Research Article CODEN: IJRPJK ISSN: 2319 – 9563

International Journal of Research in Pharmaceutical and Nano Sciences

Journal homepage: www.ijrpns.com

INVESTIGATING ANTINOCICEPTIVE AND SKELETAL MUSCLE RELAXANT PROPERTIES OF ETHANOL SEED EXTRACT OF *SENNA OCCIDENTALIS* LINN. (FABACEAE) IN WISTAR RATS

Cletus Anes Ukwubile*[1], Francis James Ireebanije[1], Monday Iganga Elijah[1], Nathaniel S. Muwa[1]

[1*]Department of Basic and Applied Sciences / Science Laboratory Technology, Federal Polytechnic, Bali, Nigeria.

ABSTRACT

Background: *Senna occidentalis* has been used in the Nigerian traditional and complementary medicine since decades to treat various diseases. It has been used to treat sexually transmitted infection, sore throat, muscular injuries, back pain, and epilepsy in folklore medicine. **Objectives:** This study was carried out to determine the effects of seed extract of *S. occidentalis* on skeletal muscles. **Methods:** Phytochemical screening was carried out on the ethanol seed extract, and the seed extract of *S. occidentalis* was further evaluated for its action on skeletal muscles and compared with a standard drug diazepam in Swiss albino mice. 50 mice of either sexes were grouped into five groups of 10 animals each. Group I was considered as normal control, group II was diazepam control while groups III, IV and V where administered with doses of 100 mg/kg, 250 mg/kg and 500 mg/kg b.w. ESESO via oral route and observed for 12 h. **Results:** Preliminary phytochemical screening revealed that ESESO contained various classes of glycosides such as anthraquinones, cardiac glycosides as well as alkaloids and carbohydrates. At doses of 100 and 250 mg/kg b.w. ESESO there was significant reduction in the time spent by the animals on revolving rod when compared to control ($p<0.05$, one way ANOVA). A dose dependent increase in muscle relaxation was seen in all the groups. **Conclusion:** The study showed that ethanol seed extract of *Senna occidentalis* has muscle relaxant effects as well as other parameters investigated in mice by reduction of pains (antinociceptive) due to the presence of these secondary metabolites.

KEYWORDS

Senna occidentalis, Phytochemical, Diazepam, Muscle relaxant and Skeletal muscle.

Author for Correspondence:

Cletus Anes Ukwubile,
Department of Science Laboratory Technology,
Federal Polytechnic Bali, Nigeria.

Email: doccletus@yahoo.com

INTRODUCTION

Senna occidentalis is also known as stinking weed, miki palaoa, hedionda, bucho, pico de pájaro, furrusca, bois puante, fedegoso, and many other names[1]. The species varies from a semi-woody annual herb in warm temperate areas to a woody annual shrub or sometimes a short-lived perennial shrub in frost-free areas[1,2]. Its height ranges from

1.0 to 1.5 m high during which the pods matures. In Brazil It is reported to reach 5 to 8 m in height[3]. Senna produces a hard, woody taproot with relatively few laterals. The stem can vary from purple to dark-green especially during dry season.

Young stems are four-angled, becoming rounded with age. The crushed foliage has an unpleasant odor. The leaves are compound and alternate with 4-6 pairs hairless leaflets. The pods are flat and slightly curved about 10 to 15 cm in length containing many seeds[4]. It grows throughout the tropics and subtropics including the United States from Texas to Iowa eastward, Hawaii, the Pacific Island Territories, Puerto Rico, and the U.S. Virgin Islands[4]. Ecologically, Senna is said to be most common in fertile, cultivated areas[5] and on fertile soils. The species occurs on pinelands in Florida and grows better on near-neutral soils than acid (pH 4.7) soils[6]. Coffee Senna as it popularly called in the USA grows in moist areas (mean annual rainfall from about 1000 to 2200 mm) in Puerto Rio and predominant in Northern and Easter Nigeria States of Taraba, Kaduna, Kano, Enugu, Nsukka, Ogurugu, etc[6]. It flowers and fruits throughout the year or seasonally, depending on rainfall and cold seasons.

Scarification is necessary for good seed germination. Mechanical scarification, acid treatment, and immersing in boiling water all worked well, giving 82 to 100 percent germination[6]. The seeds are dispersed by grazing animals. In seasonally cold or dry climates, the life cycle of coffee Senna is complete in 6 to 9 months, but in warm conditions. Perhaps in areas where extraordinary heights are reached, the species may live a third or fourth year where its growth is moderately rapid[7]. The species can be controlled with broadleaf herbicides[8]. The plant contains many secondary metabolites which are beneficial to humans in traditional medicine. Extracts or powdered leaves are used as an analgesic, antibacterial, anti-hepatotoxic, antifungal, anti-inflammatory, antiseptic, antispasmodic, antiparasitic, antiviral, carminative, diaphoretic, emmenagogue, febrifuge, insecticidal, immune stimulant, laxative, purgative, sudorific, and vermifuge. Several of these effects have been demonstrated in laboratory and clinical tests[8]. The leaves are toxic and generally avoided by livestock[9]. Ingestion of large amounts of seeds has been implicated in deaths of cows, horses, and goats. Poisoning of pigs fed coffee Senna seeds resulted in muscle necrosis[9]. This present study was designed to investigate its anti-nociceptive and skeletal muscle relaxant properties in mice.

MATERIAL AND METHODS

Plant Material and its Authentication

The seeds of *Senna occidentalis* were collected from the dried pods in August 2013 from a forest in Bali Community, Taraba State, Nigeria. They were later on grind using electronic blender (*Sonik-190457H*) at the Biology Laboratory of the Department of Basic and Applied Sciences, Federal Polytechnic, Bali. The ground plant seeds were the weighed and extracted in high grade ethanol using cold maceration techniques. It was evaporated and then stored for further use in desiccators.

Drugs and Chemicals

The chemicals used in this study were of analytical grade from trusted suppliers. Ethanol used was double-distilled to remove any possible impurities, standard drugs used were obtained from NAFDAC registered pharmacy shop.

Preparation of Extract

The powdered plant material was first defatted by using petroleum ether (70-90°C). The defatted plant material (100 g) was extracted with 99 % absolute

ethanol (400 mL) by boiling under reflux for 90 minutes. The extract was filtered and evaporated to dryness to yield the dry extract (ESESO, yield: 16.44 %). The dry extract was kept in desiccators until use.

Preliminary phytochemical studies

Phytochemical screening was carried out according to the previous methods described by Sofowora[10] as well as Trease and Evans[11], to test for the presence of saponins, flavonoids, alkaloids, cardiac glycosides, tannins and anthraquinones in the seed extract of *Senna occidentalis*.

Determination of Saponins

Two tests were carried out (*frothing test and haemolysis* test).

Frothing test

Small quantity of the extracts were each dissolved in 10mL of distilled water and shaken vigorously for thirty seconds and allowed to stand for about thirty minutes. Foam which persisted for more than thirty minutes indicate saponins.

***Haemolysis* test**

The extracts were each put in two test tubes containing 2ml of 1.8% aqueous NaCl solution. 2mL of the extract was put into one test tube and other in 2mL of distilled water. 3 drops of mice blood was added to each test tube and observed for thirty minutes. Occurrence of haemolysis in the tube containing the extract indicates the presence of saponins.

Determination of flavonoids

Sodium hydroxide test (NaOH)

0.5g of the extracts was added to 2 drops of aqueous NaOH solution. Yellow coloration was taken for presence of flavonoids.

Shinoda's test

This was carried out by heating the extract in 2mL of 50% methanol and then adding metallic magnesium plus four drops of concentrated HCl solution. Orange colour showed the presence of flavonoids.

Determination of Tannins

Ferric chloride test

0.5g of the extracts were each stirred with 10ml of distilled water and then filtered. 2 drops of 5% $FeCl_3$ was then added. A green ppt. was taken for the presence of condensed tannins.

Lead acetate test

0.5g of extract solutions were added to lead acetate solution, a brown colour was taken for the presence of tannins.

Goldbeater's skin test

A small piece of Goldbeater's skin was soaked in 2% HCl and rinsed with distilled water. It was then placed in the extract solutions for 5min and washed with distilled water. The skin was then transferred to a 1% solution of ferrous sulphate ($FeSO_4$). Presence of black colour on the skin was taken to confirm the presence of tannins[11].

Determination of Alkaloids

***Dragendorff's* test**

Few drops of *Dragendorff's* reagent were added to 0.5g of the extracts. A rose–red ppt. was taken to indicate the presence of alkaloids.

Meyer's test

Few drops of this reagent were added to sample extract in test tube, cloudy or creamy precipitate indicates alkaloids.

Wagner's test

Few drops of this reagent were added to sample extract in test tube, whitish precipitate indicates alkaloids.

Picric acid test

Two drops of picric acid solution was added to the 5 mL solution of extract and shake lightly. A yellow colored solution indicates the presence of alkaloids.

Tannic acid test

Few drops of tannic acid solution were added to the extract, black precipitate indicates alkaloids[11].

Determination of Anthraquinones

Borntrager's test (For free anthracenes)

Exactly 0.5g of the extracts was shaken with 10mL of chloroform and filtered. After this, 5mL of 10 % solution of ammonia was added to the filtrate and stirred. A pink-red colour was taken for the presence of free anthraquinones.

Modified Borntrager's test (For combined anthracenes)

Combined anthracenes were also tested for by boiling the extracts with 5mL of 10 % HCl solution for 3min, this will hydrolyze the glycosides to yield aglycones which are soluble in hot water only. The solution was filtered hot, and filtrates allowed to cool. The filtrates were the extracted with 5ml of benzene. The benzene layer was filtered off and shaken gently with half its volume with 10 % ammonia solution. Cherry-red colour indicates combined anthracenes[11].

Experimental animals

Adult Swiss albino mice of either sex weighing 18 ± 2 g were used in the study. They were divided into groups, and kept into separate cages with not more than three animals per cage and maintained under standard laboratory conditions. The animals were allowed *ad libitum* feed and water. They then kept in the laboratory for one week so as to acclimatized to the environment prior to the start of the study.

Acute toxicity study

Acute toxicity studies were conducted by using albino mice of either sex weighing 18-22 g and of 90 days age. The mice were denied access to feed and water prior to this study phase. The method of high and low doses (OECD) was used to determine the dose[12,13]. The median lethal dose of the extracts having muscle relaxant activity was determined by administering 50, 100, 200,400, 800, 1000 and 2000 mg/kg body weight oral dose and observed for signs of behavioral, neurological toxicity and mortality. The procedure was followed according Lorke's guidelines[14]. LD_{50} was calculated as per least dosage that the animals died to highest dosage in which the animal survived taken in geometrical means[14].

Selection of dose for pharmacological screening

The pure extract of *Senna occidentalis* seeds were found to be toxic at the dose of 2000 mg/kg and caused deaths of animals, therefore it is not as safe at this dosage. Hence, doses of 200 mg/kg body weight, 250 mg/kg and.500 mg/kg were used for the evaluation of muscle relaxant activity[14].

Evaluation of anti-nociceptive (analgesic) activity (tail flick test)

Prior to treatment, the reaction time for each mouse to absorbed heat was determined by placing the tip of the tail of each mouse on the heat source. The quantity of heat energy that is passing through the naked nichrome wire was maintained at 4A. The tail flicking responses time was recorded and taken as nociceptive (analgesic) terminal end. Also, mice that do not to withdraw its tail at 2 to 5 sec were not included in the study. The initial tested mice within these times (i.e. 2-5 secs) were grouped into three groups of six mice. The first two groups received the ESESO at the doses of 250 and 500 mg/kg body weight i.p. respectively while the control group were administered 5 mg/kg b.w sub-cutaneous (s.c). Response from all groups of mice were recorded in 5, 15, 30, 60, and 90 min of administration of extract by tail flicking, similarly as mentioned above, and the latent times were recorded after some seconds. Each mouse served as its own control. The mean reaction times for each group were then calculated[15].

Evaluation of locomotors activity

The central nervous system (CNS) depressant activity of ESESO was evaluated by studying locomotors activity of mice using an actophotometer (Techno, China). Three groups of six mice were used. In this method, the actophotometer was switched on and mouse from each group was placed individually in the activity cage for 10 min. After this, the behavior of all the mice were closely observed and noted. The control group was administered 3 mg/kg b.w. i.p. chlorpromazine hydrochloride while, the remaining two groups received the ESESO at the doses of 250 and 500 mg/kg body weight i.p., respectively. Activity scores were then tested in 30 and 60 min after treatments[16]. Percentage motor activity reduction was calculated for each mouse using the relationship:

% Reduction in motor activity = $(W_1 - W_2/W_1) \times 100$

Where, W_1 and W_2 are the mean activity scores before and after treatment, respectively.

Evaluation of Skeletal Muscle Relaxant Property

The muscle relaxant property of ESESO was evaluated by studying neurological deficit of mice using rotarod apparatus. The mice were divided into three groups of six. The equipment was turned on and adjusted to appropriate speed (20 rpm). Mouse from each group was individually placed on the rotarod, and the fall off time of all the animals were recorded when the mouse falls from the rotating rod. Then, the first two groups received the ESESO at the doses of 250 and 500 mg/kg body weight i.p., respectively. The positive control group was administered 4mg/kg b.w. (i.p.) diazepam, and the mice were further tested for the time to fall off in 30 and 60 min after the extract administration. Each animal served as its own control[17]. The reduction in time of fall was determined for each mouse from the relation below:

% Reduction time of fall off = $(W_1 - W_2/W_1) \times 100$

Where, W_1 and W_2 are the mean fall off times before and after treatment, respectively.

Evaluation of phenobarbitone-induced sleep potentiation

In this aspect, mice were grouped into 4 groups of six mice per group. Group I which served as the positive was administered 5 mL normal saline i.p. Groups II and III received the ESESO at the doses of 250 and 500 mg/kg body weight i.p., respectively. Group IV was administered 3mg/kg b.w. chlorpromazine hydrochloride i.p. After 30 min of treatment, each mouse was given 20mg/kg b.w. phenobarbitone sodium i.p. and each mouse was observed for various reflexes and recorded[18].

Statistical Analysis

In this study, data obtained were expressed as mean ± SE. The results were analyzed for statistical significance by Students'' test. $P<0.05$ was considered as statistically significant.

RESULTS

Preliminary phytochemical screening revealed the presence of anthraquinones, alkaloids, cardiac glycosides in ESESO (Table No.1). The ESESO was found to be toxic in Wistar rats at the dose of 2000 mg/kg body weight p.o. LD_{50} was determined to be 3250 mg/kg.

Results of antinociceptive activity, i.e., tail flick test showed Table No.2, that ESESO at both doses exhibited significant ($p<0.05$, after 5 to 90 min) and dose-dependent steady increase in reaction time of mice up to 60 minutes of administration followed by minor decrease in activities, observed at both doses. Peak analgesic effect was observed at 30 to 60 min indicating maximum increase in reaction times.

In locomotors activity study, it was found that ESESO significantly ($p<0.05$) depressed the

locomotors activity in mice in a dose and time-dependent manner. The activities increased as time approached to 60 min. The results are tabulated below in Table No.3.

In muscle relaxant study, the ESESO at both doses significantly (p<0.05) and dose dependently decreased the fall off time in mice demonstrating its skeletal muscle relaxant property. The effect was most prominent after 60 minutes of administration in Table No.4.

The results of phenobarbitone sodium-induced sleeping time are presented in table no.5. Here, ESESO at all doses pretreatment exhibited significant (p<0.05) and potentiation of phenobarbitone-induced sleeping time in mice by hastening the onset of sleep (dose dependent) and delaying recovering the animals from sleep (non-dose dependent) as compared with the vehicle control group.

DISCUSSION

From the result of the preliminary phytochemical screening, the most dominant constituents of the seeds are anthraquinones and alkaloids, and are responsible for the biological activities investigated[19] Table No.1. The present study examined some important neuro-pharmacological activities (anti-nociceptive, skeletal muscle relaxant properties and sedative effects) of ethanol seed extract of *S. occidentalis* (ESESO) in Wistar rats Table No.2. The acute toxicity results revealed that the plant was considered slightly-toxic.

Antinociceptive activity of *S. occidentalis* extract (ESESO) was evaluated by tail flick method in mice to assess central (narcotic) analgesic activity[20]. The results of tail flick study clearly indicated that the ESESO had significant central antinociceptive action revealing the involvement of the CNS in antinociception. This implies that the ESESO exerted analgesic activity interfering the central mechanisms for the transmission of painful messages in mice. The tail flick test is thermally induced nociception model where radiant heat is used as a source of pain. Here, radiant heat (through a hot nichrome wire) is applied to the tail of mice and the withdrawal of tail from the radiant heat source (hot nichrome wire) is considered as flicking response to thermally induced pain. The flicking reaction which is the end point of this test may be mediated as a spinal reflex. Analgesics of only narcotic type, e.g., morphine, pethidine, pentazocine, etc., can increase the tail flick latency period indicating antinociception[20,21].

Most of the centrally acting analgesics have certain CNS depressant effects. The locomotors activity was evaluated to assess the CNS-depressant property of ESESO on the motor activity in mice (Table No.3). Most of the centrally active analgesic agents influence the locomotors activities in human beings and rodents mainly by reducing the motor activity because of their CNS depressant property[22]. Locomotors activity is considered as an index of wakefulness or alertness of mental activity and a decrease may lead to calming and sedation as a result of reduced excitability of the CNS. The results of the present study showed significant influence in locomotors activity of mice by ESESO treatment demonstrating decrease in locomotors activity and hence indicating its CNS depressant property in the mice.

In muscle relaxant evaluation, the ESESO-induced decrease in fall off tine was due to the loss of muscle grip implying skeletal muscle relaxation[22] (Table No.4). Demonstration of marked muscle relaxant effect by the rotarod study indicated that ESESO induced neurological deficit accompanied with taming or calming effect in mice, thereby further supporting its CNS-depressant effect.

Barbiturates are putative sedatives inducing sleep in human beings and animals by depressing the CNS[23]. Phenobarbitone, although a long-acting barbiturate, at lower doses, it can serve as short to intermediate acting barbiturate[23]. In the present study, in the saline control group of mice, phenobarbitone (3 mg/kg) produced intermediate onset and duration of sleep as indicated by the loss of righting reflex (inability to maintain posture) and awakening or regaining righting reflex subsequently[23]. ESESO pretreatment remarkably reduced the sleep induction time in mice in a dose-dependent manner. It markedly and significantly prolonged the duration of sleep in phenobarbitone-induced rats; however, here the observed effect was not found dose dependent. Potentiation of phenobarbitone induced sleeping time by ESESO indicated the anxiolytic or sedative property of ESESO, thereby confirming its CNS depressant role in mice (Table No.5). Compounds like anthraquinones, alkaloids, and cardiac glycosides, found in higher plants have been reported to possess multiple biological effects[24]. Previous research showed that plants containing alkaloids, anthracenes, and glycosides are useful in many CNS disorders[24]. Therefore, the presence of these phytoconstituents may be responsible for its CNS depressant properties (Table No.1 and Table No.5).

Table No.1: Phytochemical screening of ethanol seed extract of *Senna occidentalis* (ESESO)

S.No	Class of Compound	Inference
1	Alkaloids	+++
2	Flavonoids	-
3	Saponins	-
4	Tannins	-
5	Anthraquinones	+++
6	Cardiac glycosides	+
7	Amino acids	++

Note :+(slightly present), ++ (moderately present), +++ (highly present), - (not present).

Table No.2: Effect of ESESO on tail flick test in mice

S.No	Treatment	Dose (mg/kg)	Mean Reaction Time (Seconds)					
			Before Treatment	5	15	30	60	90
1	ESESO	200	1.66±0.14	4.74±0.45	6.27±0.18	8.34±0.21	9.12±0.24	7.21±0.19
2	ESESO	250	1.84±0.10	3.84±0.40	8.98±0.28	>10	9.73±0.31	8.60±0.24
3	ESESO	500	1.90±0.32	4.83±0.47	6.78±0.52	>10	9.82±0.42	8.86±0.28
4	Morphine **S**	5	1.60±0.28	3.83±0.30	8.16±0.19	9.67±0.28	>10*	>10*

*a cut-off time of 10 seconds was taken as maximum analgesic response to avoid tail damage as of heat,' S' denotes sulphate, data are mean ± SE.

Table No.3: Effects of ESESO on locomotors activity in Swiss albino mice

S.No	Treatment	Dose (mg/kg)	Mean Motor Activity Score in 10 mints			% Reduction in Motor Activity in 10 min	
			Before Treatment	After Treatment		After	After
				30	60	30	60
1	Diazepam	2	394.01±22.08	102.37±19.10	71.14±8.23*	74.02	81.94
2	ESESO	250	415.33±33.80	120.2±19.21	95.66±14.83*	71.11	76.94
3	ESESO	500	436.33±24.96	126.33±29.01*	33.0±8.12	71.05	92.44

Data are expressed as mean ± SE (n = 6), *p< 0.05 compared with control (mice before treatment), Student's t-test.

Table No.4: Effects of ESESO on skeletal muscle relaxant in Swiss albino mice

S.No	Treatment	Dose (mg/kg)	Mean fall-off time in min			% Decrease in fall-off time in 10 min	
			Before Treatment	After Treatment		After	After
				30	60	30	60
1	Diazepam	4	15.23±2.14	4.01±0.76*	2.83±0.45*	73.67	81.42
2	ESESO	250	14.13±3.88	4.13±0.65*	7.76±0.80*	70.79	69.27
3	ESESO	500	13.80±1.34	4.26±0.79*	5.80±0.96*	69.13	57.97

Data are expressed as mean ± SE (n = 6), *p< 0.05 compared with control (mice before treatment), Student's t test.

Table No.5: Effect of ESESO on phenobarbitone induced sleeping time in mice

S.No	Treatment	Dose (mg/kg)	Onset of action (min)	Duration of action (min)
1	Normal saline	2	57.50±0.12	58.50±0.12
2	ESESO	250	20.33±3.65*	108.0±4.97*
3	ESESO	500	11.33±1.97*	108.0±1.29*
4	Chlorpromazine HCl	3	15.85±2.05*	148.65±2.83*

Data are expressed as mean ± SE (n = 6), *p< 0.05 compared with normal saline control, Student's t-test.

CONCLUSION

Our study showed that the ethanol seed extract of *Senna occidentalis* possessed promising centrally mediated antinociceptive, locomotors depressant, skeletal muscle relaxant, and sedative potentiating effects in the experimental rodent models demonstrating its prominent depressant action on the CNS, as manifested by these important neuro-pharmacological properties in the mice, and justifies its use in folkloric medicine for treating related sickness. However, the use of ethanol seed extract as an ethno medicinal prescription should be done in dose-wise fashion since the extract was toxic in experimental animals at high doses. Further studies may reveal the exact mechanisms and the major phytoconstituents responsible for the observed neuro-pharmacological properties of the seed extract in the mice.

ACKNOWLEDGMENT

The authors are grateful to the authority of the National Institute for Pharmaceutical Research and Development Abuja and to the Department of Basic and Applied Science, Federal Polytechnic Bali Taraba State Nigeria, for providing necessary facilities used in this study.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Please cite this article in press as: Cletus Anes Ukwubile *et al.* Investigating antinociceptive and skeletal muscle relaxant properties of ethanol seed extract of *senna occidentalis* linn. (fabaceae) in wistar rats, *International Journal of Research in Pharmaceutical and Nano Sciences,* 2(4), 2013, 485-494.