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Antinociceptive and Anti-Inflammatory Effects of *Stachytarpheta jamaicensis* (L.) Vahl (Verbenaceae) in Experimental Animal Models

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Key Words
Stachytarpheta jamaicensis · Antinociceptive activity · Anti-inflammatory activity · Opioid mechanism

Abstract
Objective: The present study was carried out to explore the antinociceptive as well as the anti-inflammatory effects of an ethanol extract of *Stachytarpheta jamaicensis* (L.) Vahl (EESJ) using 3 models of nociception and 2 models of inflammation in experimental animals. ***Materials and Methods:*** EESJ was prepared by overnight soaking of the oven-dried (50°C; 72 h) ground leaves (500 g) in 80% ethanol (1:5; w/v). The filtrate was evaporated to dryness (50°C), resuspended in distilled water at concentrations to provide the desired doses of 50, 100 and 150 mg/kg. For antinociceptive effects, 3 models were used: acetic acid-induced abdominal writhing, hot-plate- and formalin-induced paw-licking tests; for anti-inflammatory effects, 2 models were used – carrageenan-induced paw edema and cotton-pellet-induced granuloma tests. Appropriate doses were administered intraperitoneally (i.p.) to mice/rats prior to each test. The mechanisms of antinociceptive action of the extract were also investigated by pretreatment with naloxone (5 mg/kg, i.p.). ***Results:*** The extract exhibited significant ($p < 0.05$) antinociceptive activity in all nociceptive models tested with dose-dependent activity observed using the abdominal writhing and formalin tests. Pretreatment with naloxone partially, but significantly ($p < 0.05$) reversed the antinociceptive activity of the extract when assessed using the abdominal-writhing- and formalin-induced paw-licking tests, and completely inhibited its activity when the hot-plate test was used. The extract also showed significant ($p < 0.05$) anti-inflammatory activity in both the acute (carrageenan-induced paw edema test) and the chronic (cotton-pellet granuloma test) tests. ***Conclusion:*** This study showed the potential of EESJ to exert antinociceptive and anti-inflammatory activities, the former being modulated via peripheral and central mechanisms and involving, in part, activation of the opioid receptor system.

Introduction

Contemporary analgesics such as opiates and nonsteroidal anti-inflammatory drugs may not always be suitable for all patients, particularly those with chronic pain, due to limitations of potency, side effects and lack of tolerability, hence the continuing search for other alternatives [1, 2]. Medicinal plants and herbs were the earliest sources of substances used to produce therapeutic effects.

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Herbal medicine has been found to have some impressive credentials, which have no equivalent in modern medicine [3]. Therefore, research into plants that are employed as pain relievers in traditional ethno-medicine is one of the productive and logical strategies in the search for new analgesic drugs.

In Malaysia, many plants and their by-products are still being extensively used as an alternative in traditional treatments. *Stachytarpheta jamaicensis* (L.) Vahl, a member of the Verbenaceae family, is one of the plants claimed to possess medicinal properties that have been traditionally used for centuries in various parts of Southeast Asia, including Malaysia, Indonesia, the Philippines and Brunei, to treat malaria and rhinitis, reduce fever and suppress coughs and sores [4]. Known locally to the Malays as 'selasih dandi', *S. jamaicensis* is also used as a remedy for pain and in Sabah, Malaysia, the entire plant is boiled and the decoction applied to treat sprains [5]. The leaves of *S. jamaicensis* have also been used in some Nigerian communities for treating ailments such as diabetes, hypertension and bacterial infections [6].

S. jamaicensis has been shown to possess antihemorrhagic and inhibitory effects against snake venoms [7], inhibit the extracellular release of oxygen radicals and production of nitric oxide [8]; it was also demonstrated to have O_2-scavenging activity [8]. Other reported pharmacological effects include reduction of motor activity, sedation, ataxia, analgesia and anesthesia, and its active compounds iridoid ipolamiide and verbascoside have been successfully isolated from its leaves [9]. *S. cayennensis* leaves have also been reported to exhibit anti-inflammatory and antinociceptive activities elicited by two compounds, iridoid ipolamiide and acetoside, isolated from the leaves of the plant [10]. *S. jamaicensis* [6] has also been found to cause mild non-dose-dependent systemic activity in some specific tissues (liver, kidney, lung and testis) with no effects seen in the brain, eyes, intestine and heart tissues. *S. jamaicensis* extracts have also been demonstrated to cause toxicity to the *Aedes aegypti* mosquito [11].

Although the reported analgesic effect of aqueous extracts of the leaves of *S. jamaicensis* [9] is believed to be caused by the polar/hydrophilic compounds, the present study was carried out on ethanol extracts. These contain more lipophilic compounds, as we previously reported for *Channa striatus* extract [12]. Other than the mechanism of action of this plant extract observed by Melita Rodriguez and Castro [9], no further investigations have been undertaken on this topic, particularly regarding the involvement of opioid receptors. Hence, the present study was carried out to investigate the antinociceptive and anti-inflammatory effects of ethanol extracts of *S. jamaicensis* (EESJ) and to determine the possible involvement of opioid receptors in their activity.

Materials and Methods

Preparation of Extract

Fresh whole plants of *S. jamaicensis* were collected from their natural habitat in Sitiawan, Perak. The plants were cleaned with tap water to remove soil and left to dry for 1 week in the shade. The leaves were separated from the stems and carefully air-dried in an oven at a temperature of 50°C for 3 days. The dried leaves were then ground into a powder and 500 g of the powdered leaves were soaked overnight in 80% ethanol (1:5; w/v) at room temperature. The resultant mixture was filtered using Whatman No. 1 filter paper and the filtrate was concentrated to dryness in vacuo at 50°C using a Buchi Rotavapor R-200 to yield approximately 6% (w/w) of crude dried extract. The thick dark green paste of EESJ was kept refrigerated (4°C). Immediately before use, the EESJ was dissolved in distilled water at concentrations required to produce doses of 50, 100 and 150 mg/kg and administered (10 ml/kg, i.p.) before subjecting animals to the respective assays.

Experimental Animals

Adult male Balb/c albino strain mice (weighing between 25–35 g) and male Sprague-Dawley rats (weighing between 160–220 g) were kept in standard cages at 22 ± 2°C, 70–80% humidity on a 12/12-hour light/dark cycle in the animal house of the Faculty of Medicine and Health Sciences, UPM. The animals were first acclimatized to the laboratory environment for at least 1 week before being used in the experiments. They were provided a pellet diet and tap water ad libitum.

Drugs and Reagents

Drugs and fine chemicals were purchased from Sigma-Aldrich, USA. All preparations were freshly made in distilled water prior to the experiments.

Antinociceptive Assays

Acetic-Acid-Induced Abdominal Writhing Test. The abdominal writhing test was carried out using slight modifications of the procedure described previously by Dambisya and Lee [13]. Selected mice (n = 10) were each injected with normal saline, acetylsalicylic acid (ASA; 100 mg/kg, i.p.) or EESJ (50, 100 or 150 mg/kg). A separate group was pretreated with naloxone (5 mg/kg, i.p.) 10 min prior to the administration of the EESJ (150 mg/kg, i.p.). The 5 mg/kg naloxone dose chosen for the present study had been previously proven in our laboratory to cause no significant effect when administered alone or following pretreatment with 100 mg/kg ASA, but was found to significantly reverse the 5 mg/kg morphine antinociceptive activity assessed by the abdominal-writhing and hot-plate tests (data not shown). Thirty minutes after the pretreatment, each group was administred 0.6% acetic acid (10 ml/kg) and the mice were placed in transparent Perspex observation boxes. After a 5-min lag period following the administration of acetic acid, the presence of contractions of the abdomi-

nal muscle together with stretching of hind limbs (writhing effect) were cumulatively counted for 25 min. The number of writhing and stretching movements was used to express the percentage of analgesia using the following ratio:

$$\text{Percentage of analgesia} = \frac{(\text{control group}) - (\text{test group mean})}{\text{control group mean}} \times 100$$

Hot-Plate Test. Before the experiment was carried out, a group of mice was subjected to the selection process in which untreated mice were placed on the Ugo Basile 7280 hot-plate set at 53 ± 0.5°C. Only those mice with response latency times between 5 and 7 s were selected for this study. The hot-plate test was performed with some modifications using the method previously described [14]. The selected mice (n = 6) were treated with normal saline, ASA (100 mg/kg, i.p.), morphine (5 mg/kg) or EESJ (50, 100 or 150 mg/kg) 30 min prior to the test. A separate group of mice was pre-treated with naloxone (5 mg/kg, i.p.) 10 min prior to the administration of 150 mg/kg EESJ and 30 min later was subjected to the test. The latency time of response to discomfort, observed when the animal started licking their fore- or hind-paws or jumped when placed on the hot plate, was recorded immediately (0 min) and at 30, 60, 90, 120, 150, 180 and 210 min after the administration of each test solution. Prolongation of the latency time was taken as an indicator of antinociceptive activity. A cut-off time of 20 s was used to prevent excessive tissue damage to the animals. The prolongation of the latency times, compared with values of the control animals, was used for statistical comparison.

Formalin-Induced Paw-Licking Test. The method described by Adzu et al. [15] was used with slight modifications. Rats were placed in the observation chamber for an initial 20 min of accommodation to familiarize them with their surroundings. Selected rats (n = 6) were treated with normal saline, ASA (100 mg/kg, i.p.), morphine (5 mg/kg) or EESJ (50, 100 or 150 mg/kg) 30 min prior to the intraplantar injection of 50 μl of 2.5% formalin into the rat's right hind paw. A separate group was pretreated with naloxone (5 mg/kg, i.p.) 10 min prior to the administration of EESJ (150 mg/kg, i.p.). The amount of time the animal spent licking and biting the injected paw was counted following the injection of formalin and this was taken as a measure of pain response. Responses were recorded for the early and late phases, which were between 0–5 and 15–30 min, respectively, after formalin administration. The percentage of analgesia was calculated by the formula given below:

$$\text{Percentage of analgesia} = \frac{(\text{control group}) - (\text{test group mean})}{\text{control group mean}} \times 100$$

Anti-Inflammatory Assays

Carrageenan-Induced Paw Edema Test. A slight modification of the procedure previously described by Winter et al. [16] was used. Rats were divided into 5 groups (n = 6) and received normal saline, ASA (100 mg/kg, i.p.), or EESJ (50, 100 and 150 mg/kg) followed 30 min later by intraplantar administration of 0.05 ml of 1% carrageenan suspension into the right hind paw. Paw volume was measured before (V_0) and at 1, 2, 3, 4 and 5 h (V_f) following the carrageenan injection using a plethysmometer (Model 7140; Ugo Basile, Italy). The degree of inflammation was quantified by measuring the volume displaced by the paw between the final volume (V_f) and the initial volume (V_0). The percentage of anti-inflammation was calculated using the formula given below:

$$\text{Percentage of anti-inflammation} = \frac{(V_f - V_o)_{\text{control}} - (V_f - V_o)_{\text{treated}}}{(V_f - V_o)_{\text{control}}} \times 100$$

Cotton-Pellet-Induced Granuloma Test. The procedure described by Gupta et al. [17] was used with slight modifications. Cotton pellets, weighing around 50 mg each, were autoclaved for 2 h. The autoclaved cotton pellets were implanted aseptically at intrascapular distance under the skin on the shaved backs of anesthetized rats, divided into 5 groups (n = 6) which received normal saline, ASA (100 mg/kg, i.p.) or EESJ (50, 100 or 150 mg/kg). All test solutions were given once a day for a period of 7 days and the rats were sacrificed on the 8th day. The moist cotton pellets, surrounded by granuloma tissue were dissected, weighed, dried at 60°C and weighed again. The percentage of anti-inflammatory activity of EESJ, indicated by its ability to reduce the development of granuloma tissue, was calculated using the equation:

$$\text{Percentage of anti-inflammation} = \frac{(T_C - T_T)}{T_C} \times 100$$

where T_C = weight of granuloma tissue of control group and T_T = weight of granuloma tissue of treated group.

Statistical Analysis

The results were expressed as means ± SEM. All data were subjected to one-way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test among groups, and Student's t test for comparison between two groups. p values <0.05 ($p < 0.05$) were taken as the limit of significance in both cases. All statistical analyses were carried out using the SPSS 11.0.0 for Windows (SPSS Inc., Chicago., Ill., USA).

Results

Acetic-Acid-Induced Abdominal Writhing Test

The results of the acetic-acid-induced abdominal writhing test are given in table 1. All doses of EESJ used exhibited significant ($p < 0.05$) dose-dependent antinociceptive activity, as indicated by the reduction in the number of abdominal writhing movements. A minimum level of analgesia (34.9% inhibition of writhing) was observed at the 50 mg/kg dose of EESJ, while maximum analgesia (79.2% inhibition of writhing) was found with a dose of 150 mg/kg EESJ. In addition, the antinociception produced by 100 mg/kg EESJ was as effective as the 100 mg/kg ASA with the percentages inhibition of abdominal writhing being 57.8 and 61.5%, respectively. On

Table 1. Antinociceptive activity of EESJ assessed using the acetic-acid-induced abdominal writhing test

Treatment	Dose, mg/kg	Writhing movements, mean ± SEM (n = 6)	Inhibition, %
Saline	–	123.9 ± 8.00	–
ASA	100	47.7 ± 9.3[a]	61.5
	50	80.7 ± 11.2[a]	34.9
EESJ	100	52.3 ± 6.5[a]	57.8
	150	25.8 ± 5.5[a]	79.2
Naloxone 5 mg/kg followed by EESJ 150 mg/kg		60.5 ± 8.8[a, b]	51.2

[a] Significant difference ($p < 0.05$) compared to the saline control group.
[b] Significant difference ($p < 0.05$) compared to the 150 mg/kg EESJ-treated group.

Table 2. Antinociceptive activity of EESJ assessed using the hot-plate test

Treatment	Dose, mg/kg	Latency time of discomfort reaction, s, mean ± SEM (n = 6)							
		0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min
Saline	–	5.9 ± 0.4	5.9 ± 0.3	6.4 ± 0.2	6.2 ± 0.2	6.7 ± 0.3	6.5 ± 0.3	6.0 ± 0.3	6.3 ± 0.2
ASA	100	6.5 ± 0.4	6.5 ± 0.3	6.6 ± 0.2	7.1 ± 0.2	7.3 ± 0.4	7.3 ± 0.3	7.4 ± 0.36	7.7 ± 0.5
Morphine	5	5.7 ± 0.2	15.7 ± 1.1[a]	17.6 ± 1.1[a]	18.3 ± 0.8[a]	16.3 ± 1.1[a]	13.8 ± 1.5[a]	11.3 ± 1.2[a]	10.4 ± 0.7[a]
EESJ	50	6.8 ± 0.3	6.6 ± 0.7	6.9 ± 0.4	6.9 ± 0.3	6.6 ± 0.5	7.00 ± 0.4	8.0 ± 0.5	7.8 ± 0.2
	100	5.8 ± 0.3	7.6 ± 0.7	7.4 ± 0.4	7.7 ± 0.3[a]	8.4 ± 0.5	7.3 ± 0.4	8.6 ± 0.4[a]	7.8 ± 0.9
	150	6.1 ± 0.4	8.5 ± 0.4[a]	9.0 ± 0.5[a]	8.7 ± 0.5[a]	9.7 ± 0.9[a]	9.5 ± 0.7[a]	9.8 ± 0.6[a]	8.1 ± 0.7
Naloxone 5 mg/kg followed by EESJ 150 mg/kg		6.4 ± 0.4	6.5 ± 0.3[b]	7.1 ± 0.3[b]	7.4 ± 0.3	7.6 ± 0.4[b]	7.0 ± 0.4[b]	7.3 ± 0.5[b]	7.2 ± 0.5

[a] Significant difference ($p < 0.05$) compared to the saline control group.
[b] Significant difference ($p < 0.05$) compared to the 150 mg/kg EESJ-treated group.

the other hand, pretreatment of the 150 mg/kg dose of EESJ with 5 mg/kg naloxone significantly reduced the antinociceptive effect of EESJ from 79.2 to 51.2% ($p < 0.05$) .

Hot-Plate Test

The results obtained from the hot-plate test are shown in table 2. Administration of 50 mg/kg EESJ into mice failed to produce any significant antinociceptive effect, while the 100 mg/kg EESJ exhibited inconsistent activity in which the significant ($p < 0.05$) activity was observed only at the intervals of 90 and 180 min. Only the 150 mg/kg dose of EESJ caused significant ($p < 0.05$) increments in the latency times of reaction when administered to mice, which started at 30 min and lasted until 180 min after the EESJ administration. The antinociceptive activity of 150 mg/kg EESJ was also partially suppressed after pretreatment with 5 mg/kg naloxone. The highest latency time of reaction was produced by morphine (5 mg/kg), which lasted until the end of the experiment. The maximal antinociceptive effect of this dose of morphine was obtained 90 min after its administration to mice. On the other hand, treatment with 100 mg/kg ASA failed to show any significant increment in latency time to discomfort.

Formalin-Induced Paw-Licking Test

The results of the formalin-induced paw-licking test are shown in table 3. The antinociceptive activity of the extract was observed in both the early and late phases. In the early phase, the 100 and 150 mg/kg doses of EESJ significantly ($p < 0.05$) reduced the paw-licking time, 54.6

and 64.1%, respectively, while the 50 mg/kg dose of EESJ failed to produce any statistically significant antinociceptive effect during this phase. In the late phase, all the doses of EESJ used showed significant ($p < 0.05$) analgesic effects, as reflected in reductions in the paw-licking latency. The reductions at 50, 100 and 150 mg/kg were 66.1, 75.4 and 92.8%, respectively. In addition, the extent of analgesia produced by the 150 mg/kg dose of extract following pretreatment with 5 mg/kg naloxone was reduced in both the early and late phases. During the early phase, it was reduced from 64.1 to 46.2% while during the late phase the reduction ranged from 92.8 to 69.5%. The 5 mg/kg dose of morphine was effective in both phases (65.0 and 95.8% analgesia, in the early and late phases, respectively). In contrast, administration of 100 mg/kg ASA was only effective in the late phase (66.5% analgesic effect).

Table 3. Antinociceptive activity of EESJ assessed using the formalin-induced paw-licking test

Treatment	Dose, mg/kg	Licking time, s, mean ± SEM (n = 6)		Analgesia, %	
		early phase (0–5th min)	late phase (15–30th min)	early phase	late phase
Saline	–	46.4 ± 6.1	116.6 ± 20.00	–	–
ASA	100	35.4 ± 10.5	39.1 ± 20.3[a]	23.6	66.5[a]
Morphine	5	16.2 ± 0.9[a]	4.9 ± 0.7[a]	65.0[a]	95.8[a]
EESJ	50	30.2 ± 6.6	39.5 ± 16.4[a]	35.0	66.1[a]
	100	21.1 ± 3.7[a]	28.7 ± 11.5[a]	54.6[a]	75.4[a]
	150	16.7 ± 6.8[a]	8.4 ± 3.8[a]	64.1[a]	92.8[a]
Naloxone 5 mg/kg followed by EESJ 150 mg/kg		25.0 ± 6.6[b]	35.6 ± 21.1[a, b]	46.2[b]	69.5[a, b]

[a] Significant difference ($p < 0.05$) compared to the saline control group.

[b] Significant difference ($p < 0.05$) compared to the group treated with 150 mg/kg EESJ.

Carrageenan-Induced Paw Edema Test

The anti-inflammatory activity of EESJ against an acute model of inflammation (the carrageenan-induced paw edema test) is shown in table 4. All doses of EESJ exhibited significantly ($p < 0.05$) dose-dependent anti-inflammatory activity, which was observed 30 min after the administration of extract. While the EESJ anti-inflammatory activity at the dose of 50 mg/kg subsided by the 5th interval time, the 100 and 150 mg/kg doses of extract remained above 50% until the end of the experiment.

Cotton-Pellet-Induced Granuloma Test

The anti-inflammatory activity of EESJ against a chronic model of inflammation (the cotton-pellet-induced granuloma test) is shown in table 5. Of all doses used, only the 150 mg/kg dose of EESJ exhibited significant ($p < 0.05$) activity against chronic inflammation, as indicated by the reduction in the weight of the granuloma-bearing cotton pellets. The percentage anti-inflammatory activity recorded after treatment with the 150 mg/kg dose of EESJ was 18.4% (as measured by the percent inhibition of granuloma accumulation), while ASA (100 mg/kg) produced a corresponding inhibition of approximately 23%.

Discussion

The present study was aimed at providing a scientific authentication for the traditional use of *S. jamaicensis* (L.) Vahl to alleviate pain. The antinociceptive activity of the *S. jamaicensis* crude ethanol extract was investigated

Table 4. Anti-inflammatory activity of EESJ assessed using the carrageenan-induced paw edema test

Treatment	Dose, mg/kg	Edematous paw volume, ml, mean ± SEM (n = 6)					Anti-inflammation, %				
		1 h	2 h	3 h	4 h	5h	1 h	2 h	3 h	4 h	5 h
Saline	–	1.03 ± 0.07	1.10 ± 0.10	1.07 ± 0.11	1.00 ± 0.09	0.81 ± 0.12	–	–	–	–	–
ASA	100	0.60 ± 0.09[a]	0.58 ± 0.08[a]	0.71 ± 0.09[a]	0.77 ± 0.13[a]	0.49 ± 0.10[a]	41.8	47.3	33.6	23.0	39.5
EESJ	50	0.65 ± 0.07[a]	0.72 ± 0.07[a]	0.76 ± 0.06[a]	0.81 ± 0.04[a]	0.77 ± 0.05	36.9	34.6	29.0	19.0	4.9
	100	0.40 ± 0.05[a]	0.48 ± 0.03[a]	0.48 ± 0.07[a]	0.43 ± 0.08[a]	0.30 ± 0.08[a]	61.2	56.4	55.1	57.0	63.0
	150	0.35 ± 0.06[a]	0.43 ± 0.05[a]	0.43 ± 0.05[a]	0.41 ± 0.08[a]	0.37 ± 0.06[a]	66.0	60.9	59.8	59.0	54.3

[a] Significantly different ($p < 0.05$) when compared to the control group (saline-treated) at their respective time interval.

Table 5. Anti-inflammatory activity of EESJ assessed using the cotton-pellet-induced granuloma test

Treatment	Dose, mg/kg	Weight of cotton pellet, mg, mean ± SEM (n = 6)	Inhibition, %
Saline	–	83.3 ± 4.5	–
ASA	100	64.3 ± 1.7[a]	22.8
EESJ	50	76.3 ± 8.3	8.4
	100	73.5 ± 6.3	11.8
	150	68.0 ± 4.4[a]	18.4

[a] Significantly different ($p < 0.05$) when compared to the saline control group.

using experimental models that employed chemical- or thermal-induced nociception, which at the same time were used to determine the effectiveness of the extract on inflammatory-mediated nociception (abdominal writhing test), non-inflammatory-mediated nociception (hot-plate test) or both types of nociception (the formalin-induced paw-licking test). The acetic-acid-induced abdominal writhing test is a very sensitive method to detect antinociceptive effects of compounds at dose levels that may appear ineffective in other models [1, 2]. However, this test shows poor specificity because the abdominal writhing response can be suppressed by muscle relaxants and other types of drugs, which could lead to misinterpretation of the results [18]. Thus, additional testing using other pain models was considered necessary. The hot-plate test is a well-known method for assessing acute heat pain sensitivity in rodents. This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics [1, 2]. The heat stimulation sensitizes peripheral nerve endings and the impulses generated propagate to the brain via the spinal cord. Hence, this test is primarily used to evaluate the capability of a substance to inhibit pain of central origin [18]. The formalin-induced paw-licking test was carried out to further strengthen the evidence of the antinociceptive activity of the extract seen in both the abdominal writhing and hot-plate tests. The formalin test is considered as a valid and reliable model of persistent nociception [19] and involves two distinct phases, a neurogenic pain that corresponds to the early phase, followed by an inflammatory pain that is accompanied by the release of inflammatory mediators designated as the late phase [20]. Drugs that act primarily on the CNS (such as morphine) inhibit both phases equally, while peripherally acting drugs such as ASA inhibit only the late phase [15]. Based on our observations, EESJ possesses antinociceptive activity against chemically and thermally induced nociception, and against both inflammation- and non-inflammation-mediated nociception.

Several mechanisms of antinociceptive activity could be suggested based on the antinociceptive effects demonstrated by EESJ. Prostaglandin may be one of the nociceptive mediators inhibited by EESJ at both peripheral and central levels, since the extract affected the abdominal writhing test, which has been linked to prostaglandin (PGE_2 and $PGF_2\alpha$)-mediated nociception [18]. The hot-plate test was shown partly to involve the centrally synthesized prostaglandins or cyclo-oxygenase (COX) [21, 22]. Furthermore, the ability of EESJ to affect both peripheral and central antinociceptive mechanisms was confirmed by its positive effects in the hot-plate test [23] and in both phases of the formalin test [24]. In addition, the selective opioid antagonist naloxone was found to partially inhibit the antinociceptive activity of the extract, thus suggesting the involvement, at least in part, of opioid receptors in the extract's antinociceptive activity [23, 24]. The opioid-dependent component of EESJ is suggested to be mediated at both peripheral and central levels based on the ability of naloxone to partially inhibit the activity of the extract in the three antinociceptive models [25, 26].

EESJ was also found to exhibit anti-inflammatory activity in both acute and chronic models of inflammation. The carrageenan-induced rat paw edema test, which is considered to be an acute model of inflammation [27], has been widely used to study the potential anti-edematous properties of natural products/drugs [16, 25]. Gamache et al. [28] have earlier reported that the carrageenan-induced inflammation is a COX-dependent response and is more effectively controlled with inhibitors of arachidonate COX, but not arachidonate lipo-oxygenase inhibitors. This was supported by the finding of Damas et al. [29] that carrageenan induced the release of kinins, polymorphonuclear leukocytes as well as proinflammatory factors (e.g. prostaglandins) that contribute to the development of edema. The significant anti-edematous activity exhibited by EESJ seems to suggest that the extract can inhibit arachidonate COX [28], which is consistent with its activity in the abdominal writhing test, a prostaglandin-mediated nociceptive process. Furthermore, this finding is in agreement with the claim made by Attaway and Zaborsky [30] that compounds with anti-inflammatory activity also possess antinociceptive activity. The cotton-pellet granuloma model was established to study

the chronic inflammatory reaction and has also been employed to assess the transudative and proliferative components of chronic inflammation [31]. During the tissue repair processes associated with inflammation, there is proliferation of macrophages, neutrophils, fibroblasts and multiplication of small blood vessels that lead to the formation of a highly vascularized reddish mass, termed 'granulation tissue' [31]. The ability of EESJ to reduce the weight of granulomatous pellets suggests its potential for the treatment of chronic inflammation and as a new source of compounds with anti-arthritic activity.

Although the chemical nature of the compounds responsible for the antinociceptive and anti-inflammatory activities of the ethanol extract of *S. jamaicensis* remains speculative, scientific evidence obtained from the aqueous extract of *S. jamaicensis* leaves, which revealed the presence of analgesic-producing iridoid ipolamiide and verbascoside [9], could be involved in the observed activity. Schapoval et al. [10] have also isolated iridoid ipolamiide and acetoside from the leaves of another plant of the same genus, *S. cayennensis*, and this plant was also found to exhibit anti-inflammatory and antinociceptive activities [10].

Conclusion

The present study demonstrated the antinociceptive activity of EESJ at both peripheral and central levels as well as its ability to exert anti-inflammatory effects in both acute and chronic models of inflammation.

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