Anti-inflammatory Activity of *Saccharum Officinarum* Linn (Poaceae) Juice in Animal Models

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**ABSTRACT**

*Saccharum officinarum* (SOC) juice has been used for treatment and management of several conditions including urinary tract infection, diabetes, constipation, tooth decay and bad breath according to folklore. This study was carried out to explore the anti-inflammatory effects of the juice of *Saccharum officinarum* in doses of 2.5, 5.0, 10.0 and 20.0 mL/kg using the following models in rats - carrageenan, histamine, serotonin, formalin-induced paw oedema, and cotton-pellet granuloma. Also employing the following models in mice - castor oil-induced diarrhea and xylene-induced ear oedema. Animals were pretreated with aspirin (100 mg/kg; p.o), cyproheptadine (10 mg/kg; p.o.), atropine (1 mg/kg p.o), dexamethasone (5 mg/kg; p.o) and celecoxib (20 mg/kg p.o). Oral administration of SOC juice significantly (p<0.001) reduced the right hind paw circumference induced by carrageenan, histamine, serotonin, formalin compared to control group. Furthermore, it inhibited xylene-induced ear oedema with peak effect at 20 mL/kg, as well as castor oil-induced diarrhea and cotton pellet granuloma with peak effect at 10 mL/kg. The anti-inflammatory activities of SOC were significant (p<0.05) when compared with aspirin, cyproheptadine and dexamethasone. The study revealed the potent anti-inflammatory activities of the juice of *S. officinarum*.

**Keywords:** *Saccharum officinarum*, anti-inflammatory, carrageenan, aspirin, histamine, rat
INTRODUCTION

Inflammation is a generic body response, a part of the protective mechanisms involving a complex array of biochemical processes such as enzyme activation, mediator release and extravasation of fluid, cell migration, and tissue damage and repair (1, 2) in response to harmful stimuli, such as pathogens, damaged cells, or irritants. It is a mechanism of innate immunity involving immune cells, blood vessels, and molecular mediators, as compared to adaptive immunity, which is specific for each pathogen. Too little inflammation could lead to progressive tissue destruction by the harmful stimulus such as bacteria and compromise the survival of the organism. If not arrested or treated, acute inflammation progresses to chronic, which may lead to a host of diseases, such as hay fever, periodontitis, atherosclerosis, rheumatoid arthritis, and even cancer (gallbladder carcinoma). Inflammation therefore functions to eliminate the initial cause of cell injury, clears out necrotic cells and tissues damaged from the original insult and the inflammatory process, and initiate tissue repair (3). It is a process normally closely regulated by the body (4).

Despite the availability of conventional drugs, both old and new, the side effects of anti-inflammatory agents including gastrointestinal upset, gastric ulcer, bleeding, immunosuppression and organo-toxicity are a major concern in clinical use; which has therefore called for a continuous search for safe and effective newer agents. One of such research areas is the screening of medicinal plants with folkloric anti-inflammatory claims.

*S. officinarum* (Linn.) commonly known as sugarcane is a large, strong-growing species of grass belonging to the family Poaceae. India is the second largest producer of sugarcane, after Brazil (5). It is a tropical, perennial grass that forms lateral shoots at the base to produce multiple stems, typically 3-4m high and about 5cm in diameter. Sugarcane originated from tropical South and Southeast Asia, but different species are found in different locations (6).

Sugarcane has been used in various parts of the world for curing inflammatory diseases and other ailments. In the Ayurvedic system of medicine, sugarcane is used either as a single remedy or in combination with some other plant materials (7). Some natives and traditional healers of the world have recommended sugarcane juice for its diuretic property (8). It is thought that regular use of sugarcane juice will keep the urinary flow clear and fast, which will further help the kidneys to perform their function properly. It is also used as aphrodisiac, laxative, coolant, demulcent, antiseptic, and as a tonic (9). According to the Unani system of medicine, sugarcane juice is considered good for patients with jaundice. Most of the ethnobotanical uses have not been scientifically evaluated. Efforts in this study were aimed at evaluating the acute toxicity profile, phytochemical constituents and the anti-inflammatory activity of the juice of *S. officinarum*. The possible pathway of its anti-inflammatory activity was also explored.

MATERIALS AND METHODS

Plant Materials

Collection and Preparation

The whole plant of *Saccharum officinarum* was obtained from the vegetable farm at Idi-araba, Surulere LGA, Lagos State, Nigeria, for the purposes of identification and authentication, which were carried out at the Herbarium of the Department of Botany and Microbiology, Faculty of Science, University Of Lagos by Mr G.I. Nodza. The plant specimen was deposited in the herbarium and a voucher specimen number: LUH7935 was issued.

A juice extractor was used to squeeze out the juice from peeled fresh stems weighing 139.42 g. The juice was 84.39 g (80 mL) after extraction and always used fresh.

Animals

Eighty-eight (88) male Wistar albino rats weighing between 100-120 g and 52 albino mice weighing between 20-25 g were obtained from the Laboratory Animal Center, College of Medicine, University of Lagos. The animals were
maintained under standard laboratory conditions, having free access to standard rodent diet. All the animals were allowed to acclimatize for 10 days before commencement of experiment, and fasted 12 hours prior each experiment. All experimental procedures were carried out in compliance with the United States National Institute of Health Guidelines for care and use of Laboratory animals in biomedical research (NIH) and recommendation of ISAP (10).

Materials
Atropine, carrageenan, serotonin, histamine, formalin, xylene and celecoxib were obtained from Sigma chemical company in USA. Castor oil, aspirin, cyproheptadine and dexamethasone were purchased from a standard pharmacy shop at Idr-araba, Lagos, Nigeria.

Acute toxicity test
Fasted male mice were randomly selected into groups (n =3), and each group dosed with 15, 20, 30 and 40 mL/kg of SOC juice intraperitoneally, while 50 mL/kg was administered orally. LD₅₀ was scored accordingly (11).

PHARMACOLOGICAL INVESTIGATIONS
Carrageenan-induced paw edema test
Experimental animals were divided into 6 groups of 4 animals each. Group 1- received distilled water (10 mL/kg p.o); Groups 2,3,4 and 5 SOC juice doses; 2.5, 5, 10, and 20 mL/kg per oral and Group 6- aspirin (100 mg/kg p.o). One hour post-treatment, edema was induced by injection of carrageenan (0.1 mL, 1% w/v in saline) into the sub-plantar tissue of the right hind paw (12, 13). The linear paw circumference was measured using a digital vernier caliper. Measurements were made immediately before the injection and then at a 30 minutes interval for 6 hours and the percentage inhibition was calculated.

% inhibition = \frac{[Ct−Co]_{control}−[Ct−Co]_{test}}{[Ct−Co]_{control}} \times 100

Ct – circumference of paw of rat treated with agent at time t
Co – circumference of paw of rat with initial paw circumference

Histamine-induced paw edema test
Experimental animals were divided into 3 groups of 4 animals each. Group 1 received distilled water (10 mL/kg p.o), Group 2 SOC juice dose (20 mL/kg p.o) and Group 3- cyproheptadine (10 mg/kg p.o). One hour post-treatment, edema was induced by injection of histamine into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured using a digital vernier caliper. Measurements were made immediately before the injection of histamine and at a 30 minutes interval for 3 hours and the percentage inhibition was calculated (14, 15).

% inhibition = \frac{[Ct−Co]_{control}−[Ct−Co]_{test}}{[Ct−Co]_{control}} \times 100

Ct – circumference of paw of rat treated with agent at time t
Co – circumference of paw of rat with initial paw circumference

Serotonin-induced paw edema test
Experimental rats were divided into 3 groups of 4 animals each. Group 1 received distilled water (10 mL/kg p.o), Group 2 SOC juice dose (20 mL/kg p.o) and Group 3- cyproheptadine (10 mg/kg p.o). One hour post-treatment, edema was induced by injection of serotonin into the sub-plantar tissue of the right hind paw. The linear paw circumference was measured using a digital Vernier caliper. Measurements were made immediately before the injection of serotonin and at a 30 minute interval for 3 hours and the percentage inhibition was calculated (14, 15).

% inhibition = \frac{[Ct−Co]_{control}−[Ct−Co]_{test}}{[Ct−Co]_{control}} \times 100

Ct – circumference of paw of rat treated with agent at time t
Co – circumference of paw of rat with initial paw circumference

Castor-oil induced diarrhea in mice
Experimental mice were randomly distributed into 4 groups of 4 animals each. Group I received distilled water (10 mL/kg), groups II and III received SOC juice (10 mL/kg and 20 mL/kg respectively), while group IV received atropine (1 mg/kg, s.c), 30 min prior to the oral
administration of castor oil (0.2 mL) (16). All other treatments were orally administered. Following castor oil treatment, the animals were placed on a filter paper covered with a funnel in order to restrict movement. Defecation was observed for 5 hours.

**Formalin-induced rat Paw edema**

Experimental rats were distributed into 6 groups of 4 animals each. Group 1- distilled water (10 mL/kg), Groups 2 and 3 SOC juice doses at 10 mL/kg and 20 mL/kg, Group 4- aspirin (100 mg/kg), Group 5- dexamethasone (5 mg/kg) and Group 6- aspirin + SOC juice dose 20 mL/kg. One-hour post-treatment, edema was induced by formalin (0.02 mL, 2%) in the right hind paw of the rats. The paw thickness was measured using a vernier caliper, 1 hour after formalin injection. The drug treatment was continued for 7 days and formalin was also induced on the 3rd day. The increase in paw thickness and percentage inhibition were calculated (15).

**Xylene-induced ear edema test**

Experimental mice were allotted to 4 groups of 4 animals each. Group 1- distilled water (10 mL/kg), Groups 2 and 3 received SOC juice doses at 10 mL/kg and 20 mL/kg and Group 4- dexamethasone (5 mg/kg). One-hour post-treatment, edema was induced in each mouse by applying 0.1 mL of xylene to the inner surface of the right ear. The animals were then sacrificed by cervical dislocation after 30 minutes, both ears were then cut off to approximately equal size and weighed. The mean differences between the right and left ears were determined for each group and percentage inhibition was calculated respectively (13).

\[
\text{\% inhibition} = \frac{(\text{Granuloma (control)} - \text{Granuloma (test)})}{\text{Granuloma (control)}} \times 100
\]

**Cotton pellets granuloma in rats**

Experimental rats were divided into four groups of four rats each. Adsorbent cotton wool was cut into pieces weighing 20±1 mg and made into pellets. The abdomen of the rats was shaved, swabbed with 70 % ethanol and two sterilized cotton pellets were implanted subcutaneously, one on each side of the abdomen of the animal under light ether anaesthesia. Test drugs; distilled water (10 mL/kg), *Saccharum officinarum juice* (10 and 20 mL/kg), and celecoxib (20 mg/kg) were administered once daily throughout the experimental period of 7 days. On the 8th day after implantation, rats were anaesthetized with pentobarbital sodium. The pellets were dissected and dried at 60° for 18 h, weighed after cooling. The mean weight of the cotton pellets of the control group as well as of the test groups was calculated. The wet and dry weights, granuloma formation and percent granuloma inhibition of the test compound were calculated (17).

\[
\text{\% inhibition} = \frac{(\text{Granuloma (control)} - \text{Granuloma (test)})}{\text{Granuloma (control)}} \times 100
\]

**Phytochemical screening**

The juice of SOC was subjected to preliminary phytochemical screening following standard procedures (18 – 20).

**Anti-oxidant assay of aqueous extract of *Saccharum officinarum juice***

**Nitric oxide radical scavenging assay**

Nitric oxide (NO) generated from sodium nitroprusside (SNP) was measured (21). Briefly, the reaction mixture (5.0 mL) containing SNP (5 mM) in phosphate buffered saline (pH 7.3), with *S. officinarum juice* at different concentrations, was incubated at 25°C for 180 min in front of a visible polychromatic light source (25W tungsten lamp). The NO radical thus generated interacted with oxygen to produce the nitrite ion (NO\(^{-}\)) which was assayed at 30 min intervals by mixing 1.0 mL of incubation mixture with an equal amount of Griess reagent (1% sulfanilamide in 5% phosphoric acid and 0.1% naphthyethylene-diamine-dichloride). The absorbance of the chromophore (purple azo dye) formed during the diazotisation of nitrite ions with sulphanilamide and subsequent coupling with naphthyethyleneediaminedi-hydrochloride was measured at 546 nm. The
nitrite generated in the presence or absence of the plant extract was estimated using a standard curve based on sodium nitrite solutions of known concentrations. Each experiment was carried out two times and the data presented as an average of two independent determinations.

**DPPH (2,2-Diphenyl-1-picrylhydrazyl)**

The free radical scavenging activity of *S. officinarum* was measured with stable DPPH in terms of hydrogen donating or radical scavenging activity. 100μL of DPPH solution (0.36 mm DPPH in methanol) was added to 1 mL extract (100-1000 μg/mL in methanol), vortexed thoroughly and kept in the dark at room temperature for 30 min, after which the decrease in absorbance was measured at 517 nm using ultraviolet-visible (UV-VIS) spectrometer. Ascorbic acid was used as the positive control (22).

The scavenging effect was calculated using:

\[
\text{Percent inhibition} \% = \left( \frac{A_0 - A_1}{A_0} \right) \times 100,
\]

where \( A_0 \) is the absorbance of the control solution and \( A_1 \) is the absorbance in the presence of aqueous leaf extract of *S. officinarum* and standard.

**Ferric reducing power assay**

The ferric reducing antioxidant power (FRAP) of the different solvent fractions was assessed (23). A mixture containing 2.5 mL of 0.2 M phosphate buffer pH 6.6 and 2.5 mL 1% potassium hexacyanoferrate was added to 1.0 mL of the different solvent extracts and standards (25 μg/mL to 400 μg/mL). The resulting mixture was incubated for 20 min at 50°C. After incubation, 2.5 mL of 10% TCA (w/v) was added to terminate the reaction and centrifuged for 10 min at 3000 rpm. 2.5 mL of the supernatant was removed and mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% freshly prepared FeCl₂, FeCl₃, was added. The mixture was left to stand for 10 min, and the absorbance was read at 700 nm. A mixture of the buffer instead of the sample served as control. Increased absorbance of the reaction mixture indicated higher reducing power of the plant fractions. The results were reported as μg of ascorbic acid equivalents (AAE) per mL.

**RESULTS**

**Phytochemical screening**

The results of the phytochemical tests revealed the presence of alkaloids, saponins, flavonoids, steroids, quinone, coumarin, tannins, phenols, glycosides and terpenoids.

**Acute Toxicity**

Intraperitoneal administration of 15, 30 and 40 mL/kg showed 0 %, 66.67 % and 100 % mortality respectively. The juice was found to elicit these effects; itching, grooming and tachypnea.

### TABLE 1: Acute toxicity test (Intraperitoneal route)

| Dose (mL/kg) | Log dose | Mortality | % mortality | Probit |
|--------------|----------|-----------|-------------|--------|
| 15           | 1.176    | 0/3       | 0           | 0      |
| 30           | 1.477    | 2/3       | 66.67       | 5.52   |
| 40           | 1.602    | 3/3       | 100         | 8.09   |

LD₅₀ was calculated to be 25 mL/kg of juice dose.

**PHARMACOLOGICAL INVESTIGATIONS**

**Carragenan-induced paw oedema in rats**

For the control group, carrageenan produced a progressive swelling of the rat’s paw 7.74±0.05 mm at 30 minutes, which gradually increased to the peak at 240 minutes (4th hour) 8.30±0.03mm (Table 2). Administration of the juice 2.5, 5, 10 and 20 mL/kg after 30 minutes showed significant inhibition (p<0.001) of edema similar to the standard drug (aspirin). *S. officinarum* juice dose at 20 mL/kg produced the highest percentage inhibition of 83.20 %, which compared effectively with the standard drug (82.40 %).
TABLE 2: EFFECT OF S. OFFICINARUM ON CARRAGEENAN-INDUCED PAW OEDEMA IN RATS

| Treatments | Dose mL/kg | 0 | 30 | 60 | 90 | 120 | 150 | 180 | 210 | 240 | 270 | 300 | 330 | 360 | 440 |
|------------|------------|---|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Control (DW) | 10 | 6.13±0.09 | 7.74±0.05 | 7.79±0.03 | 7.93±0.04 | 7.95±0.04 | 7.97±0.06 | 8.23±0.11 | 8.27±0.13 | 8.30±0.03 | 8.20±0.02 | 8.20±0.02 | 8.16±0.05 | 8.01±0.03 | 7.09±0.00 |
| SO | 2.5 | 5.38±0.06 | 6.37±0.01* | 6.64±0.16* | 6.73±0.18* | 6.99±0.09 | 6.76±0.05 | 6.58±0.06 | 6.49±0.03 | 6.46±0.05 | 6.45±0.06 | 6.44±0.05 | 6.44±0.05 | 6.44±0.05 | 6.17±0.01 |
| | 5 | 5.46±0.11 | 6.18±0.11* | 6.50±0.17* | 6.77±0.09 | 6.98±0.16 | 6.83±0.15 | 6.73±0.11 | 6.58±0.12 | 6.54±0.11 | 6.50±0.12 | 6.47±0.11 | 6.01±0.10 | 6.01±0.10 | 5.88±0.10 |
| | 10 | 5.98±0.06 | 6.63±0.13* | 6.81±0.15* | 6.94±0.15 | 6.95±0.04 | 7.00±0.14 | 6.83±0.15 | 6.80±0.15 | 6.69±0.15 | 6.68±0.15 | 6.61±0.15 | 6.49±0.15 | 6.31±0.05 | 6.31±0.05 |
| | 20 | 5.68±0.21 | 5.99±0.16* | 6.09±0.15 | 6.32±0.15 | 6.43±0.15* | 6.68±0.15 | 6.78±0.15 | 6.66±0.15 | 6.59±0.15 | 6.39±0.15 | 6.31±0.15 | 6.21±0.15 | 5.75±0.17 | 5.75±0.17 |
| Aspirin | 100 mg/kg | 5.53±0.03 | 5.88±0.02* | 5.89±0.02 | 5.92±0.02 | 5.91±0.02 | 5.87±0.03 | 5.97±0.03 | 5.96±0.03 | 5.94±0.03 | 5.92±0.03 | 5.93±0.04 | 5.89±0.04 | 5.86±0.05 | 5.79±0.05 |

Results are expressed as mean± SEM (n=4); SOC: Saccharum officinarum; DW: Distilled Water; p<0.001, a p<0.01 and *p<0.05 statistical significance compared to control (Two-Way ANOVA followed by Bonferroni post-test). Percentage inhibition of oedema in parenthesis.

Histamine- induced paw oedema in rats
The extract at 20 mL/kg dose produced a high percentage inhibition of 60.71% at 180 minutes, while the standard drug recorded 85.71% percentage inhibition at that same time (Fig. 1).

Serotonin- induced paw oedema in rats
The dose 20 mL/kg of SOC juice after 60 minutes produced a significant (p<0.05) and a comparatively effective inhibition 70.1% of paw edema in rats with aspirin recording 77.6% (Fig.2).
Figure 2: Paw size of rats in serotonin-induced paw oedema. Results are expressed as mean± SEM (n=4); SOC Saccharum officinarum; DW: Distilled Water; *p<0.05 statistical significance compared to control (Two-Way ANOVA followed by Bonferonni post-test).

Castor oil-induced diarrhea in mice
Pre-treatment of mice with the different doses of SOC juice caused a significant and a dose dependent decrease in the frequency of purging (reduction of number of wet stool) and, weight of wet stools (Table 3).

| Treatment     | Dose     | Onset of diarrhea (min) | No of wet stool | Weight of wet stool (g) | No of semi-solid stool | Weight of semi-solid stool (g) | No of solid stool | Weight of solid stool (g) |
|---------------|----------|-------------------------|----------------|------------------------|------------------------|-------------------------------|----------------|-------------------------|
| Control DW    | 10 mL/kg | 11.50± 5.68             | 7.50±2.06      | 0.22± 0.05             | 6.50±1.56              | 0.23± 0.05                    | 3.50±1.44 | 0.09± 0.04              |
| S.O 10 mL/kg  |          | 53.25±16.62             | 6.00±2.35      | 0.22± 0.04             | 4.25±1.49              | 0.10± 0.04                    | 3.25±1.25 | 0.06± 0.02              |
| S.O 20 mL/kg  |          | 26.25±13.93             | 4.50±1.56      | 0.22± 0.06             | 6.25±2.00              | 0.20± 0.07                    | 4.50±1.44 | 0.06± 0.03              |
| Atropine      | 1 mg/kg  | 42.50±22.46             | 11.25±1.80     | 0.37± 0.13             | 8.25±2.25              | 0.24± 0.10                    | 4.25±1.65 | 0.12± 0.05              |

Results are expressed as mean± SEM (n=4); S.O: Saccharum officinarum; DW: Distilled Water; *p<0.001 and *p<0.01 statistical significance compared to control (Two-Way ANOVA followed by Bonferonni post-test).

Formalin-induced paw oedema in rats
The SOC juice at a dose of 10 mL/kg produced a significant inhibition of paw edema (p<0.05 and 0.01 respectively) on the 5th and 6th day. Also, the standard drug, aspirin produced a significant inhibition of paw edema (p<0.05) on the 2nd, 3rd, 4th and 6th days. Aspirin and 20 mL/kg juice dose showed a significant inhibition (p<0.01) on the 1st and 2nd day. Dexamethasone showed a significant inhibition (p<0.05) on the 4th day. On the 7th day, the juice at a dose of 10 mL/kg produced a percentage inhibition (72.09%) that was higher than that of the standard drugs (68.60% and 69.77% for aspirin and dexamethasone respectively) (fig 4).
**Figure 4:** Paw size of rats in formalin induced paw oedema. Results are expressed as mean± SEM (n=4); SOC: *Saccharum officinarum*; DW: Distilled Water; *a p<0.01 and *p<0.05* statistical significance compared to control (Two-Way ANOVA followed by Bonferonni post-test).

### Xylene-induced ear edema in mice

In this experiment; xylene produced an immediate swelling of the right ear which revealed the difference between both ears in the control group. (Fig 5).

**FIGURE 5:** Xylene-induced ear oedema

### Cotton pellet-induced granuloma

The oral administration of SOC juice produced significant (*p< 0.05 and p<0.01*) reduction in inflammation when compared to control group. Figure 6 shows the results obtained in the cotton pellet-induced granuloma model in which the juice 10 mL/kg produced a significant (*p<0.001*) reduction with 36.56 % inhibition when compared to the control group. Celecoxib 20 mg/kg showed the highest inhibition of 37.63 % which was significant (*p<.0.01*) when compared to control after 7 days drug treatment. The anti-inflammatory effect produced by the SOC juice was low dose-dependent with the highest inhibition produced by a dose of 10 mL/kg.

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**Table:**

| Treatments | ear weight |
|------------|------------|
| Control DW 10 mL/kg | 0.15       |
| S. officinarum 10 mL/kg | 0.10       |
| S. officinarum 20 mL/kg | 0.05       |
| Dexamethasone 5 mg/kg | 0.00       |
Antioxidant capacity of SOC juice

The juice of *S. officinarum* exhibited good nitric oxide scavenging activity leading to the reduction of the nitrite concentration in the assay medium. The nitric oxide scavenging capacity was concentration dependent with 100 µg/mL scavenging most efficiently. The juice of *S. officinarum* significantly inhibited (p < 0.05) the accumulation of nitrite, a stable oxidation product of nitric oxide liberated compared to the standard ascorbic acid (Figure 7). The result of effects of *S. officinarum* on DPPH radical scavenging activity is presented in Fig 8 which indicated potent radical scavenging activities. The reducing power of *S. officinarum* juice as well as the standard Ascorbic acid increased progressively in a concentration-dependent manner. The reducing power of Ascorbic acid observed was greater than *S. officinarum* juice. The reduction capability of *S. officinarum* juice when compared to Ascorbic acid is shown in Figure 9 and showed potency. Values are expressed as Mean ± SEM.

**DISCUSSION**

Anti-inflammatory models employed in this investigation included xylene induced ear edema in mice, carrageenan-induced rat paw edema, formalin induced rat paw oedema (sub-acute), cotton pellet-induced granuloma and in the process of elucidating mechanism of action of extract, the following models were used: serotonin and histamine induced rat paw oedema, and castor oil induced diarrhoea for the biosynthesis of prostaglandins. Anti-inflammatory activity is determined by analyzing the reduction in oedema size and calculating % inhibition of oedema. A mean reduction in oedema when compared with control and increase % inhibition in the treated groups is an indication of anti-inflammatory activity.

The carrageenan induced is based on the principle of release of various inflammatory
mediators. Oedema formation due to carrageenan is a biphasic event. The initial phase is attributed to the release of histamine, serotonin and kinins while the second phase is due to the release of prostaglandin, protease and lysosome (24 - 25; 15). The juice of SOC (2.5, 5, 10, and 10 mL/kg) in a dose-dependent manner significantly inhibited the carrageenan induced paw edema in rats, with peak inhibition of 83.23 %. The inhibition of induced paw edema produced by SOC was dose dependent with peak inhibition of 83.23% obtained at a dose of 20 mL/kg. Interestingly, this effect was comparable to that produced by 100 mg/kg of aspirin (82.40 %), a non-steroidal anti-inflammatory (NSAIDs) drugs.

![Graph](image_url)

**Figure 7:** Nitric oxide scavenging activity of *S. officinarum* juice in comparison with ascorbic acid.

![Graph](image_url)

**Figure 8:** DPPH scavenging activity of *S. officinarum* juice in comparison with ascorbic acid.
Figure 9: Reducing power activity of *S. officinarum* juice in comparison with ascorbic acid.

Xylen induced ear oedema model is partially associated with substance P which is an undecapeptide that is widely distributed in the central and peripheral nervous system and it functions as a neurotransmitter or neuromodulator in a variety of physiological processes (26). Release of substance P from the sensory neurons causes vasodilation and plasma extravasations suggesting its role in neurogenous inflammation. Thus, it causes the swelling of ear in mice. This test distinguishes NSAIDs and steroidal anti-inflammatory drugs. The juice of SOC (20 mL/kg) showed a significant dose dependent inhibition of inflammation when compared with distilled water control. Peak inhibitory effect was obtained at a dose of 20 mL/kg of the juice (50 %) and has a better effect to that produced by 5 mg/kg dexamethasone (40 %). This showed that SOC also possesses mild steroidal anti-inflammatory property.

The effect of the juice on formalin-induced paw oedema in mice suggests its anti-arthritic effect. The juice of SOC showed a significant inhibition of inflammation induced by formalin compared to control. The extract at a dose of 10 mL/kg produced 77.70 % inhibition of formalin on day 7, and this was however comparable to acetylsalicylic acid (aspirin) and dexamethasone at doses of 100 mg/kg and 5 mg/kg which produced inhibition of 68.60 % and 69.77 % respectively on the same day 7. Co-administration of SOJ and aspirin produced 31.40 % inhibition on day 7.

The effect of formalin is biphasic, an early neurogenic component followed by a later tissue-mediated response. In the first phase, there is a release of histamine, 5HT, and kinin, while the second phase is related to the release of prostaglandin (27). The peak inhibition of formalin on day 7 by the juice suggests the inhibition of both phase 1 (histamine production) and phase 2 (prostaglandin production) by the extract, an effect which was dose dependent.

In order to elucidate the mechanism of action of the juice of SOC, selected anti-inflammatory mediators were used, especially, those produced by carrageenan (serotonin, histamine, and prostaglandin). The juice of SOC (20 mL/kg) showed a significant inhibition of serotonin when compared with the control. The peak inhibition (70.15 %) was obtained at 60 minutes post induction compared to that of 10 mg/kg cyproheptadine (77.61%). This showed that juice of *S. officinarum* has a good anti-serotonergic effect as a result of inhibition of serotonin. On the other hand, the same dose of
the extract (20 mL/kg) showed a significant inhibition of histamine when compared with the control (10 mL/kg distilled water). The peak inhibition (60.71%) was obtained at 180 minutes compared to 10 mg/kg cyproheptadine (85.71%). This showed that the juice is equally a mild inhibitor of histamine.

To further investigate the mechanism that might account for the anti-inflammatory action of SOC juice, castor oil-induced diarrhoea, which results in biosynthesis of prostaglandin in rat was used (16). The juice (10 mL/kg), produced a significant delayed onset of diarrhoea (53 minutes) when compared to the control (11 minutes). Moreover, there was a marked reduction in the production of wet stools with juice dose 20 mL/kg. This showed an inhibition of prostaglandin synthesis, which accounted for the characteristic diarrhoea seen in control group with its rapid onset (11 minutes) and an increased number of wet stools with a decrease in the number of solid stool. The delayed onset of diarrhea (53 minutes) observed in SOC treated animals is comparable to that of aspirin (43 minutes).

The results of phytochemical analysis revealed the presence of flavonoids, glycosides and quinine in high amounts. Kim et al., (2004) (28) reported that flavonoids possess anti-inflammatory property. Ahmadiani et al, (2000) (29) also reported that flavonoids were found to have anti-inflammatory and anti-nociceptive activity, therefore flavonoid found in SOC could have contributed to its observed anti-inflammatory property. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception (15). Glycosides, the active component in willow bark have also been found to exhibit anti-inflammatory effects (30, 15).

From the foregoing, SOC has been established to possess anti-inflammatory property, which compared effectively with both standard steroidal and non-steroidal anti-inflammatory drugs in both the acute and sub-acute models employed in this study. Furthermore, the scope of the present investigation has elucidated as part of its mechanism of action, its inhibitory involvement in prostaglandins synthesis as well as on serotonin and histamine, which are inflammatory mediators.

Reactive oxygen species and free radicals, which are products of normal cellular metabolism are thought to act indirectly as cellular messengers and elicit an inflammatory response.

The juice was evaluated to determine its possible radical scavenging activity, a known mechanism by which antioxidants inhibit lipid oxidation. A balance between free radicals and antioxidants is necessary for proper physiological function in the body (31).

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) is scavenged by antioxidants through the donation of hydrogen forming a reduced DPPH, which can be quantified by its decrease of absorbance. The DPPH radical scavenging of the juice indicated potent radical scavenging that suggested antioxidant property, which compared effectively with ascorbic acid. The ferric ion reducing power of SOC was also evaluated. This measures the electron donating activity of the extract. Reducing agents would cause a reduction of Fe$^{3+}$ to Fe$^{2+}$. The ferric ion reducing power was concentration dependent when compared to ascorbic acid. Nitric oxide (NO) is generated from amino acid L-arginine by vascular endothelial cells, phagocytes, and certain cells of the brain. Nitric oxide is classified as a free radical because of its unpaired electron and displays important reactivity with certain types of proteins and other free radicals. The toxicity of NO becomes adverse when it reacts with superoxide radical, forming a highly reactive peroxynitrite anion (ONOO$^-$). The nitric oxide radical scavenging of the juice indicates a potent radical scavenging that was concentration dependent suggesting antioxidant property.

**CONCLUSION**

In conclusion, the investigation carried out demonstrated that the juice of SOC possesses
anti-inflammatory activities. Further studies are needed to elucidate the mechanism of action and therapeutic value in the treatment of inflammation. Also, it can be concluded that the use of SOC in the treatment of chronic inflammation is not as effective as its use in acute and sub-acute conditions, howbeit, the juice at 10 and 20 mL/kg doses, compared well with the standard drugs aspirin, dexamethasone, and celecoxib, and it is speculated to act via inhibition of serotonin, histamine, prostaglandins and through antioxidation.

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