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Full Length Research Paper

Preliminary studies on anti-inflammatory and analgesic activities of *Securinega virosa* (Euphorbiaceae) in experimental animal models

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Securinega virosa **is a commonly used medicinal plant in West Africa sub-region for the management of painful and inflammatory conditions. In the present study, the analgesic and anti-inflammatory activities of methanol root bark extract of *S. virosa* (SV) were investigated. The anti-inflammatory activity was evaluated using carrageenan-induced rat paw oedema while analgesic activity was tested by acetic acid-induced writhing response and hot plate tests in Swiss albino mice, and formalin-induced pain in Wistar rats. The methanolic root bark extract of SV (6.25 - 25 mg·kg^{-1} body weight, *i.p.*) significantly ($P < 0.05$) inhibited acetic acid-induced abdominal constrictions and attenuated the neurogenic pain (phase 2) induced by formalin. The extract also significantly ($P < 0.01$) prolonged the reaction latency to pain thermally-induced in mice by the hot plate. The extract at the doses (6.25, 12.5 and 25 mg·kg^{-1}) tested afforded 12, 52 and 52% inhibition of paw oedema respectively at the end of third hour. The intraperitoneal and oral LD_{50} values in mice were found to be 774.6 and greater than 5000 mg·kg^{-1} respectively, while the preliminary phytochemical screening reveals the presence of alkaloid, tannins, saponins and flavonoids. The relatively high oral median lethal dose (> 5000 mg·kg^{-1}) suggests that the extract is relatively non toxic when taken orally. The present study indicates that SV has significant anti-inflammatory and analgesic properties and lend pharmacological support to suggested folkloric uses of the plant in the management of painful and inflammatory conditions.**

Key words: *Securinega virosa;* Analgesia; writhing; Anti-inflammation; Carrageenan.

INTRODUCTION

WHO estimated that 80% of the people of the world living in developing countries rely on medicinal plants for primary health care needs (Farnsworth, 1998). The high cost of acquiring synthetic drugs, their inadequate supplies, the side effects associated with their uses, and the belief that plants hold cure to many disease conditions (including painful and inflammatory conditions) have led to a reawakening of interest in the utilization of plants and plant products in recent years. There is a need to intensify research into medicinal flora especially those claimed to have beneficial effects in serious disorders.

Securinega virosa is one of the great African medicinal plants described as a true “cure all”, of which all parts are used as remedies, particularly the root. It is a dense, low branching, many branched shrub, sometimes a small spreading tree up to about 6 m high, although, more commonly 2 to 3 m, evergreen or deciduous (Neuwinger, 1996). It is widely distributed throughout tropical Africa, also in India, Malaya, China and Australia (Dalziel, 1936). The root is used in many parts of Africa in the treatment of fever, body pain, stomach ache rheumatism, diar-

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diarrhoea,pneumonia and epilepsy (Neuwinger, 1996). The alcoholic extract of *S. virosa* root exhibited antibacterial and antifungal activities (Khan et al., 1980; Sawhney, 1978). The cytotoxic properties of the alcoholic leaf extract in tumor cells has been reported (Tatematsu, 1991).

The plant exhibited significant antimalarial activity against *Plasmodium falciparum*, *in vitro*, comparable to quinine used as standard drug (Gbeassor et al., 1989). The aqueous extract of the roots exhibited hypoglycemic effect (Moshi et al., 2000).

However, to the best of our knowledge, there are no scientific data available to validate the folkloric analgesic and anti-inflammatory claims of the plant. The aim of this study was to investigate the analgesic and the anti-inflammatory effects of the methanol root bark extract of the plant on animal models.

MATERIAL AND METHODS

Preparation of the extract

The whole plant, SV was collected from Samaru town, in Sabongari Local Government Area of Kaduna State, Nigeria, in May 2006. The plant was identified and authenticated at the Herbarium Section in the Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria. A voucher specimen (NO 918) was deposited for future reference. The root was cleaned and the bark removed. The root bark was air dried for 14 days and then crushed into coarse powder with a pestle and mortar. About 100 g of the powered root bark was extracted with 500 ml methanol for 72 h using soxhlet extraction apparatus. The solvent was evaporated on a water bath to give an average yield of 12.63% w/w. The extract was then stored in a desiccator. Solutions of the extract were prepared freshly for each study.

Phytochemical studies

The Methanol root bark extract of SV was screened for the presence of alkaloids, glycosides, tannins, saponins and flavonoids according to standard protocol (Trease and Evans, 1983).

Animals

Albino rats of Wister strain (150 - 200 g) and Swiss albino mice (18 - 30 g) of either sex were procured from the Animal house facility of the Department of Pharmacology and Clinical Pharmacy, Ahmadu Bello University, Zaria, Nigeria. They were housed in standard polypropylene cages and kept under controlled room temperature (25 ± 2^0C) in a 12 h light-dark cycle. The animals were fed on standard laboratory diet (Feed Master, Ilorin, Nigeria) and water *ad libitum*. Food and water were withdrawn during the experimental hours. All experimental protocols were approved by the University animal ethics committee.

Drugs

The following chemicals and drugs were used: carrageenan (Sigma-Aldrich), Acetic acid (Searle Essex, England), Ketoprofen (Lek, Slovenia), Morphine (Martinadale, Essex) and Piroxicam (Pfizer laboratories).

Acute toxicity study

LD_{50} determination was conducted using the method of (Lorke, 1983) for intraperitoneal (*i.p*) and oral routes in mice. The median lethal dose was determined by calculating the geometric mean of the lowest dose that caused death and the highest dose for which the animal survived (0/1and 1/1).

Acetic acid-induced writhing test in mice

This test was conducted employing the method described by Koster et al. (1957). Swiss albino mice were divided into 5 groups each containing 6 mice. The mice were treated with normal saline, the extract of SV (6.25, 12.5 and 25 $mg \cdot kg^{-1}$) or piroxicam (10 $mg \cdot kg^{-1}$), intraperitoneally. 30 min later, mice in all the groups were treated (0.6% 1ml acetic acid of 10 ml per kg body weight, *i.p.*). 5 min 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 min after 5 min latency. Percentage inhibition of writhing was calculated using the formula:

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

Formalin test in rats

The method used for this test was similar to that described by Dubuisson and Dennis (1977) and modified by Tjølsen et al. (1992). Adult Wista rats were divided into five groups each containing five rats. The rats were administered with normal saline (1 $ml \cdot kg^{-1}$, *i.p*), SV extract (6.25, 12.5 and 25 $mg \cdot kg^{-1}$, *i.p*) or morphine (4 $mg \cdot kg^{-1}$, *s.c*). 30 min 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. They were placed 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 was favored 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 5 min while the late phase (phase 2) was recorded during the last 45 min with a 10 min lag period in between both phases.

Hot plate test method

The hot-plate test employed in this study was as previously described by Lanhers et al. (1992) and modified by Mahomed and Ojewole (2004). A 600 ml test beaker was placed on thermostat hot plate (Gallenkamp thermostat Cat No: HL054). The temperature was regulated to 50° ± 1°C. Each mouse was placed in the beaker (on the hot plate) in order to obtain its response to electrical heat-induced nociceptive pain stimulus. Licking of the paws or jumping out of the beaker was taken as an indicator of the animal's response to heat-induced nociceptive pain stimulus. The time for each mouse to lick its paws or jump out of the beaker was taken (reaction time). Each mouse serves as its own control. Before treatment, its reaction time was taken thrice at 1 h interval. The

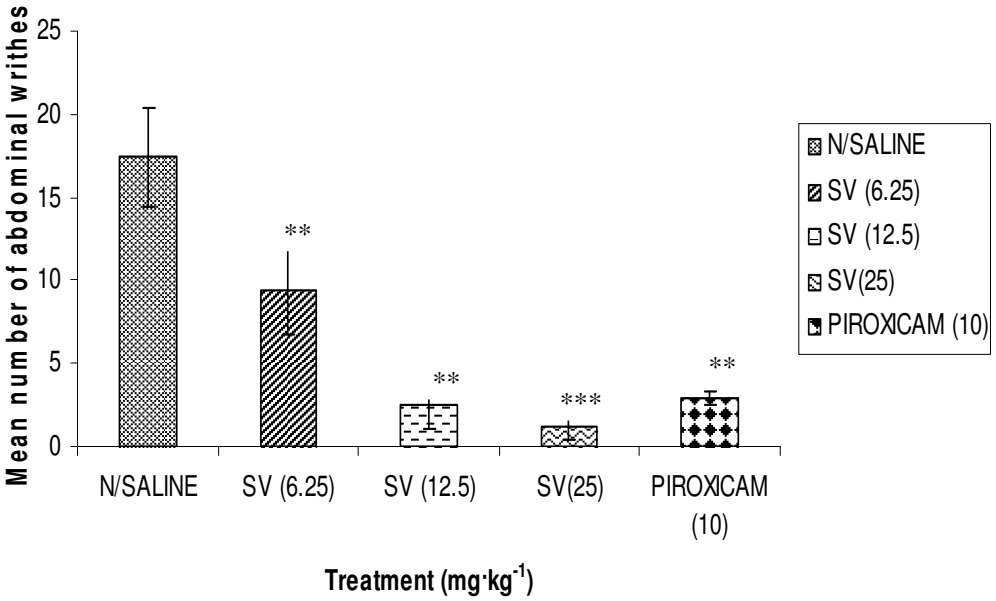

Figure 1. Effect of methanol root bark extract of *S. virosa* on acetic acid induced writhing in mice. *P < 0.05; ** P < 0.01; *** P < 0.001 (Dunnets t-test for multiple comparison); n = 5; SV = *S. virosa*.

The mean of these three determinations constituted initial reaction time before treatment of the mouse. The mean reaction time for the groups was pooled to obtain the final control mean reaction time (Tb). Each of the test mice was thereafter treated with either normal saline, methanol root bark extract of *S.virosa* (6.25 – 25 mg·kg^{-1}) *i.p*, or morphine sulphate (5 mg·kg^{-1}) subcutaneously. 30 min after treatment, the reaction time of each mouse was again evaluated but only once on this occasion. This was pooled for the mice in each treatment group and the final test mean value (Ta) for each treatment group was calculated. This final test mean (Ta) value represented the after treatment reaction time (Ta) and was subsequently used to determine the percentage thermal pain stimulus or protection by applying the formula;

% protection against thermal stimulus =

$$\frac{Test\,mean(Ta) - Control\,mean(Tb)}{Control\,mean(Tb)} \times 100$$

Carrageenan-induced paw oedema

The test was conducted according to the method described by Winter et al. (1962). Wistar rats were divided into five groups each containing five rats. The first group received normal saline [(10 ml·kg^{-1}), *i.p.*]. Groups two, three and four were given 6.25, 12.5 and 25.0 mg extract per kg body weight *i.p.* respectively, while the fifth group received 10 mg ketoprofen per kg body weight *i.p.* 30 min later, 0.1 ml of freshly prepared carrageenan suspension (1% w/v in 0.9% normal saline) was injected into the sub plantar region of the left hind paw of each rat. The paw diameter was measured with the aid of a vernier caliper at 0, 1, 2, 3, 4, 5 h, after the injection of carrageenan. 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 analyzed for statistical significance using one-way ANOVA followed by Dunnett t-test. A P value < 0.05 was considered significant.

RESULTS

The intraperitoneal and oral median lethal dose values of the extract in mice were found to be 774.6 mg·kg^{-1} and greater than 5000 mg·kg^{-1}, respectively.

The methanol root bark extract of SV significantly (P < 0.05) attenuated the acetic acid-induced abdominal writhes in mice dose dependently. The highest inhibition of abdominal constriction (P < 0.01) observed at 25 mg·kg^{-1} was greater than that of piroxicam (P < 0.01), the standard non-steroidal analgesic and anti-inflammatory drug used (Figure 1).

The methanol root bark extract of SV significantly (P < 0.05) inhibited the first phase (neurogenic pain) of the formalin induced pain in rats. However, the inhibition was not dose-dependent. The highest inhibition was obtained at 12.5 mg·kg^{-1}.The extract did not significantly protect the animals against the inflammatory pain (second phase) at the lower doses (6.25 and 12.5 mg·kg^{-1}). At the highest dose tested (25 mg·kg^{-1}); the extract significantly (P < 0.05) inhibited the inflammatory pain. Morphine, the standard drug significantly protected the animals in both phases of the model (Figure 2).

The extract significantly and dose-dependently protected the mice against thermally induced pain stimulus in mice. The reaction time at the dose of 12.5 mg·kg^{-1} (14.2 ± 1.61) was found to be twice that of the untreated group (7.1 ± 0.82). Morphine sulphate, the standard drug afforded more than 200% protection against thermally induced

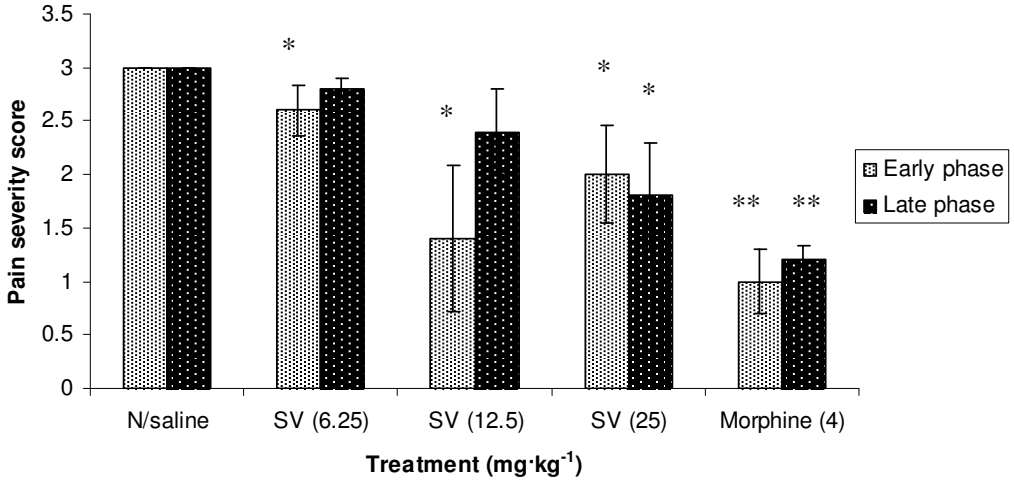

Figure 2. Effect of methanol root bark extract of *S. virosa* on formalin-induced pain in rats; *P < 0.05; ** P < 0.01; *** P < 0.001 (Dunnets t-test for multiple comparison); n = 5; SV = *S. virosa*.

Table 1. The effect of the methanolic root bark extract of *S. virosa* on thermally induced pain stimulus in mice.

Groups	Dose (route of Administation)	Mean reaction time(sec)	Protection (%)
Control Group A (Untreated)	-	7.20 ± 1.21	-
Control Group B (Normal Saline)	10ml·kg^{-1} (*i.p*)	7.10 ± 0.82	-1.39
SV	6.25mg·kg^{-1} (*i.p*)	14.11 ±1.66*	95.97
SV	12.5 mg·kg^{-1} (*i.p*)	14.20± 1.61*	97.22
SV	25 mg·kg^{-1} (*i.p*)	17.60 ± 1.08***	144.44
Morphine sulphate	5 mg·kg^{-1} (*s.c*)	25.60 ± 2.20***	255.56

Each value is mean ±SEM of 5 mice; *P < 0.05, **P < 0.01, ***P < 0.001 statistically significant compared with the treated control (normal saline).

pain stimulus in mice (Table 1).

In the normal saline treated animals, sub plantar injection of 1% carrageenan suspension produced a local oedema reaching its maximum at 3 h. The methanol root bark extract of SV significantly inhibited the paw oedema, in a dose dependent manner. The anti-inflammatory effect of the extract (25 mg·kg^{-1}) at the end of the third hour was 52% compared to 60% for ketoprofen (10 mg·kg^{-1}), the standard anti-inflammatory agent (Table 2).

The preliminary phytochemical screening of the methanol root bark extract of SV revealed the presence of flavonoids, saponins, tannins, glycosides, alkaloids and steroids.

DISCUSSION

The relatively high oral median lethal dose (LD_{50}) in mice suggests that the extract is relatively non toxic when taken orally (Lorke, 1983). 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 (Bentley et al., 1981). However, the test is not specific as it does not indicate whether the activity was central and/or peripheral (Chan et al., 1995). The intraperitoneal injection of acetic acid produces an abdominal writhing response due to sensitization chemo-sensitive nociceptors by prostaglandins (Sutharson et al., 2007).

Increased level of prostanoids, particularly PGE_2 and $PGF_{2\alpha}$ (Derardt et al., 1980) as well as lipoxygenase products (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. The formalin test has a distinctive biphasic nociceptive

Table 2. Effect of methanol root bark extract of SV on carrageenan-induced paw oedema in rats.

Groups (n = 5)	Dose ($mg \cdot kg^{-1}$)	Oedema diameter(cm)				
		1h	2h	3h	4h	5h
Normal Saline	$10ml \cdot kg^{-1}$	0.15± 0.01	0.23± 0.02	0.25± 0.03	0.19± 0.02	0.17± 0.02
SV	6.25	0.15± 0.01 ^{NS} (0)	0.22± 0.01 ^{NS} (4.35)	0.22± 0.02 ^{NS} (12.00)	0.14±0.01^{NS} (26.30)	0.11±0.01* (35.29)
SV	12.5	0.14± 0.02 ^{NS} (6.67)	0.13±0.020** (43.46)	0.12± 0.03^{NS} (52.00)	0.09±0.01** (52.63)	0.07±0.01** (58.82)
SV	25	0.09±0.010** (40.00)	0.10±0.01*** (56.52)	0.12±0.02** (52.00)	0.05±0.02** (73.68)	0.05±0.01*** (70.59)
KTP	10	0.06±0.02*** (60.00)	0.06± 0.02*** (73.91)	0.10±0.01** (60.00)	0.06±0.01*** (68.42)	0.03±0.01*** (82.35)

Each value is mean ±SEM of 5 rats; Figures in parentheses represent percentage anti-inflammatory activity; $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ compared to control NS: statistically not significant; KTP: Ketoprofen.

responses termed early and late phases. Drugs that act primarily on the CNS inhibit both phases equally while peripherally acting drugs inhibit the late phase (Chan et al., 1995). The first phase is probably a direct result of stimulation of nociceptors in the paw. Stimulation of opioid receptors has also been suggested as a possible mechanism of action against neurogenic pain (Gaertner et al., 1999). This phase, therefore reflects centrally mediated pain while the late phase is due inflammation with the release of serotonin, histamine, bradykinin and prostaglandins and at least to some degree, the sensitization of central nociceptive neurons (Tjølsen et al., 1992). The suppression of both phases as observed in the highest dose tested (25 $mg \cdot kg^{-1}$) in this study suggests the presence of both central and peripheral effects. However, the extract has greater inhibitory effect on the neurogenic pain.

Tail flick and hot plate tests are the most common tests of nociception that are based on a phasic stimulus of high intensity (Mandegary et al., 2004). Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception (Parkhouse and Pleuvry, 1979). The ability of the extract to prolong the reaction latency to pain thermally-induced in mice by the hot plate further suggests central analgesic activity.

Carrageenan-induced hind paw oedema is the standard experimental model for acute inflammation. Carrageenan is the phlogistic agent of choice for testing anti-inflammatory drugs as it is not known to be antigenic and is devoid of apparent systemic effect (Chakraborty et al., 2006). Moreover, the model exhibits a high degree of reproducibility (Winter et al., 1962). The probable mechanism of action of carrageenan-induced inflammation is biphasic, 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 the second to third hour (Brooks and Day, 1991). The coexistence of analgesic and anti-inflammatory activities is well defined for various non-steroidal anti-inflammatory drugs (NSAIDs), particularly salicylates and their congeners (Famaey, 1983). The principal therapeutic effects of NSAIDs derive from their ability to inhibit prostaglandin G/H synthase (cyclooxygenase or COX) which convert arachidonic acid to the unstable intermediates PGG_2 and PGH_2 and leads to the production of thromboxane A_2 and a variety of prostaglandins (Burke et al., 2006). Prostaglandins are also known to cause pain (Roberts and Morrow, 2001) and NSAIDs are particularly effective when inflammation has caused sensitization of pain receptors to normally painless mechanical or chemical stimuli (Burke et al., 2006). It is of interest therefore, that the extract behaved similar to the NSAIDs in this study and thus correlates well with the ethnomedical use of the plant in painful and inflammatory conditions. The ability of the extract to inhibit carrageenan induced paw oedema suggests it possesses a significant effect against acute inflammation. 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). Certain flavonoids possess potent inhibitory activity against a wide array of enzymes such as protein kinase C, protein tyrosine kinases, phospholipase A_2, phosphodiesterases and others (Middleton, 1998) There are also reports on the analgesic effects of alkaloids, essential oils and saponins (Reanmongkol et al., 2005; de Araujo et al., 2005; Choi et al., 2005). The analgesic and anti-inflammatory effect of the ex-tract may be due to the presence of flavonoids, tannins, alkaloid and saponins, either singly or in combination. However, further studies are needed to isolate the active constituents responsible for the observed effect and to reveal the possible mechanisms of action responsible for the analgesic and anti-inflammatory acti-

vities of SV.

Conclusion

These findings suggest that the methanol root bark extract of *S. virosa* (SV) contain bioactive constituents with analgesic and anti-inflammatory activities, and further support the ethnomedical claim of the use of the plant in the management of painful and inflammatory conditions.

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