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Full Length Research Paper

Analgesic and anti-inflammatory activities of the methanolic extract of the rhizomes of *Stylochiton lancifolius* pyre and Kotchy (Araceae) in rodents

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The methanolic extract of the rhizome of *Stylochiton lancifolius* (50, 100, and 200 mg/kg) was evaluated for analgesic and anti-inflammatory activities using acetic acid-induced writhing, formalin-induced pain and formalin-induced-inflammation. The methanol extract exhibited significant (P<0.001) inhibition of acetic acid-induced writhing in mice and a significant (P<0.001) reduction in paw licking time of the second phase of formalin-induced pain in rats. The methanol extract also produced a significant (P<0.001) anti-inflammatory effect in formalin-induced inflammation which is comparable to that of the reference drug Piroxicam (10 mg/kg), which is a standard analgesic and anti-inflammatory drug. The intraperitoneal (i.p) median lethal dose (LD_{50}) of the methanol extract of *S. lancifolius* was found to be greater than 5000 mg/kg in mice. The result obtained from this study showed that the methanol extract of *S. lancifolius* possesses analgesic and anti-inflammatory activities and supports the ethnomedical claim of the use of the plant in the management of pain and inflammatory conditions.

Key words: *Stylochiton lancifolius,* antinociceptive, anti-inflammatory, acute toxicity, mice and rats.

INTRODUCTION

Traditional medicine in many areas of the world relies on the use of a wide variety of plant species. In Africa, phytotherapy still plays an important role in the management of diseases, mainly among population with very low income (Geoffrey and Kirby, 1996).

Stylochiton lancifolius a small herbaceous plant with short rootstock found in savanna woodland from Senegal to Southern Nigeria and wide spread in the drier savanna of tropical Africa (Burkill, 1985). The rhizome of *S. lancifolius* has reputedly been used by the Fulani's of the Comm., 2002). In addition, the leaves are employed as analgesic and antimicrobial agent in the treatment of cellulitis of the finger commonly known as whitlow (Per. Comm., 2004). While the decoction of the root of northern Nigeria as an anti-inflammatory and analgesic agent during their customary annual "sharo" (Per. S*tylochiton natalensis* Schott is used by Zulus for the treatment of chest diseases and as an earache remedy in the Barberton district in South Africa. Furthermore, urine boiled with *Stylochiton* species is used by the Zulus as earache remedy (Watt and Breyer, 1962).

However, scientific literature substantiating its use in pain relief is lacking. The purpose of this study was to evaluate the antinociceptive and anti-inflammatory effects of *S. lancifolius* Kotschy and Peyr in mice and rats.

EXPERIMENTAL

Collection, identification and preparation of plant materials

The whole plant (aerial and the underground rhizome and roots) was collected around Samaru, village, Zaria in the month of

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September. The plant was authenticated in the herbarium, Ahmadu Bello University, Zaria, and a herbarium specimen was kept at the Department of Biological Sciences, Ahmadu Bello University, Zaria (with voucher number 1407).

The aerial part (leaves) was manually separated from the rhizome and both were air dried, powdered, sieved, weighed and stored in airtight containers and subsequently referred to as powdered leaves and rhizome.

Extraction

Powdered rhizome of *S. lancifolius* (1.3 kg) was defatted exhaustively with light petroleum ether (60 to 90°C) in a soxhlet extractor. The solvent was recovered under reduced pressure to afford a dark greenish oily mass (41 g), which was labeled as petroleum extract (RPE) and kept in the refrigerator. The resulting marc was air dried at room temperature and then exhaustively extracted with methanol using the soxhlet extractor. The methanol was also recovered under reduced pressure to yield a dark brownish waxy mass. This was labeled as methanol extract (RME) and the marc was further dried.

Animals

Swiss-strain albino mice and adult Wister rats of both sexes weighing between 23 to 30 g and 160 to 180 respectively were locally bred in the Animal House, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria. Both the rats and the mice were fed on standard rodent chow and clean drinking water was provided *ad libitum*. The animals were kept in room with controlled 12 h light and 12 h dark cycle and at an ambient temperature of 22 ± 3°C. The animal care and handling were conducted in compliance with National Regulation for Animal Research and approved by animal ethical committee of the university.

Determination of median lethal dose (LD_{50})

Acute toxicity studies (LD_{50}) in mice

LD_{50} determination was conducted using the method of Lorke (1983). In the initial phase, three groups of three mice each were treated with RME dissolved in 30% v/v tween-20 at doses 10, 100 and 1000 mg/kg body weight i. p. and observed for 24 h. In the second phase, three groups of one mouse each were injected with the methanolic extract at doses of 1600, 2900 and 5000 mg per kg body weight intraperitoneally. Mice were observed for signs and symptoms of toxicity for 24 h.

Analgesic studies

Acetic acid – induced writhing test in mice

The test was conducted by employing the method described by Koster et al. (1959). Swiss albino mice were divided into 5 groups of six mice each. The first group served as negative control and was given 10 ml normal saline per kg body weight i.p. Groups 2, 3 and 4 received 50, 100 and 200 mg extract per kg body weight i.p. respectively, while the fifth group was administered 10 mg piroxicam (Hovid Bdh, Malaysia) per kg body weight i.p. Thirty minutes later, mice in all the groups were treated with 10 ml acetic acid (0.6% v/v) per kg body weight i.p.

Five minutes after acetic acid injection, mice were placed in individual cage and the numbers of abdominal contractions were counted for each mouse for a period of 10 min. Percentage inhibition of writhing was calculated using the formula:

$$\text{Inhibition (\%)} = \frac{\text{Mean number of writhing (control)} - \text{Mean number of writhing (test)} \times 100}{\text{Mean number of writhing (control)}}$$

Formalin test in rats

The test was performed in accordance with the method described by Dubuisson and Dennis (1977). In this test, licking time in seconds was registered from 0 to 5 min (first phase) and from 20 to 25 min (second phase), after administration of formalin (50 µl of 2.5% v/v solution), under the planter surface of the right hind paw (Hunskaar et al., 1985; Hunskaar and Hole, 1987). Thirty adult Wistar rats were divided into 5 groups of six rats each. The first group received normal saline (1 ml per kg body weight i. p), the second, third and fourth received 200, 100 and 50 mg extract per kg body weight i.p. respectively, while the last group received 10 mg per kg body weight imps pentazocine (Ranbaxy, India) dissolved in distilled water. Thirty minutes later, rats in all the groups were treated with formalin.

Anti-inflammatory studies

Acute inflammation

Formaldehyde 2.5% v/v was used as inflammogen (Sayya et al., 2003). Rats were divided into five groups of six rats each. Thirty minutes before injection of 2.5% v/v formalin (50 µl volumes in the subplantar region of the left hind paw of the rat), the groups were treated i.p as follows:

Group I: received (10 mg piroxicam per kg, as positive control).
Group II: received (1 ml normal saline per kg, as negative control).
Groups III, IV and V: received the extract 200, 100 and 50 mg per kg respectively.

The paw diameter (cm) was measured using vernier caliper at interval of one hour for four hours.

RESULTS

Toxicity studies

The acute toxicity studies (LD_{50}) has shown that the methanolic extract of the rhizome has LD_{50}>5000 mg/kg because there was no death up to 5000 mg/kg.

Antinociceptive studies

Writhing test

Table 1 shows the antinociceptive effects of the RME on acetic acid induced contractions in mice. 200 mg/kg of the extract caused 60% inhibition of the acetic acid induced contraction while 100 and 50 mg/kg showed 68 and 65.6% inhibition of the abdominal contractions, respectively. The extract was found to be effective although less effective than piroxicam which inhibits the contraction by 80%. The extract decreased the number of

Table 1. Effect of the methanol extract (RME) on acetic acid induced abdominal contractions in mice.

Treatment (mg/kg)	Mean ± SEM of abdominal constrictions	% Inhibition
N/Saline	25.0±2.3	
RME (200)	10.0 ± 2.3[c]	60.0
RME (100)	8.0 ± 1.7[c]	68.0
RME (50)	8.6 ± 3.0[c]	65.6
Piroxicam (10)	4.8 ± 0.7[c]	80.8

c, represents statistical significance at P< 0.001 student t – test, n = 6.

Table 2. Effect of the methanol extract of *S. lancifolius* (RME) on formalin induced pain in rats.

Treatment (mg/kg)	First phase	Second phase
N/Saline	53.4 ± 11.2	21.6 ± 2.3
RME (200)	36.0 ± 7.4	5.3 ± 1.9[c]
RME (100)	35.0 ± 2.6	6.8 ±1.8[c]
RME (50)	38 ± 3.2	7.2± 1.9[c]
Pentazocine (10)	29.6 ± 3.8[a]	3.7 ± 0.9[c]

a, and c, represents statistical significance at P< 0.05, and 0.001 (student t – test, where, n = 6), respectively.

acetic acid induced abdominal contractions in mice and the values were found to be significant (P<0.001) in all the three administered dose when compared to the control (Table 1).

The formalin test

The formalin test on rats has shown a significant reduction in response to nociception during Phase II (P<0.001) at all the three doses. The first phase showed practical reduction in responses to nociception though statistically there is no significance (Table 2).

Anti-inflammatory studies

The result of this experiment showed that RME caused inhibition of formaldehyde-induced inflammation over a period of 4 h.

The results were statistically found to be significant to varying degree (Table 3). However, the anti-inflammatory effect was found to be more pronounced at the fourth hour, and greater than that induced by piroxicam in all the three doses.

DISCUSSION

Toxicity studies

The acute toxicity (LD_{50}) was found to be greater than 5000 mg/kg which implies that the plant is not practically toxic (Lorke, 1983). The safety of the plant rhizome allows it to be used during draught or famine as food though it requires treatment or special washings to remove the bitterness (Burkill, 1985).

Local peripheral receptors are postulated to be partly involved in the abdominal constriction response (Bentley et al., 1983). The method has been associated with prostanoids in general, example, increase levels of PGE_2 and $PGF_{2\alpha}$ in peritoneal fluids (Derardt et. al., 1980) as well as lipooxygenase products as reported by some researchers (Livini et al., 1984; Dhara et al., 2000). Therefore, the result of the acetic acid induced writhing suggests that the mechanism of action of this extract may be linked to lipooxygenases and / or cyclooxygenases (Vongtau et al., 2004).

In the formalin test, there is a distinct biphasic nociceptive response termed early and late phase. Drugs that act primarily on the central nervous system inhibit both phases equally while peripherally acting drugs inhibit the late phase (Shibata et al., 1989; Chen et al., 1995). The early phase is probably a direct result of stimulation of nociceptors in the paw and reflects centrally mediated pain while the late phase is due to inflammation with a release of serotonin, histamine, bradykinin and prostaglandins (Tjolsen et al., 1992).

The second phase (tonic pain response) of formalin phase of pain as observed with the extract (200 mg/kg) in this study lends credence to the presence of peripheral effects.

However, the observed central effects are minimal and statistically insignificant when compared to the peripheral effects that are significant at P<0.001 (Table 2).

Table 3. Effect of methanol extract of *S. lancifolius* (RME) on formaldehyde induced inflammation in rats.

Treatment (mg/kg)	Paw volume (ml) per hour				
	0 h	1 h	2 h	3 h	4 h
N/Saline	0.61±0.01	0.76±0.01	0.74±0.05	0.79±0.02	0.84±0.02
RME (200)	0.63±0.02	0.63±0.01^{c}	0.62±0.01^{a}	0.58±0.07^{b}	0.51±0.08^{c}
RME (100)	0.62±0.01	0.62±0.02^{c}	0.50±0.03^{c}	0.49±0.02^{c}	0.45±0.02^{c}
RME (50)	0.52±0.01	0.47±0.06^{c}	0.44±0.02	0.45±0.05^{c}	0.37±0.04^{c}
Piroxicam (10)	0.46±0.04^{c}	0.47±0.02^{c}	0.42±0.07^{c}	0.53±0.02^{c}	0.52±0.07^{c}

a, b, and c, represents statistical significance at P< 0.05, 0.01 and 0.001 (student t – test, n = 6) respectively.

Formalin-induced inflammation test is useful in detecting the efficacy of anti-inflammatory agents. The test is sensitive to both clinically useful steroidal and non-steroidal anti-inflammatory agents (Di Rosa et al., 1971) and it is related to Cyclooxigenase (COX) inhibition, particularly, Cyclooxigenase-2 (COX-2).

The methanol extract of *S. lancifolius* has produced strong anti-inflammatory effects on the formalin induced paw edema, which was in the same order of magnitude as that observed after piroxicam administration. In fact, the extract at 50 mg/kg has shown better activity than piroxicam at the third and fourth hours (Table 3). This implies that the extract have a slower rate of action and longer duration of action.

The antinociceptive and anti-inflammatory activities of the methanol extract could be attributed to the presence of phytosterols (Delporte et al., 1998; Calixto et al., 1998; Chechinel-Finho et al., 1996) that were detected and isolated (Pateh et al., 2009). Similarly, saponins (Hosseinzadeh and Younesi 2002; Garrido et al., 2001) that were detected (Pateh et al., 2009) and terpenoids that were reported to be present in the extract (Mukharjee et al., 1997) could possibly be responsible.

The present finding provides pharmacological support for the folkloric indication of the plant *S. lancifolius* for the relief of pain and inflammation.

Conclusion

The present experimental evidence of antinociceptive and anti-inflammatory effects of *S. lancifolius* calls for further studies to explore the active component responsible for the same.

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