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Full Length Research Paper

Antinociceptive and anti-inflammatory activities of the aqueous extract of the root bark of *Combretum sericeum* in rodents

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Combretum sericeum **(Combretacae) is used traditionally in Northern Nigeria for the treatment of fever. In this study, the activities of the aqueous extract of the root bark of this plant against nociception and inflammation were investigated in mice and rats. The studies were carried out using acetic acid writhing, hot plate algesia and carrageenan induced inflammation in rats. The results showed that the extract exhibited significant ($P < 0.001$) anti-nociceptive and anti-inflammatory activities in all the models used. Phytochemical screening of the extract revealed the presence of tannins, flavonoids, glycosides alkaloids and anthraquinones. The intraperitoneal median lethal dose (LD_{50}) of the extract was found to be 177.48 mg/kg in mice. The observed activities might be the scientific basis for the traditional use of the plant in the treatment of fever. This study also paves way for the possible development of the plant extract as a phytodrug against pain and inflammatory conditions.**

Key words: *Combretum sericeum*, anti-nociceptive, anti-inflammatory, hot plate algesia.

INTRODUCTION

The use of medicinal plants in the treatment of ailments associated with pains is well known throughout history (Almeida et al., 2001). Such plants have played important roles in drug discovery research and studies on them are logical research strategies in the search for new drugs (Elisabetsky et al., 1995). *Combretum sericeum* (Family: Combretacae) is a shrub that grows abundantly in the Savannah region of West Africa. The plant is well known locally and used for the treatment of ailments such as stomach disorders, conjunctivitis and to reduce fever (Abdullahi et al., 2003). The common name of the plant among the Hausa community of Northern Nigeria is "Taro", while the Jaba people refer to it as "Nyangbimsa". The antidiarhoeal activities of the plant have been studied (Sini et al., 2008). However, there is no scientific report that describes the pharmacological relevance of this plant in the treatment of ailments associated with pain and inflammation. This study was designed to investigate the possible anti-nociceptive and anti-inflammatory activities of the aqueous extract of *C. sericeum* root bark. Knowledge of these properties will support the understanding of the medicinal values of the extract.

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MATERIALS AND METHODS

Plant material

The roots of *C. sericeum* were collected from Kurmin-bauna village in Jaba local government area of Kaduna State, Nigeria in

November 2007. The plant was identified and authenticated at the herbarium section of the Department of Biological Sciences, Ahmadu Bello University, Zaria. A voucher specimen (Number 000135) was preserved at the herbarium. The roots were washed clean using distilled water. The washed samples were then shade dried and pulverized using mortar and pestle. About 130.4 g of the powdered plant material was packed into a thimble which was macerated in 1.2 L of distilled water and placed on a shaker overnight for 24 h. The extract was then sieved using 20 µm mesh size sieve and filtered using Whatman filter paper (20 cm). The filtrate was concentrated and the last traces of the solvent were removed by heating on a water bath at 40°C. The water extract yield was 28.50 g (21.86% w/w) and this was used for the pharmacological evaluations.

Chemicals/drugs

The chemicals used in this study were carrageenan [Fluka Chemika (Buchs Switzerland) and acetic acid (Sigma chemical Co., USA)]. The standard drugs used were piroxicam (Hovid, Malaysia) and morphine (Sigma chemical Co. USA).

Animals

Swiss albino mice and Adult Wistar rats of both sexes were used for the study. The animals were obtained from the Animal House of the Department of Pharmacology and Therapeutics of the Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. They were kept in standard animal cages at room temperature (25 ± 1°C) and subjected to a 12 h light/dark cycle. They were fed with a standard diet (Pfizer feed PLC, Lagos, Nigeria) and allowed free access to water *ad libitum*.

Acute toxicity test

The acute toxicity study was carried out based on the method describe by Lork (1983) and Sini et al. (2008).

Acetic acid-induced writhing

The acetic acid-induced abdominal constriction test (Koster et al., 1959) was used with local modification as described by Adzu et al. (2001). 30 mice were randomly divided into five groups of 6 mice each. The first group received normal saline (10 ml/kg) intraperitoneally (i.p.), the second, third and fourth groups were given 12.5, 25 and 50 mg/kg of the extract (i.p.), respectively, and the last group received piroxicam (10 mg/kg). 30 min later, mice in all the groups received 10 ml/kg of 0.7% aqueous solution of acetic acid (i.p.). Each mouse was placed in a transparent observation cage and abdominal constrictions, consisting of contortions of the abdominal muscle (stretching of hind limbs) that occurred between 5 and 15 min after acetic acid injection were counted. Activity was expressed as percent inhibition of nociception. The percentage inhibition was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Mean number of writhes (control)}}{\text{Mean no of writhes}}$$

$$\text{– mean number of writhes (test)} \over \text{(control)} \times 100$$

Hot plate test

The test was performed using a hot plate (Ugo Basile DS – 37) maintained at 55 ± 0.5°C. Mice that showed nociceptive responses within 20 s when placed on the hot plate were admitted into the study and placed into five groups of six mice each. The first group received normal saline (10 ml/kg) (i.p.), the second, third and fourth groups were given 12.5, 25 and 50 mg/kg of the extract (i.p.), respectively, and the last group received morphine (4 mg/kg) (i.p.). Each mouse was gently placed on the hot plate and the time taken by the mice to respond to the thermal stimulus was recorded. Reaction time was taken as the interval between the time the animal was placed on the plate till the moment it began to lick its paws or jump (Al-Ghamdi, 2001; Adzu et al., 2003a). 60 s was chosen as the cut off time to avoid tissue damage. Readings were taken for each mouse at time 0 and after every 30 min until the 90th min post treatment.

Carrageenan-induced paw oedema

The method of Winter et al. (1963) was used. 30 adult wistar rats of either sex were randomly divided into 5 groups of 6 mice each. The first group received normal saline (1 ml/kg) (i.p.), the second, third and fourth groups were given 12.5, 25 and 50 mg/kg of the extract (i.p.), respectively, and the last group received piroxicam (10 mg/kg). 30 min later, 0.1 ml of freshly prepared 1% carrageenan suspension was injected into the subplantar region of the left hind paw of each rat. Measurements of the paw oedema were then taken with a vernier caliper at 0, 1, 2, 3 and 4 h after the injection of carrageenan.

Data analysis

Results of the study were expressed as mean ± S.E.M. Student's t-test and one way ANOVA were used to analyze the data obtained. P values of 0.05 or lower were considered as significant.

RESULTS

Acute toxicity studies

The LD_{50} was estimated to be 177.48 mg/kg (ip) in mice (Sini et al., 2008).

Acetic acid-induced writhing

The extract significantly ($P < 0.001$) attenuated the acetic acid and induced abdominal constrictions in mice at all the doses tested (Table 1). The antinociceptive activity was observed to be dose-dependent with the lowest dose of 12.5 mg/kg, eliciting the highest activity of 91.14% inhibition of nociception. The extract's antinociceptive effect was greater than that of the standard drug piroxicam used as positive control for the experiment.

Hot plate test

The extract (at all doses tested) significantly ($P < 0.05$) increased the mean reaction time to nociception, which

Table 1. Effect of the aqueous extract of *C. sericum* roots on acetic acid-induced writhing in mice.

Treatment/dose	Mean number of writhe	Percentage inhibition
Normal saline (10 ml/kg)	24.50 ± 3.19	-
C.S. (12.5 mg/kg)	2.17 ± 1.42[a]	91.14
C.S. (25 mg/kg)	2.33 ± 1.50[a]	90.48
C.S. (50 mg/kg)	3.00 ± 2.29[a]	87.76
Piroxicam (10 mg/kg)	5.67 ± 2.70[a]	76.86

[a] Are significantly different from control at P< 0.001 (Student t test); C.S., *C. sericeum.*

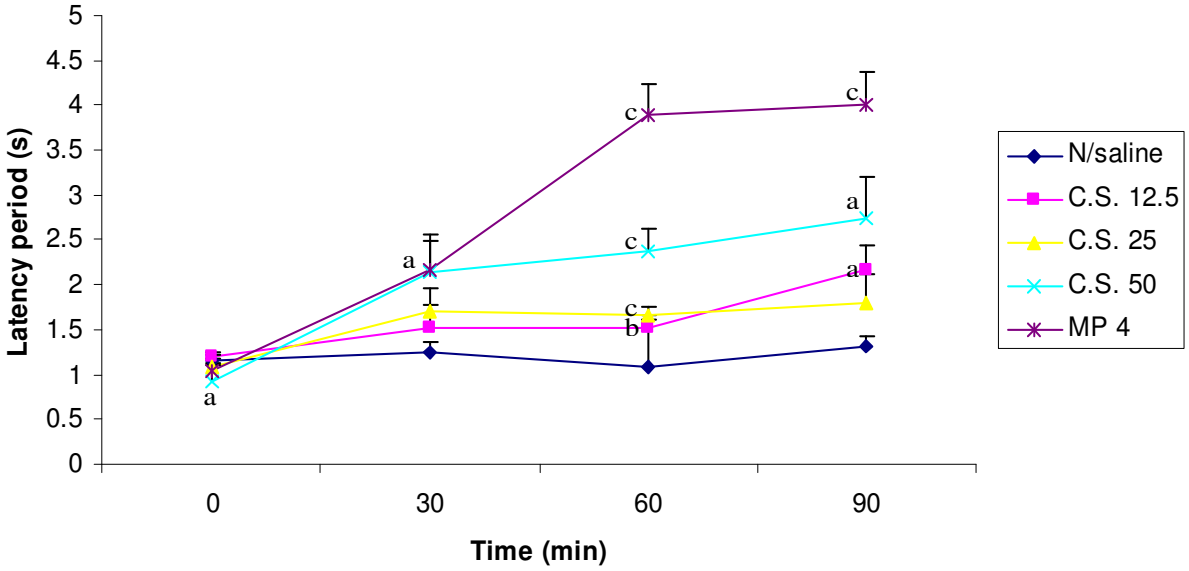

Figure 1. Effect of the aqueous extract of *C. sericum* roots on hot plate-induced pain in mice. [a]P < 0.05; [b]P< 0.05;

was caused by the hot plate in mice (Figure 1). The activity was dose-dependent with the highest activity of all doses of the extract observed at the 60th min. The standard drug exhibited greater activity than the extract.

Carrageenan-induced paw oedema

The extract caused a dose-dependent inhibition of carrageenan-induced oedema over a period of 4 h (Figure 2). The effect was significantly different from the control at P < 0.001. The peak effect of the extract's inhibition (0.04 ± 0.01) was observed at the dose of 50 mg/kg at the 120th min. At this dose, the inhibition was observed to be greater than that of the standard drug, piroxicam (10 mg/kg).

DISCUSSION

This study investigated some of the scientific reasons behind the folkloric use of *C. sericeum* in the management of ailments associated with pain and inflammation. The results showed that the extract was active against all the experimentally induced laboratory models of pain and inflammation. Acetic acid-induced writhing is a sensitive procedure for detecting analgesic effects of medicinal agents (Collier et al., 1968). The model, though widely used for the evaluation of peripheral analgesia, is non specific as central analgesics also show positive response to it (Vogel and Vogel, 1997; Trongsakul et al., 2003). The pain mechanism is believed to involve, in part, local peritoneal receptors (Bentley et al., 1983) which has been associated with increased peritoneal fluid concentration of PGE_2 and $PGF_{2\alpha}$ following intraperitoneal injection of acetic acid (Deraedt et al., 1980). The analgesic activity of the extract against this model of nociception may therefore be due either to its action on the peritoneal receptors, inhibition of the production of arachidonic acid metabolites or the inhibition of synaptic transmission of pain messengers to the central nervous system.

Since the time lag to thermal response in the mice was increased significantly by the extract, it is possible that the central mechanisms may have been involved in the

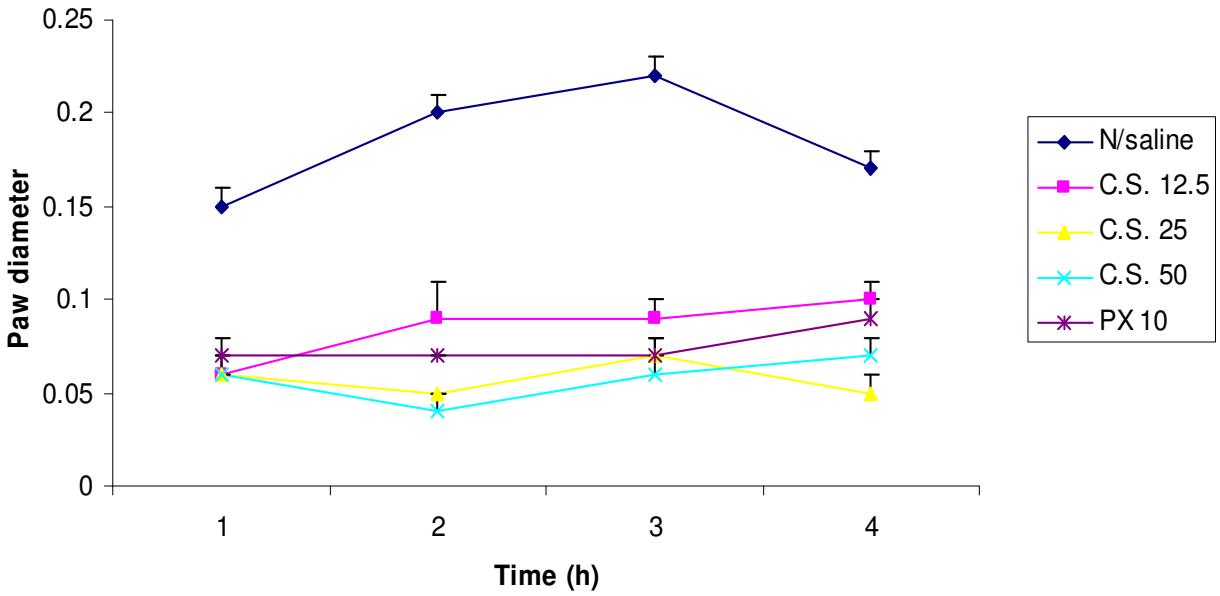

Figure 2. Effect of the aqueous extract of *C. sericum* roots on carrageenan-induced inflammation in mice. All points were significant at P < 0.001 relative to the saline control group; C.S., *C. sericeum.*

observed phenomenon which strengthens the earlier speculation that the extract could elicit activity against both models of pain. The suggestion agrees with those of Adzu et al. (2003) and Khanna et al. (1997) who observed that an increase in time lag thermal response in mice against hot plate-induced nociception could indicate the involvement of central neurons.

Carrageenan-induced inflammation is a model that is widely used in determining anti-inflammatory activities of medicinal agents and is well documented for various non-steroidal anti-inflammatory drugs (NSAIDs), particularly with salicylates and their congeners (Reuse, 1978; Beuovist and Misse, 1979; Famacy, 1983). The carrageenan model is used to detect anti-inflammatory activity in acute and sub acute inflammatory processes mediated by the release of histamine, 5HT (5-hydroxytryptamine) (Crunkhon and Meacock, 1971), kinins and prostaglandins (Castro et al., 1968; Mazumder et al., 2003). From the results of this study, the potent anti-inflammatory activity of the extract may be linked to the inhibition of histamine, 5HT prostaglandins and kinins.

Flavonoids isolated from some medicinal plants have been proven to possess antinociceptive and anti-inflammatory effects (Owoyele et al., 2008; Duke, 1992). We had earlier (Sini et al., 2008) reported that the aqeous extract of *C. sericeum* contained flavonoids. It is therefore possible that the anti-nociceptive and anti-inflammatory effect observed with the extract may be due in part to its flavonoid content.

The result of the investigation suggests that this plant possesses significant anti-nociceptive and anti-inflammatory activities in laboratory animals whose finding supports the traditional use of this plant in the treatment of ailments associated with pain and inflamemation. This study also suggests the presence of biologically active principle(s) worthy of further investigation.

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