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Original article

A neuropharmacological profile of *lycium schweinfurthii* (solanaceae) methanolic extract in mice

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Abstract: *Lycium schweinfurthii* is a shrub belonging to the Solanaceae family which widely grows in North Africa and Mediterranean regions. The plant leaves have traditionally been used for gastrointestinal diseases as peptic ulcer in Libya. This study aimed to investigate the effect of *Lycium schweinfurthii* extract on the central nervous system in mice including anticonvulsant, antidepressant and muscle relaxant activities. The methanolic extract was prepared by fractionation technique. Albino male mice weighing 22 ± 2.0 gm were used and equally divided into equal number and weight for each experiment (n = 6). The best effective pharmacologically dose of 400 mg/kg, i.p. of the methanolic extract was selected to explore the anticonvulsant activity for picrotoxin-induced convulsion in mice (5.0 mg/kg), antidepressant activity of forced swimming test of depression and muscle relaxant action by motor coordination test of hanging wire. Fluoxetine (10.0 mg/kg), imipramine (15.0 mg/kg) and diazepam (5.0 mg/kg) were used as reference compounds. *Lycium schweinfurthii* extract exhibited a significant prolonged delay in the onset time of induced convulsion and significant decrease in the frequency of convulsion as well as a significant decrease in the duration time of attacks. Pretreatment with flumazenil (2.0 mg/kg) was found to increase the frequency and duration of convulsions without profound change in the onset time produced by *Lycium schweinfurthii*. For antidepressant activity, the plant leave extract significantly decreased immobility time duration without a muscle relaxant effect. The results suggest that the methanolic extract of *Lycium schweinfurthii* leave has anticonvulsant and antidepressant-like activities without any muscle relaxant effect in mice. Thus, *Lycium schweinfurthii* may have a neuropharmacological potential use in human.

Introduction

Lycium schweinfurthii (*L.S.*) dammer is grown in the sandy and stony along the coastal line and belongs to the Solanaceae family which is widely distributed in North Africa and Mediterranean regions [1]. It can be found flowering throughout the year and it is found in some countries as Egypt, Tunisia, Libya, Portugal and Spain [1]. The leaves and fruits have traditionally been used for gastrointestinal diseases such as stomach ulcers [2]. Previous phytochemical studies have revealed presence of vital bioactive compounds in different parts of *L.S.* as flavonoids, alkaloids, saponins, glycosides and others [3]. The total flavonoids and other contents showed concentration in the leaves

more than in the other parts of the plant. Indeed, the isolation of five flavonoids from the methanolic extract of leave using different chromatographic techniques was recently reported [4]. A glucoside and five known compounds isolated from *L.S.* of total plant methanolic showed a potent inhibitory effect and lowering effect in postprandial hyperglycemia in diabetic patients [5]. 25 known with two compounds have also been isolated from *L.S.* with four showed cytotoxic effects against skin cancer cells while three showed cytotoxic effects against colon cancer cells [6].

In a recent published study [7], the phytochemical study of methanolic extract of *L.S.* indicated presences of flavonoids, tannins, saponins, phenols, cardiac glycoside, alkaloids and carbohydrates in the extract. The total flavonoids were the most content compounds in the extract. Indeed, Sherif and others reported that *L.S.* leave methanolic extract has depression, sedative and analgesic activities [6]. Several previous attempts have been made to screen some plant extracts for central neurological diseases as anticonvulsant compounds from plant-origin phytomedicines of other than *L.S.* These may provide a suggestion for developing a newer antiepileptic cmpounds with more efficacy and less side effects that can either be used alone or as an adjuvant to the existing anticonvulsant medications [8].

Depression is also considered as a major central psychiatric disorders. It is linked to severe morbidity, death and impairment in psychosocial and vocational functioning [9]. Our previous preliminary blind screening observations by Irwin primary tests for pharmacological activity of the plant extract revealed some forms of neuropharmacological responses [7] as well as to the best of our knowledge, there is no previous published data about the central pharmacological effects of *L.S.* in experimental animals. Thus, this work was aimed to explore the central effects of the methanolic extract of *L.S.* leave in Albino mice including anticonvulsant and antidepressant activities as well as muscle relaxant effect.

Materials and methods

Plant materials: Fresh plant leaves of *L.S.* were collected from Gharyan area, Northwest region of Libya, during March, 2021 (**Figures 1** and **2**). The plant was identified by two independent Botanists at the Herbarium of Department of Botany, Faculty of Science, University of Tripoli, Tripoli, Libya and a voucher specimen No: 676271 was deposited in the Herbarium [7].

Plant collection and extraction: A total weight of 387 gm of the plant leaves was collected. Then, the plant leaves were shade-dried to a constant weight and uniform powder (350 gm) was extracted by cold maceration starting with the solvent of least polarity to highest (petroleum ether, ethyl acetate and methanol 96%) and were fractionated as a first purification step for five days. Each solvent with intermittently shaken and stirred, and then finally the extract of methanol was filtered by Whatman number 1 filter paper. Then, concentrated under control reduced pressure by a rotatory evaporator at 40 °C using a vacuum pump and collected in a glass container which is left uncovered until complete dryness to obtain the crude extract. The extract was weighed (40.3 gm, yielding 11.5%) and stored in a refrigerator at 4 °C until used [7, 10].

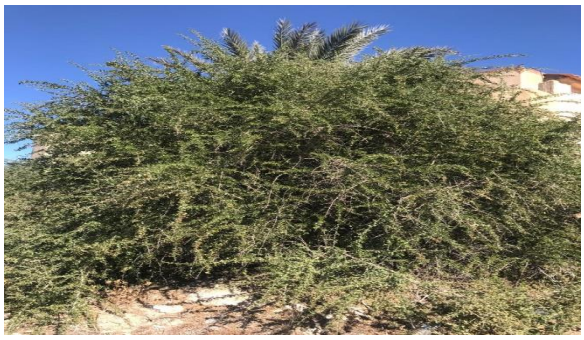

Figure 1: *Lycium schweinfurthii*

Figure 2: Flower of *Lycium schweinfurthii*

Experimental animals: Male Albino mice weighing of 22.2 ± 2.1 gm aged four months were used throughout this study. Mice were obtained from the central animal house of University of Tripoli, Tripoli, Libya (n = 114). The animals were housed in a standard animal cage (n = 6, for each cage) under controlled temperature (23 ± 2.0 °C) and humidity (70.0 – 80.0%) with a 12 hour dark/light cycle (6: 00 am - 6: 00 pm) and a free access to food and drinking water. All the animals were fasted overnight before starting the experiments but allowed a free access to tap water. All experiments were carried out between 9: 00 am and 02: 00 pm in a quiet room with low light (04.0 watt). The experimental protocols were approved by University Ethics Committee, University of Tripoli (021-2021) which is according to the international guidelines for animal use. The plant extract was prepared daily and immediately before use. All the treatments were given by an intraperitoneal route of administration.

Picrotoxin-induced convulsion: Six groups of mice (n = 6, for each group) were used in the current experiment. Group 1 administered vehicle and representing control. Group 2 was given 400 mg/kg *L.S.* methanolic plant extract [7]. Groups 3 and 4 were given diazepam in a dose of 1.0 and 5.0 mg/kg body weight, respectively, which were used as standard groups. Groups 5 and 6 were given diazepam at doses of 1.0 and 5.0 mg/kg in combination with *L.S.* extract at a dose of 400 mg/kg, respectively. After 30 min, 5.0 mg/kg of picrotoxin was injected to all the groups and mice were placed individually in the cage for a visual observation for 30 min. Induction time, frequency and duration of convulsions with type of attack and mortality were all recorded as previously reported by Adebesin and others [11].

The effect of flumazenil on picrotoxin-induced convulsion: Four groups of mice (n = 6, each group) were used in this method. Group 1 was administered only vehicle and after 30 min was given picrotoxin (5.0 mg/kg) which is used as a control. Group 2 was administered *L.S.* extract (400 mg/kg) before giving picrotoxin (5.0 mg/kg) for 30 min. Group 3 was pre-treated with flumazenil (2.0 mg/kg) for 30 min to the administration of the *L.S.* extract (400 mg/kg) and picrotoxin (5.0 mg/kg). Group 4 was treated with flumazenil (2.0 mg/kg) for 30 min before being administered with picrotoxin (5.0 mg/kg). All the parameters were recorded as reported by Ajayi and others [12].

Forced swimming test: The forced swimming test for assessing antidepressant activity was used as described previously by Prosolt et al. [13]. Thus, mice were divided into six groups (n = 6, for each group). Group 1 was administered vehicle and is used as a control. Group 2 administered 400 mg/kg *L.S.* extract. Group 3 was given fluoxetine 10.0 mg/kg and used as a standard [8]. Group 4 was treated with fluoxetine at a dose of 10.0 mg and 400 mg/kg *L.S.* extract. Group 5 administered 15.0 mg/kg of imipramine and used as a standard [14]. Group 6 received imipramine at a dose of 15.0 mg/kg with *L.S.* extract at a dose of 400 mg/kg. After 30 min, mice were individually forced to swim in an open glass cylinder (diameter of 15.0 cm and height of 25.0 cm) containing 15.0 cm of water at 25.0 ± 2.0 °C. The total duration of swimming, immobility (floating slightly hunched to keep it is head above the water) and mobility were all recorded during the last four minutes of the six minutes testing period by a video camera. Thereafter, the mice were removed from the water and wiped with towels until dry [13].

Hanging wire test: The wire-hanging test was used to assess the motor coordination and neurom-uscular tone of experimental animals as described previously by van Putten et al. [15]. The apparatus is comprised of thick metallic wire (2.0 mm diameters and 55.0 cm length), elevated 35.0 cm above the bench to prevent the mice from injury when it falls down, resting on two vertical stands. Mice were divided into three groups (n = 6, for each group). Group 1 was administration vehicle and used as a control. Group 2 was given 400 mg/kg *L.S.* extract. Group 3 administered diazepam at a dose of 5.0 mg/kg and used a standard [16]. The mice were placed by their forelimbs to the center of the wire for a period of 180 sec. When the mice fell down, it was placed again on the wire. For each mouse where the recorded falling score is set to

10.0, the reaching score is set to zero diminished or increased by one, respectively, and the time that elapsed between falls. The test ended when mice were fall 10 times or after 180 sec [16].

Statistical analysis: All data were expressed as mean ± SEM. All data were tested for parametric or non-parametric distribution by the Kolmogorov-Smirnov test. Data were analyzed by analysis of variance (one way-ANOVA) followed by Tukey's post hoc multiple comparison test. A Kruskal-Wallis test was used for non-parametric data followed by Mann-Whitney *U*-test for individual comparisons. A p value of less than 0.05 was considered statistically significant.

Results

In **Table 1**, the effect of *L.S.* methanolic extract in a dose of 400 mg/kg body weight on picrotoxin-induced convulsion in the mouse is shown. It significantly prolonged onset time of convulsion by 56.0% (induction time) and significantly decreased the frequency and duration time of convulsion by 59.0% and 55.0%, respectively. This methanolic extract dose was chosen upon a pilot and previous study of the therapeutic and toxic effects of the extract in mice [7]. While the effect of diazepam at a dose of 05.0 mg/kg produced a complete protection against the convulsion compared with plant extract. Further, the *L.S.* extract at a dose of 400 mg/kg combined with 05.0 mg/kg diazepam produced completely protection of mice from convulsion induced by picrotoxin with a similar observation as diazepam (05.0 mg/kg alone).

As shown in **Table 2**, the effect of diazepam at a dose of 01.0 mg/kg significantly increased the onset time of convulsion by 72.0% (induction time) and significantly decreased the frequency and duration of time of convulsion by 74.0% and 45.0%, respectively, without a significant change in the onset and duration of convulsion. It significantly decreased the frequency of convulsion compared to the *L.S.* extract 400 mg/kg. The plant extract of 400 mg/kg combined with diazepam 01.0 mg/kg produced a complete protection against convulsion compared *L.S.* extract alone and diazepam 01.0 mg/kg alone. Further, no death was observed in any treated group in this study. The type of attack was found to be similar to clonic convulsion.

Table 1: Effect of *Lycium schweinfurthii* on picrotoxin-induced convulsion in mice with diazepam.

Parameters / Group	**Control group**	***L.S.* extract 400 mg/kg**	**Diazepam 5.0 mg/kg**	***L.S.* extract 400 mg + diazepam 5.0 mg/kg**
Onset of convulsion (min)	11.50 ± 0.42	18.00 ± 0.68*	0.00*†	0.00*†
Frequency of convulsion	30.17 ± 0.70	12.33 ± 0.84*	0.00*†	0.00*†
Duration of convulsion (min)	05.17 ± 0.30	02.33 ± 0.33*	0.00*†	0.00*†

Data are mean ± SEM, n = 6. *L.S.* is *Lycium schweinfurthii*.
*Significant difference from the control group.
†Significant difference from *L.S.* group.

Table 2: Effect of *Lycium schweinfurthii* on picrotoxin-induced convulsion in mice with diazepam.

Parameters / Groups	**Control group**	***L.S.* extract 400 mg/kg**	**Diazepam 1.0 mg/kg**	***L.S.* extract 400 mg + diazepam 1.0 mg/kg**
Onset of convulsion (min)	11.50 ± 0.42	18.00 ± 0.68*	19.83 ± 0.60*	0.00*†‡
Frequency of convulsion	30.17 ± 0.70	12.33 ± 0.84*	07.83 ± 0.47*†	0.00*†‡
Duration of convulsion (min)	05.17 ± 0.30	02.33 ± 0.33*	02.83 ± 0.30*	0.00*†‡

Data are mean ± SEM, n = 6. *L.S.* is *Lycium schweinfurthii*.
*Significant difference from the control group,
†Significance different from *L.S.* group,
‡Significant different from diazepam group.

Table 3 shows that mice treated with *L.S.* methanolic extract at a dose of 400 mg and picrotoxin at a dose of 5.0 mg/kg significantly increase the onset time of convulsion by 50.0% and decrease the frequency and duration time of convulsion by 59.0% and 60.0%, respectively. Flumazenil at a dose of 2.0 mg with *L.S.* extract at a dose of 400 mg and at picrotoxin 5.0 mg/kg significantly increased the onset time of convulsion by 47.0%, with no significant change in the frequency and duration of convulsion as compared with the control group. At the same time, there is a significant increase in the frequency and duration of convulsion without a significant change in the onset of convulsion compared to *L.S.* methanolic extract with picrotoxin. In comparison to the effects of flumazenil (2.0 mg/kg) and picrotoxin (5.0 mg/kg), no significant change in the onset, frequency and duration of convulsion was observed in comparison to the control group. However, significant decrease in the onset of convulsion and increase in the frequency and duration time of convulsion compared to the *L.S.* extract combined with picrotoxin were noted. No significant changes in all the parameters compared to flumazenil combined with *L. S.* extract and picrotoxin were observed (**Table 3**).

Table 3: Effect of flumazenil on *Lycium schweinfurthii* extract in picrotoxin-induced convulsion in mice.

Parameters / groups	**Control picrotoxin 5.0 mg/kg**	***L. S.* extract 400 mg + picrotoxin 5.0 mg/kg**	**Flumazenil 2.0 mg + *L. S.* extract 400 mg + picrotoxin 5.0 mg/kg**	**Flumazenil 2.0 mg + picrotoxin 5.0 mg/kg**
Onset of convulsion (min)	12.00 ± 0.68	18.00 ± 0.68*	17.67 ± 0.88*	14.50 ± 1.11†
Frequency of convulsion	30.50 ± 0.67	12.33 ± 0.84*	29.33 ± 0.84†	31.33 ± 1.14†
Duration of convulsion (min)	05.83 ± 0.30	02.33 ± 0.33*	05.83 ± 0.30†	06.55 ± 0.42†

Data are mean ± SEM, n = 6. *L.S.* is *Lycium schweinfurthii*.
*Significant difference from the control group,
†Significant difference from *L.S.* group.

As shown in **Table 4** using forced swimming test, the *L.S.* methanolic extract at a dose of 400 mg/kg significantly decreased by 60.0% of the duration of immobility time in mice. In the standard group, fluoxetine at a dose of 10.0 mg/kg body weight significantly decreased by 50.0% of the duration immobility time compared to the control group with a significant change compared to *L.S.* extract alone. The methanolic extract of *L.S.* (400 mg/kg body weight) combined with fluoxetine at 10.0 mg/kg of body weight in mice produced a significant reduction in the duration of immobility time by 70.0% compared to the control and a significant change between *L.S.* methanolic extract and fluoxetine groups. In **Table 5**, imipramine at 15.0 mg/kg of body weight as another standard induced a significantly decrease by 66.0% in the duration of immobility time of mouse compared to The control group and a significant change compared to the *L.S.* extract. Whereas, the combined *L.S.* extract of 400 mg/kg with imipramine at 15.0 mg/kg showed a significant decrease in the duration of immobility time by 69.0% compared to the control. Thus, a significant difference between *L.S.* extract and imipramine with insignificant difference between imipramine and combination groups was observed (**Table 5**).

With regard to the hanging wire test, **Table 6** shows mice treated with *L.S.* extract 400 mg/kg did not induce any significant change in the score of reach, fall and in time spent on hanging wire compared to the control group. On the other hand, the group was treated with diazepam 5.0 mg/kg produced a significantly decrease in the score of reach and time spent on hanging wire and increase in the score of fall compared to the control and *L.S.* extract groups.

Table 4: Effect of *Lycium schweinfurthii* and fluoxetine on forced swimming test in mice

Group	Dose	Mobility time (sec.)	Immobility time (sec.)
Control	5.0 ml/kg	021.83 ± 1.95	219.83 ± 2.44
***L. S.* extract**	400 mg/kg	150.67 ± 3.21*	88.17 ± 3.28*
Fluoxetine	10.0 mg/kg	130.50 ± 2.04*†	108.50 ± 2.21*†
***L. S.* + fluoxetine**	400 mg + 10.0 mg/kg	174.67 ± 0.95*‡	64.50 ± 1.17*†‡

Data are mean ± SEM, n = 6. *L.S.* is *Lycium schweinfurthii* extract.
*Significant difference from the control group,
†Significant difference from *L.S.* group,
‡Significant different from fluoxetine group.

Table 5: Effect of *Lycium schweinfurthii* and imipramine on forced swimming test in mice

Group	Dose	Mobility time (sec.)	Immobility time (sec.)
Control	5.0 ml/kg	021.67 ± 2.59	217.33 ± 2.44
***L.S.* extract**	400 mg/kg	150.67 ± 3.21*	88.17 ± 3.28*
Imipramine	15.0 mg/kg	165.83 ± 4.59*†	73.67 ± 4.60*†
***L.S.* + Imipramine**	400 mg + 15.0 mg	172.33 ± 1.78*†	66.83 ± 1.55*†

Data are mean ± SEM, n = 6. *L.S.* is *Lycium schweinfurthii*.
*Significant difference from the control
†Significant difference from *L.S.* group.

Table 6: Effect of *Lycium schweinfurthii* on muscle relaxation using hanging wire test in mice

Parameters / Groups	Control (5.0 ml/kg)	*L.S.* extract 400 mg/kg	Diazepam 5.0 mg/kg
Score of reach	03.50 ± 0.34	3.83 ± 0.30	00.00 ± 0.00*†
Score of fall	10.00 ± 0.00	10.00 ± 0.00	00.00 ± 0.00*†
Time spent hanging (sec)	180.00 ± 0.00	180.00 ± 0.00	29.33 ± 1.89*†

Data are mean ± SEM, n = 6. *L.S.* is *Lycium schweinfurthii* extract.
*Significant difference from the control group
†Significant difference from *L.S.* group.

Discussion

To the best of our knowledge, this is the first study to explore the effects of *L.S.* on chemically induced convulsion in experimental animals. Picrotoxin is a non-competitive GABA-A receptor that blocks the chloride- ions channels linked to the GABA-A receptor [17]. The present findings show a change between the groups and the ability of the *L.S.* methanolic extract to prolong the onset time of convulsion (latency) and to reduce the frequency and duration time of convulsions induced by picrotoxin. The effects of *L.S.* extract is in line with the effect of diazepam at a low dose (01.0 mg/kg), as well as, the combination of the plant extract with diazepam at 01.0 mg/kg body weight produced a complete protection of convulsion. Thus, *L.S.* extract potentiates diazepam anticonvulsant effect which may indicate that *L. S.* has anticonvulsant-like activity. This may be considered as an indication that *L.S.* extract contains certain active compounds that have anticonvulsant activity as flavonoids [18 - 20]. The effect of selective GABA-A Benzodiazepam (GABA-BZD) complex receptor antagonist the flumazenil on the anticonvulsant activity of *L. S.* [21, 22]. On the

other hand, the administration of flumazenil before *L.S.* in the picrotoxin-induced convulsion in mice led to an increase in the frequency and duration of convulsions compared with *L. S.* alone group. This shows that flumazenil antagonizes the effect of plant extract. Further, no death of mice was observed in this study which indeed supports the safety use of the plant extract at least in single use. Repeated use of *L. S.* is an interest area for research to support such effect. With regard to the forced swimming test, the present method is considered to be a simple and quick method used for screening antidepressant activity [23, 24]. Our present findings show that the *L.S.* extract acts as an antidepressant in the mice and produced a significantly reduction in the duration of the immobility time and enhanced swimming and significantly more effective than fluoxetine. The co-administration of *L.S.* extract significantly enhanced the antidepressant activity of fluoxetine greater than fluoxetine alone in mice of despair. This findings opens a new area for further use of the plant in psychiatric disorders. It has been observed that no change between imipramine alone and in combination of *L.S.* extract with imipramine. This may indicate that *L.S.* has antidepressant-like effect which is more likely mediated by the serotonergic pathway rather than the noradrenergic pathway. Moreover, motor coordination and the muscle relaxant activity of *L.S.* methanolic extract in mice were identified by using a hanging wire test as a standard model. The data obtained from scores reach, fall and time spent on hanging wire, after administration of *L.S.* extract did not change the motor coordination of the mice, which is more likely no muscle relaxant activity of this extract at least in the dose used in mice. Further phytochemical studies for quantitative determination and repeated doses in experimental animals for neuropharmacological properties are ongoing for a potential therapeutic uses.

Conclusion: The crude methanolic extract of *Lycium schweinfurthii* leaves possesses anti-convulsant and anti-depressant-like activities in mice without any muscle relaxant effect. The methanolic exact of the plant leaves has some pharmacologically active compounds which can be potentially investigated for such therapeutic uses.

Author contribution: HAB & FMS conceived, designed the study, collected data and performed the analysis. MAS has contributed in analysis. All authors have drafted and revised the manuscript as well as approved the final version of the manuscript and agreed to be accountable for its contents.
Conflict of interest: The authors declare absence of any commercial or financial relationships that could be construed as a potential conflict of interest.
Ethical issues: Including plagiarism, informed consent, data fabrication or falsification and double publication or submission have completely been observed by authors.
Data availability statement: The raw data that support the findings of this article are available from the corresponding author upon reasonable request.
Author declarations: The authors confirm that all relevant ethical guidelines have been followed and any necessary IRB and/or ethics committee approvals have been obtained.

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