

Full Length Research Paper

Analgesic activities of ethanolic extract of the root of *Carpolobia lutea*

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Accepted 15 March, 2017

The analgesic activities of the aqueous extract of *Carpolobia lutea* was evaluated in mice and rats using the mouse writhing, tail flick and formalin induced pain tests. Analgesic studies were performed using three models; mouse writhing assay, formalin test and tail flick assay. The extract (1500 to 2500 mg/kg) and acetylsalicylic acid (100 mg/kg) produced a significant ($P < 0.05$) inhibition of the second phase in the formalin pain model, while the antinoceptive effect was not produced in the first phase. The extract also showed a dose dependent inhibition of acetic acid induced abdominal writhings. The tail flick latency was not enhanced by the extract. Oral administration of the extract up to 2500 mg/kg did not produce any toxic effects in the acute toxicity studies in mice. The LD₅₀ of the extract when administered orally was 3338.83 mg/kg. The data obtained shows that *C. lutea* possesses analgesic activity that is peripherally mediated.

Key words: Antinoceptive, *Carpolobia lutea*, LD50, pain model.

INTRODUCTION

The exploitation of plants for medicines has a long and honourable history, since at one time all drugs were obtained from natural sources. Natural products like plants present promise of cure as they have been the raw materials for the synthesis of drugs and as an important source of new therapeutic agents (Andreo et al., 2006).

The plant *Carpolobia lutea* G. Don (Polygalaceae) is a shrub measuring up to 15ft high (Hutchinson and Dalziel, 1954) and is widely distributed in West and Central areas of Tropical Africa (Mitaine–Offer et al., 2002). It is known by various names such as cattle stick (English), Abekpok Ibhuhu (Eket), Ikpafum, Ndiyan, Nyayanga (Ibibio), Angalagala (Igbo) and Egbo oshunshun (Yoruba) (Ettebong and Nwafor, 2009). *C. lutea* has been reported to possess anti-inflammatory and anti-arthritic properties (Iwu and Anyanwu, 1982), contain three new triterpene saponins (Mitaine–Offer et al., 2002) and possess antimicrobial activities (Phillip et al., 2005), as well as antidiarrhoeal and anti-ulcerogenic properties (Nwafor and Basse, 2007). The root is used to facilitate

childbirth, treat sterility, headache, worm infestation and also has aphrodisiac and stimulant properties (Mitaine – Offer et al., 2002). Whereas much work has been done on the antimicrobial activities of this plant, there is no documented record on the analgesic activity of the root of *C. lutea*. This study therefore, seeks to determine the analgesic properties of the roots of *C. lutea*.

METHODS

Preparation of plant extract

The plant material used in this research was collected from Akai-ubium local government area of Akwa Ibom State in May, 2007. Taxonomic identification was done by Dr(Mrs.) Basse of the Department of Botany, Faculty of science, University of Uyo, Nigeria. The roots were cleared of all debris and pulverized with mortar and pestle. The pulverized root (5000 g) was then filtered. The crude extract was concentrated to dryness in vacuo (40°C). After the concentration, a dried extract weighing 330 g was obtained and stored in the refrigerator for use throughout the study.

Drugs and chemicals

Acetylsalicylic acid solution in normal saline was used (BDH

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Chemicals, England) . The following were analytical grade chemicals from Sigma Aldrich, USA : 1% formalin, Iomotil®, distilled water, 70% ethanol, fehling solutions (1 and 11) ferric chloride chloroform, glacial acetic acid, sodium hydroxide (20%), 2% sodium nitroprusside, acetic anhydride, sulphuric acid benzene, 10% ammonia solution and ice.

Animals

Albino rats (170 to 200 g), mice (25 to 30 g) of either sex at the laboratory animal centre of the University of Uyo, Nigeria were used. All the animals were housed in standard cages under laboratory condition in the Department of Pharmacology and Toxicology University of Uyo and were fed with pellet feed (Guinea feed) and water *ad libitum*. All animal experiments were conducted in compliance with NIH guidelines for care and use of laboratory animals. This study was approved by the Ethical committee of the Faculty of Pharmacy, University of Uyo, Nigeria.

Analgesic studies

Mouse writhing assay

This was based on the method described by Koster et al. (1959). Swiss albino mice of either sex were selected and divided into five groups of five animals each. The extract 1500 to 2500 mg/kg orally, distilled water (10 ml/kg, orally) and acetylsalicylic acid (100 mg/kg subcutaneously) were administered 30 min before intraperitoneal injection of 0.6% v/v acetic acid solution in normal saline at a dose of 10 ml/1kg. Immediately after administering acetic acid, mouse – pairs were placed in transparent glass cages and the number of writhings or stretches were counted for 15 min. Reduction in the number of writhes compared to the control groups was considered as evidence of analgesic effect.

Formalin test

The method was smaller to that described previously. 20 µl of 1% formalin was injected subcutaneously into the right hind paw of mice. The time (in sec) spent in licking the paw and the biting responses of the injected paw was taken as an indicator of pain response. Responses were measured for 5 min after formalin injection (first phase) and 15 to 30 min after formalin injection (second phase). Extract 1500 to 2500 mg/kg, orally) and acetylsalicylic acid (100 mg/kg, sc) were administered 30 min prior to formalin injection. Control animals received 10 ml/kg of distilled water orally.

Tail flick assay

Rats were closely restrained in a wire mesh cage and the tails (up to 5 cm) were then dipped in a beaker of cold ice block (0 to 1°C). The time in second to withdraw the tail clearly out of the water was taken as the reaction time. All the animals were screened and those that failed to respond within 60 s were not used for the assay. Measurement of threshold was made just before (0 min) and at 30 min interval for 3 h after administration of the extract (1500 to 2500 mg/kg, orally) or Iomotil (2 mg/kg subcutaneously). Distilled water (10 ml/kg orally) served as the control.

Phytochemical test

The phytochemical analysis of the extract was carried out using

standard methods (Odebiyi and Sofowora, 1978; Trease and Evans, 1986).

Acute toxicity

Mice (4 per group) were administered orally with different doses; 2000, 3000, 3500, 4000 (mg/kg) of the extract. Mortality in each group within 24 h was recorded. LD₅₀ was estimated by probit analysis using SPSS.

Statistical analysis

Results were expressed as mean ± SEM. Statistical analysis of the data was the one using student's t-test and the results were considered significant when p < 0.05.

RESULTS

Phytochemical screening

Phytochemical screening of the extract revealed the presence of tannins, saponins, flavonoids, cardiac glycosides, anthraquinones and terpenes. Alkaloids were absent.

Acute-toxicity study

The median lethal dose (LD₅₀) was calculated to be 3340 mg/kg body weight. This was determined from probit value of 0.5 (Figure 1) by the use of statistical software SPSS version 17. Table 4 gives the values of acute toxicity testing when using manual methods of calculation. The physical signs of toxicity included initial decrease in motor activity, increase in respiratory rate, which was followed by restlessness grasping and death.

DISCUSSION

The experimental data on the study of the analgesic activity of *C. lutea* extract are summarized in Tables 1 to 3. In the mouse writhing assay, the extract was found to inhibit the acetic induce writhing in mice in dose dependent manner (Table 1). It effectively reduced the pain induced by acetic acid. Acetic acid mouse writhing assay distinguishes between central and peripheral analgesic activity. The administration of acetylsalicylic acid 30 min before intraperitoneal injection of the acetic acid solution greatly decreased the number of writhes when compared to the control. Administration of *C. lutea* aqueous extract at different doses also showed significant decreases in the number of writhes when compared to the control.

In the formalin test, the extract did not inhibit the first phase of the formalin (Table 2) but rather inhibit the late phase of the test. The formalin test measures both central and peripheral mediated analgesic activities. This

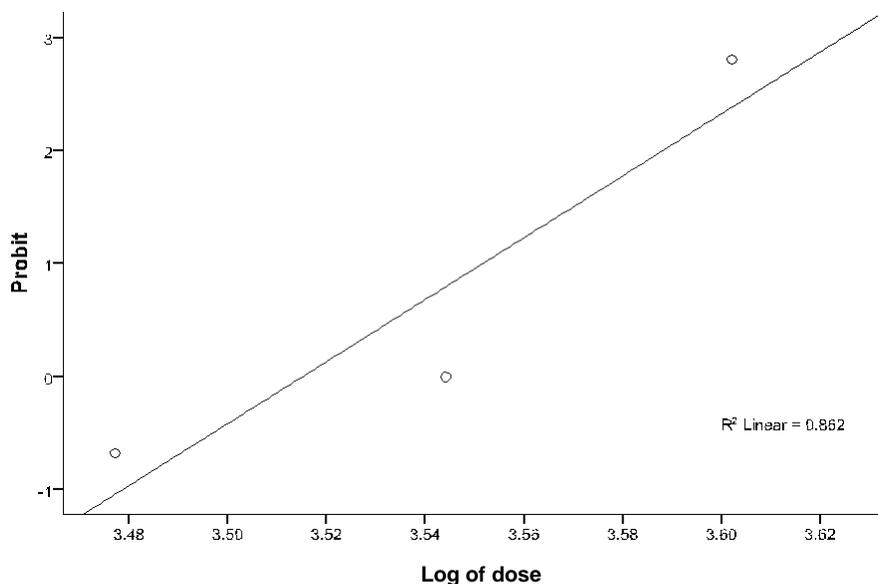


Figure 1. Probits against log dose of *C. lutea* extract.

Table 1. Effect of *C. lutea* aqueous extract on acetic acid induced writhing in mice.

Group	Dose (mg/kg)	No. of Writhes
Control		35.40±6.698
<i>C. lutea</i>	1500	22.6±3.156
	2000	13.6±1.400*
	2500	16.2±5.869
Acetyl salicylic acid	100	10.8±2.107*

Values are mean ± SEM, *p<0.05, significantly different from control (students t-test).

Table 2. Effect of *C. lutea* aqueous extract on formalin induced pain.

Group	Dose (mg/kg)	0 - 5 min	15 - 30 min
Control		34.8±5.919	33.6±5.483
<i>C. lutea</i>	1500	23.4±4.600	3.8±1.855*
	2000	21.4±2.891	0.40.400*
	2500	29.2±4.128	0.8±0.800
Acetyl salicylic acid	100	18.6±2.657	1.4±0.9798*

Values are mean ± SEM, *p<0.05, significantly different from control (students t-test).

may have been due to the absence of opioid receptors to transmit nociceptive impulses to higher structures. The inhibition of the late phase of the formalin test suggests that *C. lutea* extract effect on pain is peripherally mediated. From the data, the standard drug, acetylsalicylic acid, also exhibited similar effect as the extract.

The data obtained from the tail flick assay shows that the extract did not enhance the tail flick latency (Table 3). At the different doses of extract, there was no appreciable difference in the latency when compared with

the control group. The tail flick assay is the method for measuring centrally mediated analgesic activity. This method is selective for morphine like compound, administration of lomofil ® as a standard drug enhanced the tail flick latency when compared to the control group.

Thus, the extract lacks centrally mediated analgesic activity due to its inability to enhance the tail flick latency.

On phytochemical analysis, the following constituents were detected flavonoids, saponins, tannins, cardiac glycosides and terpenes. Alkaloids were however, absent. Flavonoids constitute one group of phytochemical

Table 3. Effect of *C. lutea* aqueous extract on Tail flick assay in rats.

Group	Dose (mg/kg)	0 h	0.5 h	1.0 h	1.5 h	2.0 h	2.5 h	3.0 h
Control		35.8±5.5	36.5±7.3	33.0±5.0	30.4±7.1	26.6±3.4	28.0±6.0	30.2±4.9
<i>C. Lutea</i>	1500	39.0±4.3	35.0±6.5	34.6±2.0	36.6±2.0	28.6±4.0	29.2±3.3	24.8±2.3
	2000	45.0±3.5	40.0±5.7	35.0±6.1	32.6±1.9	34.0±2.9	32.4±2.2	31.0±3.3
	2500	32.2±3.7	32.6±3.1	38.2±5.0	30.2±7.4	38.4±5.2	36.4±3.9	33.4±4.2
Lomotil®	2	36.6±3.3	55.0±2.2	54.4±3.7*	52.6±2.2*	54.8±2.4*	56.0±2.1*	54.2±2.9*

Values are mean ± SEM, *p<0.05, significantly different from control (students t-test).

Table 4. Acute toxicity determination of *C. lutea* extract (oral route).

Group	Dose (mg/kg)	Log dose	24 h mortality	%mortality	Probit	Approx probit
1	2000	3.301	0/4	0.00	0.04000	0
2	3000	3.477	1/4	25.00	4.3255	4.0
3	3500	3.544	2/4	50.00	5.0000	5.0
4	4000	3.602	4/4	99.75	7.8070	8.0

compounds widely associated with anti-inflammatory activity. The presence of flavonoids may have also inhibited the writhing.

In the acute toxicity studies, the LD50 was calculated to be 3340 mg/kg, which is higher than the working doses (1500, 2000 and 2500 mg/kg). In the experimental analysis, safe doses were therefore used. *C. lutea* extract does not act centrally, but exhibit peripheral analgesic activity. It would therefore, not be effective in the management of chronic pain but rather used for acute pain. This paper reports the analgesic properties of the roots of *C. lutea*.

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