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Suppression of nociception by *Solanum incanum* (Lin.) Diclomethane root extract is associated anti-inflammatory activity

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Abstract

Solanum incanum is an herb that is an important African folklore remedy for several ailments such as inflammation, pain, fever, microbial diseases, and neoplastic disorders. The herb possesses several pharmacological activities that include antinociceptive effect to thermal pain test models, antipyretic, antimicrobial and anticancer activity. However, there are no reported studies on its anti-inflammatory activity and effects on chemical pain test models. The aim of this study was to evaluate the effect of the root extract on inflammation and formalin pain test model. In the anti-inflammatory assay, white Wistar rats were injected intraperitoneally with doses of the herb diclofenac and the vehicle. Thirty minutes later the animals were injected with 50µg of 5% formalin in the sub-plantar region of the left hind paw to induce inflammation and the diameter of the paw measured using a digital caliper. The difference between the initial paw diameter and subsequent readings was quantified as the edema developed in the paw. To assess the effect of the herb on leukocyte migration, carrageenan was injected intraperitoneally into the white albino mice after thirty minutes following subcutaneous administration of the herb extracts and controls. Four hours later, normal saline was injected into the peritoneum and a peritoneal lavage performed and the total number of leukocytes in the fluid determined using a Neubauer chamber. In the antinociceptive assay, white Wistar rats were injected intraperitoneally with doses of the herb extracts. Thirty minutes later the animals were injected with 50µg of 5% formalin in the sub-plantar region of the left hind paw the total time spent in flinching, lifting, biting and licking the hind paw was quantified as the latency of nociception. The dichloromethane extract exhibited significant ($p < 0.05$) anti-inflammatory and 2rd phase antinociceptive effect, with 50 mg doses inhibiting highly significant ($p < 0.001$) effect in the early phase. Since the second phase of nociception is both inflammatory and neurogenic these results suggest that the anti-inflammatory activity of *S. incanum* plays an important role in its antinociception.

Keywords: *Solanum incanum*, Antinociceptive, Analgesic, Anti-inflammatory, Carrageenan, Leukocyte migration.

Introduction

Solanum incanum (Sodom apple in English, entulele in Maasai or mtula in Kiswahili) Solanaceae grows ubiquitously in many regions of Africa where the root is used as a folklore remedy for various ailments that includes toothache, sore throat, angina, stomachache, colic, headache and fever.[1, 2] Other uses of the plant parts include wounds healing[3] relieve of pain during menstruation, hepatic problems of onchocercal etiology,

pleurisy, pneumonia and rheumatism. It also alleviates skin problems, such as infections, whitlow, ringworm, burns, sores, rashes, wounds, warts, carbuncles, ulcers, inflammations and benign tumor cure for snake bites and sexually transmitted disease. In West Africa, leaves and fruits of certain cultivars are edible.[4] Roots boil in bone soup to add flavor by some Kenyan communities. Studies done on this plant showed that it has antinociceptive effects in the two thermal nociception test models in mice as well as antipyretic effects in rats.[5] In other studies, extracts from the herb exhibited antimicrobial[6-8], antifungal[9], spasmolytic[10], anti-diabetic[11,12], anti-schistosomal[13] and anticancer effects where an alkaloid solarmagine from the plant causes apoptosis in various tumor cell lines.[14] Though the 1:1 dichloromethane-methanol extracts from the plant showed antinociceptive effects in the thermal pain test models i.e. hot plate and tail flick, its effects on chemical induced pain test models as well as its anti-inflammatory effects have not been reported. The aim of this study was to establish whether the dichloromethane extract of the root from the herbal has anti-inflammatory effects as well as antinociceptive effects in animal models.

Materials and Methods

Plant material

The plant samples were collected 2012 in the Roysambu Kasarani district of Nairobi metropolis during the day. They were identified with the University of Nairobi Herbarium and a specimen voucher 2013/JM01 was deposited. They were air dried and ground into a powder. A 100 mg of the powder was extracted two times with dichloromethane in 24 hours at room temperature. The procedure was repeated twice in the next 48 hours. The latter procedure was repeated twice. The supernatant was filtered using Whatman No.1 paper, then the filtrate evaporated to dryness using a rotor evaporator at reduced pressure. The extract obtained weighed 0.3 grams.

Experimental Animals

Groups of white albino mice of both sexes weighing 15 to 20 grams (n =5) were used for anti-inflammatory assay, while white Wister rats weighing between 150 to 200 grams were used for both anti-inflammatory and antinociceptive assay. All the animals were placed in cages at room temperature and allowed to acclimatize for seven days before the start of the experiments. Standard commercial diet and water was provided ad libitum with a 12 hour daylight/dark cycle. All experiments were conducted in accordance with guidelines on care and use of laboratory animals.[15]

Drugs and chemicals

The following drugs and chemicals will be used in the study; diclofenac sodium, formalin, carrageenan, formalin, dexamethasone.

Drug and chemicals administration

The herbal extracts and the vehicle were injected intraperitonially (i.p.) except in leukocyte migration assay where the route of administration was subcutaneous (s.c.). Preliminary test was carried out to determine the dose-response relationship of the herbal extracts. The three lowest effective doses were used in the subsequent experiments.

Bioassays

Volume displacement method

Formalin induced paw edema was used as model for acute inflammation[16] and was used to assess the anti-inflammatory effects of *S. incanum* extracts. The white Winstar rats were injected intraperitonially with the doses of the herb extracts, 15 mg/kg diclofenac sodium and the vehicle (30% DCM in normal saline). Thirty minutes later the animals were injected with 50 µg of 5% formalin in the sub-plantar region of the left hind paw to induce inflammation. The diameter of the paw was measured using a digital vernier caliper. The paw thickness was measured before (bf) formalin injection and after every 30 minutes for the next four hours. The difference between the initial readings at subsequent times was taken as the edema developed in the hind paw of rats. The results obtained for doses of the extract and diclofenac were compared with the vehicle treated groups.

Migration of leukocytes assay

The effect of the extracts on leucocytes migration was assessed using carrageenan induced peritonitis. The controls were treated with either the vehicle (i.e. 10 ml/kg 30% DCM in normal saline) or dexamethasone, while the test groups were treated with three doses of the herb extracts. All these treatments were administered subcutaneously (s.c.). The mice were injected with 0.25 ml

of 1% carrageenan (i.p) to induce the inflammatory effect. Four hours later the mice were killed by dropping them into a jar containing cotton soaked with chloroform. Then 2 ml of modified normal saline (containing EDTA) was injected into the peritoneal cavity after which peritoneal lavage was carried out. The total white blood cell count in the lavage fluid was performed using an improved Neubauer chamber as described by Ferrandiz and Alcaraz (1991).[17] The total cell count for test doses and dexamethasone were compared with that of the vehicle treated animals. The differences in the WBC count in the lavage fluid in the vehicle & the test treated animals indicated the effect the various treatments on white blood cell migration.

Antinociceptive/ Analgesic activity assay

Evaluation of the analgesic / antinociceptive effect of the herb extract was carried out using formalin test. The pull test (a sensory motor test) was used to evaluate the muscle relaxing effect of the herb extracts. It separates muscle relaxation from sedation, catalepsy and catatonia (sensory motor impairment).[18] The pain was induced by administration of 50 micro liters of 5% formalin in the sub - plantar region of the left hind paw. The dose was chosen on the basis of previous study.[16] The rats received 15 mg/kg diclofenac and the three doses of the extracts intraperitonially prior to formalin injection. The animals were individually placed in transparent plexiglass cage observation chamber and the amount of time spent flinching, biting and licking the injected paw was considered as an indicator of pain and was recorded for 30 minutes after the formalin injection. Early (1^{st}) phase of nociception was measured between 0-5 minutes while measurement in the late (2^{nd}) phase took place between 15-30 minutes after formalin injection representing neurogenic and inflammatory pain response respectively.[19]

Statistical Analysis

The data for each set of experiment was expressed as means and standard errors of the mean. It was analysed using Windows kwikstat SDA 7.0.5 and Excel statistical software's. One way ANOVA with Scheffé post hoc test was used to compare the test and control group values. A value of $p < 0.05$ was considered significant.

Results and Discussion

In the study the *S. incanum* 10 mg doses exhibited significant ($p < 0.05$) reduction of paw edema diameter from 120 to 180 minutes after injection of formalin compared to the vehicle figure 1 and table 1. The 25 mg doses of the herb exhibited significant inhibition of paw edema diameter from 30 to 240 minutes while the 50 mg did so from 90 to 180 minutes. At 120 and 180 minutes figure 1 the 50 mg dose of the extract showed a significant ($p < 0.05$) reduction in edema diameter, which was comparable in effect, of diclofenac while the 25 mg dose exhibited a highly significant effect ($p < 0.001$) figure 1. Increase in edema is one of the three main vascular signs that results from acute inflammation reaction. Others include reduced vascular resistance resulting in increased blood flow through the tissues, increased in local hydrostatic pressure as well as ultra filtration and increased permeability of blood vessels which is associated with leukocyte migration and fluid exudates to the tissues. The latter effect causes edema and hence the enlargement of paw diameter seen in the acute inflammatory model in the study.[20]

Table 1: Shows the change in mean paw edema diameter of rats in millimeters ± sem following administration of *Solanum incanum* root extract as a function of time

Time / Dose	0 min	30 min	60 min	90 min	120 min	180 min	240 min
10 mg	0	2.03 ± 0.22	2.04 ± 0.24	2.03 ± 0.15	2.16 ± 0.28*	2.13 ± 0.24*	2.3 ± 0.34
25 mg	0	1.12±0.16*	1.51± 0.16*	1.84 ±0.10*	1.76±0.17**	1.64 ± 0.12**	1.79 ± 0.12*
50 mg	0	1.65 ±0.18*	1.92 ± 0.20	2.23 ± 0.31	2.21 ± 0.31*	2.07 ± 0.35*	2.18 ± 0.33
Diclofenac	0	2.09 ± 0.09	2.43 ± 0.1	2.13 ± 0.16	1.94 ± 0.12*	1.7±0.08*	2.51 ±0.06
Vehicle	0	2.15± 0.12	2.75 ± 0.18	2.86 ± 0.18	3.37 ± 0.19	3.35 ± 0.24	3.09 ±0.09
Baseline	0	2.83± 0.34	3.29±0.41	3.25±0.43	3.58± 0.38	3.56 ±0.38	3.22±0.09

*($p< 0.05$), ** ($p<0.001$)

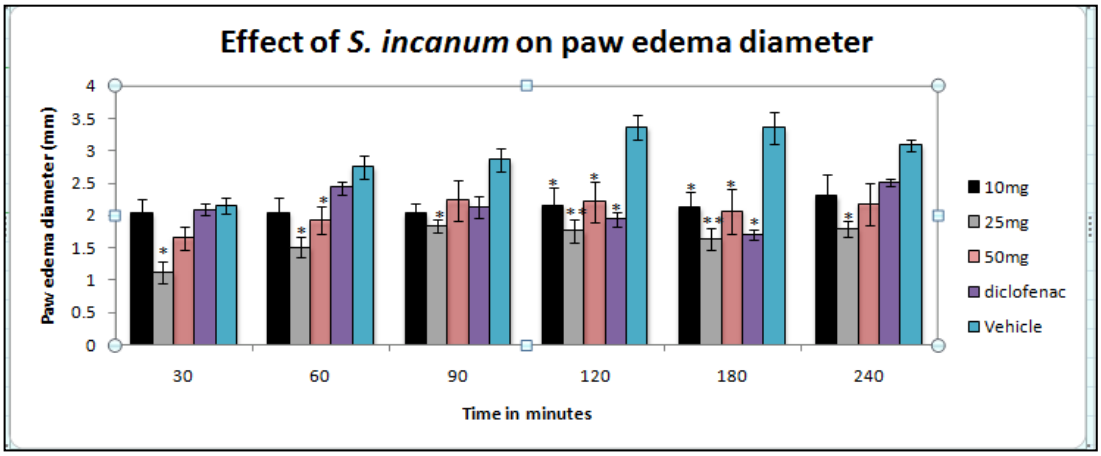

Figure 1: Shows change in paw edema diameter of rats in millimeters after injection with 5% formalin solution following administration of various doses of *Solanum incanum* root extract as a function of time

In the study, the leukocyte migration assay was carried out using carrageenan induced peritonitis and dexamethasone was used as positive control. The three doses of the herb extract exhibited significant ($p< 0.05$) inhibition of the white blood cells (WBC) migration as compared to the vehicle. The inhibition was comparable to that induced by dexamethasone. However, it was observed that there was a significant difference between the effect of the vehicle and control administered with carrageenan only figure 2 and table 2. This may be due to the effects of DMSO which was shown to inhibit leukocyte adherence to endothelial surfaces an important step in their migration.[21] In acute inflammation, reduced vascular resistance, increased blood flow and intra vascular fluid exudate is normally associated with release of pro-inflammatory cytokines like IL-I and TNF –α.[22]

Table 2: Shows the mean number of leukocytes in a peritoneal lavage fluid ± sem following treatment with various doses of *Solanum incanum* root extract

Treatment/Dose	No. of leukocytes	% inhibition
10 mg + carrageenan	256 ± 25*	40%
25 mg + carrageenan	240 ± 25 *	43 %
50 mg + carrageenan	248 ± 27 **	42%
Dexamethasone + carrageenan	292 ± 12 *	31%
Vehicle + carrageenan	424 ± 37	0%
Control Carrageenan only	1208 ± 101	-

*($p< 0.05$), ** ($p<0.001$)

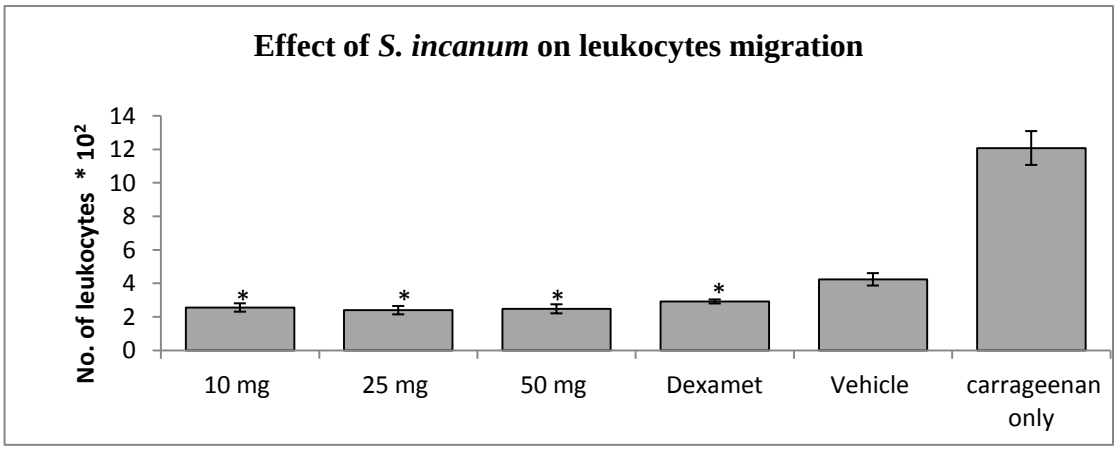

Figure 2: Shows the effect of *Solanum incanum* root extract on carrageenan induced leukocytes migration in mice

In this case, it is probable that DCM extracts of *S. incanum* caused the anti–inflammatory effect by blocking both mechanisms. Dexamethasone, a potent synthetic steroid with glucocorticoid activity and other steroids exerts their anti-inflammatory effects by inhibiting migration and degranulation of leukocytes.[22] *S. incanum* contains several phytochemicals that include steroidal alkaloids[23] and other anti-oxidants which are known to exhibit anti-inflammatory effects.[24] Therefore, it is highly likely that the anti-edema and WBC migration inhibition seen in the study may be partly due to these phytochemicals or their metabolites. In the study, the 25 mg or the median dose of the herb extract exhibited more potency than both 10 and 50 mg doses figure 3 and table 3. It is interesting to note that a higher dose (50 mg) of the herb showed lower activity as compared to 25 mg which may be attributed to higher level of competing metabolite(s) for same carrier protein or enzyme and/or higher substrate product inhibition among others.

Table 3: Shows the mean duration of nociception in minutes ± sem following pre-treated with various doses of *Solanum incanum* root extract and controls in rats

Treatment	**Early phase**	**Late phase**
10 mg extract & carrageenan	3.8 ± 0.2	4.8 ± 0.2
25 mg extract & carrageenan	3.8 ± 0.25	2.4 ± 0.6*****
50 mg extract & carrageenan	1.60 ± 0.24 **	2.2 ± 0.37 ******
Diclofenac & carrageenan	2.2 ± 0.2 **	1.4 ± 0.4 ******
Vehicle extract & carrageenan	3.4 ± 0.51	5.20 ± 0.49
Carrageenan only	5 ± 0.25	5.6 ± 0.36

*(p< 0.05), ** (p<0.001)

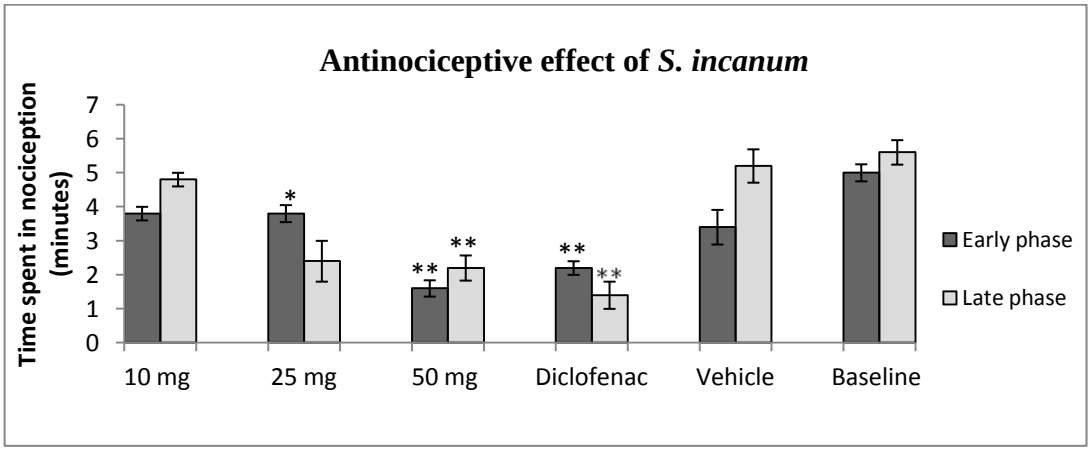

Figure 3: Shows the effect of *Solanum incanum* root extract on formalin induced nociception in rats

In the antinoceptive essay the 50 mg of the extract showed significant effect in the early (1st) phase of nociception while all the three doses exhibited very significant ($p < 0.001$) antino-ciceptive effect in the late (2nd) phase of the formalin test nociception. Therefore, it appears that the extract exerted its antinociceptive effect via inhibition of inflammation in the 2nd phase. The pro-inflammatory cytokines IL-1β and TNFα are involved in formalin induced nociception in the second phase[25], while carrageenan stimulates release of TNFα which induces the release of IL-1β and IL-6 that finally stimulates COX activity.[26] It is thought that IL-1β acts via COX-2 during the process of inflammation. Nevertheless, COX-2 is not involved in the inflammatory pain model of short duration, such as the formalin test. However, both IL-1β and TNFα are involved in inflammatory pain in the late phase of formalin test, hence selective COX-2 inhibitors usually fail to inhibit pain sensation in the early phase of nocciception.[27] The 50 mg dose of the extract significantly inhibited both the early and late phases of nociception

which means it may have inhibited both COX-1 and COX-2 activity, although this study cannot rule out the possibility of other modes of action. In an earlier study, extract of *S. incanum* exhibited significant antino-ciceptive effect to two thermal pain test model which indicated that the effect was integrated at the level of central nervous system.[5] The 2nd phase of nociception in formalin pain is both inflammatory as well as neurogenic in origin as a result of central sensitization.[19,27] It is therefore likely that the extract may have exerted its antino-ciceptive effect mainly via delay in development of the inflammation process and inhibition sensitization of central pain pathways. The other two doses of the herb extract failed to show an antinocicepive effect in the 1rst phase, which may suggest that the main cause of the herbs analgesia is due to its anti-inflammatory activity.

Conclusion

The dichlomethane extract of *Solanum incanum* root contains secondary metabolites which have both acute anti-inflammatory effects as well as antino-ciceptive effects of both acute and chronic pain models. The anti-inflammatory effect involves inhibition of edema formation and leukocyte migration while the analgesic effect may be partly due to inhibition of stimulation of nociceptors as well as anti-inflammatory and neurogenic effects.

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