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Research article

Analgesic and Anti-Inflammatory Activities of an Ethanol Extract of *Smilax krausiana* Leaf in Mice

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ABSTRACT: The effect of ethanolic extract of *Smilax krausiana* (Smilaceae) leaf was investigated for its analgesic and anti-inflammatory activities in mice. The extract significantly ($p<0.01$-0.001) inhibited in a dose – dependent fashion algesia (nociception) induced by acetic acid, formalin and hot plate. It also reduced in dose-related manner inflammation induced by fresh egg albumin, carrageenin and capsaicin. These inhibitions were statistically significant ($p<0.01 – 0.001$). Though the mechanism of action of the extract is not fully elucidated, it may in part involve suppression of capillary permeability through neurogenic and non-neurogenic pathways as well as its narcotic potential. The extract's inhibition of all the models investigated in a non-specific manner supports the folkloric use of the plant.

Key Word: *Smilax krausiana,* ethanol, leaf, analgesic, anti-inflammatory, mice.

INTRODUCTION

Smilax krausiana (Smilaceae) is a native weed of South Africa. It is an evergreen shrub or semi-shrub with climbing branches and stapler tendrils (Inyang, 2000; Inyang 2003). The leaf has reputable use in the treatment of infertility especially in human and veterinary medicine. It is also used in the treatment of joint and stomach pains. It equally reliefs insect stings (Okokon, 2005 personal communication). Nwafor *et al.,* (2006), investigated the acute toxicity potential of the leaf extract in rats, however, no anti-inflammatory or analgesic effect of the leaf has been reported earlier in literature hence the present study was undertaken to evaluate the analgesic and anti-inflammatory activities of the leaf extract in mice.

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MATERIALS AND METHODS

Preparation of extract

The plant material (leaf) was collected from Uruan, in Uruan Local Government Area of Akwa Ibom State, Nigeria in February, 2006. The plant was identified and authenticated by Dr. (Mrs.) Margaret Bassey of the Department of Botany and Ecological Studies, University of Uyo, Nigeria. A specimen voucher (UULHER, No. 44c) was made and deposited at the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, University of Uyo. The leaf was air-dried, pulverized by grinding using mortar and pestle. Thereafter, the coarse powder of air-dried leaf were subjected to successive solvent extraction by maceration for 72h using solvents of increasing polarity in hexane, chloroform, ethyl acetate and methanol. Each aqueous extract was carefully evaporated over a water bath under controlled temperature and the percentage yields of various extracts were obtained: n-hexane (2.61% w/w), chloroform (1.55%w/w) ethy lacetate (0.83% w/w) and methanol (2.96% w/w).

Phytochemical studies and median lethal dose (LD_{50})

These were earlier determined by Nwafor *et al.,* (2006).

Animal stock

Adult albino mice (weighing 8 -28g) were used in this study. All the animals were housed in a cross-ventilated room with temperature 22±2.5^{0}C, 12h light/ 12h dark cycle) and were fed with standard mash (Unfailing Veterinary Service, Uyo, Nigeria) and water al libitum. Approval for the use of animals in the study was obtained from the Animal Ethics committee, Faculty of Pharmacy, University of Uyo, Uyo, Akwa Ibom State, Nigeria.

Acetic acid-induced writing in mice

The abdominal constrictions resulting from intraperitoneal (i.p.) injection of 3% acetic acid consisting of the contraction of abdominal muscle together with the stretching of hind limbs, were carried out according to the standard procedure (Correa *et al.,* 1996; Ojewole 2005, Nwafor *et al.,* 2007). The animals were divided into six groups of six mice per group. Group 1 served as control while groups 2-4 were pretreated with 24.0, 48 and 72 mg/kg of *Smilax Krausiana* extract (i.p.) respectively. Group 5 was administered with acetyl salicylic acid [(100mg/kg; i.p (Sigma, USA)] and 30min later, was treated with extract (48mg/kg, i.p.) while group 6 received acetylsalicylic acid (100mg/kg i.p) only. The number of writhing movements by each mouse were counted for 30 min. Antinociceptive (analgesia) expressed as the reduction in the number of abdominal constrictions between control animals (acetic acid treated mice) and mice pretreated with the extract.

Formalin – induced hind paw licking in mice

The procedure used was essentially similar to that described previously by Hunskaar and Hole (1987), Sanchez – Mateo etal., (2006). The animals were used to analyze the first phase of formalin-induced licking. Twenty microlitre (20µl) of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: Nacl 137mM; KCl, 2.7mM and phosphate buffer, 10mM) was injected subcutaneously under the surface of the right hand paw. The amount of time spent licking the injected paw was timed and was considered as indicative of pain. The first of the nociceptive response normally peaks 5minn after injection and the second phase 15-30mm after formalin injection, representing the neurogenic and inflammatory pains responses respectively (Hunskaar and Hole, 1987). The animals in groups 2-4 were pretreated with extract (24; 48 and 72mg/kg, i.p. respectively). Group 5 received acetylsalicylic acid (100mg/kg. i.p.) only while group 6 received acetyl salicylic acid (100mg/kg, i.p.) plus extract 48mg/kg, i.p.) 10min later. After 30 min, all animals were challenged with buffered formalin. Group 1 served as the control and all responses were observed for 30min after the injection of formalin (i.p.) into the hind paw.

Fresh egg albumin – induced inflammation in mice

Increase in the mice hind paw linear circumference induced by sub-plantar injection of a phlogistic agent was used as the measure of acute inflammation (Winter *et al.,* 1962). The phlogistic agent employed in this study was fresh egg albunin (Nwafor *et al.,* 2007). Adult albino mice of either sex were used for this study. They were fasted for 24h before use and were only deprived of water during experiment. Inflammation (oedema) of the hind paw as induced by injecting 0.1ml of fresh egg albumin into the subplantar surface of the hind paw. Oedema (inflammation) was assessed as the difference in paw circumference between the control and 1,2,3,4 and 5h after administration of the phlogistic agent (Hess and Milonig, 1972). Animals in groups 2-4 received extract doses 24, 48 and 72mg/kg, i.p.) respectively while groups 5 acetyl salicylic acid (100mg/kg, i.p), 10 min later with extract 48mg/kg, i.p.) treatment. Group 6 received only acetyl salicylic acid. Each mice received 0.1ml of fresh egg albumin intraperitoneally (i.p.) 30 min post extract or drug treatment into the right paw. The linear circumference of the paw was measured after every hour for 5h using Vernier Callipers. Group 1 served as the control and treated with only the egg albumin.

Carrageenin – induced mice hind paw oedema

Young adult albino mice of either sex were used after 24h fast and deprived of water only during experiment. Inflammation of the hind paw was induced by injecting 0.1ml of freshly prepared 1% Carrageenin suspension into the sub-plantar surface of the rigid hind paw. The linear circumference of the injected paw was measured before and 0.5 – 5h after administration of phlogistic agent (Carrageenin) at an interval of 30min. For routine drug testing, the increase in paw circumference 0.5-5h after administration of Carragecnin was adopted as the parameter for measuring inflammation (Besra *et al.,* 1996, Nwafor *et al.,* 2002). Oedema (inflammation) was assessed as the difference in paw circumference between the control and 0.5 – 5h after administration of phlogistic agent (Hess and Milonig, 1972). The extract 24, 48 and 72mg/kg were administered intraperitoneally (i.p.) to groups 2-4 respectively 30min before induction of inflammation. Group 5 animals

received acetyl salicylic acid (100mg/kg, i.p.) and 10 min later was treated with extract (48mg/kg, i.p.) while group 6 animals received only acetyl salicylic acid (100mg/kg, i.p). Control mice received carrageenin. The average (mean oedema) was assessed by measuring with Vernier Callipers.

Capsaicin – induced inflammation in mice

To provide evidence on the possible analgesic effect of the extract on the neurogenic pain, the extract was investigated on its ability to antagonize capsaicin – induced oedema in mice. The procedure of Sakurada *et al.,* (1992) was adopted with slight modification. Oedema was assessed as the difference in paw circumference between the control and 0.5 – 5h after administration of the phlogistic agent. The extract (24 – 72mg/kg, i.p.) was administered intraperitoneally (i.p.) to groups 2 – 4, 30min before the induction of inflammation. Group 5 was injected with extract (48mg/kg, i.p) followed 30 min later with capsaicin while Group 6 received acetyl salicylic acid (100mg/kg, i.p). Group 1 received capsaicin (5µg/kg, i.p.) the average (mean) Oedema was assessed by measuring with Venier Callipers.

Thermally induced pain in mice

The effect of extract on hot plate-induced pain was investigated in adult mice. The hot plate test was used to measure the response latencies according to the method of Vaz *et al.,* (1996). In this experiment, the hot plate was maintained at 45 ± 1^0C. Animals were placed into a glass beaker of 50cm diameter on the heated surface and the time(s) between placement and shaking or licking of the paws or jumping was recorded as the index of response latency. An automatic 30 seconds cut off was used to prevent tissue damage. The mice were divided into six groups of 6 mice per group. Group 1 served as control. Groups 2 -4 were pretreated with extract 24 – 72mg/kg, i.p) respectively 30 min prior to the placement on the hot of plate. Group 5 was injected with extract (48mg/kg, i.p) followed 30min later with the acetyl salicylic acid (100mg/kg, i.p.) while group 6 received acetyl salicylic acid only 30 min prior to the placement on the hot plate.

Statistical analysis

Multiple comparisons of mean ± SEM were carried out by one way analysis of variance (ANOVA), followed by Tukey – Krammar multiple comparisons tests. A probability level of less than 5% was considered significant.

RESULTS

Acetic acid – induced writing in mice

The extract (24.0 – 72.0mg/kg) dose – dependently reduced acetic acid-induced abdominal constrictions and stretching of the hind limbs. The effects which were maximal at 30 mins were statistically significant ($p < 0.001$) (Fig. 1).

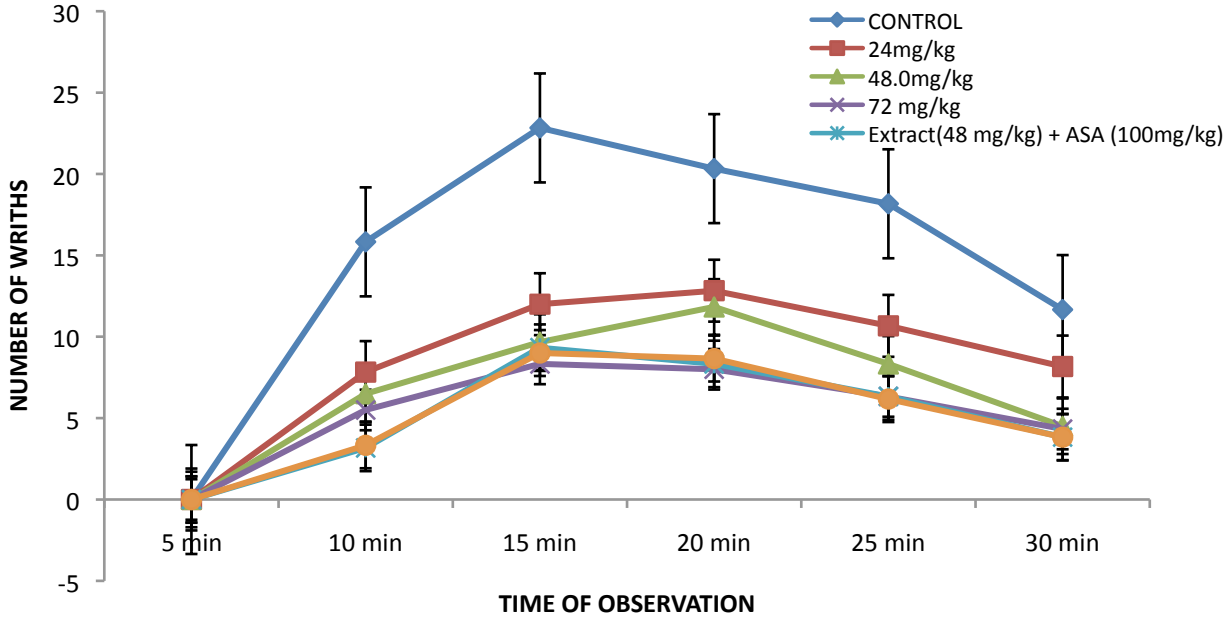

Figure 1:
Effect of methanolic extract of *Smilax kraussiana* leaf on acetic acid-induced writhing in mice. (n =6)

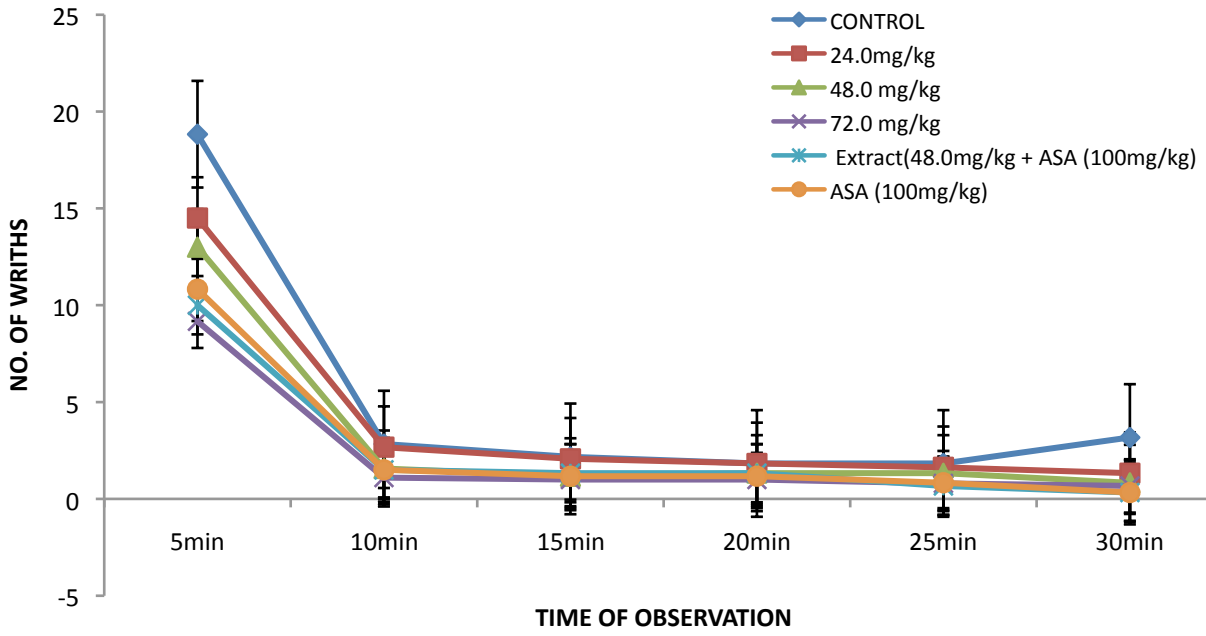

Figure 2:
Effect of methanolic extract of *Smilax kraussiana* leaf on formalin-induced hind paw licking in mice. (n = 6).

Table 1.
Effect of methanolic extract of *Smilax kraussiana* leaf on fresh egg albumin –induced inflammation in mice.

Dose(mg/kg)	Initial reading(mm)	30min	1hr	2hr	3hr	4hr	5hr
Control	0.25±0.01	0.37±0.00	0.34±0.01	0.32±0.00	0.34±0.01	0.34±0.01	0.33±0.01
24.0	0.25±0.01	0.37±0.01^{b}	0.33±0.01	0.32±0.00	0.31±0.00^{b}	0.30±0.01^{b}	0.28±0.01^{b}
48.0	0.23± 0.00	0.34±0.01^{b}	0.32±0.01	0.30±0.01^{b}	0.28±0.01^{b}	0.27±0.01^{b}	0.25±0.01^{b}
72.0	0.22±0.00	0.33±0.01^{b}	0.31±0.00^{a}	0.29±0.01^{b}	0.27±0.01^{b}	0.26±0.00^{b}	0.24±0.00^{b}
48.0+ASA	0.23±0.00	0.30±0.00^{b}	0.29±0.00^{b}	0.27±0.00^{b}	0.26±0.00^{b}	0.26±0.00^{b}	0.25±0.00^{b}
ASA(100)	0.23±0.00	0.31±0.00^{b}	0.30±0.01^{b}	0.28±0.00^{b}	0.27±0.00^{b}	0.26±0.00^{b}	0.25±0.00^{b}

Values represent Mean±S.E.M
Significance relative to control: $^{a}p < 0.01$; $^{b}p < 0.001$; ASA = Acetyl salicylic acid, (n=6)

Formalin – induced hind paw licking in mice
The extract pretreated animals showed significant ($p<0.01 – 0.001$) dose – related reduction of hind paw licking caused by formalin (Fig. 2).

Fresh egg albumin – induced inflammation in mice
The extract showed significant anti-inflammatory activity against acute inflammation induced by egg albumin ($p< 0.01 – 0.001$). This suppression was in a dose – related manner with the highest dose of extract comparable with the standard anti-inflammatory drug (acetyl salicylic acid) (Table 1).

Carrageenin – induced hind paw oedema in mice
The effect of extract on carrageenin-induced inflammation in mice is as shown in Figure 3. The inhibitory effect of the extract which was statistically significant ($p<0.05 – 0.001$) was observed in the fourth and fifth hours respectively.

Capsaicin – induced inflammation in mice
The effect of extract on capsaicin induced inflammation in mice is as shown in Table 2. These inhibitory effects which were statistically significant ($p <0.05 – 0.001$) were predominantly observed between the third and fifth hours respectively.

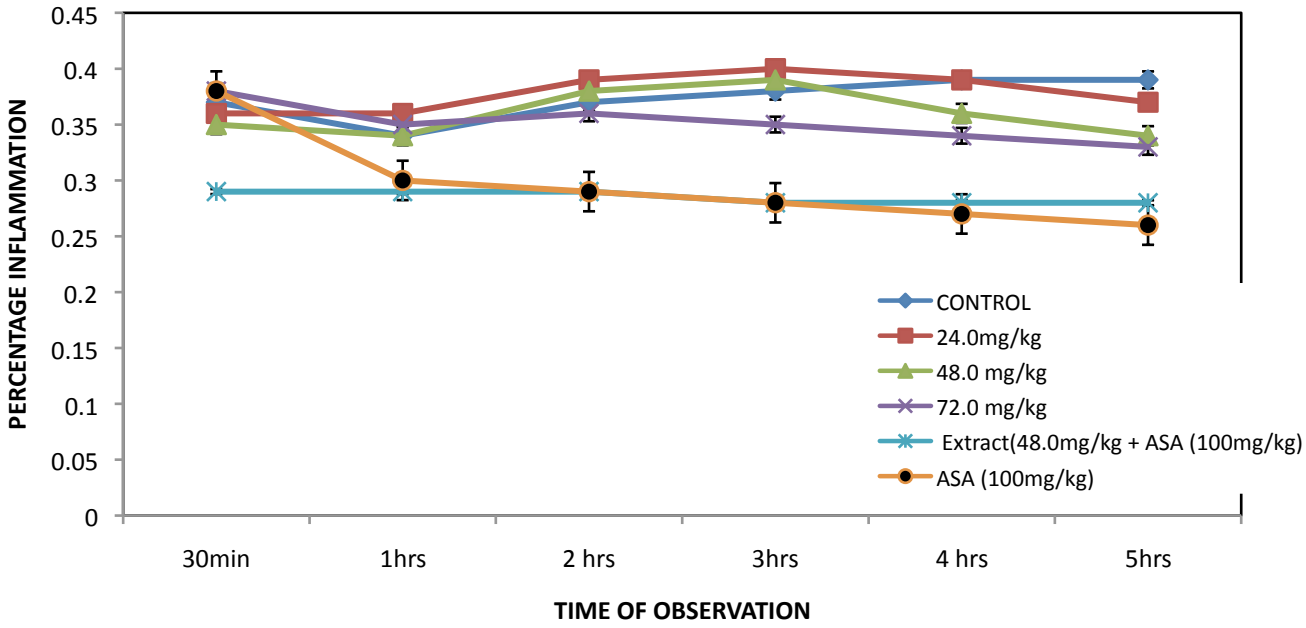

Figure 3:
Effect of methanolic fraction of *Smilax kraussiana* leaf on carragenin-induced inflammation in mice (n=6).

Table 2:
Effect of methanolic extract of *Smilax kraussiana* leaf on Capsaicin- induced inflammation in mice

Dose(mg/kg)	Initial reading(mm)	30min	1hr	2hr	3hr	4hr	5hr
Control	0.16 ±0.01	0.35±0.01	0.28±0.01	0.28±0.00	0.29±0.01	0.31±0.01	0.30±0.01
24.0	0.14 ±0.00	0.26±0.00^{c}	0.25±0.00^{c}	0.26±0.00^{c}	0.28±0.01	0.27±0.01^{c}	0.27±0.01^{c}
48.0	0.15±0.00	0.26±0.01^{c}	0.27±0.01	0.28±0.01	0.28±0.01	0.28±0.01^{c}	0.28±0.01^{c}
72.0	0.16±0.01	0.26±0.01^{c}	0.26±0.01^{b}	0.27±0.01	0.27±0.01^{c}	0.25±0.01^{c}	0.23±0.00^{c}
48 + ASA	0.15±0.00	0.25±0.01^{c}	0.23±0.01^{c}	0.22±0.00^{c}	0.21±0.01^{c}	0.21±0.00^{c}	0.20±0.00^{c}
ASA(100)	0.14±0.01	0.20±0.00^{c}	0.21±0.01^{c}	0.21±0.00^{c}	0.21±0.00^{c}	0.22±0.00^{c}	0.21±0.00^{c}

Values represent Mean±S.E.M
Significance relative to control: $^{a}p < 0.05$; $^{b}p < 0.01$; $^{c}p < 0.001$; ASA = Acetyl salicylic acid; (n = 6)

Thermally-induced pain in mice

The result of thermally-induced pain in mice is as shown in Figure 4. The extract dose – dependently reduced the thermally-induced pain in mice by increasing the pain threshold in the animals. This effect is statistically significant ($p<0.001$).

DISCUSSION

Inflammation is the response of living tissues to injury. It involves a complex array of enzyme activation, mediator release, extravasations of fluid, cell migration, tissue breakdown and repair (Vane & Bolting, 1995). It is also known that anti-inflammatory effects may be elicited by a variety of chemicals agents and that there is no remarkable correlation between their pharmacological activity and chemical structure (Sertie et al., 1990). This fact, associated with the complexity of the inflammatory process, makes the use of different experiment models essential when conducting pharmacological trial (Antonio & Souza-Briton, 1998).

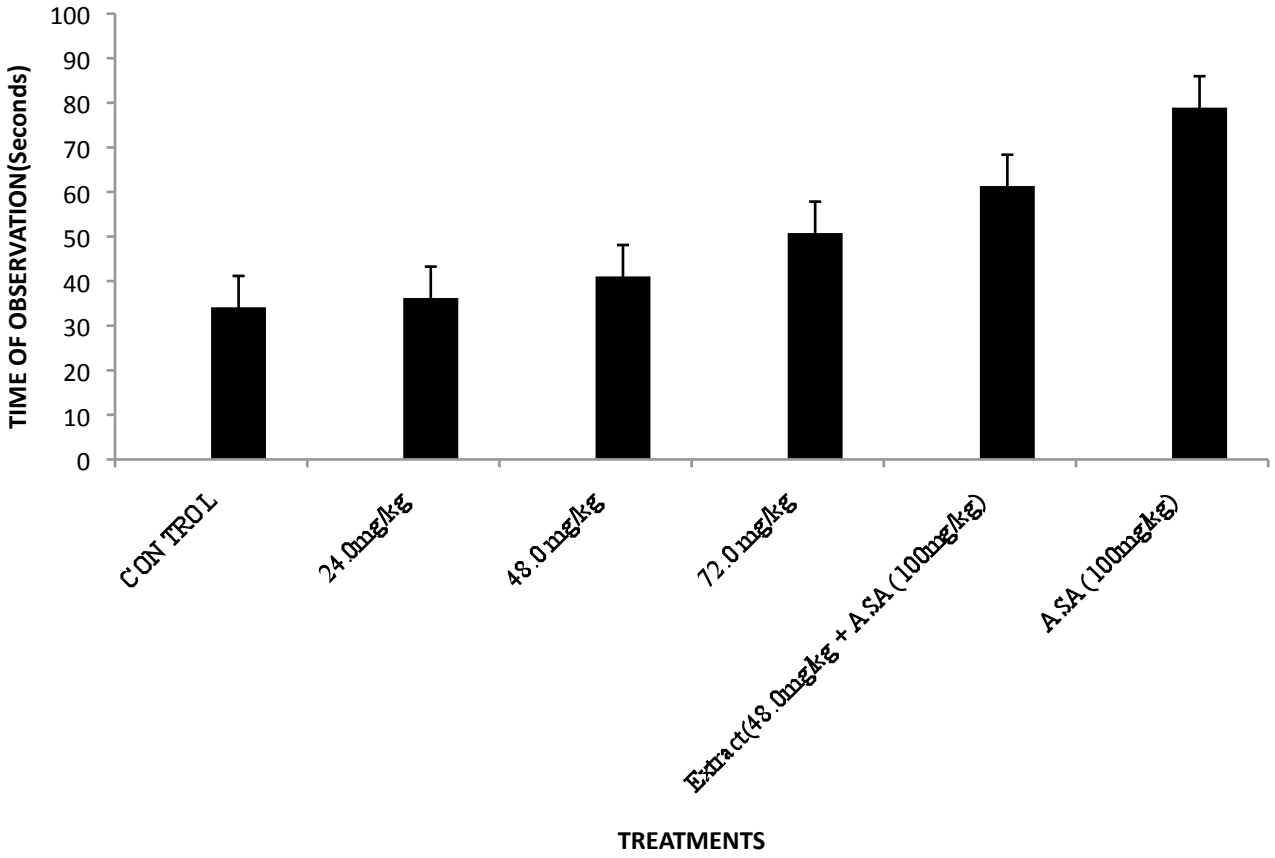

Figure 4:
Effect of *Smilax kraussiana* leaf on thermally-induced pain in mice (n = 6).

Acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Roxas *et al.,* 1984; Nwafor and Okwuasaba, 2003), formalin exhibits neurogenic and inflammatory pain (Vaz *et al.,* 1996; Nwafor et al; 2002) while capsaicin – induced oedema is primarily mediated by neuropeptides such as calcitonin gene-related pepticle, substance P. neurokinin A and vasoactive intestinal peptide, which are released from capsaicin – sensitive neurons (Blazo & Gabor, 1995). That the extract showed significant effect in these types of pain induction suggests that its analgesic effect may in part be related to its anti-inflammatory neurogenic and non-neurogenic properties.

Carrageenin-induced acute hind paw oedema in laboratory animals was first introduced by Winter *et al.,* (1962). It has been widely used to screen new anti-inflammatory drugs and remains an acceptable preliminary screening test for anti-inflammatory activity (Niemegeers *et al.,* 1975; Singh et al., 2000). It is commonly used to evaluate non-steroidal anti-inflammatory drugs (NSAID) (DiRosa and Willoughby, 1971; Boakye-Gyasi *et al.,* 2008). Its induction of inflammation involves three distinct phases of mediators release including histamine and 5-hydroxytryptamine in the first phase, Kinins in the second phase and prostaglandins in the third phase (DiRosa *et al.,* 1971). Fresh egg albumin-induced inflammation appears to be similar to carrageenin – induce inflammation in rodents (Nwafor, *et al.,* 2007) while Eddy's hot plate method indicates narcotic involvement. Non-narcotic analgesics can be differentiated from narcotic ones by their ineffectiveness in the hot plate method (Turner, 1965; Besra *et al.,* 1996). That the extract inhibited dose-dependently, inflammation induced by fresh egg – albumin and carrageenin in mice as well as thermally-induced pain indicates that its analgesic effect may in part be related to its narcotic properties as well as its anti-neurogenic potentials. Nwafor *et al.,* (2006) had shown that the phytochemical analysis of the extract contained saponins, tannins and flavonoids among others. These phytoconstituents possess anti-

inflammatory and analgesic properties (Sanchez – Mateo *et al.,* 2006).

According to Lembeck and Holzer (1979) the neurogenic component plays important role in maintaining the non-neurogenic plasma extravasation since the stimulation of peripheral neurons and subsequent release of substance P from peripheral sensory ending causes further release of histamine from mast cells. It therefore, means that the possible specific action of this extract in blocking the neurogenic component of the stimulated vascular permeability can stop the series of pathogenetic events locally evoked by noxious stimuli. Therefore, the anti-inflammatory properties of the extract strongly support the evidence of a major anti-edematous component.

In conclusion, though the exact mechanism of antinociceptive properties of the extract is not fully understood, it may not be unconnected with the present study which involves suppression of capillary permeability through neurogenic and non-neurogenic pathways as well as its narcotic potential. The observed effects confirm the folkloric use of the plant among the rural people of Uruan Local Government Area of Akwa Ibom State of Nigeria. However, further work is advocated to elucidate the exact cellular mechanism of action.

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