Original Research Article

Antinociceptive and anti-inflammatory activities of ethanolic stem bark extract of Smilax zeylanica Linn. in Wistar rats.

ABSTRACT

Background: Smilax zeylanica Linn (Smilacaceae) is a woody climbing shrub indigenous to the tropical and subtropical hills of Asia. Traditionally this plant is extensively used to treat numerous diseases. The stem bark of Smilax zeylanica was extracted with ethanol to evaluate their antinociceptive and anti-inflammatory effects.

Objective: To study the antinociceptive and anti-inflammatory activities of ethanolic stem bark extract of Smilax zeylanica Linn. in Wistar rats.

Methods: The antinociceptive activity has been assessed in mice by formalin-induced paw licking, Eddy's hot-plate, and acetic-acid induced abdominal stretching models. Carrageenan-induced paw oedema in rats has been conducted to explore the anti-inflammatory activity. Tween 80 (10ml/kg, p.o) was given to the control group, test drug group received ethanol extract of Smilax zeylanica (EESZ) orally at doses of 200 and 400 mg/kg, and standard drugs morphine (5 mg/kg, s.c), diclofenac sodium (10 mg/kg, i.p) were injected to the standard drug group.

Results: Oral administration of EESZ at test doses (200 and 400 mg/kg), produced a significant decrease of the paw licking time for 2 phases (neurogenic and inflammatory) in the formalin test, upsurge of the reaction time in Eddy's hot plate test, and significantly decreased the abdominal stretching in the acetic acid-induced writhes test. Ethanol extract of Smilax zeylanica significantly decreased the carrageenan-induced paw oedema.

Conclusion: The ethanol extract of Smilax zeylanica possesses antinociceptive, anti-inflammatory activity, probably involving central and peripheral pathways

Keywords: Smilax zeylanica, Antinociceptive activity, Anti-inflammatory activity, Inflammation

INTRODUCTION

Inflammatory cytokines and mediators sensitize primary afferent neurons to corresponding stimulation, resulting in pain as one of the general manifestations of inflammation. Many diseases manifest itself in the form of pain and inflammation. Inflammation and the inflammatory reactions cause pain, which is an intense physical and emotional sensation synonymous with actual or potential tissue damage.[1] Inflammation is a nonspecific immune response that benefits the host.[2] The prominent characteristic of inflammatory states is that typically innocuous stimuli cause discomfort or pain. Inflammation is both a defensive and a healing mechanism from the body to ensure that harmful stimuli are removed and that damaged tissue is repaired. Inflammation is usually distinguished by five symptoms: redness, swelling, heat, pain and loss of tissue function.

Most anti-inflammatory medications are effective at inhibiting inflammation at the moment, but they come with a slew of serious side effects, like stomach ulcers, gastrointestinal bleeding, and cardiovascular complications.[3] Because of these drawbacks, researchers have been working on alternative pain treatments.

Traditional plant-derived compounds have been used as medicine since ancient times, and they continue to play an important role in health care, especially in rural areas where modern medicine is scarce. Phytochemicals (bioactive compounds) found in plants have been shown to act as protective mechanisms against a variety of diseases. Natural products and their derivatives have been regarded as potential sources of new pharmaceutical agents for several years, as new therapies are often seen as critical in overcoming existing side effects in the management of pain.

Smilax zeylanica is a plant species belonging to the Smilax family. Its leaves and roots are used to treat various ailments. The plant is native to India, as well as Myanmar, Malaysia, Java, and the Solomon Islands. The plant contained chemicals such as diosgenin, smilagenin, sarsapogenin, β-sitosterol, hydroxytyrosol, trans-iso-eugenol and squalene.[4,5,6] The plant is used traditionally for treatment of diseases such as venereal diseases, skin diseases, abscesses, boils, psoriasis, rheumatism, swellings and dysentery. Smilax zeylanica has been studied previously for its Antiulcer activity[7], Catalepsy[8], Anti-oxidant[9], Anti-diabetic[10], Anthelmintic[11] and Anti-epileptic activity[12]. Cognitive Dysfunction.[13] and so on

However, there has been no studies on the stem bark of Smilax zeylanica for antinociceptive and anti-inflammatory activity. Therefore, the aim of our study is to see whether the ethanolic stem bark extract of Smilax zeylanica has antinociceptive and anti-inflammatory properties.

Figure 1: Smilax zeylanica

Figure 2: Stem of Smilax zeylanica

MATERIALS AND METHODS

Plant material: Smilax zeylanica (SZ) stem bark has been procured from the Tirupati hills, India. The stem bark was verified with a botanist, Dr. K. Madhava Chetty, S.V University, Tirupati. A voucher sample is preserved at the Department of Pharmacognosy, for further reference. The stem bark of the Smilax zeylanica was made into small slices and shade dried at room temperature. Afterward, the shade dried stem bark was crushed to a coarse powder by a grinding machine and pass-through (60-mesh sieve) to procure fine powder. The powder was kept in an airtight jar for extraction.

Preparation of the extract: The powder was extracted by ethanol using continuous hot percolation for 72 hours. After the extraction, by using a rotary evaporator the solvent was removed from the extract. The dried extract was stored for the subsequent experiment. The yield was 13% w/w. The extract has been dissolved in a vehicle (1% Tween 80) for oral administration.

Experimental animals: The study used healthy, adult male Wistar rats (8-12 weeks old) weighing between 180-230 grams and young male Swiss Albino mice 4–5 weeks old with 25–30grams weight, received from the animal house of Nizam Institute of Pharmacy. They were placed in standard cages at constant temperature (24 ± 1 ℃), humidity (60–65%), 12/12h light-dark cycle, and given standard diet and water ad libitum. The probe was ratified by CPCSEA and IAEC (Institutional animal ethical committee) with registration number (1330/AC/10/CPCSEA).

Acute toxicity study: The ethanol extract of Smilax zeylanica (EESZ) was examined for acute toxicity as per Organization of Economic Co-operation and Development (OECD 2001) guideline 425.[14] The safety of the Smilax zeylanica has been determined by conducting the limit test and for this study, five female Wistar rats were used. Rats fasted for

16 hr. before the drug administration (withheld food but not water). A female Wistar rat was given a single oral dose (2000 mg/kg) of EESZ and observed for death. If the 1st rat survives, the other four rats were sequentially administered orally so that all the five rats are tested. After injecting the drug, the rats were monitored separately for once while the first thirty minutes, then regularly in the period of the first 24 hours, (first four hours special attention should be given), and thereafter daily for fourteen days.

ANTINOCICETIVE ACTIVITY

Formalin test: The formalin test was performed to explore the antinociceptive effect of EESZ. The pain was generated by the injecting 0.05 ml of 2.5% formalin into the sub plantar surface of the left hind paw of mice. The mice were randomly classified into 4 groups each comprising of 6. The mice in group II and III were given EESZ (200 and 400 mg/kg, p.o), the mice in group I & IV were injected with 1% Tween 80 (1ml/100g) per oral, and morphine (5mg/kg) subcutaneously, one hour before the formalin injection. Mice were separately kept in a crystal clear (15×15×15cm) monitoring chamber after formalin injection. The paw licking time of each mouse was documented in both phases. Early Phase was noted in 0-5min, and late phase15–30 min. The mean of the time that the animal spent on paw licking was determined.

Hot plate test

The test described by Eddy and Leimbach,[15] was conducted for the evaluation the analgesic activity. The animals were randomized into 4 groups of each comprising 6. 1% tween 80 (10ml/kg) was given to group I, morphine (5 mg/kg, s.c) was given to group II animals. Group III and IV were given orally EESZ (200 and 400 mg/kg), mice were separately positioned on the hot plate 30 minutes after their respective treatments. The temperature has been retained at 55 ± 1 ∘ C throughout the experiment. The response (reaction time) for the individual mouse (Paw licking or jumping response) noted and reported in seconds. The cutoff time for the reaction was 20s. The response (reaction) time was noted for each mouse at 0 hr., 0.5, 1, 2, and 3 hrs. following the tween 80, morphine, and EESZ administration**.**

Percent analgesic activity = [(Ta – Tb) / (Tc-Tb)] × 100

Where: Ta= Reaction (response) time after treatment, Tb= Reaction (response) time before treatment, Tc= cut-off time.

Acetic acid induced writhes

The method reported by Koster was followed.[16] The animals were randomized into 4 groups with 6 each. The group I was administered 1% tween 80 (10ml/kg, p.o), group II & III were given orally EESZ (200 and 400 mg/kg), and group IV was injected diclofenac sodium (10mg/kg, i.p.). After thirty minutes of the drug treatment, 1% v/v acetic acid (0.1ml/10g body weight, i.p.) was given to the mice in all groups. After five min of acetic acid administration, the numbers of writhes were calculated for twenty min. percent inhibition of writhes was calculated as follows.

$$\text{Percent inhibition} = \frac{\text{Mean writhes control} - \text{Mean writhes test}}{\text{Mean writhes control}} \times 100$$

ANTI-INFLAMMATORY ACTIVITY

The methodology illustrated by Winter et al was performed to examine the anti-inflammatory effect of EESZ.[17] The rats classified into four different groups, consisting of six rats each. Oedema was induced by intraplantar injection of carrageenan (0.1ml of 1% in normal saline) to the right hind paw of each rat. The rats were treated with 1% tween 80 (10ml/kg p.o), diclofenac sodium (10mg/kg, i.p.), and ethanol extract of Smilax zeylanica (200 and 400 mg/kg, p.o) sixty min before carrageenan administration. The volume of paw edema was recorded at 0, 1st, 2nd, 3rd & 4th hour after carrageenan administration by 7140 plethysmometer (Ugo Basile, Italy). The percent inhibition of oedema was determined with below-mentioned equation:

$$\text{Percent inhibition} = \frac{Vv - Vt}{Vv} \times 100$$

Where: Vt and Vv were mean paw volume of test and vehicle groups.

Ethical Clearance: The ethical clearance has been taken prior to the commencent of the study by CPCSEA and IAEC (Institutional animal ethical committee) with registration number (1330/AC/10/CPCSEA).

STATISTICAL ANALYSIS: The data are demonstrated as the Mean ± SEM. One-way ANOVA followed by Tukey post hoc test was used for multiple comparision. p≤ 0.05 value set as statistical significance. For statistical analysis, data were computed by using GraphPad Prism 8.

OBSERVATION AND RESULTS

Treatment Group/ Dose	Paw licking time (S)		% inhibition	
	EP (0-5 min)	LP (15-30 min)	EP	LP
Tween 80 10 ml/kg	66.3333 ± 1.764	85.3333 ± 1.647	-	-
Morphine 5 mg/kg	22.6666 ± 1.838 a^3	23.16666 ± 1.352 a^3	65.995±2.138	72.933±1.141
EESZ 200 mg/kg	41.1666 ± 1.939a^3b^3	38.5 ± 1.746 a^3b^3	38.07±1.571	54.986±1.221
EESZ 400 mg/kg	26.8333 ± 1.759 a^3c^3	30.833 ± 1.887 a^3 b^1c^1	59.70±1.834	63.99±1.590

Table 1: Analgesic activity of EESZ in formalin test

Data represented as Mean ± SEM (n=6). One- way ANOVA (Tukey test). $^1P < 0.05$, $^2P < 0.01$, and $^3P < 0.001$. EP: Early Phase, LP: Late Phase
a: Tween 80 versus Morphine, EESZ 200, and EESZ 400.
b: Morphine versus EESZ 200 and EESZ 400.
c: EESZ 200 versus EESZ 400.

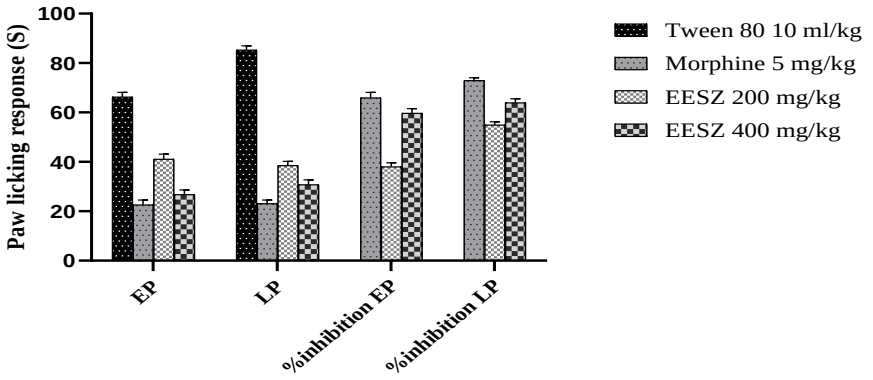

Fig. 3: Analgesic activity of EESZ in formalin test

Phytochemical screening

The phytochemical studies on Smilax zeylanica (Table 1) divulged the existence of proteins, steroids, phenols, tannins, flavonoids, glycosides, saponins, while alkaloids, carbohydrates, gums and mucilage, sterols, and terpenes are reported as absent.

Acute toxicity study

The ethanol extract of Smilax zeylanica did not shows any toxicity at 2000 mg/kg. During this study, no mortality was reported immediately as well as within the fourteen days observation. The data indicates the ethanol extract of Smilax zeylanica was safe up to a 2000 mg/kg single dose.

Formalin test

Sub plantar administration of 2.5% formalin, induced a biphasic response–Neurogenic (early phase) is an immediate intense pain response (0–5 min) and the inflammatory (late phase) is a delayed pain response (15–30 min) owing to inflammatory mediator's release. The duration of licking for the neurogenic phase was 66.3333±1.764 sec and the inflammatory phase was 85.3333±1.647 sec in the vehicle group. As presented in Table 1 and Fig. 3**,** administration of EESZ by oral, 1hr before the formalin injection produced a significant antinociceptive result. The ethanol extract of Smilax zeylanica at both test doses induce a marked reduction of the licking time in the early phase (41.1666±1.939, 37.93%, 26.8333±1.759, 61.80%) and late phase (38.5 ± 1.378, 84.33, 30.5 ± 1.517 and 64.25%), respectively. The standard drug morphine (5 mg/kg, s.c) significantly decreased licking time in both phases (22.6666±1.838, 65.83%, and 23.16666±1.352, 72.85%) respectively.

Treatment Group/Dose	Reaction time (s) (% inhibition)				
	0 h	0.5 h	1 h	2 h	3 h
Tween 80 10 ml/kg	6.50±0.7638	6.666±0.6667	6.333±0.7149	6.1666±0.6540	6.333±0.6667

Morphine 5 mg/kg	6.833±0.6009	10.1666±0.7032a[1] (25.31%)	12.1666±0.7032a[3] (40.5%)	14.5±0.6708 a[3] (55.42%)	16.667±0.5578a[3] (74.68%)
EESZ 200mg/kg	6.1666±0.7032	7.8333±0.7923 (12.04%)	9.1666±0.7032 a[1]b[1] (21.68%)	10.666±0.667 a[3] b[2] (32.53%)	11.667±0.667 a[3] b[3] (39.75%)
EESZ 400mg/kg	6.333±0.6146	8.666±0.7601 (17.07%)	10.3333±0.6146a[2] (29.26%)	11.8333±0.7032a[3] (40.24%)	13.333±0.7149 a[3] b[2] (54.87%)

Table 2: Effect of EESZ on pain induced by the hot plate method

Data represented as Mean ± SEM (n=6). One- way ANOVA (Tukey test). $^{1}P < 0.05$, $^{2}P < 0.01$, and $^{3}P < 0.001$.

a: Tween 80 versus Morphine, EESZ 200, and EESZ 400.

b: Morphine versus EESZ 200 and EESZ 400.

c: EESZ 200 versus EESZ 400.

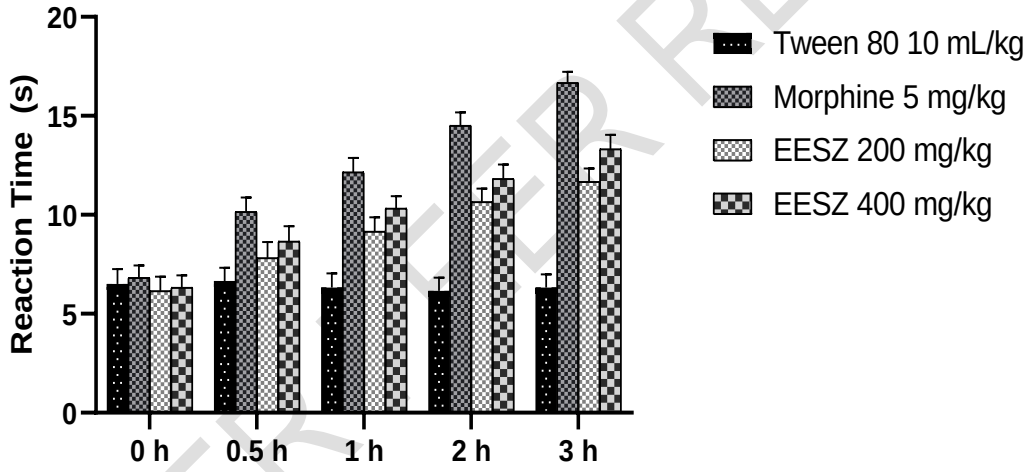

Fig. 4: Analgesic activity of EESZ on pain induced by hot plate method

Hot-plate test

The ethanol extract of the Smilax zeylanica at tested doses exhibit a significant rise in reaction time of heat sensation in mice as compared to the vehicle group (Table 2 & fig. 4). After 3 hours of drug administration, morphine increases reaction time to thermal stimuli significantly (16.667±0.5578) higher than the two doses (200 and 400 mg/kg) of EESZ, which produced mean reaction time (11.6667±0.667 and 13.333±0.7149) respectively while compared to vehicle group. The extract of the Smilax zeylanica exhibited a dose-dependent rise in reaction time in comparison with vehicle group.

Treatment Group/ Dose	Number of writhing responses	Percent inhibition
Tween 80 10 ml/kg	62.833±2.088	-
Diclofenac Sodium 10 mg/kg	16.5±1.176a[3]	73.78±1.533

EESZ 200 mg/kg	41.1666±1.167a^3b^3	34.345±1.548
EESZ 400 mg/kg	30.6666±1.333 $a^3b^3c^3$	51.175±1.491

Table 3: Effect of EESZ on acetic-acid induced writhes

Data represented as Mean ± SEM (n=6). One- way ANOVA (Tukey test). $^1P < 0.05$, $^2P < 0.01$, and $^3P < 0.001$.
a: Tween 80 versus Diclofenac Sodium 10 mg/kg, EESZ 200, and EESZ 400.
b: Diclofenac Sodium 10 mg/kg versus EESZ 200 and EESZ 400.
c: EESZ 200 versus EESZ 400.

Writhing test

The anti-nociceptive activity of Smilax zeylanica extract on acetic acid-induced abdominal constrictions showed in table 3. Vehicle group animals exhibited the highest number of writhes 62.833±2.088, treated with 1% acetic acid (i.p) in normal saline. The acetic acid-induced abdominal constrictions have been decreased significantly by Smilax zeylanica extract in a dose-dependent pattern. The percent inhibition of abdominal writhes with the EESZ at 200 and 400 mg/kg doses were 34.345±1.548 %, and 51.175±1.491% respectively, though the reference drug diclofenac sodium produced 73.78±1.533% inhibition at a dose of 10 mg/kg when compared with the vehicle (fig.5).

Treatment Group/Dose	Mean paw volume after carrageenan administration (% inhibition)				
	0 h	1 h	2 h	3 h	4 h
Tween 80 10 ml/kg	0.4733± 0.01358	0.6883±0.01222	0.9133±0.01333	1.025±0.01118	0.98833±0.01078
Diclofenac Sodium 10 mg/kg	0.48666± 0.01202	0.625±0.01384 a^1 (35.65%)	0.745±0.01118a^3 (41.28%)	0.7666±0.01687 a^3 (49.24%)	0.685±0.01607 a^3 (61.48%)
EESZ 200 mg/kg	0.47666± 0.01406	0.6733±0.01282 (8.53%)	0.8333±0.01116 a^3b^3 (18.94%)	0.895±0.01335a^3b^3 (24.17%)	0.81666±0.01022a^3 b^3 (33.98%)
EESZ 400 mg/kg	0.4683± 0.01167	0.6433±0.01542 (18.60%)	0.78833±0.01078 a^3	0.8483±0.01641 a^3b^2 (31.12%)	0.765±0.01335 a^3 b^2 c^1 (42.39%)

Table 4: Effect of EESZ on the carrageenan-induced paw edema

Data represented as Mean ± SEM (n=6). One- way ANOVA (Tukey test). $^1P < 0.05$, $^2P < 0.01$, and $^3P < 0.001$.
a: Tween 80 versus Diclofenac Sodium, EESZ 200, and EESZ 400.
b: Diclofenac Sodium versus EESZ 200 and EESZ 400.
c: EESZ 200 versus EESZ 400.

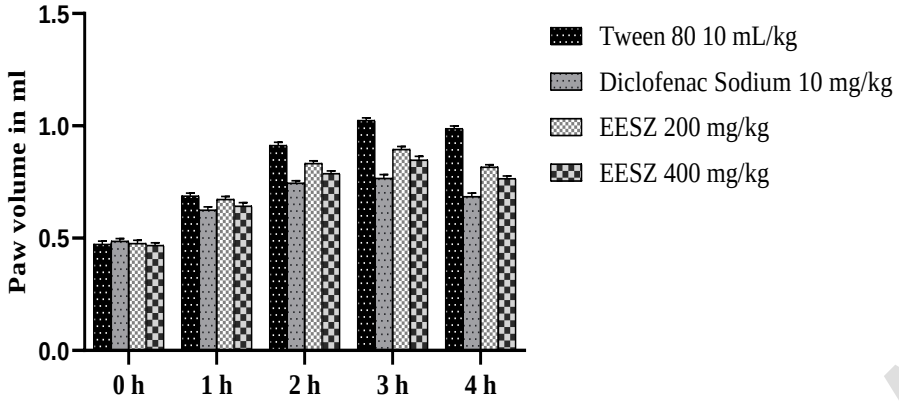

Fig. 5: Anti-inflammatory effect of EESZ in carrageenan-induced rat paw oedema test

Carrageenan-induced paw edema

Table 5 represents the anti-inflammatory effect of Smilax zeylanica ethanol extract and diclofenac sodium. Oedema paw volume in vehicle group rats was gradually increased. Although, in the test group animals, the ethanol extract of Smilax zeylanica produced a significant diminishing in paw oedema volume. As shown in (Fig. 5). Oral administrations of EESZ at doses of 200 and 400 mg/kg, one hour before carrageenan showed a dose-related inhibition of hind paw oedema. The highest inhibitory effect was observed with ethanol extract of smilax zeylanica at 400 mg /kg. The extract showed significant effects. Diclofenac sodium (10 mg/kg, i.p,) inhibited paw oedema significantly comparable to the extract. Both the extract and diclofenac showed inhibition of oedema formation 33.98%, 42.39% and, 61.48%, respectively at 4 hours after administration of carrageenan.

DISCUSSION

The quest for bioactive components that can be used as non-conventional analgesics, NSAIDs, and antipyretics has received a lot of attention recently because of the growing global need for long-term pain, inflammation, and fever treatments that are safe for humans and therefore have no side effects unlike mainstream medicine.[18] This study aimed to establish the scientific basis on which Smilax zeylanica can traditionally be used to treat pain and inflammation.

The formalin-induced pain is categorized into two phases (early and late) and serves as a useful model for studying possible mechanisms. The early phase (neurogenic pain) lasts for 0 to 5 minutes and begins directly after formalin injection due to substance P activation. The late (inflammatory pain) is due to release of numerous mediators, such as prostaglandins, serotonin, bradykinin, and histamine.[19] The licking response to formalin was the result of a combination of spinal cord sensitization and peripheral inputs. Based on the above facts the antinociceptive activity of the Smilax zeylanica extract might result from central or peripheral action.

Centrally acting analgesics inhibit both stages, while peripherally acting analgesics only inhibits the late phase. Opioids, which are centrally acting medications, work by blocking the

effects of prostaglandins released in response to inflammation, as well as their activity on the central nervous system through endogenous opioids. Acetylsalicylic acid is a peripheral analgesic that only inhibits inflammation, whereas narcotic analgesics inhibits both phases. Smilax zeylanica blocked both phases of pain, indicating that it has both peripheral and central analgesic potency. The activity of Smilax zeylanica ethanol extract may be linked to opioid receptors, cyclooxygenase, and/or lipoxygenase (arachidonic acid cascade). In the current research, an ethanol extract of Smilax zeylanica greatly reduced paw licking span in both phases, indicating the presence of compounds that function on both the peripheral and central nervous systems.

In the Hot plate test, The oral administration of Smilax zeylanica ethanol extract resulted in a strong antinociceptive effect, indicating that the action was attributed to the supraspinal mechanism. Paw licking and hopping were the components evaluated on the hot surface. Drugs that function centrally but not peripherally disband all supraspinal responses (paw licking and jumping). EESZ had an increased response time, indicating the presence of centrally acting antinociceptive constituents.

Acetic acid-induced writhing is a popular paradigm for evaluating peripheral analgesic drugs. In our study, 400 mg/kg of Smilax zeylanica extract increased reaction time to the same extent as the standard medication diclofenac. The lowest dose (200 mg/kg) did, however, yield a substantial response later in the observation period. The action of acetic acid causing writhing reaction was effectively revoked by extract of Smilax zeylanica, which may be due to blockage of prostaglandin synthesis. When contrasted to control, administration of Smilax zeylanica ethanol extract tends to decrease the abdominal constriction caused by acetic acid. This suggests that the extract has peripheral analgesic efficacy.

In the Carrageenan-induced paw oedema model which was used to test EESZ's anti-inflammatory activity. The occurrence of oedema caused by carrageenan injection resulted in a biphasic episode. In the initial phase (0 to 2 hrs.) chemical mediators such as bradykinin, serotonin and histamine played an important role, while in the late phase (post 2 hrs.) there was an increased exhibition of COX-2 and synthesis of PG’s. The 2 phases were associated by kinin release. Maximum inflammation was detected three hours after carrageenan injection, which led to the release of PG’s. COX-2 appears more during the neurogenic process, and it may later increase prostaglandin levels. In this model, the ethanol extract of Smilax zeylanica may inhibit the release of PG, an inflammatory mediator of acute inflammation. Despite the fact that the ethanol extract of Smilax zeylanica greatly reduces paw oedema in a dose-dependent manner, the inhibition was less than that of diclofenac sodium.

Divya et al.[20] investigated the anti-inflammatory activity of aqueous, ethanol, and chloroform extracts of S. zeylanica roots and rhizomes in mitigating inflammation using three different models of inflammation: carrageenan-induced acute inflammation, formalin-induced subacute inflammation, and cotton pellet-induced chronic inflammation. The ethanol and aqueous extracts exhibited a significant anti-inflammatory activity.

Research has shown that analgesic activity in many plants is due to their constituents’[20] phenols[20], steroids[20], alkaloids[20], flavonoids[21], terpenoids[22], tannins[23] and saponins[24]. As a result, the presence of steroids, phenols, tannins, saponin, and flavonoids in EESZ may justify its analgesic activity in formalin-induced pain, acetic acid-induced abdominal writhes,

and the hot plate model in our research. Thus, the study's findings suggest that an ethanol extract of Smilax zeylanica has antinociceptive and anti-inflammatory properties.

CONCLUSION

The presence of secondary metabolites such as flavonoids, glycosides, tannins, and saponins in the ethanol extract of Smilax zeylanica does explain the antinociceptive and anti-inflammatory effects. The extract elicited both peripheral and central pathways. EESZ blocked the release, synthesis or action of PG, histamine, or 5-HT.

Further studies need to be conducted in order to determine the active chemical compounds and the precise mode of action for the analgesic and anti-inflammatory activities of Smilax zeylanica ethanol extract.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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