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ACUTE TOXICITY AND ANTINOCICEPTIVE ACTIVITY OF CRUDE ETHANOL EXTRACT OF *SECURIDACA LONGEPEDUNCULATA* (FRESEN) ROOT BARK IN ALBINO RATS

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ABSTRACT

The *Securidaca longepedunculata* crude root bark extract was studied in rats to evaluate its acute toxicity and antinociceptive effects. The LD_{50} was determined using Lorke's methods while tail flick, hotplate and formalin test methods were used to evaluate the antinociceptive activities of the extract at doses of 3mg/kg and 6mg/kg intraperitoneally. The result of the LD_{50} value was 14.14mg/kg. The extract exhibited significant antinociceptive effect at 6mg/kg ($P<0.05$) as compared to the control in tail flick test and in both phases of formalin test. It was concluded that the crude ethanol extract of *S. longepedunculata* possessed dose dependent antinociceptive activities but it was a highly toxic extract in rats with intraperitoneal LD_{50} value of 14.14mg/kg.

Keywords: Acute toxicity, Antinociception, Crude ethanol extract, *Securidaca longepedunculata*.

INTRODUCTION

Securidaca longepedunculata (Fres), a member of the family polygalaceae is a highly medicinal herb commonly used in parts of Africa. It is a savannah shrub and is common in various parts of western, northern and eastern Nigeria (Iwu, 1986), and in Malaysia, Guinea, Cuba, and several Asian countries (Anonymous, 1996). It is among the medicinal plants used as a herb throughout the world (Sofowara, 1993). In Nigeria, it is locally called "gazawuro" in Kanuri and "sanya" in Hausa (Akinniyi and Sultanbawa, 1983). In English, it is known as violet tree. The root bark of *S. longepedunculata* contains methyl salicylate, steroids, tannin, and saponin related substances (Jayasekara et al, 2002; Kamba and Hassan, 2010). The root bark has been found to have anticonvulsant property (Odebiyi, 1978), antimicrobial property (Ajali and Chukwurah, 2004; Apak and Olila, 2006), anti-inflammatory activity (Okoli, *et al.,* 2006). In South Africa, the roots are inserted in to the vagina as a means of committing suicide with death occurring within 12hrs (Hagreaves, 1986). This study is therefore aimed at evaluating the acute toxicity and analgesic activities of the ethanol extract of *S. longepedunculata* in rats.

MATERIALS AND METHODS

Plant Collection and Identification

S. longependuculata root bark was obtained in the bush near Ngulde in Askira/Uba Local Government Area of Borno State. It was identified and authenticated by Prof S. S. Sanusi

of the Department of Biological Science, University of Maiduguri and a voucher sample (Vet212A2) was preserved at the Veterinary Pharmacology Laboratory, University of Maiduguri, Nigeria.

Extract Preparation

The root of the plant was collected, cleaned, and the bark removed, crushed and then air-dried at room temperature for one week. It was pulverized using a mortar and pestle and 400 g of the ground herb was soaked overnight in petroleum ether. The residue from defatted samples was extracted in 95% ethanol for 24h. The sample was filtered using Whatman filter paper No. 1 and evaporated to dryness under reduced pressure using a rotary evaporator (R201D PEC Medicals U.S.A.).

Experimental Animals

Seventy two albino rats (91g to 183g) of both sexes bred at the Veterinary Physiology Laboratory, University of Maiduguri, were used for the experiments. They were kept in clean plastic rat cages and were fed with commercial feed and water *ad libitum.*

Acute Toxicity

The method used was that described by Lorke (1983). In the first phase, nine rats (96-130g) were grouped into three with three rats per group. The rats in the groups (1-3) were administered the ethanol extract 10, 100 and 1000 mg/kg body weight i.p respectively and were observed for signs of toxicity and death within 24 hours. In the second phase, three rats (91-176g) were also grouped into three with a rat per group. The rats in the groups (1-3) were administered the ethanol extract 5, 20 and 40 mg/kg body weight i.p respectively and were observed for signs of toxicity and death within 24 hours. The LD50 value was calculated using the formula:

$LD_{50} = \sqrt{a \times b}$. Where a = least dose that killed a rat while b = highest dose that did not kill any rat

Tail Flick Test

Rat cold water tail-flick test described by (Pizziketti *et al.,* 1985) was employed. Rats were divided into four groups (A, B, C and D) of five rats each. Tail flick test was performed 30 minutes after administration of the crude ethanol extract to groups (A and B) at doses of 3 and 6 mg/kg i.p respectively, while group C received morphine 10mg/kg i.p (a known analgesic) and group D received distilled water i.p. Each rat was restrained by holding the nape and 5cm of the caudal end of the tail was dipped in a beaker of cold water (0-1°C). The time in seconds for tail withdrawal from the cold water was taken as the reaction time.

Hot Plate Test

Hot plate proposed by Eddy and Leimbach (1953) was employed. A transparent container was used to view the animal on the heated surface of the plate. Rats divided into four groups of five rats each (A, B, C and D) and received treatments as in the Tail flick method above. After 30 minutes of treatment, each rat was kept on Eddy's hot plate having a constant temperature of 50 ± 1°C and latency to respond to thermal stimulus was taken.

Formalin Test

Method modified by Hunskaar *et al*. (1986) was adopted. Four groups (A, B, C and D) of five rats each were used for the experiment. Group A received the vehicle (distilled water) and served as the control group while Group B and C received the crude ethanol extract at 3mg/kg and 6 mg/kg i.p respectively and group D were injected with piroxicam 10mg/kg i.p (a known analgesic). Thirty (30) minutes later, all the groups (A, B, C and D) were then injected with 0.05 mls of diluted formalin (1% in normal saline) under the skin of the dorsal surface of the right hind paw. Rats were observed immediately after injection of the diluted formalin and the time spent licking the injected paw (licking time) was recorded during the first five minutes in the first instance (early phase) and then during 10 - 20 minutes in the second instance (late phase).

Statistical Analysis

The values obtained were expressed as mean ± standard deviation. Graphpad InStat® (2003) computer statistical software package was used for the analysis of variance and P $\leq$0.05 was considered significant.

RESULTS

The Acute Toxicity Study

The acute toxicity test of the ethanol extract of *Securidaca longepedunculata* root bark in rats using Lorke's method is presented in Table 1. The intraperitoneal median lethal dose (LD_{50}) was calculated to be 14.14 mg/kg.

Effect of *Securidaca longepedunculatar* Root Bark Extract on induced Pain in Albino Rats

In the tail flick test (Table 2), the crude ethanol extract of *S. longepedunculata* at the dose of 3mg/kg recorded no significant antinociceptive effect in albino rats but at the dose of 6mg/kg it had a significant antinociceptive effect (P<0.05) as compared to the control. Latency of response increased significantly (p<0.05) from 25.46 ± 4.52 seconds in the control group to 46.20 ± 22.09 seconds in 6mg/kg treated group. The positive control, morphine 10mg/kg, produced no effects as compared to the negative control.

The crude ethanol extract of *S. longepedunculata* in Table 3 at the doses of 3mg/kg and 6mg/kg had no significant thermal antinociceptive effect on the albino rats. Only the morphine treated (positive control) group produced very significant (p<0.01) activity where latency of response increased from 23.34 ± 3.84 seconds in the control to 40.08 ± 10.07 seconds in the morphine treated group.The effect of ethanol extract of *S. longepedunculata* root bark on formalin induced pain in rats is presented in Table 4. At the dose 3mg/kg in both the first and the second phases, there were no significant differences (p>0.05) as compared to the control group. At the dose of 6mg/kg in the both phases, there were significant antinocicrptive effects (P<0.01) as compared to the control. At this dose the time spent in licking of the formalin injected limb decreased from 65.80 ± 9.01 seconds in the control group to 18.40 ± 22.18 seconds in the treated group (6mg/kg) in the first phase. While in the second phase the decrease was from 143.92 ± 22.78 seconds in the control group to 43.80 ± 52.39 seconds in the treated group (6mg/kg). The morphine treated group showed significant (p<0.05) decrease in the second phase only (from 143.92 ± 22.78 to 69.04 ± 31.11 seconds).

DISCUSSION

Several pain models were employed to evaluate the antinociceptive effect of the crude ethanol extract of *Securidaca longepedunculata* root bark. It is necessary to apply test which differ with respect to stimulus quality, intensity and duration to obtain as complete picture as possible of antinociceptive properties of a substance (Tjolsen *et al.,* 1992). The methods used for investigating antinociception were selected such that both central and peripheral mediated effects were evaluated. The tail flick test and the thermal test elucidated central activity, while the formalin test investigates both peripheral and central activities. In tail flick test, the extract conferred protection from pain better than morphine to the extent of which the time taken for the albino rats to flick their tail from the cold water was longer in the groups treated with the extract 6mg/kg than the group treated with morphine 10mg/kg. The antinociceptive activity recorded was achieved centrally. Pain sensations from cold are transmitted by both cold and pain receptors (Guyton and Hall, 1996) while morphine is known to influence specifically pain receptors and this could be responsible for the non significant effect of morphine in this model. This model is used to measure centrally antinociceptive activities mediated at the spinal levels (Wong *et al.,* 1994). In hot plate method, there was no significant effect on the thermal induced pain when the doses of 3mg/kg and 6mg/kg were administered. Even though the result showed statistically insignificant increase, it may be pharmacologically significant because both tail flick test and thermal test are indicative of central activity and the result obtained in the tail flick test was significant indicating central antinociceptive activity of the extract.

In the formalin test method, there was a distinctive significant protection from pain in both first and second phases at the dose of 6mg/kg of the extract which can be compared with the effect of piroxicam at 10mg/kg. It is an established fact that drugs acting centrally inhibit both phases, while peripherally acting drugs inhibit only the second phase (Tjolsen *et al.,* 1992). The effect of piroxicam (peripheral analgesic) in this study is in agreement with this assertion as it has significant activity only in the second phase while the extract may be acting centrally or peripherally since there were significant antinociceptive activities during both phases. The centrally mediated activity further supports the result from the tail flick test. In all three models carried out, there were dose dependent activities of the extract since the effect produced at the lower dose of 3mg/kg was always lower than the effect produced at the higher dose of 6mg/kg. The antinociceptive activities observed could be as a result of various phytochemicals present in the extract. The root bark of *S. longepedunculata* has been reported to contain flavonoids, steroids, saponins, terpenoids, and methyl salicylate (Jayasekara *et al.,* 2002; Kamba and Hassan, 2010).

However caution should be taken when using this extract especially via parenteral route because the result from the acute toxicity study indicated that the i.p LD_{50} of the crude ethanol extract of *S. longepedunculata* was 14.14mg/kg. Based on the LD_{50} values, toxic substances are classified into 6 classes as extremely toxic, highly toxic, moderately toxic, slightly toxic, practically non-toxic and relatively harmless (Hodge and Sterner, 1949; OECD, 2001; Saganuwan, 2012). Any substance which has LD_{50} of 5-50 mg/kg in rats is considered as highly toxic. Agbaje and Adekoya (2012) reported similar results as the i.p and oral LD_{50} values were 44.67 and 3162 mg/kg respectively. The i.p LD_{50} value of this extract falls between 5-50 mg/kg and thus is considered highly toxic.

CONCLUSION

The crude ethanol extract of *S. longepedunculata* possessed antinociceptive activities but it was a highly toxic extract in rats with i.p LD_{50} value of 14.14mg/kg.

Table 1: The intraperitoneal acute toxicity test of the ethanol extract of *S. longepedunculata* root bark in rats using Lorke's method

Phase	Dosage (mg/kg)	Clinical signs	Mortality
1	10	None	1/3, 24hr
1	100	None	3/3, 24h
1	1000	None	3/3, 24h
2	5	None	0/1
2	20	None	1/1, 24hr
2	40	None	1/1, 24hr

$$LD_{50} = \sqrt{20 \times 10} = \sqrt{200} = 14.14\text{mg/kg}$$

Table 2: Effect of ethanol extract of *Securidaca longepedunculata* root bark on tail flick test in rats

Dose	Mean ± SD (sec)
Control	25.46 ± 4.52
Extract 3mg/kg	15.95 ± 9.20
Extract 6mg/kg	46.26 ± 22.09*
Morphine 10mg/kg	8.75 ± 4.94

*=significant (P<0.05) increase as compared to control
N=5

Table 3: Effect of ethanol extract of *Securidaca longepedunculata* root bark on thermal nociception test in rats

Dose	Mean ± SD (sec)
Control	23.34 ± 3.84
Extract 3mg/kg	26.34 ± 8.76
Extract 6mg/kg	32.56 ± 3.26
Morphine 10mg/kg	40.08 ± 10.07*

*=significant (P<0.01) increase as compared to control
N=5

Table 4: Effect of ethanol extract of *Securidaca longepedunculata* root bark on formalin test in rats

	Reaction time (sec) Mean±SD	
Dose	First Phase	Second Phase
Control	65.80 ± 9.01	143.92 ± 22.78
Extract 3mg/kg	58.04 ± 15.25	117.78 ± 19.38
Extract 6mg/kg	18.40 ± 22.18**	43.80 ± 52.39**
Piroxicam 10mg/kg	47.54 ± 21.73	69.04 ± 31.11*

**=significant (P<0.01) increase as compared to control
*=significant (P<0.05) increase as compared to control
N=5

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