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Analgesic Activity of Ethanol Leaf Extract of *Saccharum officinarum*

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ABSTRACT

Pain is one of the commonest symptoms reported to physicians and health workers and causes more frequent visits to health facilities than most other symptoms. Diverse methods and substances including plant parts are being used to treat pain. *Saccharum officinarum (*sugar cane) which is used in traditional medicine to treat arthritis was therefore investigated to authenticate its ability to ameliorate pain. The ethanol leaf extract of *S. officinarum* (170, 340, 510 mg/kg) was evaluated for analgesic activity against experimentally-induced pain in mice using three standard models of acetic acid-induced writhing, formalin-induced hind paw licking and thermally-induced pain. The median lethal dose (LD_{50}) of the extract using Lorke's method, was estimated to be 173 g/kg. The leaf extract significantly ($p<0.005$-0.001) inhibited pain in all the models tested in a dose-dependent fashion. Acetic acid-induced model is used to test for pain of visceral origin. Formalin causes biphasic pain, first phase being neurogenic, while the second phase is peripheral and central. Hot plate model is used to test for pain of central origin. Acetyl salicylic acid (ASA) induces analgesia through activation of opiod receptors and can be used to test for pain of peripheral and central origin. Therefore, the apparent similarity between the results of the extract and ASA indicates that they might work in the same manner to reduce pain sensation. The findings of this study show that the leaf extract possesses analgesic activity which confirms its use in traditional medicine in the treatment of pain.

Keywords: *Saccharum officinarum,* Analgesic, Pain, Ethanol extract, Ethnomedicine.

Introduction

Saccharum officinarum (Family-Poaceae) commonly known as sugarcane is widely cultivated throughout tropical and subtropical regions. In folkloric medicine it is used in the treatment of diarrhoea, dysentery, eye infirmities, fever, arthritis, bedsores, boils, cancer, colds, cough, opacity, skin sores, sore throat, hiccups, inflammation, laryngitis, spleen, tumors, and wounds.[1] Biological activities reported on the leaf include antibacterial and anthelmintic,[2] anti-hyperglycaemic, anti-hyperlipidaemic,[3] antioxidant,[34] Diuretic and antiurolithiatic,[5] antidepressant and anticonvulsant activities.[6] Phytochemical screening of leaf extract of *S. officinarum* reported the presence of glycosides, phytosterols, saponins, tannins, flavonoids.[2] The study reports the analgesic activity of *S. officinarum* leaf extract in mice.

Materials and Methods

Plant materials

~~The fresh leaves~~ of the plant were collected in June 2018 from compounds in Uyo, Uyo Local Government Area, Akwa Ibom State, Nigeria. *S officinarum* leaves were identified and authenticated by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, Uyo, Nigeria. An herbarium specimen was deposited at the Faculty of Pharmacy Herbarium, University of Uyo, Uyo, Nigeria.

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Extraction

The leaves of *S. officinarum* were washed and air-dried on laboratory table for 2 weeks. The dried leaves (30 g) were pulverized using a pestle and mortar and then macerated in 600 ml of 95% ethanol for 72 hours. The filtrate obtained by filtration was evaporated to dryness in a rotary evaporator at 40˚C. The extract was stored in a refrigerator until used for the experiment.

Animals

Male and female Swiss mice were used for the experiments. The mice were housed in standard cages and were maintained on a standard pelleted feed (Guinea feed) with water given *ad libitum.* Approval for animal studies was obtained from the College of Health Sciences Animal Ethics Committee, University of Uyo.

Determination of median lethal dose (LD_{50})

The median lethal dose (LD_{50}) of the extract was determined in mice by intraperitoneal (i.p) route using the method of Lorke (1983).[7] The mice were administered the extract (500, 1000, 1500. 2000, 2500, 3000 mg/kg) to groups of three mice each. The animals were observed for manifestation of physical signs of toxicity such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death. The number of deaths in each group within 24 hours was recorded. The LD_{50} was calculated as geometrical means of the maximum dose producing 0% (a) and the minimum dose producing 100% mortality (b).

$LD_{50} = \sqrt{ab}$

Acetic acid-induced writhing in mice

Abdominal constrictions consisting of the contraction of abdominal muscles together with the stretching of hind limbs (writhings) resulting from intraperitoneal (i.p) injection of 2% acetic acid, was

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induced according to the procedure described.[8,9] The animals were divided into 5 groups with 6 mice per group. Group 1 served as negative control and received 10 mL/kg of normal saline, while groups 2, 3 and 4 were pre-treated with 170, 340, and 510 mg/kg doses of *S. officinarum* leaf extract intraperitoneally, and group 5 received 100 mg/kg of acetyl salicylic acid intraperitoneally. After 30 minutes, 0.2ml of 2% acetic acid was administered intraperitoneally (i.p). The number of writhing was counted for 30 minutes. Antinociception (analgesia) was expressed as the reduction of the number of writhings between control animals and mice pretreated with extract.

Formalin-induced hind paw licking in mice

The procedure adopted was as previously described[10,11] The animals were injected with 20 µL of 2.5% formalin solution (0.9% formaldehyde) made up in phosphate buffer solution (PBS concentration: NaCl 137 mM, KCl 2.7 mM and phosphate buffer, 10 mM) under the surface of the right hind paw. The time spent licking the injected paw was timed and considered as the indication of pain. Male and female adult mice (20-25 g) randomized into five groups of 6 mice each were used for the experiment. The mice were fasted for 24 hours before being used but allowed access to water. The animals in group 1 (negative control) received 10 mL/kg of normal saline, groups 2-4 received 170, 340, and 510 mg/kg doses of the extract, while group 5 received 100 mg/kg of acetyl salicylic acid (ASA) 30 minutes intraperitoneally before being challenged with buffered formalin. The responses were measured for 30 minutes after formalin injection.

Thermally induced pain in mice

The effect of extract on hot plate-induced pain was investigated in adult mice. The hot plate was used to measure the response latencies[11]. he hot plate was maintained at 45±1˚C, each animal was placed into a glass beaker of 50 cm 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. 30 second cut off time was used to prevent tissue damage. The animals were randomly divided into 5 groups of 6 mice each and fasted for 24 hours but allowed access to water. Group 1 served as negative control and received 10 mL/kg of normal saline. Groups 2, 3 and 4 were pretreated intraperitoneally with 170, 340, and 510 mg/kg doses of *S. officinarum* leaf extract respectively, while group 5 animals received 100 mg/kg of acetyl salicylic acid intraperitoneally, 30 minutes prior to the placement on the hot plate.

Statistical analysis

Data were analyzed statistically using ANOVA (One-way) followed by a post test[13] Differences between the means were considered significant at 1% and 5% level of significance, that is, $p < 0.05$.

Results and Discussion

Determination of Median lethal dose (LD_{50})

The median lethal dose (LD_{50}) was calculated to be 173 g/kg. The physical signs of toxicity included excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma which was followed by death.

Effect of ethanol crude extract of S. officinarum on acetic acid-induced writhing in mice

The administration of *S. officinarum* extract (170, 340, 510 mg/kg) demonstrated a non-dose-dependent reduction in acetic acid-induced writhing in mice. The reductions were statistically significant (p<0.05-0.001) relative to control and comparable to that of ASA. The low dose (170 mg/kg) was observed to exert the highest effect (Table 1).

Effect of ethanol extract of S. officinarum on formalin-induced hind paw licking in mice

The extract exhibited a non-dose-dependent analgesic effect on formalin-induced hind paw licking in mice. The extract prominently inhibited the two phases of formalin-induced pains with a more considerable inhibition of the second phase. These inhibitions were significant relative to the control (p< 0.05-0.001) and comparable to that of the standard drug, ASA. The low dose (170 mg/kg) was observed to exert the most prominent activity (Table 2).

Effect of ethanol crude extract of S. officinarum on thermally induced pain in mice

The extract (170, 340, 510 mg/kg) exhibited a dose-dependent effect on thermally induced pain in mice. These inhibitions were statistically significant (p<0.05-0.001) relative to the control but not comparable to that exerted by ASA (Table 3)**.** The extract significantly reduced acetic acid-induced writhing, formalin-induced hind paw licking and also delayed the reaction time of animals (mice) to thermally induced pain. Acetic acid causes inflammatory pain by inducing capillary permeability,[14,15] and in part intraperitoneally of PGE_2 and $PGF_{2\alpha}$[16, 17] The acetic acid-induced abdominal writhing is a visceral pain model in which the processor releases arachidonic acid and prostaglandin biosynthesis plays a role in the nociceptive mechanism.[18] It is used to distinguish between central and peripheral pain. The results suggest that the extract may be exerting its action partly through the cyclooxygenase system.

The inhibition of acetic acid-induced writhing by the extract at all doses given suggests an antinociceptive effect which might have resulted from the inhibition of the synthesis of arachidonic acid metabolites.

Formalin-induced pain involves two different types of pains which are in phases; neurogenic and inflammatory pains[12,19] and measure both centrally and peripherally mediated activities that are characteristic of biphasic pain responses. The first phase (0 to 5 min), named neurogenic phase is known to provoke the release of bradykinin and substance P, while the second and late phase initiated after 15 to 30 min of formalin injection usually results in the release of inflammatory mediators such as histamine and prostaglandin.[20, 21] The first phase of formalin-induced hind paw licking is selective for centrally acting analgesics such as morphine,[22] while the late phase of formalin-induced hind paw licking is peripherally mediated. The ability of the extract to inhibit both phases of formalin-induced paw licking suggests its central and peripheral activities as well as its ability to inhibit bradykinins, substance P, histamine and prostaglandins which are mediators in these pain. The study also shows that the extract significantly delayed the reaction time of the thermally induced (hot plate) test. This model is selective for centrally acting analgesics and indicates narcotic involvement [23]with opioid receptors.

Phytochemical screening of leaf extract of *S officinarum* reported the presence of lycosides, phytosterols, saponins, tannins, flavonoids,[2] Some of these phytoconstituents found to be present in the leaf extract in this study may be responsible for the observed reported activity. Flavonoids are known to act through inhibition of the cyclooxygenase and lipoxygenase pathways,[24, 25] phospholipase A_2 and phospholipase C[26] Some flavonoids exert their antinociception via opioid receptor activation activity.[27,29]

Table 1: Effect of *Saccharum officinarum* leaf extract on acetic acid-induced writhing in mice

Treatment/ Dose (mg/kg) Extract	Time Intervals (hr) 5	10	15	20	25	30	Total
Control	6.66±0.88	11.66±1.20	24.66± 1.85	17.0± 0.57	13.00±1.15	11.0± 1.00	83.98±6.65
170	7.00± 1.73	0.00±0.00 ^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00±0.00^{c}	0.00± 0.00^{c}	7.00.
340	0.00± 0.00	12.33± 3.71	9.00±1.00^{c}	13.00 ± 0.33^{c}	6.00±0.57^{a}	5.66±0.66^{a}	45.99±6.27^{c}
510	3.33± 0.33^{a}	5.33±0.33 ^{b}	5.33±0.66^{c}	5.33± 0.66^{c}	5.00±1.00^{b}	3.33± 0.20^{c}	27.65±3.18^{c}
ASA 100	1.00±0.57^{c}	2.00±0.57 ^{c}	8.00± 0.58^{c}	7.66± 0.13^{c}	6.66±0.36^{a}	4.00 ± 0.57^{c}	29.32±2.78^{c}

Data are expressed as mean ± SEM. significant at $^{a}p< 0.05$, $^{b}p < 0.01$, $^{c}p < 0.001$ when compared to control. n = 6.

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Table 2: Effect of *Saccharum officinarum* leaf extract on formalin-induced hind paw licking in mice

Treatment/ Dose (mg/kg) Extract	Time Intervals (hr) 5	10	15	20	25	30	Total
Control	16.33±0.33	18.46±0.88	20.54±0.14	16.64±0.41	12.44± 0.24	10.86±0.20	95.27±2.20
170	5.66± 0.24[c]	1.00±1.00 [c]	0.00±0.00[c]	0.00±0.00[b]	0.66±0.33[c]	0.00± 0.00[c]	7.32.
340	14.33± 2.03	6.33± 0.88	7.33±1.76[c]	8.00 ± 0.64[c]	5.00±0.57[c]	2.66±0.33[c]	43.65±6.2[c]
510	12.33± 0.88[a]	3.33±0.33[c]	0.33±0.33[c]	1.33± 0.66[c]	1.66±0.66[c]	1.00± 0.57[c]	19.98±3.43[c]
ASA 100	7.66±0.20[c]	0.33±0.33[c]	2.66± 0.88[c]	2.00± 0.00[c]	1.66±0.33[c]	3.00 ± 0.00[c]	17.31±3.58[c]

Data are expressed as mean ± SEM. significant at [a]p< 0.05, [b]p< 0.01, [c]p< 0.001 when compared to control. n = 6.

Table 3: Effect of *Saccharum officinarum* leaf extract on thermally induced pain in mice

Group	Dose Mg/kg	Reaction time (sec) (mean ± SEM)	%
Control	-	4.92 ± 0.23	
S. officinarum	170	10.66 ± 0.85 [a]	116.
	340	23.33 ± 0.50[b]	374.
	510	25.66 ± 0.72[b]	421.
ASA	100	30.00 ± 0.00[b]	

Data are expressed as mean ± SEM. Significant at [a]p< 0.05, [b] p< 0.001 when compared to control. n = 6.

Conclusion

The extract has been reported to exhibit analgesic activity. The presence of these compounds (polyphenolics and flavonoids) in this plant might account for the activity and may in part explain the mechanisms of its actions. The results of this study demonstrated that *S. officinarum* possesses analgesic properties.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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