



EVALUATION OF ANXIOLYTIC EFFECT OF METHANOL EXTRACT OF SANSEVIERA LIBERICA LEAVE

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ABSTRACT

The leaves are used Traditionally in Nigeria and some other parts of Africa for the treatment insomnia and anxiety. The anxiolytic effect of the *Sansevieria librica* leave was studied in mice by using a number of experimental paradigms of anxiety and the result compared with that of the known anxiolytic compound. The methanol extract (100–400 mg/kg, i.p.) of the *Sansevieria librica* leave was examined. The anti-anxiety effect of the extract in this study was evaluated using an elevated plus maze, light and dark method and open field test. The methanol leave extract of *Sansevieria librica* inhibited novelty-induced rearing behavior (NIR), the number of square crossed both central and peripheral increased significantly, mice spent an increased number of time in the light compartment of light and dark apparatus and also,

showed indices of open arm avoidance consistent with anti-anxiety effect. Flumazenil (10 mg/kg, i.p.) abolished the effects of methanol leave extract of *Sansevieria librica* on rearing and anxiety model. The methanol leave extract of *Sansevieria librica* possess anxiolytic activity. The blockade effect of Flumazenil of leave extract of *Sansevieria librica* on rearing and elevated plus maze suggesting that GABA receptors are mediated in the observed anxiolytic activity. In conclusion, this study confirmed the ethnomedicinal uses of the plant.

KEYWORDS: Anxiolytic, elevated plus maze test, light and dark and rearing.

INTRODUCTION

Anxiety disorders have been discovered to be one of the commonest psychiatric disorders that is affecting the society.^[1] We have different categories of Anxiety disorders, these include, Social Anxiety Disorder (Social Phobia), Panic Attack, Separation Anxiety Disorder,

Agoraphobia, and Specific Phobia. Benzodiazepines and selective serotonin-reuptake inhibitors (SSRIs) are one of the major classes of drug treatment of anxiety.^[3] However, most of these agents are reported to have various adverse effects including the following hepatotoxicity, muscle relaxation and insomnia.^[4] Because of the aforementioned undesirable effect, the need to search for other agents with fewer side effect and wider margin of safety are imperative.

Medicinal plants have been reported to be a good source where new remedies for these disorders can be found. *Sansevieria liberica*, which belong to the family Dracaenaceae has been found to belong to one of the said medicinal plant with many traditional uses. This plant is used for the following ailment traditionally: pain-killers, eye and ear treatments, haemorrhoids, small-pox, chicken-pox, measles. It is also used to treat the following condition: paralysis, epilepsy, convulsions, spasm.^[5] *Sansevieria liberica* has several stiff edged elliptical, broad leaves, marked with dark and light green bands. The fruits of the plant contained one seed and reddish in colour. The plant is found majorly in the tropical regions of the world. It is commonly called “Oja ikoko” among the Yoruba ethnic group of South West, Nigeria The plant is traditionally used in the treatment of convulsions.^[6,7] Many scientists have reported the used of the plant especially in the treatment of diarrhea, abdominal pains and in promoting wounds healing.^[8] Many other uses of the plant have been reported. Despite all these finding, not much work have been done on the central nervous system stimulation or depression, hence, we decided to look at the effect of the plant on anxiety

MATERIALS AND METHODS

Laboratory animals

Swiss albino mice (20-22g) of either sex used in the study were purchased from the Laboratory Animals center, College of health sciences, Ladoke Akintola University of Technology Ogbomosho, Nigeria. They were kept in a well-ventilated environment with free access to food and water ad libitum. The study was carried out in the Department of Pharmacology and Therapeutic, College of health sciences, Osogbo and the ethical guidelines for the handling of experimental animals were followed.

Plant material

Sansevieria librica leaves were collected from the wild near Osun Osogbo shrine, very close to Lautech College of Health Sciences Osogbo, Nigeria in the month of September. The leave

was identified by Mr Homomon of Botany department, Obafemi Awolowo University, Ile-Ife, Nigeria as *Sansevieria librica*.

Preparation of the extract

The leaves were air-dried and powdered using laboratory mill. The aerial parts of *Sansevieria liberica* (1kg) were extracted with absolute methanol at room temperature using Soxhlet apparatus according to the method previously described.^[9] The solution was evaporated to dryness using rotary evaporator at 80 °C in order to obtain concentrated extract which was later freeze-dried to dry powder. The percentage yield was 18.7%% with reference to the powdered leaves.

Assessment of the anti-anxiety effect of the methanol extract of *Sansevieria liberica* leave in mice using open field test (OFT)

A wooden box (60 × 60 × 30 cm³) with the floor divided into 16 squares (15 × 15 cm²) was used in this study. Lamp of 40-watts was suspended 120cm above the floor of the box to illuminate the inside. Five groups of animals with five animals per group were prepared for the experiment. Group 1, received normal saline (10 ml/kg), group 2, received diazepam (4 mg/kg), while groups 3-5, received methanol extract of *Sansevieria liberica* in the doses of 100, 200, and 400 mg/kg respectively. Mice were administered intraperitoneally. Each mouse was placed at the center of the field and was left for 2 minutes to acclimatize with the environment. The following parameters were observed; the number of squares, central or peripherally crossed by each mouse, rearings (number of times the mouse raised its forelimb in the air or on the wall of the container) and faecal droppings (number of faecal excreted by each mouse during experimental period). The parameters were measured for 10 minutes.^[10,11] After each test the cage was cleaned with ethanol in order to avoid any odor cues.

Assessment of the anti-anxiety effect of the methanol extract of *Sansevieria liberica* leave in mice using the elevated plus maze

The maze is consisting of four arms, two open arms with the dimension of 30 × 5 × 0.2 cm and two closed arms with the dimension of 30 × 5 × 15 cm extended from a central platform (5 × 5 cm) and was 45 cm standing above the floor. The entire maze was made of wooden material, painted in black, the inside was thoroughly cleaned with ethanol and allowed to dry before the next experimentation to avoid or eliminate any odor cues.

Five groups of animals with five animals per group were prepared for this study. They were pretreated 30 minutes with diazepam (standard drug), vehicle and the extract. Group 1 received diazepam (4 mg/kg, ip), group 2 received the vehicle (10 ml/kg) while, extract at 100, 200, or 400 mg/kg were given to groups 3, 4 and 5 respectively. All mice were administered intraperitoneally. Each mouse was used just once and for one experiment. Experiments were conducted in a well quite environment and each mouse placed at the center of the maze facing open arm and during a 5 minutes' period of observation, the number of entries and the time spent in closed and open arms were recorded. When all the four paws of the mouse are place in the opened or closed arm is an index of arm entries.

Assessment of possible mechanism of action of methanol extract of *Sansevieria liberica* leaves

To investigate some possible mechanism of action by which methanol extract of *Sansevieria liberica* exerts its neurobehavioural activity. Methanol extract of *Sansevieria liberica* (100, 200, and 400 mg/kg i.p) was administered intraperitoneally thirty minutes after the mice have been pretreated with flumazenil (10 mg/kg) as described by Meer and Raber, (2005).^[12]

Assessment of the anti-anxiety effect of the methanol extract of *Sansevieria liberica* leave in mice using Light-Dark Box Test (LDBT)

The behavioural effect of extract and diazepam on the mice inside light-dark box was performed as described by Costall et al.^[13] The test apparatus consisted of a plexi glass box with the dimension (length 45 cm × width 27 cm × height 27 cm). It has two compartments, the dark and the brightly illuminated compartments. Five groups of mice with five mice per group were prepared for this study, animal in group 1 received diazepam (4 mg/kg, ip), group 2 received the normal saline (10 ml/kg) while, animals in groups 3, 4, and 5 received the extract in the doses of 100, 200, 400 mg/kg, respectively. The mouse was centrally placed in the illuminated compartment and allowed to explore the environment. The time spent in the light and dark compartments were recorded. Increased entrance or time spent in the light compartment was taken as an index of less anxiety.^[14]

Statistical analysis

Data were analyzed using One-way analysis of variance (ANOVA) followed by post-hoc tests (Student Newman Keul's) which was used to determine the source of a significant effect. Results were expressed as Mean ± SEM., while $p < 0.05$ was taken as accepted level of significant difference from control or vehicle.

RESULTS

Effect of Methanol extract of *Sansevieria liberica* on anxiety using Elevated Plus maze

Figure 1a revealed a significant increase in the time the mice spent in open arm most especially at the doses of 200 and 400 mg/kg body weight, the number of entries into the open arm was very significant at 400 and not very significant at 200 mg/kg body weight as shown in fig 1b.

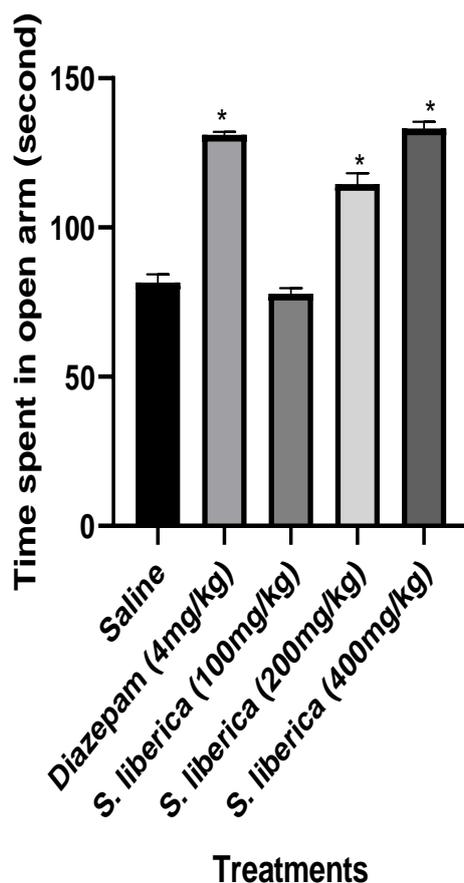


Figure 1a: Effect of methanol extract of *S. liberica* and diazepam on time spent in open arm by the mice. Each column represents the mean \pm SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test.

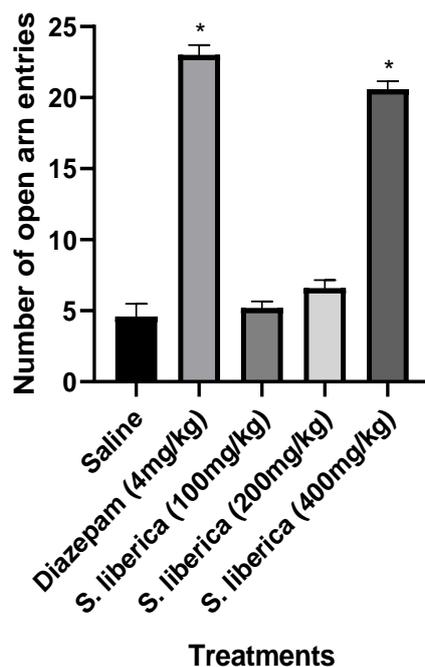


Figure 1b: Effect of methanol extract of *S. liberica* and diazepam on number of open arm entries by the mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test.

Effect of Methanol extract of *Sansevieria liberica* on anxiety using Light/dark test

The results as shown in Table 1 revealed that *Sansevieria liberica*, at the dose of 100, 200, and 400 mg/kg, demonstrated significant and dose-dependent increase in the time spent in light box and number of transitions between the two boxes as compared with the control (saline-treated) group. The time spent in the light box and the transition made in-between boxes by the animals treated with diazepam were significantly increased.

Table 1: Effect of Methanol extract of *Sansevieria liberica* on anxiety using Light and dark test.

Treatment	Dose (mg/kg)	Time spent in the light box(s)**	Number of transition **
Saline	0	72.8 ± 18.39	7.60 ± 0.91
Diazepam	4	135.7 ± 6.07*	12.41 ± 2.06*
<i>S. liberica</i>	100	110.9 ± 2.53*	10.61 ± 3.12*
<i>S. liberica</i>	200	138.5 ± 5.34*	10.87 ± 2.34*
<i>S. liberica</i>	400	174.7 ± 4.28*	13.10 ± 1.31*

**Values are recorded as means±SEM (n=5). *Values are statistically significant (p<0.05) in relation to control. One-way ANOVA follow by Newman-Keuls Multiple Comparison tests.

Effect of Methanol extract of *Sansevieria liberica* on anxiety on open field test (OFT)

Methanol extract of *S. liberica* demonstrated dose-dependent and a significant decreased in number of rearing (fig 2a) (number of times the animal raised its forelimb in the air or on the wall of container) and faecal drooping (fig 2d), but significant increase in the number of square crossing (central fig 2b and peripheral fig 2c) in open field test compared with saline-control group. Diazepam also produced significant increase in the number of squares crossed but decreased in number of rearing and faecal droppings.

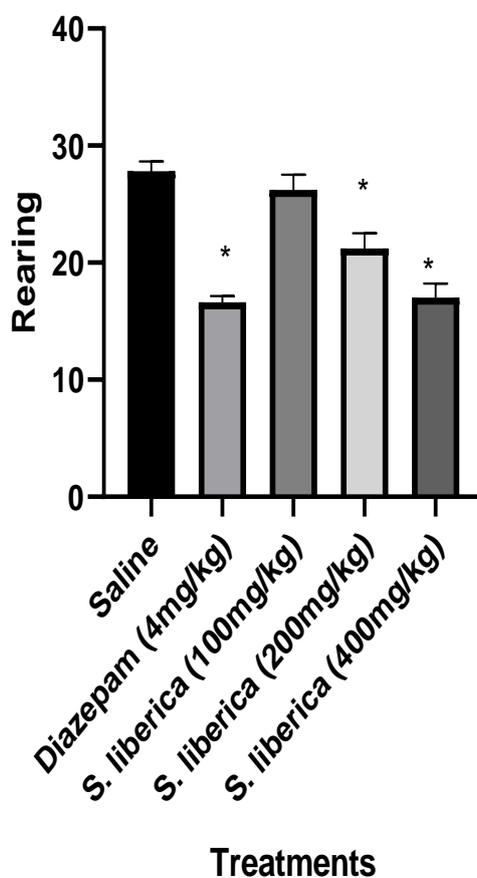


Figure 2a: Effect of methanol extract of *S. liberica* and diazepam on rearing behavior.

Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test.

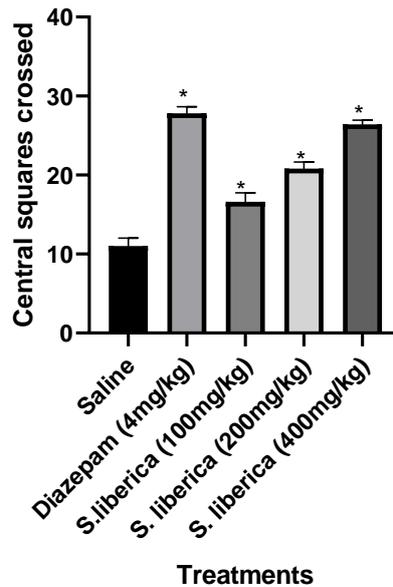


Figure 2b: Behavioural effect of methanol extract of *S. liberica* and diazepam on central squares crossed by mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test.

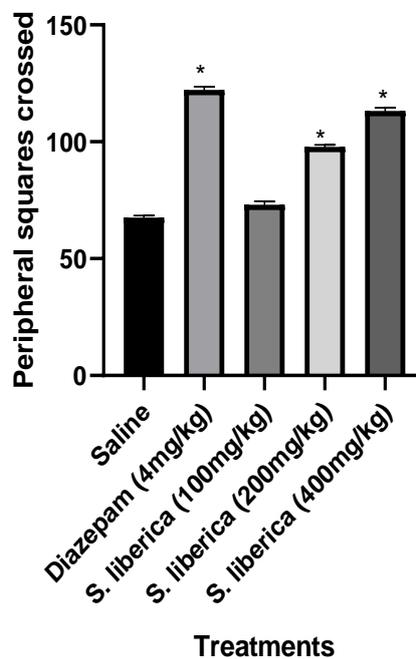


Figure 2c: Behavioural effect of methanol extract of *S. liberica* and diazepam on peripheral squares crossed by mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test.

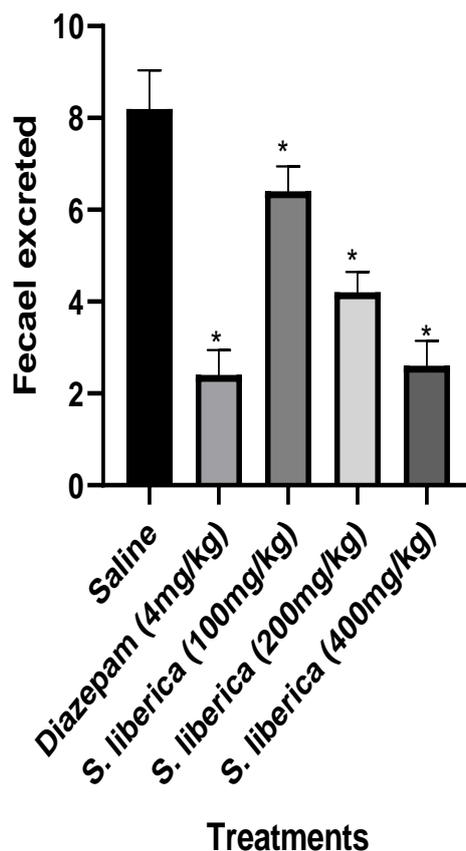


Figure 2d: Behavioural effect methanol extract of *S. liberica* and diazepam on fecal excreted by mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test.

Effect of Flumazenil Antagonism on the Anxiolytic Effect of the methanol extract of *S. liberica* leave

Flumazenil significantly nullified the effect of methanol extract of *S. liberica* leave on time spent in open arm entries while, the novelty-induced rearing in mice increased (Figs 3a and 3b).

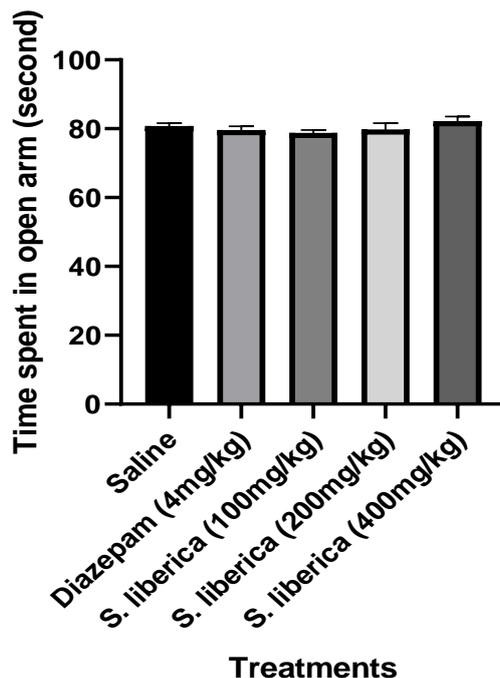


Figure 3a: Effect of methanol extract of *S. liberica* and diazepam on time spent in open arm by the mice. Each column represents the mean±SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test.

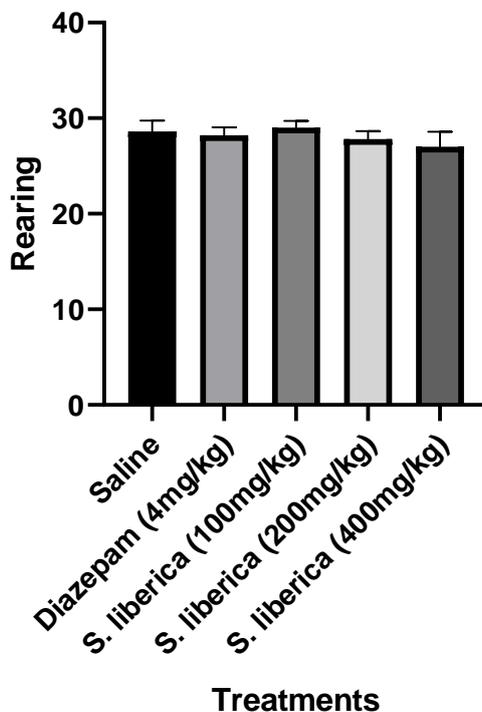


Figure 3b: Effect of methanol extract of *S. liberica* and diazepam on rearing behavior.

Each column represents the mean \pm SEM (n=5 per group). *P<0.05 compared to treated groups. ANOVA followed by Newman-Keuls Multiple Comparison test

DISCUSSION

Anxiety is known to be one of the major health problem in the society and may have serious consequent on the quality of life.^[2] There are many drugs over the counter that are available for the treatment of anxiety such as antidepressants, benzodiazepines, buspirone and cognitive behavior therapies.^[15] Many patients failed to comply with the use of most of these drugs because of their untoward side effects most especially the elderly.^[16] Based on the aforementioned condition, we therefore, decided to investigate the anxiolytic effect of *Sansevieria liberica* extract using mice as a models. Probably, most of the aforementioned adverse effects might be resolved.

The elevated plus-maze and light-dark box tests are the most used animal models of anxiety to evaluate or to search for anxiolytic drugs. When animal is exposed to an environment very new, and animals are not familiar with it, there is a natural conflict between the tendency to explore and tendency to avoid an unknown risk. The anxiolytic effect of an agents is to enhance the response of GABA-receptor to GABA, by way of increasing the opening GABAA-activated chloride channels. In the present study, we used the EPM model of anxiety to evaluate the anxiolytic effects of *Sansevieria liberica*. It was previously reported that diazepam increases the time spent and numbers of entries to the open arm.^[17] In the present study, the time spent as well as the number of entries into the open arms increased with diazepam. These confirmed the previous studies, which indicated that diazepam like other benzodiazepines exhibited anxiolytic effects in a variety of anxiolytic screening tests, including EPM. Methanol extract of *Sansevieria liberica* showed similar results to diazepam. The time spent and the number of entries in the open arms increased significantly (P<0.05) at the maximum dose (400mg/kg). Methanol extract of *Sansevieria liberica* most likely work in a similar way to diazepam. The pretreatment of mice with flumazenil, a specific antagonist of the benzodiazepine site inhibited anxiolytic effect of *Sansevieria liberica* and the diazepam. The mechanism of action of *Sansevieria liberica* may therefore, be mediated via the activation of the benzodiazepine site of the GABA receptors of the central nervous system. We may therefore hypothesize that *Sansevieria liberica* may be acting like a benzodiazepine-like substance.

It was previously reported that, in the light-dark box test, drug-induced increases in time spent in the light box and number of transitions in to the light chamber.^[18] where an increase in these parameters is considered to reflect anxiolytic-like properties. In the light-dark box test animals mostly want to spend more time in dark box as contrasted with light box on account of fear of exposure to the new environment.^[11] In the present study, the extract significantly increased the time spent in illuminated side dose-dependently suggesting anxiolytic action.

In an open field test, the locomotion of an anxious animal is strikingly diminished. The animal also would like to pay more attention in the peripheral areas, also, there is every tendency for reduction in rearing and grooming in an anxious animal.^[10] An increase in number of squares cross and decrease in the number of faeces excreted by the mice indicating anxiolytic-like activity.^[21,11] while the novelty-induced rearing behavior was reduced. The frequency of rearing in rodents can be used in assessing the extracts for both sedative property and central nervous system stimulation.^[20] In this study, methanol extract, has the principles that inhibit NIR in mice. This study suggested that methanol extract possess sedative activity. Endogenous neurotransmitters, such as acetylcholine (ACh), dopamine, serotonin (5-HT), gamma-amino butyric acid (GABA), opioid and noradrenaline was reported to regulate novelty-induced rearing behavior.^[21,22,23] The study also showed that the methanol extract at a dose of 400 mg/kg body weight was empirically equipotent to 4 mg/kg body weight of diazepam for the sedative effect in mice. In order to establish the mechanism or receptor involve in NIR behaviors inhibition, some of the receptor antagonists (atropine for muscarinic receptors and flumazenil for GABA receptors) were administered to the test animals.^[24] In this study, flumazenil (10 mg/kg, i.p.) blocked the NIR inhibition induced by the methanol extract. This study therefore concludes that the inhibition of novelty induced rearing measures the sedative property of the extract mediated via GABA receptor. Also, increased number of squares crossed and a decreased in the number of faeces excreted by the mice compared with control in the mice administered with the extract and diazepam suggested anxiolytic activity.

In conclusion, the present study establishes the anxiolytic-like activity of methanol extract of *S. liberica* in animal models of anxiety. Isolation, mechanism of action and toxicology evaluation of the active constituents would be a focus of future work.

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