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Mechanism of antinociceptive activity of the methanol leaf extract of *Senna italica* (Mill) in murine model of pain

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ABSTRACT

Background & Aim: *Senna italica* leaf (SIL) is extensively used in traditional medicine for the management of various types of pain including stomach cramps, back ache, joint pains, headache, and migraine. The current study was designed to scientifically investigate the purported uses of the leaves as an analgesic agent and to elucidate its possible mechanism of antinociceptive action.

Experimental: Phytochemical screening and oral acute toxicity studies were conducted using standard protocols. Antinociceptive potentials were evaluated using acetic acid-induced writhing and hot plate tests in mice. The possible pharmacological mechanism(s) involved in the anti-nociceptive activity were investigated by pretreating mice with Naloxone (2 mg/kg), L-arginine (50 mg/kg), Propranolol (20 mg/kg), Glibenclamide (10 mg/kg), and Prazosin (1 mg/kg)15 min prior to SIL (1000 mg/kg) administration, then assessed using acetic acid-induced writhing 1 h later. Data was analyzed using One-way Anova followed by Bonferroni post hoc test.

Results: Phytochemical screening revealed the presence of alkaloids, flavonoids, saponins, steroids and triterpenes. Oral median lethal dose of SIL was found to be greater than 5000 mg/kg. SIL at the doses of 250, 500 and 1000 mg/kg demonstrated significant ($P<0.05$) dose-dependent protection against acetic acid-induced writhes in mice. The extract at the highest dose (1000 mg/kg) also significantly ($P<0.05$) increased the reaction time of mice to thermal stimulus in the hot plate test. Pretreatment with naloxone, prazosin, L-arginine, and propranolol significantly ($P<0.05$) reduced the antinociceptive activity of the extract. However, pretreatment with glibenclamide showed no effect on its antinociceptive activity.

Recommended applications/industries: The findings of this research has validated the traditional use of the plant in the management of pain through possible involvement of opioidergic, α-adrenergic, β-adrenergic systems including the L-arginine/nitric oxide pathway.

1. Introduction

Pain a debilitating symptom of several medical conditions (Yasmen *et al.*, 2018), has been defined by the International Association for the Study of Pain (IASP, 2020), as "an unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage"

(IASP, 2020). In many countries worldwide, pain is considered as a major clinical, social, financial, and economical problem (Smith and Hillner, 2019; Russo and Sundaramurthi, 2019; Hutton *et al.*, 2023). Based on etiology, pain can be classified into nociceptive, neuropathic, and more recently nociplastic pain (Lee *et al.*, 2020; Fitzcharles *et al.*, 2021). Nociceptive pain results from injury or illness that affects somatic structures such as the skin, muscles, tendons, bones, and joints (Chen *et al.*, 2021); neuropathic pain is a direct consequence of an injury or disease affecting the somatosensorial system (Yasmen *et al.*, 2018), whilenociplastic pain is caused by continued inflammation and tissue lesions (Fitzcharles *et al.*, 2021). During tissue injury, inflammatory mediators including bradykinins, eicosanoids (prostaglandins and leukotrienes), adenosine triphosphate (ATP), histamine, pro-inflammatory cytokines, chemokines, neurotrophins and oxygen reactive species are released which act on nociceptors to bring about painful responses (Yasmen *et al.*, 2018; Ashagrie *et al.*, 2023).

Over the years, pain management has involved the use of a wide range of orthodox medicines including non-steroidal anti-inflammatory drugs (NSAIDs), opioid and non-opioid analgesics. However, the use of these drugs has been associated with limited efficacy and unwanted effects like gastrointestinal bleeding, auto-immune responses, drug-dependence, and other organ toxicities (Dragos *et al.*, 2017; Yekkirala *et al.*, 2017; Kim and Seo, 2020). Thus, the need to identify safer and effective alternative therapies for the management of pain. Worldwide more than 50% of drugs presently used clinically are natural products or their derivatives (Mokgotho *et al.*, 2013). Recent pharmacological research show that new more efficacious drugs and therapies may be developed through complete study of bioactive compounds found in medicinal plants (Arena *et al.*, 2022). This is evidenced by the discovery of morphine a potent opioid analgesic drug isolated from *Papaver somniferum* many years ago (Hughes, 1975).

Many traditional medicine practitioners in Nigeria have employed various plant species to treat pain (Eze *et al.*, 2019). One of such plants is *Senna italica*, a member of the Fabaceae family (subfamily Caesalpinaceae) (Randell and Barlow, 1998). The plant *Senna italica* with synonyms *Cassia italica* and *Acacia abovata* is used traditionally to treat many ailments such as constipation, stomach cramps, bacterial infection, back ache, joint pains, headache, and migraine (Qureshi *et al.*, 2010; Dabai *et al.*, 2012; Mahmoud and Gairola, 2013).Various sections of the plant have been documented for their biological properties, such as antioxidant (Jothi *et al.*, 2015), cytotoxic (Kuete *et al.*, 2013), antibacterial (Masoko *et al.*, 2010; Dabai *et al.*, 2012), and antiproliferative, antimicrobial, anticancer and antioxidant (Madhkour *et al.*, 2017; Khalaf *et al.*, 2019), antiobesity and hypoglycemic (Malematja *et al.*, 2018), antihelminthic (Mahmuda *et al.*, 2020) and antimalarial activities (Olorukooba *et al.*, 2022).

Previous chemical investigations on different parts of *S. italica* led to the separation and characterization of phytochemicals such as beta-sitosterol, stigmasterol, amyrin, physcion, chrysophanol, 10,10′-chrysophanol bianthrone, 3,4',5-trihydroxystilbene, 1,8-dihydroxy3-methylanthraquinone, 1,1,8,8′-tetrahydroxy-6′-methoxy-3,3′-dimethyl-10,10′-bianthracen-9,9′-dione, 1,1,8,8′-tetrahydroxy-7′-methoxy-3,3′-dimethyl, and 1,2-benzenedicarboxylic acid (El-Sayed *et al.*, 1992; Magano *et al.*, 2008; Mokgotho *et al.*, 2013; Yagi *et al.*, 2013). Gololo *et al.* (2016) also identified the phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol); 1,2-benzenedicarboxylic acid, mono (2-ethylheptyl) ester; n-tetracontane; 13-docosenamide; squalene (2,6,10,14,18,22-hexamethyltetracosane),1-heptacosanol; α-tocopherol-β-D-mannoside; 1,2-epoxynonadecane; stigmasterol; γ-sitosterol and lupeol from hexane extract of *Senna italica* Mill. leaves. Despite the increased number of pharmacological studies undertaken on various parts of *Senna italica* in recent years, more comprehensive information on its potential analgesic effect and possible mechanism(s) for the analgesic processes are lacking. The present study therefore aimed at validating the traditional use of *Senna italica* leaves in the management of pain and establish the possible mechanism(s) responsible for the antinociceptive activity using experimental animal models.

2. Materials and Methods

2.1. Drugs and chemicals

Naloxone, Prazosin, Glibenclamide, L-arginine and Propranolol (Abcam Biochemicals Plc, Cambridge, UK), Morphine sulphate (Martindale Pharmaceuticals, U.K), Glacial acetic acid (May and Baker Limited,

England), Chloroform, Methanol (Sigma Chemical Co. USA).

2.2. Experimental animals

Swiss albino mice of both sexes weighing between 20 - 24 g were obtained from the Animal House of the Department of Pharmacology and Therapeutics, Ahmadu Bello University, Zaria. The mice were kept in standard cages with access to water and food *ad libitum* and were exposed to a normal day and light cycle. Animals were utilized in accordance with the standards described in the National Institute of Health Principles for the Care and Use of Laboratory Animals, published in 1998.

2.3. Plant collection and identification

The intact plant of *Senna italica* was collected in the second week of May, 2022 from the vicinity of the Federal Medical Centre Nguru local government area of Yobe State, Nigeria. The plant was then taken to the Department of Botany, Ahmadu Bello University Zaria where it was ascertained by a Taxonomist - Mr. Namadi Sanusi. A voucher number ABU03106 was assigned to the plant.

2.5. Plant extraction

The leaves were removed, cleaned, dried in shade, then pulverized with a mortar and pestle. The powdered leaves (600 g) were subjected to cold maceration with 2.5 L of 70% *v/v* methanol (70% absolute methanol and 30% water) for 72 h with continuous mixing. The mixture was filtered using Whatman size No. 1 filter paper and the filtrate obtained was concentrated with rotary evaporator (Azwanida *et al.*, 2015). The extractive yield was calculated, and the extract was put in an airtight jar, labeled as *Senna italica* methanol leaf extract (SIL). The jar was stored in a desiccator until needed for the study.

2.6. Preparation of extract and drugs

The extract and drugs were made as stock solutions by dissolving a predetermined amount in distilled water, followed by repeated dilution to get the necessary concentrations for the studies. Extract and drug solutions were freshly prepared daily before each experiment to maintain stability.

2.7. Phytochemical screening

Qualitative phytochemical screening of SIL was carried out using standard phytochemical assays as described by Soforowa (2008) and Evans (2009).

2.7.1. Test for alkaloids

A quantity (3 mL) of the leaf extract was placed in a test tube and 1mL HCl was added to it. The mixture was then heated gently for 20 min, cooled, and filtered. Two drops of Dragendorff's reagent were added to 1 mL of concentrated extract, A rose red precipitate indicates the presence of alkaloids.

2.7.2. Test for saponins

Frothing test: Two milliliters (2 mL) of extract was placed in a test tube and shaken vigorously for 2 min and allowed to stand for 30 min, a honeycomb froth formed for more than 30 min indicates the presence of saponins.

2.7.3. Test for glycosides

Keller-killiani's test for cardiac glycoside: A small portion of the extract was dissolved in glacial acetic acid containing traces of ferric chloride. The test tube was held at an angle of 450, and 1 ml of concentrated sulphuric acid was added down the side of the test tube. A purple ring color at the interface indicates the presence of cardiac glycosides.

2.7.4. Test for tannins

Ferric chloride test for tannins: To the sample of the extract, 10 mL of distilled water was used to dissolve it which was then filtered. Few drops of ferric chloride were added to the filtrate. Formation of blue-black precipitate indicates the presence of hydrolysable tannins, and a green precipitate indicates the presence of condensed tannins.

2.7.5. Test for steroids

Five drops of concentrated H_2SO_4 were added to 1 mL of the leaf extract. Development of a red coloration is indicative of a positive reaction.

Salkowski test for steroids and triterpenes: 2 mL of chloroform water were added to small quantity of test extract and 1 mL of sulphuric acid was added to it from the side of the test tube to form a lower layer. A reddish-brown coloration at the inter-phase indicates the presence of steroidal nucleus.

2.7.6. Test for carbohydrates

Molisch's test for carbohydrates: To a small portion of the extract in a test tube, few drops of Molisch reagent (alcoholic alpha naphthol) were added and 1 ml of conc. Sulphuric acid down the side of the test tube. Formation of a red ring at the interphase indicates the presence of carbohydrates.

2.7.7. Test for anthraquinones

Bontrager's test for anthraquinones: A little quantity of the extract was boiled with 10 mL of aqueous sulphuric acid and filtered hot. The filtrate was cooled to room temperature and shaken with 5 mL of chloroform. The chloroform layer was separated, and half of its volume was taken, 10 % ammonium hydroxide was added to it. A pink, red or violet color in ammonia phase is an indication of a combined anthracene or anthraquinone derivatives.

2.7.8. Test for flavonoids

One milliliter (1 mL) of 10% NaOH was added to 3 mL of the leaf extract. The development of yellow coloration indicated a positive test.

2.8. Acute toxicity study

Acute oral toxicity profile of SIL in mice was determined using the up-and-down approach as described by the Organization for Economic Cooperation and Development (OECD) 425 guidelines. Two sets of five mice (n= 5) were employed. The first group (control group) received distilled water (10 mL/kg), whereas the second (test group) received a single dosage of SIL (5000 mg/kg) through oral gavage (p.o). Prior to treatment, the mice were denied food (but not drink) for 3–4 h. They were closely monitored for the first 24 h, then daily for the next two weeks, for signs or symptoms of toxicity such as mortality, diarrhea, noisy breathing, salivation, convulsion, injury, changes in locomotor activity, weakness, discharge from eyes and ears, coma, pain, aggressiveness, food, or water rejection. After two weeks, the median lethal dose (LD_{50}) was then estimated.

2.9. Experimental design

2.9.1. Analgesic studies

A total of fifty (50) mice were used; twenty-five (25) for the acetic acid-induced writhing test and another twenty-five (25) for the hot plate test. The mice were randomly divided into five groups of 5 mice each (n = 5). For each study, mice were pretreated with graded doses of SIL (250, 500 and 1000 mg/kg p.o) an hour before commencement (Table 1).

Table 1. Grouping for analgesic studies.

Group	Treatment administered (p.o)	Dosage
I	Distilled water	10 mL/kg
II	SIL	250 mg/kg
III	SIL	500 mg/kg
IV	SIL	1000 mg/kg
V	ASP/MOR	150/10 mg/kg

SIL = *Senna italica* methanol leaf extract; ASP = Aspirin; MOR = Morphine

2.9.2. Mechanistic studies

Forty animals were randomly grouped into eight groups of five per group. Three groups were orally administered distilled water (10 mL/kg), morphine (10 mg/kg) and extract (1000 mg/kg - the most effective analgesic dose determined from the previous studies carried out). The remaining groups were pretreated with the following antagonists as shown in Table 2 for 15 min before the oral administration of the extract.

Table 2. Grouping for mechanistic studies**.**

Group	Treatment administered (p.o)	Dosage
I	Distilled water	10 mL/kg
II	SIL	1000 mg/kg
III	PRAZ + SIL	1 + 1000 mg/kg
IV	PROP + SIL	20 + 1000 mg/kg
V	NAL + SIL	2 + 1000 mg/kg
VI	GLI + SIL	5 + 1000 mg/kg
VII	LAA + SIL	50 + 1000 mg/kg
VIII	MOR	10 mg/kg

SIL = *Senna italica* methanol leaf extract; MOR = Morphine, PRA = Prazosin; PROP = Propranolol; NAL = Naloxone; GLI = Glibenclamide; LAA = L-arginine

2.10. Antinociceptive studies

2.10.1. Acetic acid-induced writhes test

Peripheral analgesic activity of SIL was investigated using the acetic acid-induced writhing method (Koster *et al.*, 1959). Twenty-five (25) mice were randomly separated into five groups, each containing five mice. The first and last groups were given distilled water (10 ml/kg) and acetyl salicylic acid (ASP, 150 mg/kg). While the second, third and fourth groups were treated with the extract at doses of 250, 500 and 1000 mg/kg, respectively, all through the oral route. After one hour, each mouse was given 0.6 percent v/v acetic acid (10 ml/kg) intraperitoneally to cause abdominal writhes. The mice were placed in individual observation cages and the number of writhes for each mouse counted five

minutes after the acetic acid injection and recorded for a 10-minute interval. Using the formula below, the percentage (%) inhibition of writhing was computed for each mouse.

% inhibition = [Average number of writhes (control) − Average number of writhes (test) / Average number of writhes (control)] × 100

2.10.2. Hot plate test

Centrally acting analgesic activity was evaluated using the hot plate test according to the method described by Turner (1965). Hot Plate (Ugo Basile Ltd. Italy) was used to determinate the central component of nociception. Twenty-five mice were randomly divided into five groups with five animals in each group. The first and last groups were given distilled water (10 mL/kg) and morphine (10 mg/kg). While the second, third and fourth groups were treated with the extract at doses of 250, 500 and 1000 mg/kg respectively all through the oral route. One hour after administration of distilled water, drug and extract, the animals were placed on a hot plate maintained at a temperature of 45 ± 0.5 ˚C. The reaction time was then observed over a period of 120 min (readings were taken at times 0, 30, 60, 90 and 120 min) after extract and drug administration. The reaction time was considered as the time elapsed between placing a mouse on the hot plate and licking of fore paw or jumping. Only mice that showed initial nociceptive response within 30 seconds were selected and used for the study. The cut off time for the hot plate latencies was set at 20 seconds.

2.11. Evaluation of the pharmacological mechanisms of antinociceptive action of SIL

To evaluate the possible pathways involved in the analgesic action of SIL, the method as described by Rangel *et al*. (2012) was employed. Randomly selected mice (n= 5) were pretreated with the following drugs: Naloxone (2 mg/kg, *i.p.*), a competitive opioid antagonist; Prazosin (1 mg/kg, *i.p.*), a selective α_1-adrenoceptor blocking agent, L-arginine (50 mg/kg, *i.p.*), a nitric oxide precursor, Propranolol (20 mg/kg, *i.p.*), a non-selective β-adrenoceptor blocking agent and Glibenclamide (5 mg/kg, *i.p.*) an inhibitor of ATP-sensitive potassium channels (K_{ATP}); 15 min before oral administration of SIL (1000 mg/kg). In addition, the effects of the vehicle (distilled water, 10 mL/kg), morphine (10 mg/kg), and SIL (1000 mg/kg) were evaluated in three separate groups of mice (n= 5).To induce writhing, mice in all groups were given 10 mL/kg of 0.6 percent v/v acetic acid intraperitoneally. The mice were then placed in individual observation cages five minutes after receiving the acetic acid injection, and the number of abdominal writhes for each mouse was recorded for ten minutes. Percentage inhibition of writhes was calculated using the previously stated formular.

2.12. Statistical analysis

Data obtained from the study were expressed as the mean ± standard error of mean (SEM) and presented in tables and figures where appropriate. The data obtained were analyzed using one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test (acetic acid-induced writhes test) and repeated measures ANOVA followed by Bonferroni's post hoc test (hot plate test). Statistical Package for Social Scientist (SPSS 23.0) was employed for the analysis. Results were considered as statistically significant at $P<0.05$.

3. Results and discussion

3.1. Extractive value and phytochemical profile of SIL

The extraction of 600g of powdered *S. italica* leaf produced 95.5 g of extract. The percentage yield was thus calculated to be 15.9 % w/w. The preliminary phytochemical test revealed the presence of steroids, triterpenes, flavonoids, tannins, carbohydrates, alkaloids, and saponins (Table 3). Qualitative phytochemical screening of the methanol leaf extract of *S. italica* revealed the presence of several important phytoconstituent classes including flavonoids, steroids, saponins, glycosides, tannins, and alkaloids. However, Adjou *et al*. (2021) reported the presence of anthocyanins, leucoanthocyanins, catechins, tannins, flavonoids, alkaloids and anthraquinones in the leaf extract. Several studies have shown that several compounds from the aforementioned classes have antinociceptive properties (Bittar *et al.,* 2000; Calixto *et al.,* 2000; Wang *et al.,* 2009; Chen *et al.,* 2012). The presence of similar phytochemical compounds in SIL may therefore be responsible for the observed antinociceptive activity.

Table 3. Phytochemical constituents present in *Senna italica* methanol leaf extract.

Phytochemical	Test	Observation	Inference
Steroids and triterpenes	Liebermann-Burchard	Presence of purple color	Present
Saponins	Frothing	Presence of honeycomb froth	Present
Flavonoids	Shinoda	Presence of red color	Present
	Sodium chloride	Presence of yellow color	Present
Tannins	Ferric chloride	Presence of greenish black precipitate	Present
Anthraquinones	Bontrager	Bright pink color	Absent
Alkaloids	Dragendorff	Reddish brown precipitate	Present
	Wagner	Whitish precipitate	Present
Carbohydrates	Keller-Killiani	Pale green color	Present

3.2. Oral acute toxicity (LD_{50}) study of SIL in mice

Evaluating the acute toxicity effects of a compound/extract is significant in preclinical research because it determines the negative effects that may occur because of intentional or unintentional short-term exposure (Erhirhie *et al.*, 2018). From this study, no sign of toxicity or mortality was observed on administration of the extract at a dose of 5000 mg/kg in mice. During the 2-week observation period, there were no changes in food intake or other behaviors when compared to the baseline. The LD_{50} of the extract was thus estimated to be greater than 5000 mg/kg and according to the Lorke (1983) classification of LD_{50} values of chemicals, oral administration of the SIL was considered as practically safe in mice.

3.3. Effect of SIL on acetic acid-induced writhes in mice

The effect of *Senna italica* leaf extract on acetic acid-induced abdominal constrictions in mice is presented in Figure 1. The results showed that the extract (250, 500, 1000 mg/kg), and the reference drug aspirin (150 mg/kg) significantly (P<0.05) reduced abdominal writhes in mice when compared to the negative control group (mean number of writheswas reduced from 26.10 ± 0.15 in the negative group to 4.67 ± 0.02 at the dose of 1000 mg/kg). The reduction was in a dose dependent manner.

The acetic acid-induced writhing test is a widely used model for evaluating the peripherally analgesic potential of a test compound (Bentley *et al.*, 1983). The intraperitoneal injection of acetic acid in mice causes irritation and stimulates the release of noxious endogenous mediators like bradykinin, serotonin, histamine, and substance P. These substances once released cause pain which is characterized by contraction of abdominal muscles, expansion of the forelimbs and body elongation in the injected mice (Verma *et al.*, 2005). Any agent/drug that inhibits acetic acid-induced writhing has the potential to be an analgesic, via inhibiting prostaglandin production, which is recognized as a peripheral mode of pain control (Hassan *et al.*, 2014). In our study, SIL significantly reduced acetic acid-induced writhing, suggesting that the extract has peripheral analgesic effect via suppression of local peritoneal receptors or arachidonic acid pathways involving cyclooxygenases and/or lipoxygenases.

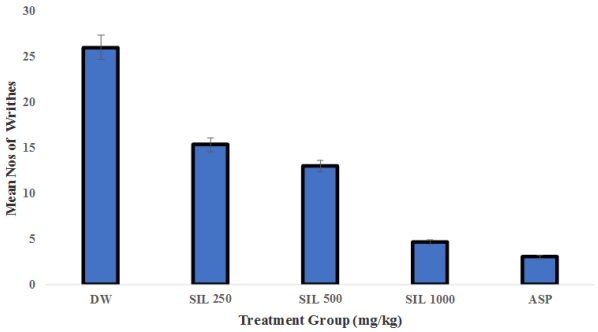

Figure 1. Effect of *Senna italica* methanol leaf extract on acetic acid-induced writhes in mice
Values represented as Mean ± SEM, * = p<0.05 versus distilled water (negative control); Data analyzed by One-way ANOVA followed by Dunnett's post hoc test; n = 5; DW = Distilled water (10 mL/kg); SIL = *Senna italica* methanol leaf extract; ASP = Aspirin

3.4 Effect of SIL on hot plate-induced pain in mice

In this model all the three test doses of SIL extract and the standard drug morphine produced significant (P<0.05) central analgesic activity by delaying the reaction time of mice to thermal stimulus at the 60- and 90-min intervals of observation when compared with the negative control (Figure 2). The maximum analgesic activities of all doses of the extract (250, 500 and 1000 mg/kg) and morphine (10 mg/kg) were observed at 90 mins interval of observation.

The hot plate method is an appropriate assay for determining the efficacy of centrally acting analgesic drugs or compounds (Nunez Guillen *et al.*, 1997). The paw licking and jumping behavioral reactions produced by the hot-plate test are both supraspinally integrated

responses (Chapman *et al.*, 1985). Opioids and other centrally acting analgesics act through supraspinal and spinal receptors to lengthen reaction times in hot plate test (Sabina *et al.*, 2009; Mondal *et al.*, 2014). The present study demonstrated that oral administration of *Senna italica* leaf extract elevated pain threshold by prolonging the response latency period to heat stimulus in a dose dependent manner. The standard drug – morphine (a renowned central analgesic which exerts its effect through binding with the opioid receptors μ, κ, and δ located in the post and pre synaptic membrane) also increased mean reaction time to thermal-induced stimulus (Mondal *et al.*, 2014). Spinal, supraspinal, and peripheral analgesia have all been linked to the activation of these receptors (Fürst, 1999). In the present study, the relatively enhanced activity of the1000 mg/kg dose at all observation times may be attributed to the presence of higher concentration of active metabolites. Findings from our study thus demonstrate that SIL possesses analgesic activity mediated via central mechanism.

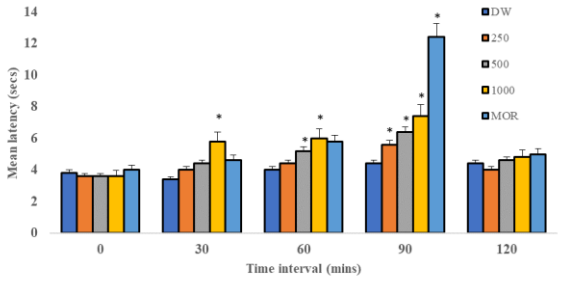

Figure 2: Effect of *Senna italica* methanol leaf extract on Hot Plate Latency Time in Mice**.**
Values represented as Mean ± SEM, *= P<0.05 versus distilled water (negative control); Data analyzed by repeated measures ANOVA followed by Bonferonni's post hoc test; n= 5; DW = Distilled water; SIL = *Senna italica* methanol leaf extract (mg/kg); MOR = Morphine (10 mg/kg).

The 1000 mg/kg dose of SIL produced maximum analgesic effect in both acetic acid-induced and hot plate tests; the dose was thus selected for the mechanistic studies.

3.5 Mechanistic study

Administration of SIL significantly ($P< 0.05$) reduced the mean number of writhes in mice when compared to control group. The pretreatment of animals with glibenclamide + SIL did not produce a significant ($P< 0.05$) increase or decrease in the antinociceptive activity of the extract when compared to SIL-alone treated group. However, the pretreatment of animals with naloxone + SIL. prazosin + SIL, L-arginine + SIL and propranolol + SIL significantly ($P<0.05$) reduced the analgesic activity of the SIL-alone treated group (Figure 3).

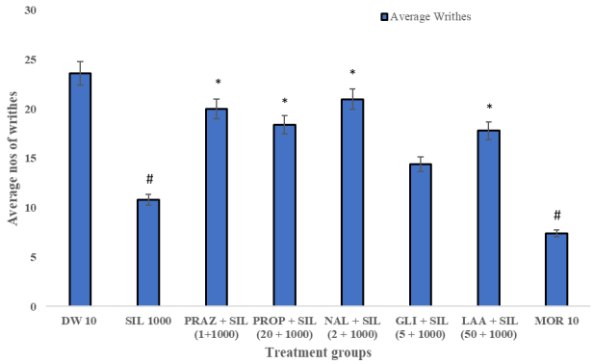

Figure 3: Effect of different receptor blockers on the analgesic activity of *Senna italica* methanol leaf extract on acetic acid-induced writhes in mice.
Values represented as Mean ± SEM, # = $P<0.05$ versus distilled water (negative control); * = $P<0.05$ versus SIL; Data analyzed by One-way ANOVA followed by Dunnett's post hoc test; n = 5; DW = Distilled water; SIL = *Senna italica* methanol leaf extract; MOR = Morphine, PRA = Prazosin; PROP = Propranolol; NAL = Naloxone; GLI = Glibenclamide; LAA = L –arginine.

The acetic acid-induced writhing test was used to investigate the possible mechanisms of analgesia of the extract in mice (Pa vao-de-Souza *et al.*, 2012; Rangel *et al.*, 2012). The study used the most effective dose of SIL (1000 mg/kg) obtained from previous experiments. Intraperitoneal injection of acetic acid in mice stimulates pain peripherally by releasing endogenous substances such as serotonin, histamine, prostaglandins, bradykinins, and substance P (Mishra *et al.*, 2010). Acetic acid also stimulates central pain by triggering the release of mitogen-activated protein (MAP) kinases and microglia in the spinal cord; in addition to several interactions involving the opiate, dopaminergic, descending noradrenergic and serotonergic systems (Mishra *et al.*, 2010, Zhang *et al.*, 2011). The process of pain in the central and peripheral nervous systems are mediated via several neurotransmitters and receptor systems including potassium ion (K^+) channels (Ocana *et al.*, 2004), adrenergic (Han *et al.*, 2004), glutamatergic (Osikowicz *et al.*, 2013) and opioidergic systems

(Rangel *et al.*, 2012). In the present study, pretreatment of mice with naloxone, prazosin, L-arginine and propranolol significantly increased the mean number of abdominal writhes when compared to SIL alone treated group. This suggests that opioidergic and α_1-adrenergic, β-adrenergic and nitric oxide pathways are possibly involved in the antinociceptive activity of SIL. By contrast, pretreatment with glibenclamide (ATP-sensitive potassium (K_{ATP}) channel blocker), did not significantly change the analgesic action of SIL suggesting that K_{ATP} channel may not be implicated in the extract's analgesic activity.

Naloxone a competitive non-selective opioid antagonist of μ, δ and κ receptors (Trescot *et al.*, 2008) was used to investigate the involvement of the endogenous opioidergic system on the analgesic mechanism exerted by SIL. Our findings revealed that the antinociceptive activity produced by the administration of SIL was significantly reversed by the pre-treatment of mice with naloxone. This suggests that SIL may be acting through the opioidergic pathway. Several investigations have revealed the involvement of the adrenergic system in pain mechanisms mediated by the alpha1 and 2 (α1 and α2) receptors (Millan, 2002; Roczniak *et al.*, 2013). Studies have also shown that alpha1 adrenoceptors play a crucial modulatory function in opioid analgesia and withdrawal (Pinardi *et al.*, 2001; Ozdoğan *et al.*, 2003; Drummond *et al.*, 2015). Pre-treatment of mice with prazosin (an α1-blocker) reversed the analgesic activity of the extract thus suggesting the involvement of the α adrenergic system in the analgesic action of SIL. Nitric oxide (NO), which is produced from L-arginine, has a function in nociception due to the presence of neuronal nitric oxide synthase (nNOS) in the superficial dorsal horn and intermediolateral cell column of the spinal cord (Al-Tahan *et al.*, 2011). The increased number of abdominal writhes observed after pretreatment with L-arginine suggests the involvement of the L-arginine/nitric oxide/cGMP pathway in the antinociceptive activity of the extract. β-adrenergic receptors mediate antinociceptive action via signal transduction pathways involving adenylyl cyclase and protein kinase. Propranolol a non-selective β-adrenergic blocker reversed the antinociceptive effect of SIL, this shows that the suggests the β-adrenergic receptors may be implicated in analgesic activity of SIL.

4. Conclusion

The findings from this research revealed the possible involvement of opioidergic, α adrenergic, β-adrenergic systems including the L-arginine/nitric oxide pathway in the analgesic activity of *Senna italica* leaf, thus supporting the use of the plant in folk medicine in the treatment of painful conditions.

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6. References

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