

Analgesic activity of the aqueous leaf extract of *Byrsocarpus coccineus*.

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ABSTRACT

Byrsocarpus coccineus (Schum. and Thonn.) is used as an herbal remedy for earache, muscular and rheumatic pains in West Africa. To validate the claim of the users, the analgesic effect of the aqueous leaf extract of the plant was studied in mice and rats using acetic acid-induced writhing, formalin, tail immersion, and cold-water tail flick pain tests. The extract (50 - 400mg/kg; p.o) showed a dose dependent and significant ($p < 0.05$) inhibition of pain in the acetic acid-induced writhing, tail immersion, tail flick and the formalin (second phase) tests. The extract (400mg/kg) gave a significantly ($p < 0.05$) higher inhibition than acetylsalicylic acid, ASA, (100mg/kg; p.o) in the acetic acid-induced writhing test. Its effect on the second phase of the formalin test was comparable to that of ASA. The elevation of pain threshold at 60 minutes post-treatment produced by 400mg/kg of the extract in the tail immersion and tail flick tests were slightly lower than that of morphine (2mg/kg; s.c). The results suggest that the aqueous leaf extract of *Byrsocarpus coccineus* possesses effective analgesic activity mediated via peripheral and central mechanisms.

KEY WORDS: *Byrsocarpus coccineus*, Analgesic, Herbal remedy.

INTRODUCTION

Byrsocarpus coccineus Schum. and Thonn. (Connaraceae) is a scandent shrub widely dispersed across West Africa. Apart from its ornamental value, its use as an herbal remedy for diverse conditions in the sub-continent is well documented¹. A decoction of the leaves of the plant is widely used to treat sores (mouth and skin), swellings, and tumours. The use of the plant as remedy for earache, muscular and rheumatic pains is the motivation for this work, in view of the fact that limited investigations have been done to verify these claims.

In this study, the analgesic activity of the aqueous leaf extract was evaluated using the acetic acid-induced writhing, formalin, tail immersion, and cold-water tail flick pain tests.

MATERIALS AND METHODS

Preparation of Plant Extract

The fresh plant was collected from Iju-Ogundimu in Ifako/Ijaiye Local Government Area of Lagos State, Nigeria. Botanical identification and authentication was done by Professor J. D.

Olowokudejo of the Department of Botany, Faculty of Science, University of Lagos, Lagos, Nigeria and Mr. T. K. Odewo, a Senior Superintendent of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria. Voucher specimen (FHI 106623) was deposited in the herbarium of the Institute.

Fresh leaves of *B. coccineus* were air dried until a constant weight was obtained. After pounding, the dried material was macerated in distilled water (10g in 1L) and the liquid was decanted 24 hours later. The filtrate was evaporated to dryness in an oven set at 40°C giving a dark brown solid with a yield of 12.91%. The dried extract was weighed and dissolved in distilled water (pH 6.8), to a concentration of 200mg/ml just before use.

Animals

Albino rats (150 – 200g) and mice (20-25g) of either sex obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Lagos, Nigeria, were used. The animals were maintained under standard environmental conditions and had free access to standard diet and water *ad libitum*.

Analgesic Activity**Acetic Acid-induced Writhing Test**

This was carried out according to the method described by Koster et al.² and Singh and Majumdar³. The extract (50-400mg/kg, orally), distilled water (10ml/kg, orally) and the standard drug, acetylsalicylic acid (100mg/kg, orally), were administered to mice 60min. before intraperitoneal injection of acetic acid (0.6% v/v in normal saline, 10ml/kg). The number of writhes was counted from 5min. after acetic acid administration for 30min. (n = 8).

Formalin Test

A similar method to that described by Shibata et al.⁴ and Vianna et al.⁵ was employed. The extract (50-400mg/kg, orally), distilled water (10ml/kg, orally), and acetylsalicylic acid (100mg/kg, orally) were administered to mice 30min. prior to formalin (20 microlitres of 1%) injection subcutaneously into the right hind paw. The time (seconds) spent in licking and biting responses of the injected paw was taken as an indicator of pain response.

Responses were measured for 5min. after formalin injection, first phase, and 15-30min. after formalin injection, second phase (n = 8).

Tail Immersion Test

This was based on the method described by Singh and Majumdar³. The extract (50-400mg/kg, orally), distilled water (10ml/kg, orally), and the standard drug, morphine (2mg/kg, s.c), were administered to the mice. The tail (up to 5cm) was then dipped into a water bath maintained at 55± 0.5° C. The time (seconds) to withdraw the tail clearly out of the water was taken as the reaction time. Readings were taken 30, 60, 90, 120 and 150min. after administration. The mice used were initially screened and those that did not attempt to withdraw the tail in 10sec. were discarded (n = 8).

Cold water Tail Flick Test

The method for this test was based on that described by Pizziketti et al.⁶ and Clark et al.⁷. Rats were closely restrained in a wire mesh cage and the lower half of their tails immersed in a beaker of cold water (0 - 1°C). The time (seconds) for tail withdrawal from the water was taken as the reaction time. Initial screening of animals was carried out and those failing to respond after 60sec. of tail immersion were discarded. For each animal, nociceptive threshold was taken twice before administration of test drugs and averaged to obtain a baseline (control reading). Measurement of threshold was then made 30, 60, 90, 120 and 150min. after administration of extract (50-400mg/kg, orally) or morphine (2mg/kg, sc). Control animals received distilled water, 10ml/kg orally (n = 8).

Preliminary Phytochemical Screening

Preliminary phytochemical analysis of the extract was carried out for various constituents (alkaloids, saponins, tannins, phlobatannins, anthraquinones, glycosides, simple sugars etc.) following the method described by Odebiyi and Sofowora⁸.

Acute Toxicity Test

Mice were fasted for 12hr., and administered with the extract up to 10g/kg, orally. Also, the extract (50, 100, 200, 400 & 800mg/kg) was administered to another set of groups of mice (5 per group), intraperitoneally. Mortality in each group within 24hr. was recorded. The LD₅₀ was estimated using the method described by Miller and Tainter⁹.

Statistical Analysis

Results obtained are expressed as mean ± SEM. The data were analysed statistically using the Student's *t*-test and results were considered significant when *p*<0.05.

Drugs

The drugs used in this work were acetylsalicylic acid (Sigma Chemical Company, St Louis, USA), morphine sulphate (Evans Medical Ltd., England), and sodium chloride (BDH Chemicals Ltd, Poole England).

RESULTS**Acetic Acid-induced Writhing Test**

B. coccineus (50-400mg/kg) dose dependently reduced the number of writhes induced by 0.6% acetic acid solution. The highest dose (400mg/kg) produced a significant (*p*<0.05) percentage inhibition of 55.6% while acetylsalicylic acid (100mg/kg) gave 44.8%, a value slightly lower than that of the extract (as shown Table 1).

Formalin Test

In the first phase, the extract (50-400mg/kg) elicited a non-significant dose dependent inhibition of pain. The highest dose (400mg/kg) produced a comparable percentage inhibition (14.3%) to that produced by acetylsalicylic acid, 100mg/kg (12.3%). However, in the second phase of the test, the extract (50 - 200mg/kg) produced a significant (*p*<0.05) and dose dependent inhibition, percentage inhibition being 17.4%, 31.3% and 34.8% for 50, 100 and 200mg/kg respectively. The level of inhibition obtained with 200mg/kg (34.8%) was comparable to that of the standard drug (36.1%) as shown in Table 2.

Tail Immersion Test

B. coccineus at the different post-treatment times induced a dose-dependent increase in latency

Table 1: Effect of *B. coccineus* aqueous extract on acetic acid-induced writhing in mice.

Group	Dose (mg/kg)	No. of writhings	% Inhibition
Control	–	55.1 ± 4.6	–
<i>B. coccineus</i>	50	42.2 ± 4.5	32.5 ± 3.2
	100	33.4 ± 4.9*	36.5 ± 8.5
	200	25.3 ± 4.8*	52.2 ± 7.9
	400	22.9 ± 3.2 *	55.6 ± 7.7
Acetylsalicylic acid	100	29.6 ± 5.7*	44.8 ± 9.3

Values are mean ± SEM. *p<0.05, significantly different from control; Student's t-test (n=8).

Table 2: Effect of *B. coccineus* aqueous extract on formalin-induced pain in mice.

Group	Dose (mg/kg)	0-5min (s)	% Inhibition	15-30min. (s)	% Inhibition
Control	–	78.3 ± 4.8	–	137.8 ± 13.1	–
<i>B. coccineus</i>	50	91.8 ± 3.2	4.9 ± 4.4	117.8 ± 19.2	17.4 ± 6.4
	100	75.8 ± 3.4	7.9 ± 4.2	94.0 ± 11.1*	31.3 ± 6.4
	200	69.5 ± 2.1	12.7 ± 4.1	91.4 ± 13.3*	34.8 ± 9.2
	400	72.1 ± 4.3	14.3 ± 5.2	101.8 ± 12.0	24.7 ± 6.6
Acetylsalicylic acid	100	67.7 ± 4.1	12.3 ± 4.4	91.5 ± 6.3*	36.1 ± 7.7

Values are mean ± SEM. *p<0.05, significantly different from control; Student's t-test (n=8).

for removal of tail. The maximum latency (highest significant, p<0.05, percentage inhibition irrespective of post-treatment time) for the extract at 50, 100, 200 and 400mg/kg were 1.3, 9.2, 10.9 and 15.7% respectively compared to a value of 24.4 % for morphine. Morphine produced greater percentage inhibition compared to the extract (400mg/kg) at all post-treatment times except at 90min. when the extract elicited a non-significant value of 21.3% as against 8.0% for the standard drug. Peak antinociceptive effect for the extract occurred at about

120min. post-treatment (as shown in Table 3).

Cold water Tail Flick Test

In this test, a dose dependent antinociceptive effect was produced by the extract (50-400mg/kg). The maximum latency (highest significant, p<0.05, percentage inhibition, irrespective of post – treatment time) for the extract at 50, 100, 200 and 400mg/kg were 18.2, 69.3, 77.5 and 65.5% respectively while morphine (2mg/kg) elicited a value of 86.3%. The standard drug produced greater percentage inhibition

Table 3: Effect of *B. coccineus* aqueous extract on tail immersion test in mice.

Group	Dose (mg/kg)	Pre-Treat-ment(s)	+30min(s)	%Inhi-bition	+60 min(s)	%Inhi-bition	+90min(s)	%Inhi-bition	+120min(s)	%Inhi-bition	+150min(s)	%Inhi-bition
Control	–	1.4±0.1	1.3±0.1	0.1±0.1	1.3±0.1	0.9±0.4	1.4±0.1	0.5±0.2	1.3±1.0	0.8±0.3	1.4±0.1	0.9±0.3
<i>B. coccineus</i>	50	1.0±0.2	0.8±0.0	0.0±0.0	1.0±0.1	1.0±0.7	0.9±0.1	0.2±0.2	1.0±0.2	0.1±0.0	1.0±0.0	1.3±0.9
	100	1.3±0.2	1.6±0.2	4.0±1.7*	1.6±0.1	4.1±1.4*	2.0±0.3	7.9±1.5*	2.1±0.2	9.2±2.1*	1.8±0.2	6.1±1.4*
	200	1.7±0.2	1.8±0.2	2.6±0.9*	2.2±0.4	6.1±2.4	2.2±0.3	8.0±2.8*	2.6±0.4	10.9±3.0*	2.8±0.7	13.9±8.3
	400	1.5±0.2	1.8±0.2	4.5±1.9*	2.8±0.4	15.7±3.5*	3.2±0.9	21.3±10.6	2.4±0.3	10.9±2.1*	2.3±0.2	9.2±2.1*
Morphine	2	1.3±0.1	2.3±0.3	11.8±3.2*	3.2±0.3	21.7±3.4*	2.0±0.3	8.0±3.4*	3.4±1.0	24.4±10.8*	2.5±1.0	14.1±1.2

Values are mean±SEM. *p<0.05 significantly different from control; Student's t-test (n=8).

Table 4: Effect of *B. coccineus* aqueous extract on cold-water tail flick test in rats.

Group	Dose (mg/kg)	Pre-Treat-ment(s)	+30min(s)	%Inhi-bition	+60 min(s)	%Inhi-bition	+90min(s)	%Inhi-bition	+120min(s)	%Inhi-bition	+150min(s)	%Inhi-bition
Control	-	8.7±1.7	9.2±2.2	3.1±1.5	9.2±2.3	3.6±1.9	11.4±2.2	6.2±2.0	9.7±2.1	3.1±1.0	9.3±2.4	2.5±1.3
<i>B. coccineus</i>	50	6.0±0.5	6.9±0.5	1.7±0.3	11.2±2.3	9.7±3.8	14.1±2.6	15.1±4.5	15.8±2.2	18.2±3.7*	13.6±2.6	13.9±5.5
	100	12.1±1.8	20.4±3.1	17.9±4.4*	30.9±5.5	40.0±10.2*	38.7±7.1	57.8±13.9*	44.5±7.2	69.3±14.2*	32.2±6.7	44.2±13.4*
	200	15.6±2.5	36.9±6.1	51.3±10.2*	48.6±5.7	77.5±10.7*	46.6±6.5	71.7±13.8*	41.5±4.7	59.5±10.6*	27.5±4.6	27.1±9.5*
	400	13.0±2.7	26.2±4.0	29.0±6.2*	31.6±5.6	42.0±10.7*	39.9±6.3	59.0±13.0*	42.5±6.9	65.5±13.7*	39.4±7.6	59.7±14.9*
Morphine	2	10.8±1.2	53.5±5.9	86.3±12.5*	51.4±5.2	81.6±10.9*	37.8±6.0	54.6±12.1*	27.1±6.2	32.0±12.8*	17.7±2.7	13.8±5.4

Values are mean±SEM. *p<0.05 significantly different from control; Student's t-test (n=6).

relative to the extract only up to 60min. post-treatment. Peak antinociceptive effect for the extract was attained at about 120min. post-treatment (as shown in Table 4).

Phytochemical Tests

Preliminary phytochemical analysis indicated the presence of alkaloids, tannins, saponins, reducing sugars, glycosides and anthraquinones.

Acute Toxicity Test

No mortality was observed with the extract given orally up to 10g/kg, but the LD₅₀ was 141.3mg/kg when administered intraperitoneally. Mice that were administered with the extract orally were behaviourally comparable to control except for intermittent reduction in locomotion and a higher frequency of rearing, which latter disappeared. Behavioural observations in respect of intraperitoneal administration, especially at the highest dose, included the display of signs of discomfort like immobility and seclusion.

DISCUSSION

Results from the present study indicate that *B. coccineus* produced a dose dependent inhibition of pain in the acetic acid induced writhing, tail immersion, tail-flick and the formalin tests. Writhing induced by chemical substances (e.g. acetic acid, phenylbenzoquinone) injected i.p. are due to sensitization of nociceptors by prostaglandins^{10,11} and this test is useful for the evaluation of mild analgesic non-steroidal anti-inflammatory compounds^{12,13} which act peripherally.

The inhibition of writhing in mice by the extract suggest a peripheral mechanism of action possibly mediated via inhibition of prostaglandins among several possibilities. This fact is corroborated by the

significant inhibition of the second phase of the formalin test. The formalin-induced pain model is very useful for elucidating the mechanism of pain and analgesia^{11,14,15}. Drugs that act mainly centrally, such as narcotics, inhibit both phases of formalin-induced pain while peripherally acting drugs, such as aspirin, only inhibit the late (second) phase^{15,16}. The effect of the extract was higher than that of the standard drug, acetylsalicylic acid, in the writhing test while being comparable in respect of the formalin test (Table 1 and 2). Peak antinociceptive effect was observed at a dose of 200mg/kg extract in the formalin model (second phase), unlike the case in the writhing test. In order to further confirm the antinociceptive action of the extract, the cold-water tail flick and tail immersion tests were carried out. The effects of the extract in the cold-water tail flick and tail immersion methods confirmed its analgesic action. This goes further to suggest also a central mechanism of action for the extract. It is known that centrally acting analgesic drugs elevate the pain threshold of mice towards heat and pressure³ and the cold-water tail flick test is specific for opioid induced antinociceptive effect^{7,15}. In the tail immersion test, the effects of the extract were lower than that of the standard drug, morphine (Table 3). The extract, however, showed a more prolonged effect than morphine in the cold water tail flick test (Table 4). Compounds detected in the plant extract, from preliminary phytochemical screening, include alkaloids, tannins, saponins, reducing sugars, glycosides, and anthraquinones. One or a combination of these components might be responsible for the established analgesic activity of *B. coccineus*. The safety of the plant extract when taken orally is justified by the fact that oral administration, up to 10g/kg, did not produce any mortality and visible toxic signs. However, the LD₅₀ was 141.3mg/kg when administered intraperitoneally.

In conclusion, the aqueous extract of *B. coccineus* possesses effective analgesic activity mediated via peripheral (probably through inhibition of prostaglandin synthesis) and central mechanisms. This supports the use of the plant in ethnomedicine to alleviate pain.

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