



BJP

Bangladesh Journal of Pharmacology

Research Article

*Salvia officinalis* induces antidepressant-like effect, anxiolytic activity and learning improvement in hippocampal lesioned and intact adult rats

## ***Salvia officinalis* induces antidepressant-like effect, anxiolytic activity and learning improvement in hippocampal lesioned and intact adult rats**

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### **Article Info**

Received: 21 September 2018  
Accepted: 30 November 2018  
Available Online: 21 December 2018  
DOI: 10.3329/bjp.v13i4.38375

Cite this article:

Gabbas ZE, Bezza K, Laadraoui J, Makbal R, Aboufatima R, Chait A. *Salvia officinalis* induces antidepressant-like effect, anxiolytic activity and learning improvement in hippocampal lesioned and intact adult rats. *Bangladesh J Pharmacol*. 2018; 13: 367-78.

### **Abstract**

The anxiolytic and antidepressant like effects of *Salvia officinalis* extract (50, 100 and 200 mg/kg) were evaluated using marble burying, forced swimming and open-field tests in intact and hippocampal lesioned rats. Additionally, *S. officinalis* was evaluated on rat's memory using conditioned learning test and we screened the methanolic extract for anti-oxidant activity, phytochemical and high performance liquid chromatography analyses. The administration of sage extract showed a significant reduction of immobility time in lesioned and intact animals during the forced swim test and anxiolytic effect in marble burying test. In the case of conditioned learning paradigm, memory enhancement was observed in sage treated group which indicates a cognition improvement. These activities seem to be related to the anti-oxidant capacity and the phytochemicals (phenolic, flavonoid, and tannin) detected into the extract of *S. officinalis*. The findings show that the methanolic extract of sage possess antidepressant-like effect, anxiolytic activity and also may contain bioactive compounds that stimulate learning in rat.

### **Introduction**

Several synthetic and chemical drugs have been introduced to treat patients with various neurological disorders, but their therapeutic effects have modest efficacy and most of them are associated with several adverse effects indicating need of better-tolerated and more efficacious treatments (Kim and Oh, 2012). Medicinal plants have been a major source of natural active compounds that can provide important options for developing drugs for the treatment of different mood and neurological disorders.

*Salvia officinalis* L. (Lamiaceae, common sage) is an aromatic and medicinal plant native to the Medi-

tean region, extensively used since ancient times in folk medicine (Penso, 1983). It is known in Arabic as "almufassih" because it helps who take it to talk fluently, and in Morocco, it is also called Salmiya, commonly used in the food especially to flavor the tea and for the treatment of various diseases. The leaves of *S. officinalis* have a very old reputation as traditional herbal remedies and have been recorded to be used as cognition-enhancing agents (Kennedy and Scholey, 2006; Perry et al., 1999). Experimental evidence supports several biological activities for different types of *S. officinalis* extracts, including anti-oxidant (Lu and Yeap Foo, 2001), antihyperglycaemic (Alarcon-Aguilar et al., 2002; Behradmanesh et al., 2013), gastroprotective (Mayer et



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al., 2009), anti-inflammatory and also anti-nociceptive activities (Rodrigues et al., 2012). Systematic and mechanistic studies of the effects of *S. officinalis* extracts have revealed multiple activities potentially relevant to brain function, and some reports showed that ethanolic extract of *S. officinalis* potentiates the memory retention and interacts with muscarinic and nicotinic cholinergic systems involved in the memory retention process (Eidi et al., 2006). Even the efficacy of the leaves of *S. officinalis* extract in the management of Alzheimer's disease, the treatment of depression, and memory disorders have been reported (Akhondzadeh et al., 2003; Howes et al., 2003; Perry et al., 2002; Savelev et al., 2004).

With regard to the possible effects of *S. officinalis* on behavior, this work aimed to investigate the effect of methanolic extract of *S. officinalis* on depression, anxiety and learning in hippocampal lesioned and normal adult rats.

## Materials and Methods

### Plant collection and identification

*S. officinalis* leaves were collected from the Ourika region, Morocco in March, 2013 and the plant's identity was confirmed by Prof. Ouhammou in the Faculty of Sciences Semlalia, University Cadi Ayyad Marrakech, Morocco. A voucher specimen of the plant has been deposited at the herbarium of the Faculty under the number MARK-10004.

The plant was cleaned and shed dried at 40°C. The dried leaves of the plant were triturated in order to obtain a powder.

### Preparation of methanolic extract

Dried and ground leaves (110 g) were used for extraction with methanol (600 mL) in a soxhlet apparatus for 72 hours. After extraction the solvent was evaporated by a rotavapor (Stuart® RE300, Bibby Scientific, UK). The final weight of the extract was 34.45 g.

### Determination of total polyphenols content

The total phenolic content of the extract was determined using Folin Ciocalteu method as described previously (Singleton and Rossi, 1965). Briefly, 100 µL of the properly diluted extract was mixed with 3.9 mL of distilled water followed by 100 µL of Folin-Ciocalteu reagent. After 3 min, 1 mL of 20% sodium carbonate ( $\text{Na}_2\text{CO}_3$ ) was added. The mixture was shaken and incubated for 1 hour at room temperature in the dark. The absorbance was measured at 725 nm against a blank using a UV-vis spectrophotometer. The total phenolic concentration is expressed as mg equivalent gallic acid per gram of the dry matter. All assays were carried out in triplicate.

### Determination of total flavonoids

The flavonoids content was determined by aluminum trichloride method using catechin as reference compound (Zhishen et al., 1999). A volume of 200 µL of extract was mixed with 800 µL of distilled water added to 60 µL of 5%  $\text{NaNO}_2$  solution. The mixture was allowed to stand for 6 min, then 40 µL of aluminum trichloride (10%) was added and incubated for 5 min, followed by the addition of 400 µL of 1M NaOH. 500 µL of distilled water are added to the reaction medium. Immediately, the mixture was agitated in order to homogenize the content. After 15 min of incubation, the absorbance of the pinkish mixture was measured at 510 nm. The content of total flavonoids of studied extract was expressed as mg equivalent catechin per gram of the DM.

### Total condensed tannin contents

The quantities of condensed tannins are estimated using the method to vanillin in acid medium (Xu and Chang, 2007). A volume of 200 µL of the extract is introduced in two test tubes separated (one for the sample and the other for the witness). Then, 2 mL of a solution of vanillin (4% in methanol) and 1 mL of concentrated hydrochloric acid are added. After 15 min incubation of the obtained mixture, the absorbance was read at 500 nm with the aid of a spectrophotometer UV-vis. The results were expressed as mg equivalent catechin per g of the dry matter.

### DPPH Free radical-scavenging activity

The total anti-oxidant capacity measures the free radical scavenging capacity of *S. officinalis* extract. An aliquot of 100 µL of different sample or standard concentrations (butylated hydroxytoluene, quercetin, ascorbic acid and  $\alpha$ -tocopherol) was added to 2.9 mL of the methanolic DPPH solution (0.004%) freshly prepared. In parallel, the negative control was prepared by mixing 100 µL of methanol with 2.9 mL of methanolic DPPH at the same concentration used. The mixture was shaken and kept in the dark at room temperature for 30 min and the absorbance was recorded at 517 nm against a blank containing all reagents except the test sample. Assays were carried out in triplicate. The following equation was used to determine the percentage of the radical scavenging activity of each extract.

$$\text{Inhibition (\%)} = [(\text{control absorbance} - \text{sample absorbance})/\text{control absorbance}] \times 100$$

### $\beta$ -Carotene/linoleic acid bleaching assay

The anti-oxidant activity of the samples was determined using the  $\beta$ -carotene/linoleic acid test. The assay was carried out as described previously (Miraliakbari and Shahidi, 2008). A stock solution of  $\beta$ -carotene and linoleic acid was prepared by adding together 0.5 mg  $\beta$ -carotene in 1 mL chloroform, 25 µL

linoleic acid and 200 mg Tween 20. The chloroform was removed using a rotary evaporator, and distilled water (50 mL) was subsequently added to the residue slowly with vigorous agitation, to form an emulsion. 350  $\mu$ L of *S. officinalis* extract solution or reference anti-oxidant (butylated hydroxytoluene) at various concentrations were added to 2.5 mL of the above emulsion. The test and control (containing water in place of sample) tubes were capped and incubated at 50°C for 2 hours. The absorbance of the emulsion at 470 nm was determined. All determinations were performed in triplicate.

Anti-oxidant activity (AA) was calculated as percent inhibition relative to the control:

$$I\% = [(A_{\text{sample } 2 \text{ hours}} - A_{\text{blank } 2 \text{ hours}}) / (A_{\text{initial blank}} - A_{\text{blank } 2 \text{ hours}})] \times 100.$$

Where,  $A_{\text{sample } 2 \text{ hours}}$ ,  $A_{\text{blank } 2 \text{ hours}}$  are the absorbance of the test compound and control respectively after 2 hours assay and a initial blank is the absorbance of control at the beginning of the experiment

The sample concentration providing 50% inhibition ( $IC_{50}$ ) was measured by plotting inhibition percentages against the sample concentrations.

#### **Iron (II) chelating activity**

The ability of *S. Officinalis* extracts to reduce  $FeCl_3$  to  $FeCl_2$  was investigated using the method (Oyaizu, 1986). 1 mL of the tested sample (*S. officinalis* extract or standard (butylated hydroxytoluene, quercetin,  $\alpha$ -tocopherol and ascorbic acid) was mixed with the phosphate buffer (2.5 mL, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 mL, 1%). The mixture was then incubated at 50°C for 20 min. Subsequently, 2.5 mL of trichloroacetic acid (10%) was added to the mixture, which was then centrifuged for 10 min at 3,000 rpm. Finally, the upper layer of the solution (2.5 mL) was mixed with distilled water (2.5 mL) and  $FeCl_3$  (0.5 mL, 0.1%), and the absorbance was measured at 700 nm in a spectrophotometer.

#### **HPLC analysis of *S. Officinalis* extract**

The identification of phenolic monomers was conducted using a high-performance liquid chromatography (Knauer) equipped with a (K-1001) pump and a PDA detector (200-700 UV-vis) operating at 280 nm. The column was (4.6 × 250 mm) (Eurospher II 100-5), and the temperature was set at 25°C. The flow rate was 1 mL/min and the sample volume injected was 2 mL. Acidified water (A) and acetonitrile (B) mixture were chosen as the optimal mobile phase for a total running time of 60 min. The identification of phenolic compounds was executed by comparison of retention times and UV-vis spectra with the standards.

#### **Experimental animals**

Male Sprague-Dawley rats, from the animal facility of

the faculty of Sciences Semlalia, Marrakech, Morocco weighing 300–350 g were used in this study. The animals, housed in individual plastic cages, were kept at constant room temperature (21 ± 2°C) and relative humidity (60%) with a 12 hours light/dark cycle (dark from 7 P.M.) and had free access to water and food.

#### **Acute toxicity test**

Acute toxicity test was performed on mice weighing about 17 to 25 g were divided equally into different groups (n=6) and fasted overnight, but provided with water *ad libitum*. On the test day, the methanolic extract was given by gavage in the doses of 1, 2, 3, 4 and 5 g/kg (10 mL/kg). To detect signs of toxicity and death, mice were observed for 2, 4, 6, 8, 12 and 24 hours post-extract administration. The mice were also observed daily for up to 14 days to detect any possible delayed death.

#### **Drugs and treatments**

The drugs administered to the rats were imipramine (Novartis, 30 mg/kg), diazepam (F. Hoffmann-La Roche, Switzerland, 1 mg/kg), and *S. Officinalis* methanolic extract (50 mg/kg). Imipramine was selected as standard drug (positive control) for depression in the forced swim test, diazepam as reference drug (positive control) for anxiolytic activity and saline (0.9% NaCl) as control. All drugs and vehicle were injected intraperitoneally. The injection volume was 0.1 mL/100 g body weight.

Thirty min after intraperitoneal treatment, the animals were submitted to behavioral tests (forced swim test, marble burying test and conditioned learning test).

#### **Hippocampal lesion**

After the acclimatization to the laboratory conditions, all rats were divided into different groups:

A control group [control-saline, n=6, positive-control, (n=6)] remained free of any surgical manipulation, treated group with sage (n=6). In the sham group (n=6), animals underwent the same surgical procedure at the same coordinates to those employed in the lesioned group [lesioned (n=6), lesioned plus sage (n=6)] except the electric current.

For the hippocampal lesion and after thiopental anesthesia (60 mg/kg body weight), the rats were mounted on a stereotaxic frame (Horseley-Clark). The hippocampus was damaged at one anteroposterior site in relation to the interaural zero point. All coordinates were obtained from the rat brain atlas (Paxinos and Watson, 1998) with anteroposterior, mediolateral and dorsoventral positions referenced from Bregma: antero-posterior, -5.2 mm, mediolateral, +4.8 mm, dorsoventral, +4.8 mm. The unilateral electrolytic lesion was made with a lesion-generating device (GRASS, D.C.

LM5A, USA) by passing 2 mA DC cathodal current for 15 sec, (Ramos, 2008) through a monopolar stainless steel electrode insulated with INSL-X except for the 0.5 mm tip. The rats in the sham group underwent the same surgical procedure at the same coordinates except the electric current.

#### *Antidepressant-like activity of *S. officinalis* extract*

The forced swim test is one of the most used assays of depressant-like activity in rodent. It was performed according to the method described previously (Porsolt et al., 1977). A vertical plexiglas cylinder (40 cm high, 20 cm in diameter and depth of 20 cm) was filled with water at 25°C. The rats were first subjected to pre-swim by placing each of them in the cylinder for 15 min. The day of test, and 24 hours after the pre swim, each rat was forced to swim in the cylinder for 6 min and the duration of floating (i.e. the time during which the rat made only the small movements necessary to keep its heads above water) was scored. The soiled water was changed between the tests.

#### *Effect of *S. Officinalis* extract on locomotor activity*

In order to evaluate the possible effects of *S. officinalis* on locomotor activities, a group of tested rats was evaluated in the open-field test as previously described (Katz et al., 1981). Rats were individually placed in a wooden box (80 x 80 x 40 cm) divided into 25 squares of equal areas. The number of crossings defined as the rectangles crossed by the animal with its four paws was registered during a 5 min period. During the test time, the animal's movements in the field were quantified by counting the number of crossings (at least three paws in a square) and the number of rearing which was defined

as the animal standing upright on its hind legs (wall rears and free rears). The number of crossings was considered as indicative of locomotor activity. The floor of the open-field apparatus was cleaned with 10% ethanol at the end of each test to remove any olfactory cues.

#### *Effect of *S. officinalis* extract on learning*

In this experiment, the testing was performed into two equally sized compartments (right and left) (30 x 25 x 50 cm) connected by an opaque wood door (with one hole). The box was covered by a hinged roof of clear Plexi-glass with numerous holes to allow ventilation and the floor was made of stainless steel rods (2.5 mm in diameter) that were separated by a distance of 1 cm. The experimental room was illuminated and quit.

All rats were trained initially to the box for 10 min and immediately followed by a series of 10 trials = 1 sequence. Each trial consisted of a 3 sec of sound stimulation (conditioned stimulus), which was an 80 db tone presented through a bell centrally mounted about 20 cm above the box. The conditioned stimulus was followed by an electrical stimulation 1, 2 mA foot-shock of 30 sec as unconditioned stimulus delivered through the grid floor. The unconditioned stimulus was terminated when the animal crossed to the other compartment. If the rat crosses to the other compartment during conditioned stimulus, before unconditioned stimulus onset, successful acquisition of learning response was recorded.

Each animal was tested for 10 sequences and 30 min after methanolic extract injection.

#### **Box 1: Marble Burying Test**

##### **Principle**

The lesion(s) in the hippocampus and septum reduce the digging

##### **Uses**

This test is an useful model of anxiety, obsessive compulsive disorder and neophobia. The test has predictive validity for screening of novel anxiolytic or antidepressant.

##### **Requirements**

Glass toy marbles; Polycarbonate rat cage; Wood chip bedding

##### **Preparation of the cages**

1. The polycarbonate rat cage (26 x 48 x 20 cm) with fitted filter-top cover was filled with wood chip bedding (about 5 cm depth), lightly tamped down to make a flat and even surface.
2. A regular pattern of glass toy marbles were placed on the surface, evenly spaced (about 4 cm apart).
3. Use a stop watch for counting the time (30 min for each

test)

##### **Test**

1. Place one rat into a corner of a cage on bedding carefully and left for 30 min
2. The number of marbles buried up to 2/3 their depth within the bedding was counted

##### **Advantages**

1. Readily available to most laboratories and is not expensive
2. Simple to apply
3. Easy to score reliably

##### **Limitation**

The neuronal circuitry of the behavior is not clearly elucidated.

##### **References**

Ho et al., 2002; Nicolas et al., 2006; Gyertyán, 1995; Hedlund and Sutcliffe, 2007; Dekeyne, 2005; Harasawa et al., 2006; Palucha and Pilc, 2007; Shimazaki et al., 2004; Deacon and Rawlins, 2005; Dringenberg et al., 2006

### Histological verification

At the end of all behavioral experiments, rats were anesthetized with thiopental (60 mg/kg i.p.) and perfused transcardially with 50 mL of 0.9% saline followed by 3.2% paraformaldehyde. After perfusion, the brains were removed and post-fixed for 24 hours in 3.2% paraformaldehyde, then stored in 30% sucrose. Coronal sections (50 µm) were sectioning with the cryostat (Leica Microsystems, Germany), stained with 0.5% toluidine blue, mounted on glass microscope slides and examined under a microscope (Leica Microsystems, Germany) where the lesions and probe placement could be seen. Any subjects with misplaced cannula or significant damage around the injection site were excluded from the subsequent statistical analyses of the behavioral data.

### Statistical analysis

All experimental results are given as the mean  $\pm$  S.E.M. For the behavioral measures in the forced swim, marble burying and conditioned learning tests, the time spent immobile and sequences for all groups were analyzed using a one-way analysis of variance (ANOVA). If the ANOVA revealed a significant main effect, a Tukey post-hoc analysis was performed to compare between the specific groups. Statistical significance was set to  $p < 0.05$ . The SigmaPlot 12.5 software was used for statistical analysis.

## Results

### Total polyphenols, flavonoids, condensed tannin contents

The results showed (Figure 1) that *S. Officinalis* extract

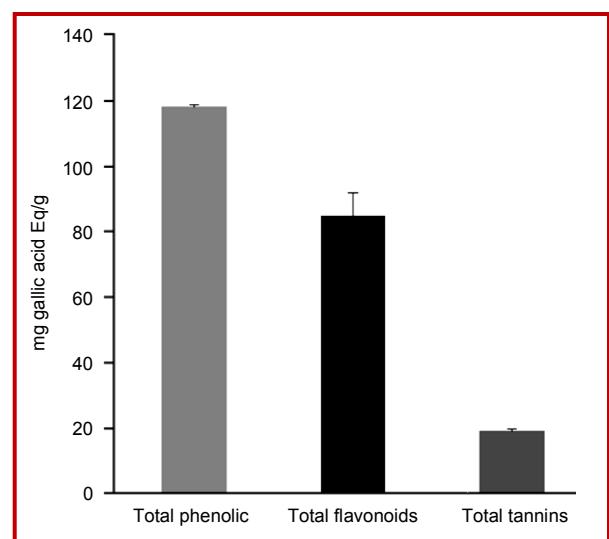


Figure 1: Total phenols, total flavonoids and condensed tannins in *S. officinalis* methanolic extract. The data represent the mean  $\pm$  SD

demonstrated the highest total phenol content with more than  $118.2 \pm 0.7$  mg gallic acid Eq/g dry matter, the flavonoid concentration ( $84.7 \pm 7.0$  mg gallic acid Eq/g dry matter) and the tannins content was estimated at  $19.1 \pm 0.7$  mg catechin Eq/g dry matter.

### DPPH Free radical scavenging activity

The effects of methanolic extract of *S. officinalis* in the DPPH, iron (II) chelating activity and  $\beta$ -carotene/linoleic acid bleaching assay are shown in Figure 2, which was comparable to that of the standard anti-oxidant butylated hydroxytoluene, quercetin, vitamin C and E. As the better anti-oxidant activity was reflected by the lower IC<sub>50</sub> values, the results showed that methanolic extract exhibited the highest anti-oxidant activity. The lowest IC<sub>50</sub> was obtained with reducing power assay: IC<sub>50</sub> values of  $0.334 \pm 0.007$  mg/mL, followed by  $\beta$ -carotene/linoleic acid bleaching assay

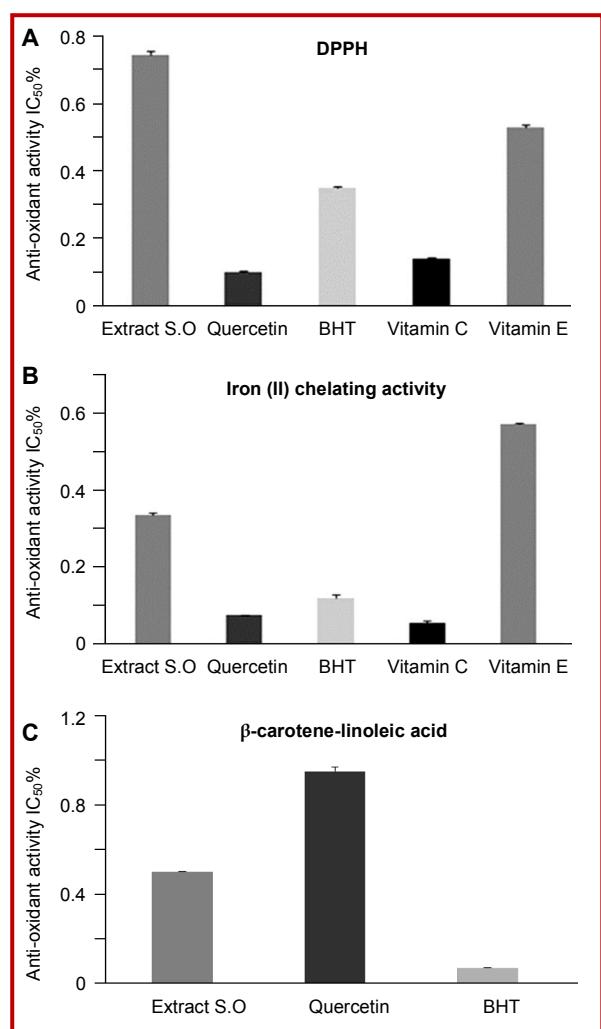


Figure 2: Anti-oxidant activity of *S. officinalis* extract by DPPH (A), Iron (II) chelating activity (B) and  $\beta$ -carotene/Linoleic Acid Bleaching assay (C). Each value represents a mean  $\pm$  SD (n = 3)

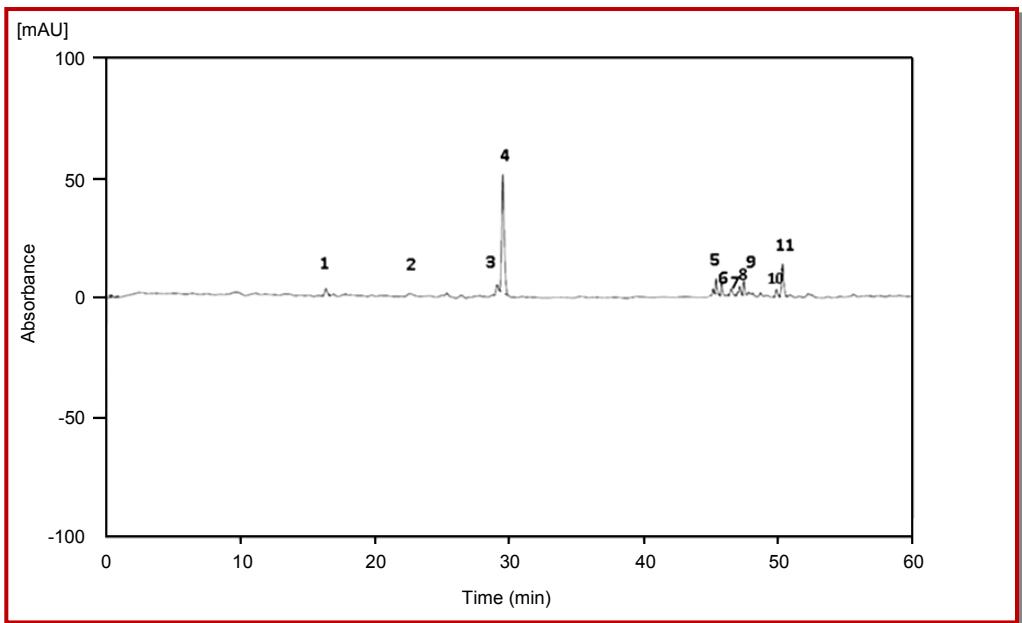


Figure 3. HPLC chromatogram of at 280 overlaid. Peaks: 1, caffeic acid; 2,p-coumaric acid; 3, Rutin; 4, rosmarinic acid; 5,Quercetin; 6, luteolin; 7,apigenin; 8, carnosol; 9, carnosic acid; 10, 11,not identified

$IC_{50}$  values  $0.5 \pm 0.002$  mg/mL and then DPPH free radical scavenging test system ( $IC_{50} = 0.741 \pm 0.011$   $\mu\text{g}/\text{mL}$ ).

#### HPLC analysis of *S. Officinalis* extract

According to the HPLC data shown in Figure 3, the extract of *S. officinalis* contained flavonoids and phenolic acid derivatives in different proportions. Eleven phenolic compounds were identified. Among the compounds, rosmarinic and caffeic acid were the most abundant.

#### Acute toxicity

The oral administration of *S. officinalis* extract at doses up to 5 g/kg did not produce any mortality symptoms of toxicity in mice during the study period of 14 days.

#### Lesion site verification

The histological analysis by Nissl staining technique done on rat brains sections from the lesioned animals revealed the lesions affected the dorsal hippocampus unilateral damage of dorsal hippocampus. Picture of

**Table I**

#### Effects of *S. officinalis* extract in the presence or absence of hippocampus lesion

	Sequences									
	1	2	3	4	5	6	7	8	9	10
Control	$0.3 \pm 0.2$	$1.5 \pm 0.3$	$3.8 \pm 0.3$	$4.8 \pm 0.2$	$5.2 \pm 0.2$	$5.8 \pm 0.3$	$6.8 \pm 0.3$	$7.0 \pm 0.0$	$7.3 \pm 0.3$	$8.3 \pm 0.3$
Sham	$0.2 \pm 0.2$	$1.2 \pm 0.5$	$4.0 \pm 0.4$	$5.2 \pm 0.2$	$8.2 \pm 0.5$	$10.0 \pm 0.0$	$9.8 \pm 0.2$	$8.0 \pm 0.0$	$7.0 \pm 0.0$	$8.3 \pm 0.3$
Sage (50 mg/kg)	$2.3 \pm 0.4$	$2.8 \pm 0.5$	$3.8 \pm 0.3$	$5.2 \pm 0.2$	$6.2 \pm 0.2$	$6.7 \pm 0.3$	$6.8 \pm 0.3$	$7.5 \pm 0.3$	$8.2 \pm 0.3$	$8.7 \pm 0.3$
Sage (100 mg/kg)	$3.0 \pm 0.4$	$3.3 \pm 0.3$	$4.3 \pm 0.3$	$5.3 \pm 0.2$	$6.8 \pm 0.2^a$	$6.8 \pm 0.3$	$7.0 \pm 0.0^a$	$7.7 \pm 0.3$	$8.7 \pm 0.3$	$8.9 \pm 0.3$
Sage (200 mg/kg)	$2.7 \pm 0.5$	$4.7 \pm 0.2$	$4.2 \pm 0.3$	$5.7 \pm 0.2$	$7.0 \pm 0.3$	$6.7 \pm 0.3^a$	$7.2 \pm 0.3$	$7.8 \pm 0.3$	$8.5 \pm 0.3$	$9.2 \pm 0.3$
Lesioned	$0.2 \pm 0.2^a$	$0.3 \pm 0.2$	$1.7 \pm 0.2$	$3.0 \pm 0.3^b$	$3.5 \pm 0.2^b$	$4.7 \pm 0.3$	$4.5 \pm 0.3$	$4.3 \pm 0.4^a$	$4.0 \pm 0.3$	$4.5 \pm 0.3$
Sage (50 mg/kg) + Lesioned	$5.7 \pm 0.4^{b,d}$	$5.7 \pm 0.4^{b,d}$	$6.0 \pm 0.4^{b,d}$	$6.3 \pm 0.2^{b,d}$	$6.5 \pm 0.2^{b,d}$	$6.5 \pm 0.3$	$6.7 \pm 0.3^+$	$6.8 \pm 0.3$	$7.2 \pm 0.3$	$7.3 \pm 0.3$
Sage (100 mg/kg) + Lesioned	$6.2 \pm 0.3^{b,d}$	$5.8 \pm 0.3^{b,d}$	$6.5 \pm 0.2^{b,d}$	$6.7 \pm 0.2^{bd}$	$6.7 \pm 0.2^{bd}$	$6.7 \pm 0.3^c$	$6.8 \pm 0.3^c$	$7.3 \pm 0.3^+$	$7.2 \pm 0.3^c$	$7.7 \pm 0.3^c$
Sage (200 mg/kg) + Lesioned	$6.2 \pm 0.3^{b,d}$	$6.0 \pm 0.3^{b,d}$	$6.5 \pm 0.2^{b,d}$	$6.8 \pm 0.2$	$6.8 \pm 0.2^c$	$6.8 \pm 0.3^c$	$7.0 \pm 0.0^c$	$7.5 \pm 0.3^+$	$8.2 \pm 0.4^+$	$7.7 \pm 0.3^c$

Statistical significance test was done by ANOVA followed by Tukey's test (n=6), values are mean  $\pm$  SEM of 6 animals per group <sup>a</sup>p<.05, <sup>b</sup>p<.001 vs. control group. <sup>c</sup>p<.05, <sup>d</sup>p<.001 vs. lesioned group

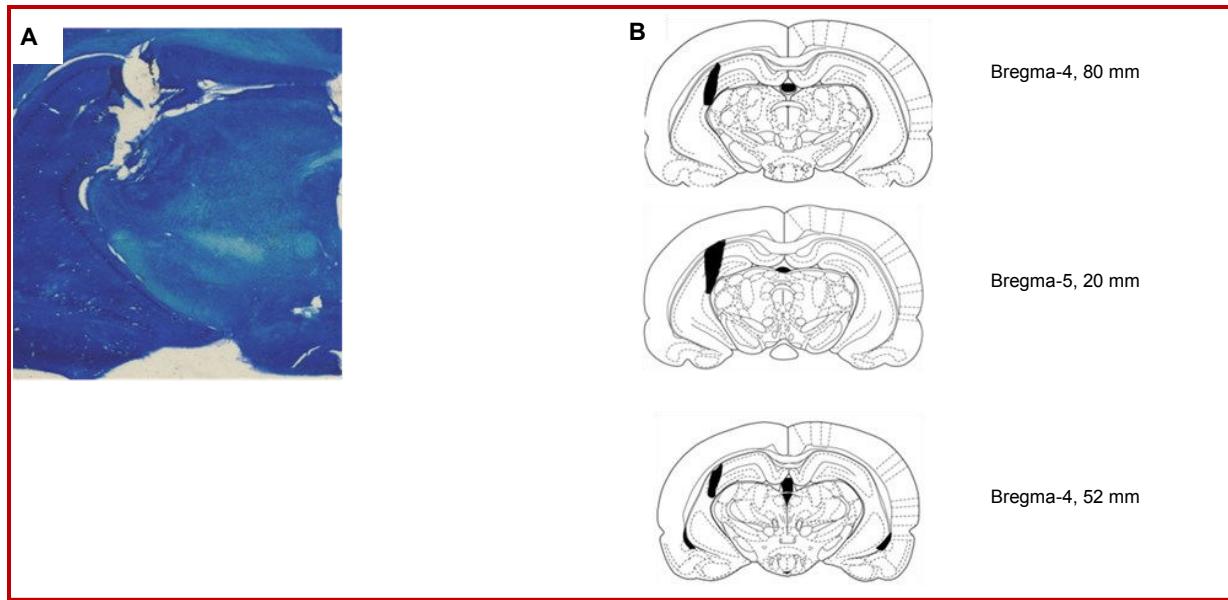


Figure 4: (A) Photomicrographs of coronal Nissl-stained sections from a representative hippocampus lesioned rat. (B). Serial histological reconstructions of representative extent (Black) of electrolytic lesions in hippocampus in the rat according to Paxinos and Watson, 1998. Numbers indicate distance posterior from bregma according to Paxinos and Watson, 1998.

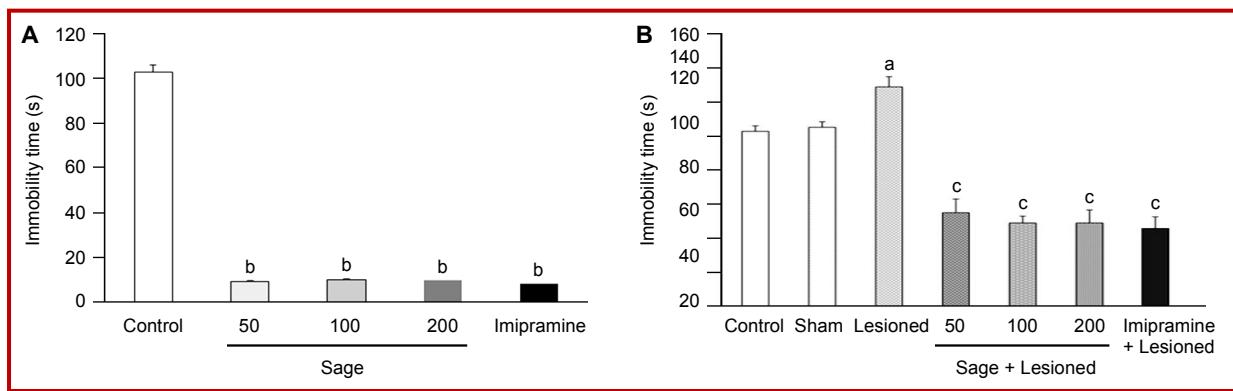


Figure 5. Effect of *S. officinalis* methanolic extract (50, 100 and 200 mg/kg i.p.) or imipramine (30 mg/kg i.p.) on forced swim test immobility time in intact and hippocampal-lesioned rats.

Sage methanolic extract or imipramine was injected 30 min before the test. Control group received saline 30 min before the test. Graph A and B shows the duration of immobility in the different tested groups, after treatment with Sage extract or imipramine. Data represent means  $\pm$  S.E.M. of immobility during the 6-min test session ( $n=6$  rats per group). Comparisons were made by using a one-way ANOVA followed by post hoc Tukey test: \* $p<0.05$ , <sup>b</sup> $p<0.001$  compared with control group, <sup>c</sup> $p<0.001$  compared with lesioned group.

the representative lesions are presented in Figure 4A and representative schematic drawings of the brains of the animals with lesions are shown in Figure 4B.

Four rats from lesioned and sage plus lesioned group were excluded from statistical analysis for the forced swimming test, whereas two rats from each group. The lesioned group and sage plus lesioned group were excluded from statistical analysis for the conditioned learning.

#### Antidepressant-like activity of *S. officinalis* extract

In the forced swim test, we investigated the anti-

depressant like effect of sage extract in both intact and hippocampal-lesioned animals.

The results presented in Figure 5 (A) show that the immobility duration of treated groups with sage at different doses (50, 100 and 200 mg/kg) or imipramine (30 mg/Kg, intraperitoneally), was shorter than the control group, [H(10.6)=29.7;  $p=<0.001$ ], and the treatment with sage extract reached roughly the same result observed in positive control treated with imipramine (30 mg/kg, intraperitoneally).

Post hoc comparisons revealed a significant higher total time of immobility in lesioned group as compared with

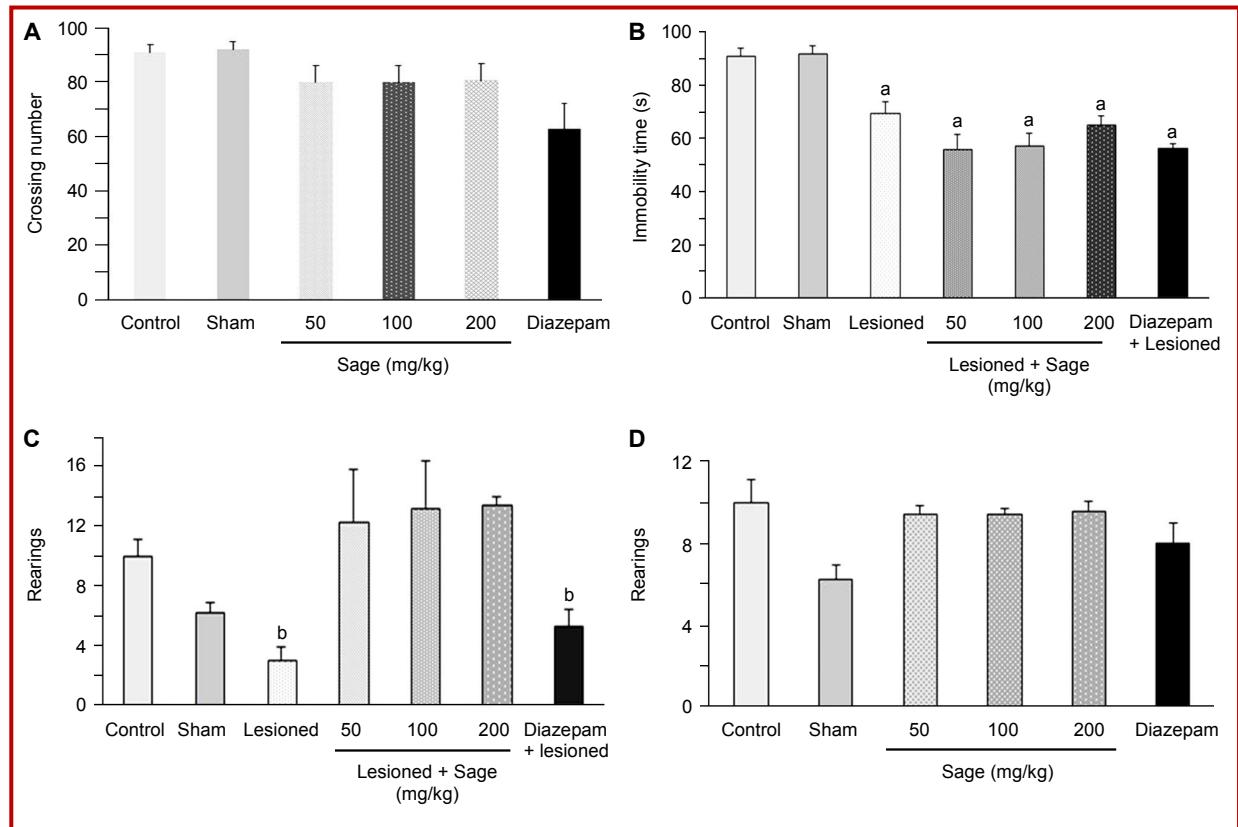


Figure 6: Open-field activity of rats. Intact and hippocampal-lesioned were treated with *S. officinalis* (50, 100 and 200 mg/kg i.p) or diazepam (1 mg/kg i.p) (A, B); Number of crossings during the 5 min session. (C,D) Number of rearings during the 5 min session. Values are the means  $\pm$  S.E.M. ( $n = 6$ ). <sup>a</sup>p<0.001, <sup>b</sup>p<0.05 as compared to the normal control group

normal control ( $p<0.05$ ) while in the lesioned animals and treated with sage extract (50, 100 and 200 mg/kg) or imipramine (30 mg/kg, intraperitoneally) a reduction of the same parameter was observed as compared to lesioned group ( $p<0.001$ ) (Figure 5B).

#### Effects on the number of crossing and rearing in the open-field test

The locomotor activity of extract is evaluated by its effect on open field test. In this test, our results show that treatment with sage or diazepam had no effect on the locomotor activity in open-field test compared to the lesioned animals. Post hoc analysis indicated a significant reduction in the locomotor activity in both lesioned and lesioned treated with sage or diazepam, as compared to the normal control group  $F(10.55)=12.20$ ;  $p<0.001$  (Figure 6A,B). In the vertical activity (number of rearings), the lesioned and lesioned rat treated with diazepam (1 mg/kg, intraperitoneally) showed a significant decrease in the number of rearing as compared to the normal control group,  $H(10.55)=29.04$ ;  $p<0.05$ . The sage treatment did not show any significant effect on these behavioral measurements (Figure 6C,D).

#### Anxiolytic activity of *S. officinalis* extract

The treatment of normal rats with sage extract (50, 100

and 200 mg/kg) or diazepam (1 mg/kg) reduced significantly the marble-burying behavior compared with the control group  $F(10.55)=78.06$ ,  $p<0.001$  (Figure 7A). On the other hand, comparisons using the Tukey's test revealed that lesioned rats buried a significant higher number of marbles as compared to the normal control group,  $p <0.001$ . However, when the lesioned animals are treated with diazepam or sage extract a dose-dependent decrease in marble burying behavior was observed ( $p <0.001$ ) (Figure 7B).

#### Effect of *S. officinalis* extract on learning

In this experiment, the animals were tested using 10 sequences of classic conditioning.

Results of conditioned learning performance represented in Table I indicate that the administration of sage extract (50, 100 and 200 mg/kg) showed a significant increase in the number of avoidance response as compared to the normal group which indicated that methanolic extract enhanced acquisition of the task since the seven sequences (sequence 7;  $F(10.55)=30.10$ ;  $p=0.039$ ). Therefore, statistical analysis revealed that the learning performance of lesioned animals is less than the control group (sequence 3;  $F(10.55)=32.67$ ;  $p<0.001$ ) which is reflected by the decrease of conditioned

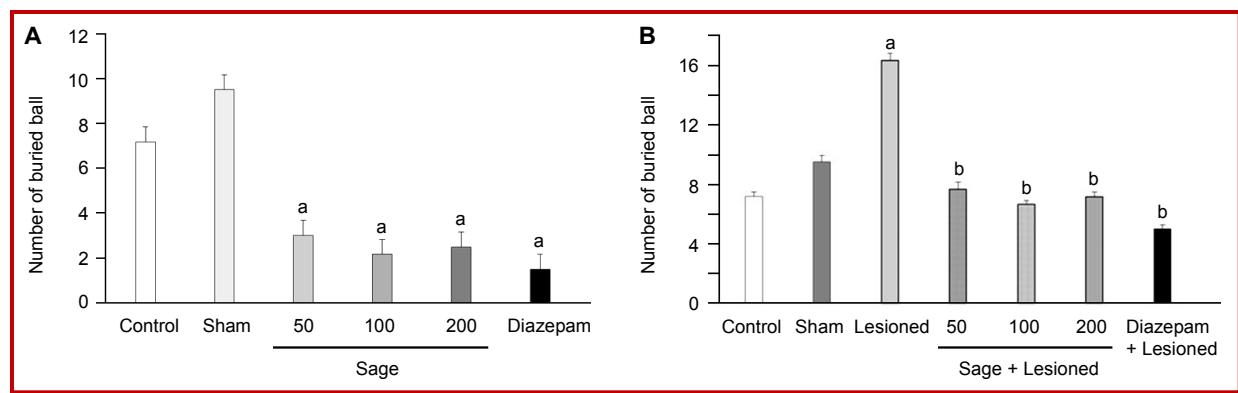


Figure 7: Effects of *S. officinalis*, methanolic extract (50, 100 and 200 mg/kg i.p.) or diazepam (1 mg/kg i.p.) in the presence or absence of hippocampus lesion on marble-burying behaviour in rats during 30 min. Results are expressed as means  $\pm$  S.E.M. ( $n = 6$  per group)

reactions number during all of the tested sequences. However, the treatment of lesioned animals with three doses 50, 100 and 200 mg/kg of sage extract a highly significant gradual increase in number of conditioned reactions as compared to the lesioned animals (sequence 4;  $F(10.55)=37.27$ ;  $p<0.001$ ). No significant differences between the sham group and the other tested groups ( $p>0.05$ ).

## Discussion

Major depression, memory loss and atrophy of the hippocampus are the most deterioration observed in Alzheimer disease (Byers and Yaffe, 2011; Schweitzer et al., 2002), and none of the current treatment can successfully cure (Alzheimer disease) at an early stage. On the other hand, many herbs have pharmacological activities relevant to dementia therapy, like *S. officinalis* (Howes et al., 2003). It hence the need to evaluate the antidepressant and anxiolytic-like activity of *S. officinalis* and the effective effect on learning in the presence or absence of hippocampus lesion.

Our present study has shown, for the first time the antidepressant-like effect of methanolic extract from the leaves of *S. officinalis* in the forced swim test in rats. The i.p. injection of plant extract produced a marked reduction in immobility time. This effect was comparable to the reference antidepressant drug imipramine. These findings proved the antidepressant like effect of this plant at the different tested doses (50, 100, 200 mg/kg), which could be due to their riches in polyphenolics compounds particularly rosmarinic acid and caffeic acid, confirmed by our HPLC analysis and supported by a previous study (Farhat et al., 2013). On the other hand, further results showed that rosmarinic acid and caffeic acid the major phenol compounds of sage, exerts anti-depressant like effect in an animal model of depression, and reduced the duration of immobility in forced swimming test (Kondo et al., 2015; Takeda et al., 2002). Furthermore, our data showed that methanolic

extract possess anti-depressant like effect even in the presence of electrolytic lesion of hippocampus, and could be explained by neuroprotective effect of rosmarinic acid against corticosterone (Sasaki et al., 2013), and in earlier studies rosmarinic acid has antidepressant activity via regulation of Mkp-1, which the expression in hippocampus could be increased by the high glucocorticoid levels in depression (Kim et al., 2005), and modulation of dopamine and corticosterone synthesis (Kondo et al., 2015). On the other hand, the extract had no significant effect on the motor activity as assessed by the open-field test. These data demonstrate that the sage antidepressant-like effect is specific, not due to the psychostimulant effect, which might be considered a false positive result in the forced swimming test (Borsini et al., 1988).

Using the marble burying test, the treatment with sage extract showed anxiolytic effects in hippocampal lesioned and intact rats which was reflected by the reduced number of buried marbles. The defensive burying behaviors in the marble burying test represent an active coping strategy in response to a discrete threat including the predictive validity for anxiety (Gorton et al., 2010), there are some evidence about the possible effects of *S. officinalis* on anxiety. The results of a study on 30 healthy participants showed improvement in mood and reduced anxiety effect following the administration of single doses of *S. officinalis* due to the cholinesterase inhibiting properties of this plant (Kennedy et al., 2006). Some studies about anxiolytic effects of other salvia species showed that *S. leriiifolia*, *S. reuteriana* Boiss and *S. eleganspocess* induces anxiolytic effect in rats using EPM model (Herrera-Ruiz et al., 2006; Hosseinzadeh et al., 2008; Mora et al., 2006; Rabbani et al., 2005).

In addition, the present study indicate that methanolic extract of *S. officinalis* enhance and improve learning of rats and offsetting the negative effect of electrolytic lesion in the hippocampus. These data are in agreement with some other studies reporting that ethanolic extract

of *S. officinalis* leaves possess a mnemonic effect on adult male rats (Eidi et al., 2006; Tildesley et al., 2005). In our study, these effects were revealed by increase the number of conditioned responses in learning and suggested that *S. officinalis* exhibits central nervous system acetylcholine receptor activity, including nicotinic and muscarinic binding properties (Wake et al., 2000). In addition, several clinical and experimental studies (Eidi et al., 2003; Hasselmo, 2006; Herholz et al., 2005; Muir, 1997; Tang et al., 1997; Winters and Bussey, 2005) have demonstrated the important role of the cholinergic system in learning, memory and attention. The degeneration of the cholinergic system is responsible for reduced acetylcholine esterase and acetylcholine transferase levels and changed distribution of cholinoreceptors in brains of patients with Alzheimer disease (Mufson et al., 2008).

Moreover, the bioactive compounds of sage possess anticholinesterase activity (Perry et al., 1996), and the interaction of rosmarinic acid properties like anticholinesterase, neuroprotective, and anti-oxidant which has been confirmed through the DPPH,  $\beta$ -carotene and the reducing power methods, may all be responsible for the revealed effects of *S. Officinalis* (Hasanein et al., 2016; Hasanein and Mahtaj, 2015).

These data supply further evidence that the efficacy of *S. officinalis* extract can be appropriate to the treatment of cognitive disorders (Houghton and Howes, 2005; Sallam et al., 2016) and might potentially provide natural treatment for Alzheimer disease (Akhondzadeh et al., 2003).

## Conclusion

The methanolic extract of *S. Officinalis* leaves reveal beneficial effects on depression, anxiety and learning of rats in the presence or absence of hippocampus electrolytic lesion.

## Ethical Issue

All experiments were carried out in accordance with the European Community Guidelines (EEC Directive of 24 November 1986; 86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

## Conflict of Interest

No potential conflict of interest was reported by the authors.

## Acknowledgement

The authors are grateful to Mr. A. Regragui for his helpful giving animals and drugs.

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