In northern Nigeria, the plant has been used for treatment of pain and malaria for many years and its efficacy is widely acclaimed among the Hausa communities (Audu, 1989).

Previous studies using crude methanolic extract of *C. febrifuga* revealed that it contains biologically active substances with potential values in the treatment of trypanosomiasis, malaria and *Staph aureus* infection (Hostettmann et al., 2000; Yusuf et al., 2004).

Apart from the aforementioned, there are no documented data to our knowledge, on the activity profile of this important medicinal plant. The present study was designed to evaluate its analgesic, anti-inflammatory, anti-pyretic and anti-plasmodial properties in rodent so as to scientifically describe the potential values of the plant, which is already in common use in traditional medicine.

MATERIALS AND METHODS

Collection plant material

Fresh stem bark of *C. febrifuga* was collected from Suleja, Niger State, Nigeria, between the months of October and December, 2004. It was identified and authenticated by Mallam Ibrahim Muazzam of the Department of Medicinal Plant Research and Traditional Medicine, National Institute for Pharmaceutical Research and Development (NIPRD), Abuja where herbarium specimen (voucher number 4041) was made and deposited.

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Preparation of the plant extract

The stem bark was cleaned, air-dried for seven (7) days and crushed into coarse powder using a pestle and mortar. 500 g of the coarse powder was cold macerated with 2.5 l of 70% $^{v}/_{v}$ methanol in water for 72 h with constant shaking using the GFL shaker (No. 3017MbH, Germany). The resultant mixture was filtered using Whatman filter paper (No.1) and the filtrate concentrated to dryness *in vacuo* at 40°C using Rotary Evaporator to give a yield of 20%$^{w}/_{w}$ of the extract.

Phytochemical screening

Standard screening tests (Trease and Evans, 1983) for detecting the presence of different chemical constituents were employed. Secondary metabolites tested for include: alkaloids, tannins, saponnins, flavonoids, glycosides anthraquinones, carbohydrates, monossacharides, free reducing sugar, combined reducing sugars, tannins, combined terpenes and phenols

Animals

Swiss albino mice (18 – 25 g each) and Wistar rats (180 – 250 g) of either sex maintained at the Animal Facility Centre of NIPRD, Abuja, were used. The animals were housed under standard conditions of temperature, (25 ± 2°C) and light, (approximately 12/12h light-dark cycle) and fed on standard diet and given water *ad libitum*. These animals were approved for use by the AFC committee after reviewing the protocol. The studies were carried out following the principles of good laboratory practice and animal handling (National Institutes of Health Guide for the Care and use for Laboratory animals; Publication No. 85-23, revised 1985).

Acute toxicity (LD_{50}) study

The median lethal dose (LD50) of the methanolic extract was determined in mice intraperitoneally (*i.p.*) using the method described by Lorke (1983) with modifications. Briefly, mice of either sex were fasted overnight and the evaluation of the LD_{50} was carried out in 2 stages. In the first stage, 3 groups of 3 mice each were treated with the extract at doses of 10, 100 and 1000 mg/kg, *i.p.* in order to determine the range in which the LD_{50} falls. In the second stage another 4 groups of 3 mice each were further treated with the extract at doses 140, 225, 370 and 600 mg/kg. Animals were observed for 24 h after treatment for signs and symptoms of toxicity. The number of deaths in each group within 24 h was recorded and the final LD_{50} values were calculated as the geometric mean of the highest non-lethal dose (with no deaths) and the lowest lethal dose (where deaths occurred).

Analgesic studies

The following experimental models for pain were employed in an attempt to evaluate antinociceptive effects of the crude extract.

Acetic acid-induced writhes in mice

The method described by Koster et al. (1959) was used with some modifications. The mice were randomly-divided into five test groups of six mice each. The first three groups were pre-treated with 25, 50 and 100 mg/kg, *i.p.,* of the extract, respectively. Two other groups received normal saline 3 ml/kg i.p. and acetyl salicylic acid 150 mg/kg orally (p.o.), respectively, to serve as controls. Thirty minutes after pre-treatment, each mouse was injected with 10 ml/kg of an aqueous solution of 0.7% acetic acid i.p. and placed in a transparent Perspex observation box. After a five minute lag period, the number of writhes (a syndrome characterized by a wave of contraction of the abdominal musculature followed by extension of hind limbs) for each mouse was counted for five minutes at 30 and 60th min respectively.

The dose of acetyl salicylic acid used was selected based on documented studies reported in literature (Dhara et al., 2000). The number of acetic acid-induced writhes recorded in the extract and acetylsalicylic acid pre-treated mice was compared with those in the normal saline-treated group (control) mice.

Analgesic study

The study involved the use of analgesymeter (Model 7200, Ugo Basile, Italy), which exerts force at a constantly- increasing rate on the rat paw. The force is monitored by a pointer moving along a linear scale and the rat's response is taken to be the point at which its struggles or its paw slips off the plinth of the instrument. Rats were divided into four groups of six rats each and pre-treated as follows: Groups I, II and III received the extract at doses of 25, 50 and 100 mg/kg i.p, respectively, while groups IV received normal saline. Thirty minutes after treatment, readings were taken at thirty minutes intervals for 120 min.

Anti-inflammatory Studies

Hind paw oedema in rats

This test was carried out using a modification of Winter et al. (1963) as described by Akah and Nwambie (1994). The rats were divided into five groups of six rats of either sex and pre-treated as follows: Group I (control) received normal saline (3 ml/kg), groups II, III and IV received 25, 50 and 100 mg/kg of the extract i.p. respectively, while group V rats received acetyl salicylic acid (150 mg/kg, p.o.) After 30 min of post-drug administration, rats in each group were injected with 0.5 ml/kg raw egg albumin (a cheap phlogistic agent) in the sub-plantar surface of the left hind-paw thereby inducing pedal oedema after 7 min following the fresh egg albumin injection. A digital plethysmometer (Letica, Spain LE7500) was used to measure the volume of paw oedema (volume dis-placement) for a period of 120 minutes, with readings taken at 20 min intervals, i.e. 20, 40, 60, 80, 100 and 120 min after albumin administration. *Pedal oedema (inflammation) was always evident within 5 - 8 min following fresh egg albumin (0.5 ml/kg) injection.

Antipyretic studies

Effect on yeast-induced pyrexia

The procedure described by Al-Ghamdi (2001) was adopted for this study. The body temperature of each albino Wistar rat was recorded by measuring rectal temperature (RT) at predetermined intervals. Fever was induced in the rats by injecting 15%w/v suspension of Brewer's yeast (*Saccharomyces cerevisiae*) at a dosage of 1 ml/kg body weight subcutaneously. The rectal temperature of each rat was again recorded after 24 h of yeast administration. Rats that did not show a minimum increase of 0.5°C in temperature 24 h after yeast injection were discarded. Twenty-five selected rats were grouped into five and immediately treated as follows: group I received normal saline, group II, 20% Dipyrone while groups III, IV and V received 25, 50 and 100 mg extract /kg respectively i.p. Rectal temperature of all the rats was then recorded by inserting digital thermometer (Omron Digital Fever Thermometer, Omron® Healthcare, China) into the rectum of each rat at thirty minutes in-

Table 1. Effect of *C. febrifuga* on acetic acid induced writhes in mice.

Drug/Dose (mg/kg)	Number of Writhes (min)	
	30	60
Control	28±1.5	24.16±2.8
25	9.67±2.9*	9.0±4.5*
50	3.3±2.0*	1.0±0.5*
100	2.3±0.89*	1.5±0.6*
ASA (150)	5.5±2.8*	1.67±0.75*

*Significantly different from the control at P<0.05

tervals for 120 min.

Anti-plasmodial studies

Test on established infection (Rane test)

A modified method of Saidu et al. (2000) was used. Thirty mice were selected, inoculated with $1x10^7$ *Plasmodium berghei* infected erythrocytes i.p. and divided into five groups (n = 6) on day three(D3). The rats were then treated as follows: Group I received normal saline; Groups II – IV received the extract at 25, 50 and 100 mg/kg respectively while Group V received 5 mg chloroquine /kg i.p. The treatment continued daily until D7. Thick and thin blood smear films were collected daily from tail blood, fixed with methanol, stained with 4% Giemsa at pH 7.2 for 45 min and examined under microscope (Nikon YS2-H, Japan). The number of infected and uninfected red blood cells (RBCs) were counted in 5 different fields using a tally counter. After the seventh day, the mice were further observed for 30 days. Any death that occurred during this period was recorded and used to determine the mean survival time.

Statistical analysis

Results were expressed as Mean ± Standard Error of Mean (SEM). The data was analyzed using student's t-test. P<0.05 was considered significant.

RESULTS

Phytochemical Screening

A phytochemical analysis of the crude extract gave a positive reaction for each of the following secondary metabolites: Carbohydrates, monossacharides, free reducing sugar, combined reducing Sugars, Tannins, combined Anthraquinones, Saponins, Terpenes, Flavo-noids and Phenols.

Acute toxicity tests

The behavioural signs of toxicity exhibited by mice that received 100 mg extract/kg and above are decreased respiratory rate, inactivity, increased abdominal contractions. The intraperitoneal LD_{50} of the extract in mice was estimated to be 774 mg/Kg.

Acetic acid-induced writhes

The extract significantly decreased the number of acetic acid-induced writhes in mice in a dose-related manner. The extract provided analgesia over the 60 min monitoring period of the study and the effect increased with time. Acetyl salicylic acid also exhibited significant inhibition of acetic acid-induced writhes (Table 1).

Analgesic study

This study, which employed mechanically-induced pain, revealed significant and dose-dependent increase in pain threshold by the extract compared to control in the mice. The effect was also time-related (Table 2).

Anti-inflammatory studies

The extract significantly attenuated albumin-induced oedema over a period of 120 min. The effects were dose-dependent. Acetyl salicylic acid also caused a significant inhibition of albumin-induced oedema (Table 3).

Anti-pyretic studies

The extract caused a dose-dependent decrease in rectal temperature. The effect became significant at 30 and 60 min at the highest dose of 100 mg/kg. However, Dipyrone caused a significant reduction in rectal tempe-rature (Table 4).

Anti-plasmodial activity

The extract dose-dependently reduced the mean parasitic count in mice. The values were found to be significant at all doses tested. Chloroquine also exhibited a significant reduction in the parasite count. The effect of chloroquine on the parasite was more than the effect of 25 and 50 mg extract/kg, but less than that of the highest dose (100 mg/kg) of the extract (Table 5).

DISCUSSION

The data presented here suggests that the methanolic extract of *C. febrifuga* possesses anti-nociceptive, anti-inflammatory, antipyretic, and antiplasmodial activities.
The extract at the doses tested was shown to possess anti-nociceptive activity evident in both the nociceptive models, signifying it possesses both central and peripherally mediated activities. The abdominal constriction response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics (Gene et al., 1998). In general, acetic acid causes pain by liberating endogenous substances such as serotonin, histamine, prostaglandins (PGs), bradykinins and substance P,

Table 2. Effect of *C. febrifuga* on pain threshold using analgesymeter.

Drug/Dose (mg/kg)	Time (min)				
	0	30	60	90	120
Control	117.5±16.2	134.16±28.2	108.5±20.8	146.33±7.2	113.0±12.4
25	107.0±18.2	204. ±22.1*	254.16±20.6*	226.5±23.8*	183±16.4*
50	113.25±12.4	228.67±20.6*	303.67±25.4*	247.5±27.3*	199.5±18.6*
100	97.5±13.6	275±11.2*	328.33±26.0*	266.5±9.5*	245±26.5*

Significantly different from the control at P<0.05

Table 3. Anti-inflammatory properties of *C. febrifuga*.

Drug (mg/kg)	Volume of Oedema (cm^3)/Time (min)					
	20	40	60	80	100	120
Control	0.53±0.06	0.65±0.07	0.51±0.5	0.58±0.02	0.61±0.04	0.58±0.02
25	0.30±0.02*	0.29±0.08*	0.24±0.06*	0.31±0.04*	0.26±0.06*	0.22±0.05*
50	0.22±0.04*	0.22±0.04*	0.26±0.02*	0.24±0.02*	0.27±0.02*	0.23±0.02*
100	0.30±0.04*	0.21±0.04*	0.31±0.04*	0.29±0.01*	0.26±0.02*	0.25±0.02*
ASA (150)	0.26±0.03*	0.35±0.04*	0.46±0.07*	0.41±0.03*	0.45±0.05*	0.34±0.06*

* Significantly different from the control at P<0.05

Table 4. Anti-pyretic properties of *C. febrifuga*.

Group	Dose (mg/kg)	Rectal temperature (^OC)**					
		BBT	0.0h	0.5h	1.0h	1.5h	2.0h
1	Control	36.98 ± 1.01	38.38 ± 0.58	38.15 ± 0.51	38.35 ± 0.34	38.18 ± 0.45	38.35 ± 0.73
2	25	37.48 ± 0.74	38.2 ± 0.58	37.75 ± 0.33	37.98 ± 0.19	39.12 ± 0.18	37.73 ± 0.70
3	50	37.83 ± 0.64	38.6 ± 0.36	37.53 ± 0.55	37.73 ± 0.83	38.28 ± 0.66	37.43 ± 0.70
4	100	36.93 ± 0.69	38.38 ± 0.48	37.3 ± 0.36*	37.6 ± 0.60*	37.95 ± 0.49	38.13 ± 0.86
Dipyrone	20%	38.2 ± 0.21	38.58 ± 0.42	37.13 ± 0.35*	37.3 ± 0.49*	37.48 ± 0.57*	37.98 ± 0.42

* Significantly different from the control at P<0.05
BBT: Basal body temperature

which stimulate nerve endings. Local peritoneal receptors are postulated to be involved in the abdominal constrictions response (Bentley et al., 1983). The method has also been associated with prostanoids in general, that is, increased levels of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids (Dredt et al., 1980), as well as lipoxygenase products (Insel, 1996). The significant reduction in acetic acid-induced writhes by the methanolic extract of *C. febrifuga* suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs (Koster et al., 1959) and other endogenous substances.

The analgesymeter antinociceptive test is useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level (Vongtau et al., 2004). The significant increase in pain threshold produced by the methanolic extract of *C. febrifuga* in the analgesymeter test suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems (Bensreti and Sewel, 1983; Headley and Oshaughnessy, 1985; Wigdor and Wilcox, 1987; Pasero et al., 1999; German and Bonica, 2000). The analgesic effect produced by the extract may be via central mechanisms involving opiate, dopaminergic, descending noradrenergic and serotonergic systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain.

Albumin-induced hind paw oedema test is useful in detecting activity in acute inflammation. The significant inhibition of albumin-induced hind paw oedema in rats suggest that the methanolic extract of *C. febrifuga* may contain biologically active substances with anti-inflammatory property. The co-existence of antinociceptive and

Table 5. Anti-Plasmodial Activity of *C. febrifuga*

Drug/Dose (mg/kg)	Mean Parasitemia	Percentage Inhibition (%)
Control	65.5 ± 25.6	-
25	12.0 ± 4.4*	81.7
50	22.33 ± 10.7*	65.9
100	10.0 ± 5.1*	84.7
CQ (100)	15.33 ± 7.8*	76.6

* Significantly different from the control at P<0.05

anti-inflammatory activities observed in this study, are properties shared by most non-steroidal anti-inflammatory drugs (NSAIDs), particularly the salicylates and their derivatives.The therapeutic benefits of traditional remedies are often attributed to a combination of active constituents (Chindo et al., 2003). For instance, flavonoids are known to target PGs involved in late phase of acute inflammation and pain perception. It is therefore, probable that the biologically active flavonoid components of the extract might contribute in part to anti-inflammatory and analgesic activities of the extract.

The extract produced a significant reduction in yeast-induced pyrexia in rats dose-dependently and its effect is comparable to that of the standard anti-pyretic drug (dipyrone) used in this study. Pyrexia is a result of secondary impact of infection, tissue damage, inflammation, graft rejection, malignancy or other diseased states. The infected or damaged tissue initiates the enhanced formation of pro-inflammatory mediators (cytokines like interleukin 1β, α, β and TNF-α) which increase the syn-thesis of PGE_2 near pre-optic hypothalamus area thereby triggering the hypothalamus to elevate the body temperature (Spacer and Breder, 1994). Most of the anti-pyretic drugs inhibit COX-2 expression thus inhibiting PGE_2 biosynthesis to reduce elevated body temperature. They are however toxic to the hepatic cells, glomeruli, cortex of the brain and heart muscle. A natural PGE_2 inhibitory anti-pyretic remedy like *C. febrifuga* with minimal toxicity is therefore essential.

The extract elicited potent activity against the rodent malaria parasite used in this study. The results of this study suggest that *C. febrifuga* contains active substances with potential value in the treatment of malaria, fever, pain and inflammation. These findings lend pharmacological support to the reported folkloric uses of the plant in the treatment of painful and inflammatory conditions. The results of the preliminary phytochemical analysis of the crude extract revealed the presence of tannins, combined anthraquinones, saponins, terpenes, flavonoids and Phenols. It is therefore probable that the multiplicity of actions reflected by the broad spectrum of activity

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