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ANALGESIC AND ANTI-INFLAMMATORY ACTIVITIES OF THE METHANOLIC LEAVES EXTRACT OF *SECURINEGA VIROSA* (EUPHORBIACEAE)

¹*M. Yerima, ¹M.G.Magaji, ²A.H.Yaro, ³Y.Tanko and ¹M.M. Mohammed

¹Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria

²Department of Pharmacology, Faculty of Medicine, Bayero University, Kano, Nigeria

³Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria, Nigeria

*Author for Correspondence: pharmyerima@yahoo.com, 234-8035960302

ABSTRACT

The methanolic leaf extract of *Securinega virosa* was evaluated for possible analgesic and anti-inflammatory activities in rodents. Acetic acid induced writhing test in mice and formalin-induced pain in rats were used to study the analgesic effect, while the effect of the extract on acute inflammation was investigated on carrageenan-induced paw oedema in rats. The extract significantly ($P < 0.01$) inhibited acetic acid induced writhing in mice and significantly ($P < 0.05$) attenuated the neurogenic phase of formalin-induced pain in rats at the highest dose tested. The extract also produced a moderate anti-inflammatory activity which was found to be significant ($P < 0.05$) at all the doses tested. Preliminary phytochemical screening of the extract revealed the presence of alkaloids, tannins, saponins, cardiac glycosides, flavonoids and resins. The intraperitoneal median lethal dose (LD₅₀) of the extract in mice was found to be 1,265 mg/kg suggesting that the extract may be relatively safe at the analgesic doses. The results obtained in this study lend credence to the ethnomedical use of the plant in the management of pain and inflammatory conditions. Thus, supporting the development of the biologically active substances as analgesics and anti-inflammatory agents.

Key words: Analgesic, Anti-inflammatory, *Securinega virosa*, Carrageenan, Formalin

INTRODUCTION

The long historical use of medicinal plants in many traditional medical practices, including experience passed from generation to generation has demonstrated the safety and efficacy of traditional medicine (Rukangira, 2001). However, scientific evaluation is needed to provide evidences of their safety and efficacy (WHO, 2000). *Securinega virosa* is a low branching, dioecious shrub, or a small tree, distributed throughout Tropical Africa (Dalziel, 1936). The plant has enjoyed wide patronage among the people of Tropical Africa and its efficacy is widely acclaimed

(Neuwinger, 1996). The decoction of the leaves and roots is used for abdominal pain in Tanzania while the leave decoction is drunk for fever by the Yorubas of South western Nigeria. The decoction of the leaves with other herbs is used in Northern Nigeria for treatment of painful swellings. (Neuwinger, 1996).

Narcotic analgesics are associated with addictive properties and numerous side effects including respiratory depression, drowsiness, decreased gastrointestinal motility, nausea and alterations of endocrine and autonomic nervous system activities (Almeida *et al.*, 2001). The search for pharmacological agents

to overcome these shortcomings has become a major goal in pain research. In view of this and on account of the ethnomedical claim of the usefulness of the plant in the management of pain and inflammatory conditions, which to our knowledge has not been scientifically

investigated, this present study was aimed at investigating the analgesic and anti-inflammatory activities of the methanolic leaf extract of *Securinega virosa* in laboratory animals.

MATERIALS AND METHODS

Collection Material

Fresh leaves of *Securinega virosa* were collected from Basawa town, Sabon-Gari Local Government Area of Kaduna State, Nigeria in July, 2006. The plant was authenticated at the Herbarium Section in the Department of Biological Sciences, Ahmadu Bello University (ABU), Zaria, Kaduna state, Nigeria. A voucher specimen (No 918) was deposited at the herbarium for future reference.

Preparation of Extract

The leaves were air dried under shade for twenty one days and then size-reduced into powder with a pestle and mortar. About 100g of the powdered leaves was macerated with 500ml methanol for 72hour with occasional shaking. The extract was concentrated *in vacuo* affording a yield of 13.2 %w/w and subsequently referred to as methanolic leaves extract of *Securinega virosa* (SVMLE). Solutions of the extract were prepared freshly for each study.

Phytochemical Screening

The screening was carried out in accordance with the standard protocol as described by Trease and Evans (1983).

Experimental animals

Swiss albino mice of either sex (weighing 20-30g) were obtained from the animal house facilities of the Department of Pharmacology and clinical Pharmacy, Ahmadu Bello

University, Zaria. The mice were maintained on standard laboratory animal feed and water *ad libitum*, and housed in polypropylene cages at room temperature throughout the study. These studies were carried out in Ahmadu Bello University in accordance with the rules governing the use of laboratory animals as accepted internationally.

Drugs

The following chemicals and drugs were used: carrageenan (Sigma-Aldrich), Acetic acid (Ranbaxy Laboratories Ltd., Punjab), Ketoprofen (Lek, Slovenia), Morphine (Martinadale, Essex) and Piroxicam (Pfizer laboratories, Pakistan). The methanolic leaves extract of *Securinega virosa* (SVMLE) (25, 50, 100mg/kg).

Acute Toxicity study

The intraperitoneal LD₅₀ of the extract in mice was conducted according to the method of Lorke (1983). Briefly; the method was divided into two phases. In the initial phase, 3 groups of three mice each were treated with the methanolic leaves extract of the plant at doses of 10,100 and 1000mg/kg body weight *i.p.* and observed for signs of toxicity and death for 24 hours. In the second phase, 4 groups each containing one mouse was injected with four more specific doses of the extract based on the result of the first phase. The LD₅₀ value was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1 and 1/1).

Phytochemical screening of M. angolensis

The phytochemical screening of *M. angolensis* was carried out on the methanolic root bark extract using standard protocol (Trease and Evans, 1983).

Acetic acid induced writhing test in mice

Mice were divided into five groups each containing 6 mice. The control group received Normal saline (10ml/kg, *i.p.*). The test groups were treated with 25, 50, 100 mg/kg/*i.p.* of SVMLE while the fifth group received piroxicam at the dose of 10mg/ kg, *i.p.* After 30 minutes of drug administration, the mice were treated with 0.6% acetic acid at 10ml/kg body weight, *i.p.* (Koster *et al.*, 1959). Five minutes after acetic acid injection, mice were placed in individual cage and the number of abdominal contractions was counted for each mouse for a period of 10 minutes after 5 minutes latency, and the percentage inhibition of writhing was calculated.

Formalin test in rats

The rats were divided into five groups each containing 5 rats and were administered with either normal saline (1ml/kg, *i.p.*), methanolic leaves extract (25, 50 and 100 mg/kg, *i.p.*) or Morphine (4 mg/kg, *s.c.*). Thirty minutes after this treatment; 50 μ l 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

RESULTS

The preliminary phytochemical screening of SVMLE revealed the presence of flavonoids, saponins, tannins, glycosides, alkaloids and steroids (Table 1). The intraperitoneal median lethal dose value of the extract in mice was found to be 1,265mg/kg. The extract significantly attenuated acetic acid induced writhing in mice ($P < 0.001$). The activity was greater than that of piroxicam, the standard

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; Tjølsen *et al.*, 1992).

Carrageenan-induced paw oedema

The rats were divided into five groups each containing 5 rats. Acute inflammation was induced by injecting 0.1 ml of (1%) carrageenan into plantar surface of rat hind paw (Winter *et al.*, 1962). The methanolic leaf extract (25, 50 and 100 mg/kg), normal saline (1ml/kg) and ketoprofen (10 mg/kg) as reference agent were administered 30 min before carrageenan injection. The paw volume was measured at 0, 1, 2, 3, 4 and 5h using a vernier caliper to determine the diameter of oedema. The difference between the readings at time 0 h and different time interval was taken as the thickness of oedema.

Statistical analysis

The results were expressed as Mean \pm SEM and analysed using Student's t-test. A P value < 0.05 was considered significant.

agent, at the higher doses (Table 2). The highest dose of the extract (100mg/kg) significantly ($P < 0.05$) inhibited the first phase of formalin-induced pain. Morphine, the standard agent inhibited both phases (Table 3). The extract significantly ($P < 0.05$) inhibited carrageenan-induced paw oedema at the third hour. However, this was considerably lower than that of ketoprofen which afforded 91.3% protection (Table 4).

Table 1: Phytochemical screening of methanolic leaf extract of *Securinaga virosa*

Constituents	Remark
Tannins	+
Saponins	+
Flavonoids	+
Alkaloids	+
Cardiac glycosides	+
Cyanogenic glycosides	+
Resins	+
Steroid/Terpenoids	+
Carbohydrates	+
Anthraquinone	-

Note: + Present, - Absent

Table 2: Effect of methanolic leaf extract of *Securinaga virosa* on acetic acid induced writhing in mice

Treatment(mg/kg)	Mean no.of writhes	% inhibition
Normal saline	16.5 ± 4.0	
SVMLE 25mg/kg	5.3 ± 2.2*	67.9
SVMLE 50mg/kg	2.8 ± 0.9*	83.0
SVMLE 100mg/kg	3.0 ± 2.1*	81.8
Piroxicam (10mg/kg)	4.8 ± 1.7*	70.9

Each value represents mean ± SEM. **P* < 0.001 compared with control (Student's t-test). SVMLE= Methanolic leaf extract of *S. virosa*; n=6

Table 3: Effect of Methanolic leaf extract of *Securinega virosa* on Formalin-induced pain in rats

Treatment(mg/kg)	Mean Pain Scores	
	First Phase	Second Phase
Normal saline	3.0 ± 0.0	3.0 ± 0.0
SVMLE 25mg/kg	2.4 ± 0.2*	3.0 ± 0.0
SVMLE 50mg/kg	2.4 ± 0.4	2.0 ± 0.6
SVMLE 100mg/kg	1.4 ± 0.7*	2.0 ± 0.6
Morphine(5mg/kg)	1.2 ± 0.4**	1.4 ± 0.2**

Each value represents mean ± SEM. **P* < 0.05 ***P* < 0.001 compared with control (Student's t-test) SVMLE= Methanolic leaf extract of *S. virosa*. n=5

Table 4: Effect of Methanolic leaf extract of *Securinaga virosa* on Carrageenan-induced paw oedema in rats

Treatment Groups	Mean Paw Diameter (cm)				
	Time (h)				
	1	2	3	4	5
N/Saline 1ml/kg	0.19 ± 0.01	0.25 ± 0.03	0.23 ± 0.02	0.20 ± 0.03	0.20 ± 0.02
SVMLE 25mg/kg	0.16 ± 0.02 (15.8)	0.19 ± 0.02 (24.0)	0.16 ± 0.02* (30.4)	0.13 ± 0.02* (35.0)	0.12 ± 0.02* (40.0)
SVMLE 50mg/kg	0.11 ± 0.01** (42.1)	0.16 ± 0.03* (36.0)	0.14 ± 0.02* (39.1)	0.12 ± 0.02** (40.0)	0.10 ± 0.02* (50.0)
SVMLE 100mg/kg	0.10 ± 0.01** (47.4)	0.15 ± 0.01* (40.0)	0.14 ± 0.02* (39.1)	0.09 ± 0.02** (55.0)	0.08 ± 0.01** (60.0)
Ketoprofen 10mg/kg	0.08 ± 0.02*** (57.9)	0.07 ± 0.03*** (72.0)	0.09 ± 0.03*** (91.3)	0.10 ± 0.02** (50.0)	0.09 ± 0.03** (55.0)

Each value represents mean ± SEM. * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$, compared with control values for corresponding hours. Figures in parentheses are percentage inhibition of inflammation.

SVMLE= Methanolic leaf extract of *S. virosa*. n= 5

DISCUSSION

The acetic acid –induced writhing test is very sensitive and able to detect anti-nociceptive effects of compounds at dose levels that may appear inactive in other methods like tail flick test (Collier *et al.*, 1968; Bentley *et al.*, 1981). Increased level of prostanoids, particularly PGE₂ and PGF_{2a} (Derardt *et al.*,1980) as well as lipoxygenase products (Levini *et al.*,1984; Dhara *et al.*,2000) have been found in the peritoneal fluid after intraperitoneal injection of acetic acid. The analgesic effect of the

extract may therefore be due either to its action on visceral receptors sensitive to acetic acid, to the inhibition of the production of algogenic substances or the inhibition at the central level of the transmission of painful messages. However, this model may not be able to indicate the mechanism of analgesic effect of the extract because other agents such as antihistamines (Naik *et al.*, 2000) and myorelaxant (Koyama *et al.*, 1997) are able to reduce the pain induced by acetic acid. Formalin test is a well established valid model for the study of central sensitization events at

the spinal level after peripheral inflammatory state (Diaz and Dickeson, 1997). The two distinct phases in formalin test are due to direct effect of formalin on nociception and due to inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degree, the sensitization of central nociceptive neurons (Dubuisson and Dennis, 1977; Huskaar and Hole, 1987; Tjøsen *et al.*, 1992). Stimulation of opioid receptors has also been suggested as a possible mechanism of action against neurogenic pain (Gaertner *et al.*, 1999). The ability of the extract to inhibit the second phase of formalin-induced pain suggests that it may possess central analgesic activity. The probable mechanism of action of carrageenan-induced inflammation is bi-phasic, the first phase is attributed to the release of histamine, serotonin and kinins in the first hour; while the second phase is attributed to the release of prostaglandins and lysosome enzymes in 2 to

CONCLUSION

These findings suggest that the methanolic leaf extract of *Securinega virosa* may contain bioactive constituents with analgesic and anti-

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- 3 hours (Brooks and Day, 1991). The extract moderately inhibited the carrageenan-induced inflammation in the 3rd hour. Analgesic and anti-inflammatory effects have been observed in flavonoids as well as tannins (Ahmadiani *et al.*, 2000). Flavonoids such as quercetin are known to be effective in acute inflammation (Rajnarayana *et al.*, 2001). There are also reports on the analgesics effects of alkaloids, essential oils and saponins (Choi *et al.*, 2005; de Araujo *et al.*, 2005; Reanmongkol *et al.*, 2005).
- The analgesic and anti-inflammatory effect of the extract may therefore, be due to the presence of flavonoids, tannins, alkaloid or saponins. However, further studies are in progress in our laboratory to isolate the active constituents responsible for the observed effect, and to elucidate the possible mechanisms of action responsible for the analgesic and anti-inflammatory activities of the methanolic leaf extract.
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