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Original article

Toxicity and analgesic studies of leaf methanolic extract of *maytenus senegalensis* (Lam.) Exell (*celastraceae*)

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ABSTRACT

The leaf methanolic extract of Maytenus senegalensis (Lam.) Exell was investigated for toxicity and analgesic effects. Acute toxicity was investigated via intraperitoneal route using mice and rats, analgesic activity was investigated using two models; acetic acid-induced writhing in mice and formalin induced pain in rats. The extract was determined to have LD (50) of 1264.91 mg/kg in both mice and rats. It significantly (0.001) inhibited acetic acid-induced writhes in mice at all doses administered (75, 150 and 300 mg/kg) in a dose dependent manner and significantly (0.01) inhibited formalin induced pain in rats, in a non-dose dependent manner. The inhibitory effect observed was higher in the extract compared to the standard used (piroxicam). The results obtained suggested the extract to be relatively toxic in both mice and rats with a dose dependent anti-nociceptive activity.

1. Introduction

Maytenus senegalensis (Lam.) Exell belongs to the family Celastraceae, and is commonly known as confetti tree in English and by the Hausa people of Northern Nigeria as 'Namijin Tsada', 'Kunkushewa', 'Kambun-shafu',

'Kyalbuwa', 'Mangaladi' 'Kurunkushewa', or 'Kurunkushiya'. It ranges from a shrub to a small tree, and widely available in tropical Africa (Burkill, 1985). It has a long history of usage by the 'Hausa' community in traditional medicine and known to exhibit a wide range of biological activities such as anti-inflammatory, analgesic, antibacterial activities, etc.

In the present study, an attempt was made to investigate the toxicity and anti-analgesic effect of this plant.

2. Materials and methods

Plant Collection and Identification The plant material was collected from Ahmadu Bello University Dam, Samaru, Zaria, Nigeria. It was identified at the herbarium unit of Biological Science Department, of the same University, where a voucher specimen No. 900144 has been deposited.

Extraction The leaf powder was extracted with aqueous methanol (70:30 v/v) using maceration method for 4 days. The extract was concentrated using water bath at the temperature of 450C.

2.1. Experimental animals

Male wistar rats weighing between 200-280g and Swiss albino mice of both sexes weighing between 20-30g were used for the experiments. The animals were obtained from Animal House Facility of the University of Jos. They were kept under room conditions of temperature and light. The animals were fed on laboratory animal feed.

2.2. Acute toxicity study (LD50)

Determination of LD50 was conducted following the method of Lorke (1983), using the intraperitoneal route. In the initial phase, mice/rats were divided into 3 groups of three and treated with the methanolic extract of the leaf at doses of 10, 100 and 1000 mg/ kg body weight. The animals were then observed for 24 hours for signs of toxicity and death. In the final phase, mice/rats were divided into 4 groups of one mouse each and treated with the extract at the doses of 600, 1000, 1600 and 2900 mg/kg i.p. The final LD50 was calculated as the square root of the product of the lowest lethal dose and the highest non-lethal dose, i.e. the geometric mean of the consecutive doses at which 0% and 100% survival rates were recorded.

Tests for anti-analgesic studies Acetic acid induced writhing in mice The Acetic acid induced writhing test in mice as described by Koster et al. (1959) was employed. Mice were divided into 5 groups of 6 mice each. The first group was given 1 ml/kg of Normal saline (i.p.) and served as control, groups 2, 3 and 4 received 75, 150 and 300 mg extract/kg body weight (i.p.) respectively, group five was treated with piroxicam (10 mg/kg) i.p. Thirty minutes later, mice in all the groups were treated with Acetic acid (0.6%v/v), 1ml per 100g body weight (i.p.). Five minutes after acetic acid injection, mice were placed in individual cages and the number of abdominal contractions was counted for each mouse for a period of 10 minutes.

Formalin induced pain in rats. The rats were divided into five groups each containing 6 rats. Group 1 was administered with normal saline (1ml/kg, i.p.), group 2, 3 and 4 with methanolic leaf extract (75, 150 and 300 mg/kg, i.p) and group five with piroxicam (10 mg/kg, i.p). Thirty minutes after this treatment; 50 µl of a freshly prepared 2.5% solution of formalin was injected subcutaneously under the plantar surface of the left hind paw of each rat. The rats were placed individually in an observation chamber and monitored for one hour. The severity of pain response was recorded for each rat based on the following scale: (0) rat walked or stood firmly on the injected paw; (1) the injected paw was favoured or partially elevated; (2) the injected paw was clearly lifted off the floor; (3) the rat licked, chewed or shook the injected paw. Anti-nociceptive effect was determined in two phases. The early phase (phase 1) was recorded during the first 5minutes, while the late phase (phase 2) was recorded during the last 45 minutes with a 10 minutes lag period in-between both phases. (Dubuisson and Dennis, 1977; TJölsen et al., 1992).

2.3. Statistical analysis

Results were statistically significant when p value is less than 0.05 ($p<0.05$) as described by Duncan et al., (1977). p value was obtained using post hoc test.

3. Results and discussion

The methanolic leaf extract was found to have median lethal dose (LD50) of 1264.91mg/kg in both rats and mice. The LD50 indicated the leaf extract to be slightly toxic to the experimental models. This is supported by Matsumma (1975) who classified chemicals based on their LD50 and suggested LD50 range 500-5000mg/kg to be slightly toxic. Martin (2002) defined toxicity as the degree to which a substance is poisonous and therefore, it was suggested by Ibrahim et al (2011) that plant crude drug evaluation for any possible pharmacological action must involve determination of its possible toxicity and/or safety margin.

(a) Phase 1

Table 1 (a-b)
LD50 Determination of M. senegalensis Methanolic Leaf Extract in mice/rats.

Doses (mg/kg)	Results
10	0/3
100	0/3
1000	1/3

(b) Phase 2

Doses (mg/kg)	Results
600	0/3
1000	0/3
1600	1/3
2900	1/3

LD50= √1000 x 1600 = 1264.91 mg/kg i.p.

The extract at doses of 150 and 300 mg/kg significantly ($P < 0.001$) reduced the number of acetic-acid-induced abdominal constrictions in a dose dependent manner.

The highest inhibition of abdominal constriction ($P < 0.001$) observed at 300 mg/kg was higher than that of Piroxicam ($P < 0.001$), the standard non-steroidal analgesic and anti-inflammatory drug used. Bentley et al (1983) suggested the abdominal constriction to partly involve peritoneal receptors. Therefore, the extract may have interfered with these receptors to bring about analgesia.

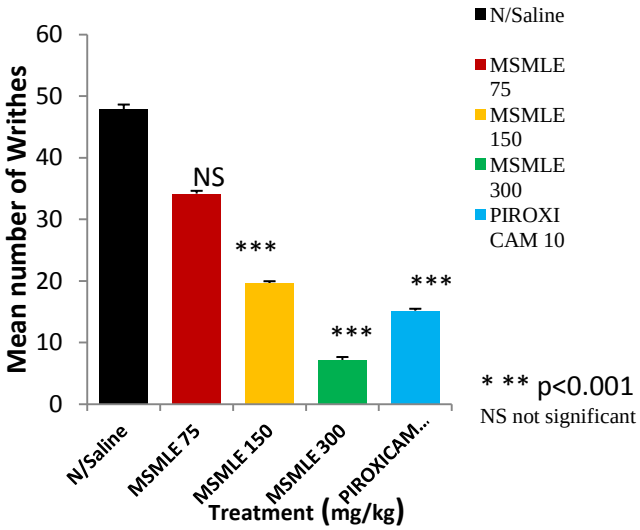

Fig. 1. Effect of leaf methanolic extract on acetic acid induced writhes in mice. Data expressed as meant + SEM for animals/group, significantly different from the control at P<0.001.

In the first phase of formalin induced pain, the extract was able to significantly ($p < 0.01$) reduce pain at all doses (75, 150 and 300mg/kg) administered, although, not in a dose dependent manner. Higher inhibition was

seen at the lowest dose (75mg/kg) administered. In the second phase, the extract at the dose of 300 mg/kg significantly ($p < 0.01$) inhibited formalin induced pain. Inhibition by the extract was higher than that observed with piroxicam (10mg/kg). The drugs that are capable of inhibiting both phases are said to exhibit central activity while those that are capable of inhibiting only the late phase are said to exhibit peripheral activity (Chant et al., 1995). The first phase may be as a result of stimulation of nociceptors in the paw, or stimulation of opioid receptors as suggested by Gaertner et al., (1999). Therefore, the first phase reflects centrally mediated pain. The last phase may be as a result of inflammation with the release of serotonin, histamine, bradykinin, prostaglandins and sensitization of central nociceptive neurons (Tjölsen et al., 1992). The ability of the leaf methanolic extract tested to suppress the two phases in this research suggests the extract to have both central and peripheral activities.

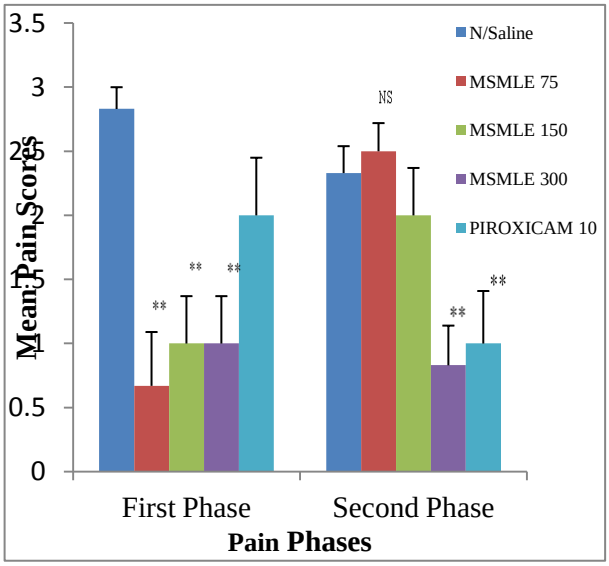

Fig. 2. Effect of leaf methanolic extract on formalin induced pain in rats.
Data expressed as meant + SEM for 6 animals/group, significantly different from the control at P<0.01.

4. Conclusion

By this research, it has been established that the leaf methanolic extracts do possess significant anti-nociceptive effect in laboratory animals at the doses investigated. The results support the traditional use of the plant in the management of pain.

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