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Research Article

Antidepressant-like effect of ethanol extract of *Salvia fruticosa* leaves (*Labiatae*) in a mouse model of depression; Role for 5-HT_2receptors

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Abstract

Salvia fruticosa L. (Labiatae) is a well-known plant for its medicinal values in several medical disorders. In the present study, S. fruticosa, at the i.p. doses 125 and 250 mg/kg, showed significant antidepressant effect against both forced swimming test (FST) and tail suspension test (TST) induced immobility behave in mice, with a maximum protective values, respectively, of (FST; 51.9% and 72.3% decrease; P<0.001; TST; 36.8% and 45.4% decrease, P<0.05). In addition, in FST, S. fruticosa significantly increased active swimming by 75.1% (P<0.001) compared with fluoxetine value of 66.7%, but produced no significant changes in climbing tries against a cylinder walls. The extract also showed insignificant effect on locomotor activity of mice. Moreover, the antidepressant effect of S. fruticosa, in TST, was significantly abrogated in mice pretreated with cyproheptadine (3mg/kg, i.p), indicating a role for 5HT_2 receptors in this protective effect. Phytochemical screening of the extract showed the presence of saponin, steroids, alkaloids and phenolic compounds. These findings demonstrate that S. fruticosa ethanol extract has a potential antidepressant-like effect that seems to be mediated by serotonergic system, via stimulation of 5HT_2 receptors, as well as providing a scientific evidence for its use in folk medicine.

Keywords: Depression; Serotonin; Fluoxetine; Salvia fruticosa; Forced swimming test; Tail suspension test; Immobility; Mice

Introduction

Depression, or in other word hopelessness, is an emotional disorder characterized by alteration in mood, lack of attention in the surroundings and psychomotor retardation. It has been estimated that the number of people living with depression in the world is more than 320 million and its prevalence rates vary by age, peaking in older adulthood "above 7.5% among females aged 55-74 years, and above 5.5% among males" (Park *et al.*, 2011, WHO, 2017). In addition, clinical studies have showed that suicide is one of the most common depression outcomes (Shahmoradi and Saadat, 2018, WHO, 2001) Currently, a number of drugs are used in the treatment of depression but since the efficacies of the available antidepressant drugs are very limited and the problematic side-effects are in a significant number of patients, there is search for more efficacious and better-tolerated regimens. In this consequence, the use of alternative therapy, traditional medicine included, in particular medicinal plants, for the treatment of psychiatric and behavioral illness has been intensively increased. A foremost example is St John's WortL., Hypericaceae (Blumenthal, 2002).

The use of medicinal plants as cures is as ancient as human civilization. In recent times, crude plant extracts are authenticated worldwide as a great source of phytochemicals having plentiful biological activities. In this regard, various *Salvia* species are investigated after widely used in traditional medicine. *Salvia fruticosa* L. of the family *Lamiaceae* (*Labiatae)* is an aromatic perennial herb distributed in different regions of the world; Central and

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South America; South Africa and South-Eastern Asia. Moreover, it is more concentrated in Europe "in countries bordering the Mediterranean Sea" and known as a Mediterranean sage (Gali-Muhtasib, 2006). *S. fruticosa* is an evergreen shrub plant growing up to 1 meter. The leaf has an oval shape and its edge is 8-50mm long and 4-20mm wide. In folk medicine, *S. fruticosa* is commonly used by herbalists and people either as inhaled in steam baths, internally as drinks or even applied externally. Indeed, *S. fruticosa* has been reported to possess diverse medicinal properties. *S. fruticosa* leaves are boiled as a tea for the relief of colds, influenza and pains (Boukhary *et al.*, 2016). Preclinical studies have shown that *S. fruticosa* exhibits hypoglycemic effect and it improves memory (Bassil *et al.*, 2015, Gali-Muhtasib, 2006, Raafat *et al.*, 2013). Boukhary and co-workers (2016) have demonstrated that *S. fruticosa* possess strong radical scavenging activity and potential anti-inflammatory effects in mice (Boukhary *et al.*, 2016). Furthermore, it was shown that *S. fruticosa* organic extract did inhibit the spontaneous movement of rabbit ileum, and to inhibit acetylcholine induced contractions of the rabbit ileum (Al-khalil and Suleiman, 1992), and to modulate $GABA_A$ receptors (Abdelhalim *et al.*, 2014). However, despite the widespread uses of this plant, no scientific work is reported in literature regarding the effect of *S. fruticosa* leaves against depression like states. Nonetheless, several *Salvia* species is reported to have antidepressant activity and its leaves are used in traditional medicine for the treatment of different central nervous system diseases, principally depression (Herrera-Ruiz *et al.*, 2006).

The genus *Salvia* L. comprises more than 500 species, mainly perennial herbs growing wildly throughout the world. Hence, the pharmacological activities of *S. fruticosa* (Boukhary *et al.*, 2016) and other species of the family *Labiatae* (Raafat *et al.*, 2013) was the subjects of several studies, antidepressant activities included (Mora *et al.*, 2006). Animal model studies have showed that *Salvia elegans* could be able to protect mice from depression (Mora *et al.*, 2006). *S. miltiorrhiza* f. *alba* Wu and Li, used in Chinese traditional medicine, showed neuro protective effect, due to the NMDA receptor antagonistic activity (Sun *et al.*, 2003), *S. reuterana* Boiss showed anxiolytic effect in mice (Rabbani *et al.*, 2005) and *S officinalis* possesses metabolites with benzodiazepine-like effects (Kavvadias *et al.*, 2003). So, in order to explore the rich biodiversity of the country and discover natural products, and to provide more scientific evidences, since until nowadays there is no preclinical evidence indicating the antidepressant action of *S. fruticosa*, the present study, therefore, was aimed, to evaluate the possible antidepressant activity of *S. fruticosa* ethanol extract using two mouse models, forced swimming test and tail suspension test. In addition, to investigate whether the serotonin pathway has a role in this antidepressant effect we functionally blocked 5-HT_2 receptors.

Material and methods

Drugs and chemicals

All chemicals used during this study were of analytical grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA). Fluoxetine was purchased from Ely-Lilly Co. (Indianapolis, USA). Cyproheptadine (a $5HT_2$ receptor antagonist) was purchased

from Ibn Al Baytar (Carthage, Tunisia) S/L Hikma Pharmaceutical, Jordan.

Animals

Albino mice (adult ♂, weighing 18–38g and aged 2-3 months) were obtained from the animal house of Tripoli University, and used throughout the whole experiments. Animals were kept at room temperature (25-27 °C) with free access to water and food" *ad libitum"* under a 12:12 h light: dark cycle. Six mice, one mouse from each group, were housed per cage in 4 groups (n=24) and were familiarized to the laboratory for 6days before starting the studies. Experiments were performed between 9:00 am and 14:30 pm. The study protocol was accepted and approved by the University Ethics Committee for Research Programs under Ref. No. Pharm. 2011/2012. Experiments were conducted in accordance with the Animal Care Committee counsels of Tripoli University. The doses used for *S. fruticosa* extract (125mg/kg and 250mg/kg) were based on previous related studies (Herrera-Ruiz *et al.*, 2006).The estimated LD_{50} of the *S. fruticosa* plant, in mice, is 4g/kg (Moharram *et al.*, 2006).

Source of S. fruticosa

S. fruticosa leaves were collected from Kalet Al-Ebeshstate, Tripoli city, and it was authenticated, with the help of local flora, by Dr Fathi ELrateeb, taxonomist (Prof. Department of Botany, Faculty of Science, Tripoli University). Voucher specimen of the aerial part, merely leaves, (No. 25112011) of *S. fruticosa* was deposited in the herbarium for upcoming reference.

Extraction and preliminary phytochemical screening

The leaves of *S. fruticosa* were chopped into small pieces, washed with water and then air-dried, at room temperature, for 3 weeks in the shade, away from direct sun light in the herbarium of Department of Pharmacognosy, Faculty of Pharmacy, Tripoli University. The dried leaves were coarsely powdered using an electrical grinder Wiley mill. The powdered material "100g" was subjected to extraction with 250ml of 70% ethanol at 50°C for 10 hours using a soxhlet apparatus. The extraction was lasted when the defatting material had taken place. The extracts were filtered through whatman paper. After, the filtrates were collected together, dried at 40°Cup to semisolid materials in a rotatory evaporator (Buchi Rotavapor, Model R-210) for ~4-5 hours. Next, the filtrate was concentrated over a water-bath at 50°C. The final yield of dried extract was 28.4% (w/w). *S. fruticosa* ethanol leaves extract was then stored in a refrigerator (4-8°C), in a tightly desiccated dark container to maintain its stability for subsequent phytochemical and pharmacological evaluations.

The ethanol extract of *S. fruticosa* was subjected to standard qualitative phytochemical screening tests, according to the color test procedure previously mentioned elsewhere, to detect the presence of secondary metabolites like flavonoids, alkaloids, saponin, steroids and phenolic compounds. Briefly are as follows:

- **Test for flavonoid**
  - The test: to 1ml of extract, few fragments of magnesium ribbon and few drops of concentrated hydrochloric acid were added. Appearance of a pink or red color after few minutes indicates the presence of flavonoids (Tiwari *et al.*, 2011).
- **Test for alkaloids**
  - The test: 5ml of the extract was added to 2ml of hydrochloric acid in a test tube.

After, the mixture was treated with 1ml dragendorffs reagent (a solution of potassium bismuth iodide). Formation of orange or red precipitate indicates the presence of alkaloids (Tiwari *et al.*, 2011).

- **Test for steroids**
  - The test: 2ml of extract was added to 2ml of chloroform, 2ml of acetic acid and 1ml of concentrated H_2SO_4. Appearance of a greenish color indicates the presence of steroids (Ajiboye *et al.*, 2013).
- **Test for saponin**
  - The test: to 2ml of extract, 5 ml distilled water was added in a test tube. After vigorous shaking for 30 seconds, formation of stable persistent foam indicates the presence of saponin (Siddiqui and Ali, 1997).
- **Test for phenolic compounds**
  - The test: 2ml of extract was mixed with 1ml of ferric chloride. Appearance of a greenish black precipitate indicates the presence of phenolic compounds (Kumar *et al.*, 2012).

Animal treatments

Mice were randomly scattered into four groups of six animals each. The ethanol extract of *S. fruticosa* was freshly dissolved in normal saline and i.p. injected at doses 125mg/kg and 250mg/kg. Fluoxetine (20mg/kg) (Taher *et al.*, 2018), used as a positive control, was dissolved in normal saline and injected i.p. in a volume of 0.2ml per 20g body weight. Negative control group received an equivalent volume of normal saline. After 30 min. mice were subjected to behavioral studies, to measure their despair state, expressed by immobility condition. The decrease in duration of immobility was taken as indicator of the antidepressant activity.

To study the possible antidepressant mechanism of action, cyproheptadine (a $5HT_2$ receptor antagonist, 3mg/kg; dissolved in normal saline) (Ulak *et al.*, 2010) was given i.p. 30 min before the administration of *S. fruticosa* extract and fluoxetine. After 30 min. duration of immobility was measured, using tail suspension test.

Test for CNS depressant activity

Forced Swimming Test (FST)

FST was performed according to the method described previously in rats (Porsolt *et al.*, 1977) with a slight adjustment in mice (Taher *et al.*, 2018). Duration of immobility in the FST was measured to estimate the antidepressant potential of medication. Briefly, animal experimental session included two comparable trials. Mice were placed freely in a plastic container (22cm diameter x 23cm height) filled with tap water (22-25ºC) to a depth of 15cm; at this deepness the mouse cannot uphold itself by placing paws or tail on the bottom of container. Water was altered each time, to eliminate urine, excrement and fur, before the next mouse was pushed to swim in the tank. During the 1st session, pretest session, mice were kindly placed in the water container for few minutes (conditioning session). After, animals were towel dried and returned to their home cages. On the 2nd day (test session) and after 30 minutes of drug injections, mice were forced to swim in the container for six minutes. The incidence of immobility (passive/immobile behavior) and the total time of immobility "floating of animal in water with all four extremities motionless, except that movements required to keep its nose above water surface", during the last four minutes of observation period,

were determined by a trained observer. At the same time, active/mobile behaviors "swimming, with all four legs dabbling", were also measured using a digital hand stop watch. The total number of escape-oriented climbing behaviors "vigorous whipping movements with forepaws directed against the walls of the container" was also counted.

Tail suspension test (TST)

Tail suspension test is a rapid, consistent and simple method used to evaluate the antidepressants activity in rodents, in particular mice. The duration of immobility produced by a mouse due to a tail suspension was determined as previously postulated (Taher *et al.*, 2018, Steru *et al.*, 1985). Briefly, 30 minutes post-treatments with saline, fluoxetine or *S. fruticosa* ethanol extract, each mouse was suspended independently from its tail, using an adhesive tape fixed at one cm from the tip of the tail, on 40cm horizontal metal rod distanced half a meter above the bench and 1.20 meter from the ground. Initially, a mouse was tried to escape from its situation by creating series of twisting movements. After-ward, the mouse showed a behavioral despair illustrated by inert movement and immobility, a clue of hopelessness state. Immobility time was recorded during experimental period. Each mouse was suspended for six minutes. The immobility behave, mouse hung-down passively and absolutely motionless, was observed and the duration of immobility, the total time, in seconds, that a mouse displayed no movement, was totaled throughout the last four minutes testing period using a digital hand stop watch. As a facile means of evaluating potential antidepressive activity the total time of immobility produced by mice treated with *S. fruticosa* ethanol extract was compared to relative control treatment groups.

In another set of experiments, in order to investigate the possible involvement of the serotonergic system to the effect of *S. fruticosa* ethanol extract in TST, mice were pretreated with cyproheptadine (3mg/kg, a 5-HT_2 receptor antagonist), or vehicle, since that this receptor found to play a role in mood disorders, depression included (Binfare *et al.*, 2009, Clenet *et al.*, 2001), and after 30 minutes, they received the extract of *S. fruticosa* (125 or 250mg/kg), fluoxetine or normal saline, before being exposed to the TST method 30 minutes later.

Spontaneous motor activity

The ambulatory behavior activity of individual mouse was measured in the open-field test as described previously by Taher and his colloquies (Taher *et al.*, 2018). Briefly, the open field box (Opto-Varimex monitor, Columbus Instruments, USA) is four-sided box constituted of 44x44cm ground floor and 18cm high walls. The left side of the box releases infrared beams located horizontally at 2cm above the floor and spaced along the longitudinal axis. Interruptions of beams by a mouse signify its location in the box. At the beginning of experiment, all mice were habituated the open field test box for 2h. The locomotor activity counts were recorded 30 minutes after injections of normal saline, fluoxetine or the ethanol extract of *S. fruticosa* (125 and 250mg/kg, i.p, n=6). Briefly, in a sound less and dark environment, each mouse was placed in the center of the box and allowed to move freely for a period of four minutes. The overall distance walked by each mouse was measured automatically by a digital recorder that counts every photocell beam interruptions. The Opto-Varimex-Minor

activity box was cleaned and wiped between animal observations with alcohol (70%) and allowed to dry to remove any smell

Statistical analysis

Data are presented as mean response time in sec ± SEM, unless otherwise stated. Statistical differences between groups were proved by one way analysis of variance (ANOVA) followed by Tukey's *Post hoc* test, to find out the position of difference between groups. A probability value was considered statistically significant when it is equal to 0.05 or less. Analysis of data was executed by GraphPad software (version 3.0, San Diego, USA).

Results

Phytochemical screening

The phytochemical screening of the ethanol extract of *S. fruticosa* leaves has revealed the presence of saponin, steroids, alkaloids and phenolic compounds. The flavonoids were absent (table 1).

Effect of S. fruticosa extract on FST

In this study, ethanol extract of *S. fruticosa* showed antidepressant-like effect comparable to that of the selective serotonin reuptake inhibitor, fluoxetine. In pretest session all animals exhibited signs of desperation after 250 sec of swimming among stall control sand *S. fruticosa* ethanol extract treated-groups (p=0.45, fig. 1). Throughout the test session, 24 hrs after pretest session, mice that had received normal saline displayed similar duration of immobility behavior (P=0.88; paired *t*-test) compared with pretest session. Compared to the duration of immobility showed by control group, injection of 125 and 250 mg/kg of ethanol extract of *S. fruticosa* in a mouse model of depression induced respectively, significant reduction in duration of immobility by 51.9% and 72.3%, ($p<0.001$, n=6 each, fig. 1) indicating that the extract has an acute antidepressant-like effect. In addition, there was a significant dose dependent effect on the duration of floating observed in the doses studied, respectively (127±15.6 sec vs. 73.3±12.9; $P<0.05$). The reference drug, fluoxetine, efficiently produced a significant decrease in duration of immobility towards the forced swimming induced depression in this acute model compared with control mice (63.0% decrease, $p<0.001$, fig. 1). In this FST model, the antidepressive effect of 250mg/kg of ethanol extract of *S. fruticosa* was more prominent, and was higher than that induced by fluoxetine (73.3±12.9 sec vs. 97.8±10.9 sec total immobility time, respectively, p= 0.16, fig. 1).

The mean response time of swimming, in seconds, and number of climbing trials produced by mice during FST are illustrated in Fig. 2. Swimming time and climbing trials were found to be similar in control group among the pre and post-test sessions. Mice treated with ethanol extract of *S. Fruticosa* exhibited a great enhancement in the swimming time by 66.7% increase, ($p<0.001$) compared with control mice. The ethanol extract of *S. fruticosa* at dose of 125mk/kg and 250mg/kg significantly, in a dose dependent manner (p=0.03), increased swimming time in mice, respectively, from 95.5±5.05 sec to 233±15.6 and 286.7±12.9 sec compared with control group (fig. 2). Fluoxetine, the reference drug, produced significant increase in the swimming time by 63.6% ($p<0.001$) compared with control mice. In addition, 250mg/kg of ethanol extract of *S. fruticosa* induced a slightly higher swimming time (not significant, p=0.18) than that produced by fluoxetine. In contrast, none of the two *S. fruticosa* ethanol

extracts doses, nor fluoxetine did change the climbing behavior in mice (fig. 2)

Effect of S. fruticosa extract on TST

Findings of the TST are presented in figure 3. Overall, mice displayed natural behavior before tail suspension among all controls and treated animals (data not shown). After half an hour, mice that had received normal saline showed, after tail suspension, distinct aberrant behavior expressed by overall motion less for 150.2±15.4 sec as ascertained by the total immobility time during the experiment (fig.3). Mice treated with ethanol extract of *S. fruticosa* significantly decreased the duration of immobility compared with control group. 125mg/kg of *S. fruticosa* extract showed significant suppression in the duration of immobility by 36.8 %, from 150.2±15.4 to 95.0±5.2 ($P<0.01$). Also, 250mg/kg of *S. fruticosa* extract induced a significant reduction in the event of tail suspension induced episodes of motionless behavior from 150.2±15.4 to 82.0±5.6, and the percentage increase in the mobility time was 54.6% ($P<0.05$) for respective group. The reference drug, fluoxetine, effectively suppressed the duration of immobility significantly by 53.2 % ($P<0.001$) compared with control mice (fig. 3).

Figure 3, also shows the influence of pretreatment of mice with cyproheptadine (a 5-HT_2 receptor antagonist) on the effect of ethanol extract of *S. fruticosa* and fluoxetine in TST method. Compared with control group, cyproheptadine treatment alone did not change the duration of immobility induced by tail suspension. Importantly, blocked of 5-HT_2 receptor by pretreatment of mice with cyproheptadine significantly antagonized the suppression induration of immobility produced by 125mg/kg ethanol extract of *S. fruticosa* (38.7% reversal, $P<0.05$) and 250mg/kg (47.9% reversal, $P<0.001$) compared with that seen in mice treated only with *S. fruticosa* extract (fig.3). Fluoxetine significantly reduced the duration of immobility by 53.2% ($P<0.001$) compared with those seen in control-treated mice. Blocked of 5-HT_2 receptors considerably abrogated the suppression in duration of immobility produced by fluoxetine (42.7 %reversal, $P<0.05$, fig. 3). So, these findings clearly demonstrate that 5-HT_2 receptors have a role in the anti-depressant-like effect of ethanol extract of *S. fruticosa* in mice.

Effect of S. fruticosa extract on locomotor activity of mice

Indeed, the measurement of the general locomotor activity of mice was performed to avoid the possibility that the drop in the duration of immobility induced by the treatment with the ethanol extract of *S. fruticosa* was due to a psychostimulant effect, such as produced by amphetamine (Porsolt *et al.*, 1977) and anticholinergic drugs (Herman *et al.*, 1981). The present study showed that, compared to control group, there were no significant changes in the ambulatory movements, as measured by photoelectrical cells method, seen in *S. fruticosa* treated mice as expressed by number of squares crossed among all treatment groups (p= 0.57, fig. 1). These findings proved that mice treated with ethanol extract of *S. fruticosa* did not exhibit any psychostimulant effects.

Discussion

Despite that *S. fruticosa* herb of the Mediterranean region has long time popular use, in folk medicine for treatment of numerous diseases, depending on their experience and local herbalist dealing with

this plant, however, pharmacological studies addressing its antidepressant effects in related-human models are remained limited. So, herein, for the goal of assessment of antidepressant activity of *S. fruticosa* we use two animal models, the FST and TST in mice. These rodent models are the most widely validated tests used for preclinical screening of supposed antidepressant compounds (Bach-Rojecky *et al.*, 2004). The immobility state exhibited by mice after exposed to force swimming or tail suspension is believed to produce a state of despair and dropped mood, which are agreed to reflect depressive disorders in humans. The duration of immobility was showed reduced after treatment with antidepressant drugs. Also, a considerable correlation was observed between clinical efficiency of antidepressant drugs and their potency in these animal models (Porsolt, 2000). So, the findings obtained from FST and TST established that the duration of immobility observed in the test reproduced a state of hopelessness and decreased mood in animals.

In this study, our pre-clinical data provide convincing evidence that the extract of *S. fruticosa* produce specific antidepressant-like effect in mice. In the two behavioral models, FST and TST, it was clearly observed that *S. fruticosa* leaves extract significantly and dose dependently produced antidepressant-like effect, which indicating that the constituents existing in the extract possess alike mode of action as that of fluoxetine extent, as established by a mean reduction in duration of immobility from baseline values. This behavioral effects are consistent with previous findings by Herrera and colloquies (2006), using other Salvia species as *Salvia elegans* (Herrera-Ruiz *et al.*, 2006). Indeed, so far, literature data have revealed that other herb of the family Lamiaceae, *Salvia divinorum,* used for centuries in healing ceremonies by the Mazatec Indians of Oaxaca, Mexico, has displayed clinically beneficial effects in treatment of resistant depression(Hanes, 2001). The reduction in the duration of immobility observed in this study was accompanied with arise in mice swimming time. Besides, the effect obtained after single administration of the extract was found to be dose-dependent, that are, of duration of immobility and swimming times depending on the concentration administered, were obtained with the FST and TST. In addition, the results obtained in this study, that use two models of different stress situations to induce states of despair, elucidated that the effect of the extract on the duration of immobility was expressed more strongly in the FST model than in the TST. Our present findings are in line with other authors' observation and indicate that these models could present differential sensitivity (Bai *et al.*, 2001) and that FST is more sensitive and better displays the state of depression than TST (Bach-Rojecky *et al.*, 2004). However, the decrease in the duration of immobility produced by the reference drug, fluoxetine, in both behavioral models validates these behavioral techniques. Also, the ability of these methods to detect the antidepressant effects of drugs, from a variety of classes, after acute administration, allows for rapid assessment of novel drugs (Cryan *et al.*, 2005). Furthermore, the observed effect was without modifying the spontaneous motor activity of mice.

A large number of evidence indicates that swimming behavior is sensitive to drugs acting on serotoninergic system (Cryan and

Lucki, 2000, Taher *et al.*, 2018). Fluoxetine, acts by inhibiting serotonin reuptake and has been used as a reference drug in majority of studies. In addition, the implication of $5HT_2$ receptors in the pathophysiology of depression has been suggested by experimental studies which show that 5-$HT_{2A/2C}$ antagonism has a role in the mechanism underlying the antidepressant effect of certain antidepressants in the FST (Cryan and Lucki, 2000) and in the TST (Binfare *et al.*, 2009). Our findings in this study are in agreement with our previous studies (Taher *et al*., 2018) showing that the reduction in immobility induced by fluoxetine was parallel with an increase in active swimming, whereas climbing trials was not changed. On the same hand, the pattern of effects observed for the *S. fruticosa* extract is qualitatively comparable to that observed with fluoxetine. Hence, it should be noted that the extract elicited its effect *via* a mechanism similar to that of fluoxetine, considering that *S. fruticosa* extract induced increased swimming time which is equal to that of fluoxetine. These findings are in agreement with other reports illustrating that the antidepressant effect of *Salvia elegans* is characterized by increased swimming time and involvement of serotonergic system (Mora *et al.*, 2006). Although it cannot be totally exclude that stimulation of noradrenergic and dopaminergic mediators, in particular noradrenaline and dopamine, are involved in the antidepressant effect of *S. fruticosa*, the observation that our extract augmented swimming time, and did not change the climbing trials, during test session, indicates that the effectiveness of *S. fruticosa* is undoubtedly mediated through its interference with serotonergic system (Cryan and Lucki, 2000, Detke *et al.*, 1995). This conclusion is further supported by our findings that cyproheptadine, a $5HT_2$ receptor antagonist; considerably reversed the extract action. The latter effect further indicates that the antidepressant-like effect of the extract mediated by stimulation of $5HT_2$ receptors. These outcomes are in agreement with literature data showing that $5HT_2$ receptors play a role in the mechanism of antidepressant drugs (Machado *et al.*, 2007).

In this study, the antidepressant effect produced by *S. fruticosa* extract was dose-dependent. Higher dose of the plant extract was more effective than smaller dose, both in TST and FST. We observed that increased doses of the extract did produce significant changes in the animal behaviors. The mechanism underlying this effect remains unclear. One possible explanation for this might be due to the concentration of various constituents in the ethanol extract. It is probably that increasing the chemical constituents of the extract did increase the effect of the active constituent which shows anti-depressant-like effect. In addition, the extract was lacking any signs of toxicity when administered to mice. Though chronic toxicity was not evaluated in this study, absence of acute harmfulness symptoms explains its common use in traditional medicine. As the estimated LD_{50} of *S. fruticosa* was 4g/kg (Moharram *et al.*, 2006), 16-32 fold higher than the efficient doses, used in this study, it reveals that the doses required to induce the therapeutic actions are lower than the toxic dose.

Since, the reduction in immobility was under the changes of motor behavior, we also verified the effect of the extract on this performance. It is significant step in

evaluating drug effect on CNS to examine its effect on locomotor activity of the animal. The present study showed that the extract did not produce significant changes of mice ambulatory motor activity, although it renders them more active and less depressed. Open field test is a widely used method for evaluating of neuro pharmacological activity (Taher *et al.*, 2018). So, herein, we used a photoelectric cell method to assess the effect of the extract on the locomotor activity of mice. The increase in mobility effect elicited by the administration of *S. fruticosa* extract in FST and TST seems not to be interrelated to motor effects since animals treated with *S. fruticosa* extract do not exhibit changes in ambulation and number of squares crossed, when assessed in the open-field for general locomotor activity. Hence, it appears clearly that the observed effect of the extract is a real antidepressant liked activity (Novas *et al.*, 1988). This observation strongly verifies that the psych-stimulant effect is not at play and further strengthens that the observed antidepressant effect is central and specific. This could provide a rationale for the traditional use of this plant in state of depressant.

The preliminary characterization, according to our results, reveals that the phytochemical constituents present in the ethanol extract might be responsible for the extracts' effect by interfering with the synthesis, release, and/or antagonizing the action of the depressor neuro-mediators at the target sites. Herein, the phytochemical screening showed that the ethanol extract, prepared from dried leaves of *S. fruticosa* plant, is a multipart of secondary metabolite products. The major ingredients found are saponin, steroids, alkaloids and phenolic compounds. The exact phytoconstituent responsible for this effect cannot be concluded at present, since; any of these components can be responsible for the observed antidepressant action of the extract. According to literature data, several studies had reported that some of these components produce antidepressant-like action. Therefore, the antidepressant activity may be attributed to the presence of alkaloids (Kukuia *et al.*, 2018). It also reported that the antidepressant effect could arise from the presence of phenolic compounds, through attenuation of oxidative stress produced during depression (Boukhary *et al.*, 2016). In addition, a number of studies have showed that saponin has an antidepressant action (Liang *et al.*, 2016). Therefore, although it is possibly that the antidepressant efficacy induced by ethanolic extract is attributed to the synergistic effect elicited by the individual active phyto constituents and conceivably *via* varying mechanisms of action, nevertheless, the active constituent by which *S. fruticosa* specifically induce antidepressant-like effect, in mice, remains to be elucidated.

Conclusion

In summary, the present study is the first novel research to demonstrate the anti-depressant like activity of *S. fruticosa* in an animal model predictive of antidepressant properties. It can be concludes that *S. fruticosa* is a therapeutically potential herb has antidepressant-like effect as well as neuropharmacological potential without affecting psychostimulant deeds. The antidepressant action was dose-dependent. Furthermore, it seems that the serotoninergic system is triggered by *S. fruticosa* extract, *via* stimulation of $5HT_2$ receptors. The leaves of *S. fruticosa* plant contain nature

antidepressant bioactive substances. This justifies its conventional use in the handling of mental illness. In addition, provide an evidence for rationality its use in folk medicine. Further studies are needed to isolate and describe the active constituents present in the extract, and further elucidate other likely mechanisms of action before extend these results for application in human, as antidepressant.

Disclosure

A part of these studies was presented in abstract form at the 1st Libyan Medical Sciences Conference March 2nd, 2017 [abstract]: S08; Page 25-26.

Conflict of Interests

No conflict of interests was existed before and after running of this study.

Table 1. Phytochemical constituents of ethanol extract of *S. fruticosa* leaves

Constituents	Ethanol extract
Saponin	+
Flavonoids	-
Steroids	+
Alkaloids	+
Phenolic compounds	+

'+' Indicate presence, '-'Indicate absence

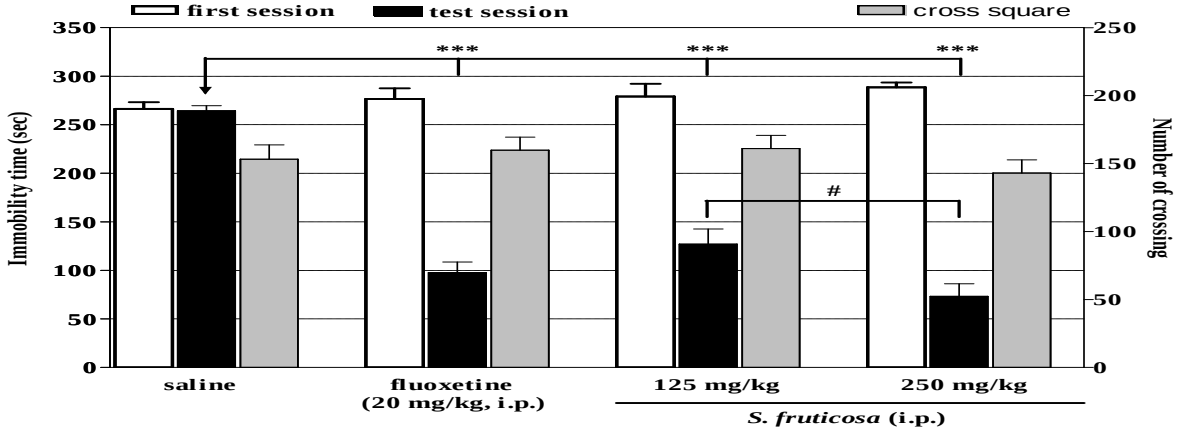

Figure 1: Effects of fluoxetine (20mg/kg) and *S. fruticosa* ethanol extract (125mg/kg and 250mg/kg) on duration of immobility in FST and on total locomotor activity. Fluoxetine and *S. fruticosa* extract were i. p injected 30 min. prior to the test. Mice were forced to swim or left freely moved in open filed test for six min. Each column represents the mean values ± S.E./4 min. Six mice were tested per group. $^{***}P < 0.001$ compared with control-treated mice. $^{\#}P < 0.05$ compared with *S. fruticosa* extract125mg/kg -treated mice.

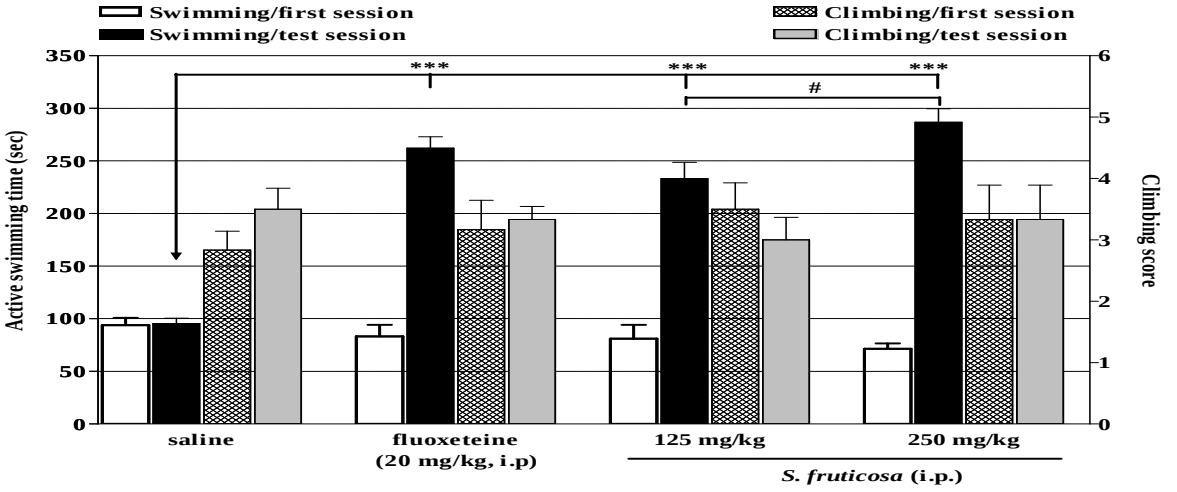

Figure 2: Effects of fluoxetine (20mg/kg) and *S. fruticosa* ethanol extract (125mg/kg and 250mg/kg) on active swimming and number of climbing in a mouse FST model. Fluoxetine and *S. fruticosa* extract were i. p

injected 30 min prior to the test. Mice were forced to swim for six min. Each column represents the mean values ± S.E/4 min. Six mice were tested per group. $^{***}P < 0.001$ compared with control-treated mice. $^{\#}P < 0.05$ compared with *S. fruticosa* extract 125mg/kg -treated mice.

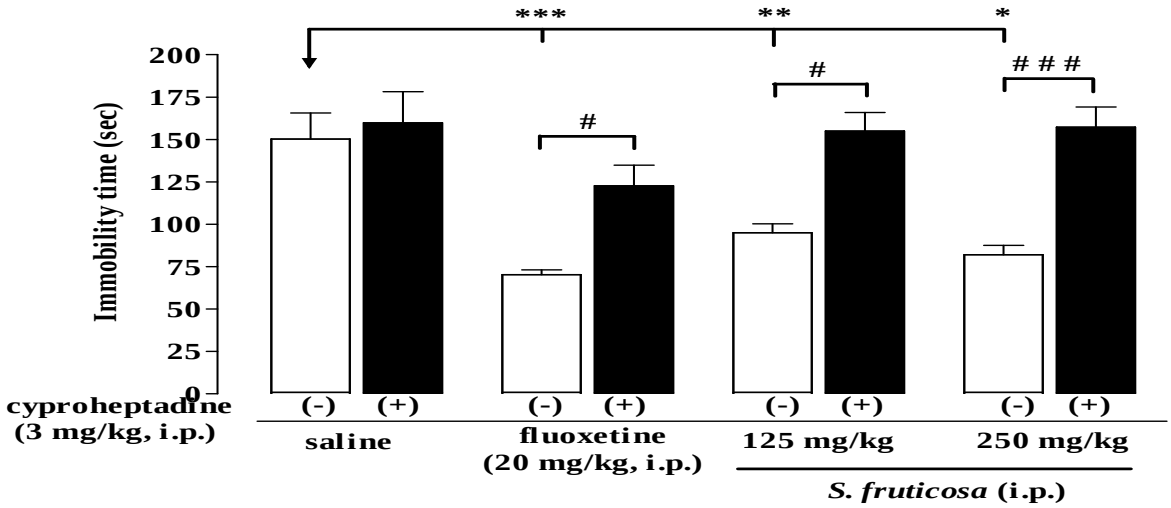

Figure 3: Effects of fluoxetine (20mg/kg) and *S. fruticosa* ethanol extract (125mg/kg and 250mg/kg) alone, and in presence of cyproheptadine, a $5HT_2$ receptor antagonist, on duration of immobility in TST. Cyproheptadine was administered i.p. one hr prior the injection of the extract. Mice were tested 30 min. after *S. fruticosa* extract or fluoxetine treatments. Mice were tail suspended for six min. Each column represents the mean values ± S.E/4 min. Six mice were tested per group. $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$compared with control-treated mice. $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ compared with respective group.

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