Pharmacogn. Mag.
A multifaceted peer reviewed journal in the field of Pharmacognosy and Natural Products
www.phcog.com | www.phcog.net

ORIGINAL ARTICLE

Behavioral and Physiological Assessments to Evaluate the Effect of *Acacia senegal* and *Acacia seyal* in Albino Mice

Gadah Albasher[1], Nahed S. Alharthi[2], Saad Alkahtani[1], Nada Aljarba[3], Nouf Al Sultan[1], Ahad A. Alsaiari[4], Omaima Nasir[5], May Bin-Jumah[3], Wedad S. Alqahtani[1]

[1]Department of Zoology, College of Science, King Saud University, [2]Department of Medical Laboratory Sciences, College of Applied Medical Sciences, Prince Saatam bin Abdul Aziz University, Al-Kharj, [3]Department of Biology, College of Sciences, Princess Nourah Bint Abdulrahman University, Riyadh, [4]Department of Clinical Laboratory Science, College of Applied Medical Sciences, [5]Department of Biology, Turabah College, Taif University, Taif, Saudi Arabia

Submitted: 15-Feb-2020 Revised: 28-Mar-2020 Accepted: 21-Apr-2020 Published: 28-Aug-2020

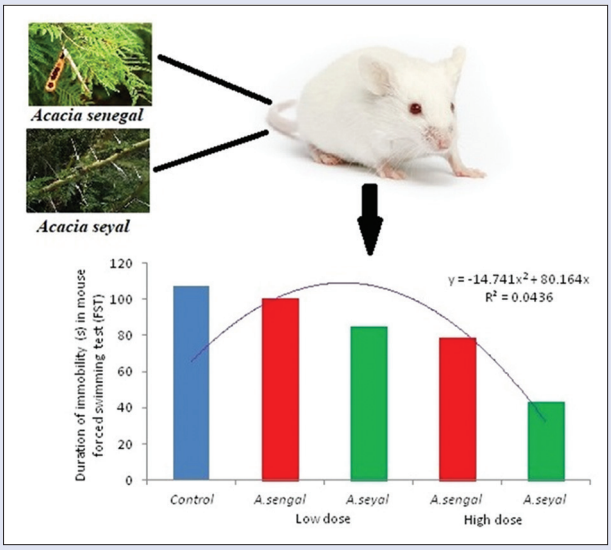
ABSTRACT

Background: *Acacia senegal* and *Acacia seyal* (family Leguminosae) are the sources of Gum Arabic that is widely used in food-processing and pharmaceutical industries. The neuropharmacological activities of *A. senegal* and *A. seyal* remain unexplored. **Objective:** The objective of the study was to investigate the neuropharmacological activities of *A. senegal* and *A. seyal*. **Materials and Methods:** Behavior, variations in selected blood parameters, acetylcholinesterase (AChE) activity, and lipid peroxidation level were studied in normal Albino mice that received *A. senegal* and *A. seyal* extracts. **Results:** The results revealed a significant decrease in the body weight of mice after the treatment with the extract of *A. senegal* and *A. seyal* compared to the control group. The locomotor activity in the treated mice decreased as well. In forced swimming test (FST), the treated mice showed a marked reduction in immobility respective to increase in the dose. The anxiety reflex test (FRT) revealed an increase in the percentage of time spent in the open arms of the maze. The treatment resulted in a notable decrease in motion balance and function. The active avoidance test revealed a dose-dependent reduction in avoidance. The study provided the evidence that *A. senegal* and *A. seyal* contributed to reduction in plasma cholesterol and glucose levels in treated mice, with no marked variations in hemoglobin level. Estimation of AChE resulted in minor decline in the activity in the treated group of mice. *A. senegal* was safer than *A. seyal,* but exerted toxicity at high dose. **Conclusions:** Low doses of *A. senegal* have the potential to be used for the development of the treatment for neurological disorders.

Key words: *Acacia senegal*, *Acacia seyal*, acetylcholinesterase, immobility, lipid peroxidation

SUMMARY

- *Acacia senegal* can be a potential candidate for developing a treatment for neurological disorders at their low dose. However, histological analyses revealed marked changes in liver and brain tissues upon treatment with *Acacia senegal* and *Acacia seyal* at their high concentrations, for which a further extensive study is needed to explore the exact mechanism of action of the two plant extracts. Further investigation on the neurological properties of *Acacia senegal* is needed to explore if it is possible to use this plant in the treatment of central nervous system disorders or not.



Abbreviations used: AChE: Acetylcholinesterase; CS: Conditional stimulus; CV: Central vein; CCV: Congested central vein; EPM: Elevated plus maze; FST: Forced swimming test; F: Fissure; GABAA: γ-aminobutyric acid A, GL: Granular layer; GA: Gum Arabic; HC: Hepatocyte; HS: Hepatic sinusoid; MDA: Malondialdehyde; ML: Molecular layer; MAO: Monoamine oxidase; MeN: Medulla neurons; NCH: Neurocyte chromatolysis; PD: Postnatal day; PYC: Pyramidal cell; PKC: Pyknosis; PC: Purkinje cell; UCS: Unconditional stimulus; WM: White matter.

Correspondence:

Prof. Gadah Albasher,
Department of Zoology, College of Science,
King Saud University, Riyadh, Saudi Arabia.
E-mail: galbeshr@ksu.edu.sa

DOI: 10.4103/pm.pm_20_20

Access this article online
Website: www.phcog.com
Quick Response Code:

INTRODUCTION

Acacia senegal and *Acacia seyal* (family Leguminosae) are the sources of natural gum, commonly known as gum Arabic (GA).[1] More than 80% of GA are cultivated in Sudan.[2] GA is obtained from dried exudates of stems and branches of *A. senegal* and *A. seyal*. Polysaccharides, oligosaccharides, and glycoproteins are the key components of GA.[3] The constituent sugars include D-galactose, L-arabinose, rhamnose, and glucuronic. *A. senegal* and *A. seyal* have a different level of each sugar. The polysaccharide fraction is composed of a linear chain of β-1,3-linked

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

Cite this article as: Albasher G, Alharthi NS, Alkahtani S, Aljarba N, Al Sultan N, Alsaiari AA, *et al*. Behavioral and physiological assessments to evaluate the effect of *Acacia senegal* and *Acacia seyal* in albino mice. Phcog Mag 2020;16:S410-7.

© 2020 Pharmacognosy Magazine | Published by Wolters Kluwer - Medknow

galactose.[4] The amino acid content in both the species is the same, but the content is in *A. senegal* (2.7%) compared to *A. seyal* (1.0%).[5]

GA has a wide application in food-processing, pharmaceutical, and other industries. GA serves as a stabilizer, a thickening agent, or an emulsifier.[2] It is a rich source of water-soluble dietary fibers. The medicinal values of *Acacia* are recognized globally for antimicrobial, anti-helminthic, antiulcer, antioxidant, hypoglycemic, analgesic, antipyretic, cardiovascular, renal, and central nervous system (CNS) activities.[6,7] Earlier studies demonstrated the antioxidant, fetal hemoglobin-inducing, and hypolipidemic effects of GA.[8] The protective effects of GA are related to different mechanisms, including its ability to decrease systolic blood pressure, decrease blood urea and phosphate levels, increase urinary antidiuretic hormone excretion and creatinine clearance, decrease Na^+ excretion, increase renal and intestinal excretion of Mg^{2+} and Ca^{2+}, and enhance remineralization of the teeth.[9,10]

In recent decades, researchers focused their attention on the assessment of the behavioral effects of this plant extract and its influence on motor activity. Plant extracts tend to have psychoactive properties inducing variations in mood sensation, thinking process, and behavioral patterns. Studies on the neuropharmacological effects of plant extracts use a mouse as a model for testing due to its close resemblance with humans in terms of body functions.[11] A number of studies reported increase or decrease of locomotor activity and antidepressant and other psychotropic effects caused by different plant extracts.[12,13] The fear and anxiety-like behavior in rats is regulated by the medial prefrontal cortex of the brain.[14]

The present study investigated the neuropharmacological effects of *A. senegal* and *A. seyal* on behavior, variations in blood parameters, acetylcholinesterase (AChE) activity, and lipid peroxidation level in normal Albino mice.

MATERIALS AND METHODS

Plant material and extract preparation

A. senegal or *A. seyal* powder was purchased from Dar Savanna Ltd., Khartoum, Sudan. The quality of the powder conformed to the standards set by the Food and Agriculture Organization of the United Nations (FAO), British Pharmacopoeia, United States Pharmacopoeia, and Joint FAO/WHO Expert Committee on Food Additives.

Plant extracts were prepared by macerating 5 g of dry powder of *A. senegal* and *A. seyal* in 500 mL water. The mixture was centrifuged (5000 rpm for 5 min), and the supernatants were evaporated to dryness under reduced pressure at 30°C. The yield extract was 32% (w/w).

Animal housing

The experiments were carried out on male and female white Albino mice of 8–9 weeks old (n = 40) in the Animal House of Biology, Faculty at Princess Noruah bint Abdulrahman University (PNU), Riyadh, Saudi Arabia. Animals were housed in plastic cages (two males and two females) measuring 30 cm × 12 cm × 11 cm. The animals were bred multiple times to get a clean generation of offspring. The animals were maintained in a well-ventilated room under controlled environmental conditions (22°C–24°C, 50%–70% humidity, and a 12-h light/dark cycle). Throughout the study, the mice were fed with standard pelleted food and drinking water containing *A. senegal* or *A. seyal* extract. The experiments were ethically approved by the PNU number H-01-R-059/20-0133.

Experimental procedure

To study the effects of *A. senegal* and *A. seyal* on the behavior of mice, the experimental sessions were conducted between 8 a.m. and 8 p.m. The animals were grouped and assigned to treatment conditions in counterbalanced order. The home cage rack was taken to the test room at least 30 min before each experiment. Dry surfaces of apparatus were thoroughly cleaned with 70% ethanol before releasing the animals.

The mice were divided into five treatment groups either with *A. senegal* or *A. seyal* with low dose (10 mg/mL water) and high dose (25 mg/mL water). The control group was given normal tap water during the period of the experiment.

The experiment extended over a period of 35 days and was done in the following order:

Body weight

Body weight is the indicator used to determine the development of mice. Mice from the control and treated groups were weighed at an interval of 3 days from postnatal day 1 to postnatal day 21.

Locomotion activity test

The locomotor activity was evaluated by an open-field method. This test was conducted for PD 22 mice. The control and treated groups of mice were subjected to a locomotor activity test in a wooden chamber measuring 80 cm × 80 cm × 30 cm with floor demarcated into 64 equal-sized squares.[15] Behavioral elements included in the experiment were the number of squares crossed, duration of locomotion, and duration of immobility. The mice were placed at the center, and all their movements and activities were recorded for 5 min.[16]

Behavioral assessment by forced swimming test

The behavioral evaluation was carried out 60 min post administration in PD 28 mice. The antidepressant effects of *A. senegal* and A. *seyal* were evaluated using forced swim test (FST). Each animal was placed individually in a glass chamber (25 cm × 15 cm × 25 cm) filled with water up to a height of 15 cm and maintained at 25–26°C. Water in the chamber was replaced with fresh water after each tested animal and each mouse was used only once. The animals were observed for 6 min. The duration of immobility was recorded during the last 4 min of the observation period because each animal showed vigorous movement during the first 2 min. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface.[17]

Anxiety reflex

The anxiety-like behavior was evaluated by the elevated plus maze (EPM) test. The apparatus consisted of two open and two closed arms, with the open pair perpendicular to the closed one. The maze was elevated 50 cm above the floor made of opaque black Plexiglas. The extracts were orally administered 30 min prior to the start of the experiment. The animals were individually placed at the center of the plus maze. This test was conducted on PD 25 mice. The examination time was 5 min and implied recording the time(s) spent by the animals in the open and closed arms.[18]

The motor coordination test

The motor coordination test was conducted by rotarod method on PD 30 mice. It is a well-established procedure to test the motor coordination and motor performance aspects of balancing the body. A mouse was placed on a horizontally oriented metal rod (3 cm diameter) rotating at an initial speed of 10 rpm/min. The apparatus accelerated the speed from 10 rpm to 20 rpm in 300 s. The mice naturally tried to stay on the rotating rod to avoid falling. The time spent on the rod maintaining equilibrium without falling was recorded.[19]

Active avoidance response

The memory and learning abilities of the mice were determined through active avoidance response with the help of the modified method described by Abu-Taweel *et al*.[20] PD 35 mice were examined using the shuttle-box-automated reflex conditioner (Ugo Basile, Italy). The shuttle-box consisted of two equal-sized chambers with a gate fitted in between. Each animal was acclimatized for 5 min before the onset of the experiment. A lamp and a buzzer were used as conditional stimulus (CS). Electric shock was used for unconditional stimulus (UCS). The reflux responses of the mice were recorded automatically using a recorder fitted to the shuttle box. Each animal was exposed to thirty trials with an interval of 30 s. Prior to CS, each animal was subjected to UCS for 2 s. The lamp and the bell were operated thirty times, including the electricity shocks depending on the animal's ability to learn to maintain an interval of 10 s between stimuli. Healthy animals with the ability to learn quickly moved from one room to another on seeing the light of the lamp and hearing the bell to prevent stun electric shocks. The avoidance responses were recorded on the basis of animal avoidance of electric shocks by running into the other compartment within 5 s. The time taken by an animal to enter the other compartment to avoid shocks was calculated as latency period (s). The number of inter chamber crossings during UCS and CS was also recorded.

Determination of plasma cholesterol, glucose, and hemoglobin

On the last day of the experiment, the animals were lightly anesthetized with diethylether, and approximately 150 µl of blood was withdrawn into heparinized capillaries by puncturing the retro-orbital plexus. Blood losses were replaced with 400 µl 0.9% NaCl subcutaneously. The obtained plasma was used to measure cholesterol (mg/dL),[21] glucose (mg/dL),[22] and hemoglobin (g/dL).[23]

Biochemical and histopathological analyses

Mice from each group were sacrificed by decapitation, and the whole intact brain was removed, rinsed in physiological saline, and then frozen at −80°C. Cooled block samples from the brain cortex, subcortical structures (thalamus and basal ganglia), medulla oblongata, and cerebellum were homogenized separately in cold phosphate-buffered saline (pH 7.4) and centrifuged at 20,000 ×*g* at 4°C for 10 min. The supernatant was collected and used for assaying AChE activity and lipid peroxidation. The AChE activity was estimated as per standard method[24] with slight modification in the whole-brain homogenate. Lipid peroxidation was measured using the method of Ohkawa *et al*.[25] by the estimation of monoaldehyde (MDA), the secondary products of peroxidation. The method is based on the reaction of MDA with thiobarbituric acid, which creates color change. The colored samples were measured spectrophotometrically at 530 nm. Malondialdehyde-bis-acetal was used as the standard for calibration. The amount of MDA produced was expressed in µmol/g/wet weight.

Histopathological examinations of liver and brain were performed by fixing in paraformaldehyde solution and embedding in paraffin followed by sectioning and staining in hematoxylin and eosin.

Statistical analysis

SPSS software (ver. 25) (IBM Corp, Armonk, NY, USA) was used for data analysis. Data were analyzed with repeated measures using one-way ANOVA followed by Tukey–Kramer´s *post hoc* test and Student's *t*-test. P <0.05 or <0.001 was considered statistically significant or highly significant, respectively. The results were expressed in means ± standard error of means.

RESULTS AND DISCUSSIONS

Body weight

Table 1 shows the effects of different doses of *A. senegal* and *A. seyal* on the body weight of mice. Treatment resulted in a significant increase in the body weight of mice compared to the control group. However, there was no significant difference between the effects of the dose as well as between two plant treatments with regard to the increase in the body weight. The results revealed a marked increase in the body weight for 0–3 days of treatment, maintaining a more or less constant weight for 6–15 days of treatment followed with a slight decline post 18–21 days. This finding is consistent with earlier reports;[26] however, it contradicts the previously published evidence on the increase of body weight after the 4th week of treatment.[27] These variations may be attributed to differences in the active ingredients of the natural products providing different experimental results.[28]

Locomotion activity test

Table 2 presents the effects of *A. senegal* or *A. seyal* on the various indices of locomotor activity. The locomotor activity in the treated mice showed a decrease in the number of squares crossed with respect to an increase in the concentration of the dose. The results suggested significant effects of both doses (F = 4.02; P < 0.05) and (F = 3.56; P < 0.05). However, no significant correlation between the two plant extracts was noticed. The locomotion duration (s) decreased with the increase in the concentration of the dose of *A. seyal*, whereas the locomotion duration decreased at low concentration with *A. senegal* and increased at high concentration compared to the control group. The results confirmed significant variability of locomotion duration with respect to the control group, but the effects were non-significant between the plant extracts used. The duration of immobility increased along with the increase in dose concentration (P < 0.05 and P < 0.001, respectively). Flavonoids and alkaloids, as documented by other researchers, could contribute to the behavioral actions observed in the current experiment. In agreement with the previous studies, flavonoids from different medicinal plants may cross the blood–brain barrier and affect the CNS,

Table 1: The variation of mean±standard error values of mouse body weight (n=8) during 0-21 days after supplementing drinking water with different concentrations of *Acacia senegal* and *Acacia seyal*

Days	Control	Low dose		High dose	
		Acacia senegal	*Acacia seyal*	*Acacia senegal*	*Acacia seyal*
0	2.31±0.32	2.22±0.58	2.41±0.32	2.44±0.21	2.98±0.16*
3	3.34±0.11	3.91±0.13	2.98±0.32*	2.99±0.63*	3.12±0.79
6	4.59±0.20	4.12±0.54*	3.67±0.30*	4.12±0.41*	4.34±0.44
9	4.99±0.81	5.13±0.32	4.78±0.32	4.54±0.37*	4.87±0.85
12	5.56±0.45	5.98±0.44	4.96±0.32*	5.01±0.22*	5.34±0.24
15	6.89±0.63	6.11±0.77 *	5.67±0.29*	5.32±0.65*	5.69±0.45*
18	7.11±0.72	7.00±0.11	6.11±0.52*	6.78±0.18*	6.21±0.27*
21	8.12±0.88	7.21±0.87	7.87±00.25*	6.99±0.39*	6.69±0.77*

*P<0.05, compared with control (Tukey-Kramer multiple comparison test)

producing neuroprotection, antioxidant, anxiolytic, antidepressant, and anticonvulsing outcomes, acting at the γ-aminobutyric acid A ($GABA_A$) receptors.[29,30]

The essential step in assessing the effects of agents on the CNS was the process of monitoring locomotor activity of the treated animals. The movement is a measure of the level of excitability of the CNS, and its decline may be related to sedation.[12] The *A. senegal* and *A. seyal* extracts significantly reduced locomotor activity and increased immobility time, suggesting sedating effect. Sedation may be caused by interaction with benzodiazepine-like agents. The extracts could strengthen the GABAergic inhibition in the CNS by membrane hyperpolarization, which decreases the firing rate of important neurons in the brain. Alternatively, the effect could be related to the direct activation of GABA receptor by the extracts.[31] Previous studies on phytochemical compounds and plants indicated that many polyphenols and flavonoids are ligands for $GABA_A$ receptors in the CNS, which suggested that *A. senegal* and *A. seyal* extracts could act as benzodiazepine-like agents.[32]

Behavioral assessment by forced swimming test

The FST was administered to evaluate the antidepressant effects of *A. senegal* and *A. seyal* at two recommended doses [Figure 1]. The results were in conformity to locomotory test. The treated mice showed a significant reduction in immobility in response to the increase in the dose. The lower mobility score was indicative of antidepressant-like behavior. The percentage of reduction in immobility was 10.7% and 27.1% for low and high doses, respectively, for *A. senegal* extract, and 16.2% and 56.8% for low and high doses, respectively, of *A. seyal* extract. The observed immobility could be attributed to the blockage of 5-HT reuptake or inhibition of monoamine oxidase (MAO) by active ingredients found in the extracts. In this regard, hypericin present in *Hypericum perforatum* has the potential to inhibit MAO and hyperforin is also an another active compound which is consider as 5-HT reuptake inhibitor.[33] The present results revealed more passive behavior in the treated animals compared to the control group. The result corroborates with earlier findings on depression-like behavior in mice.[34]

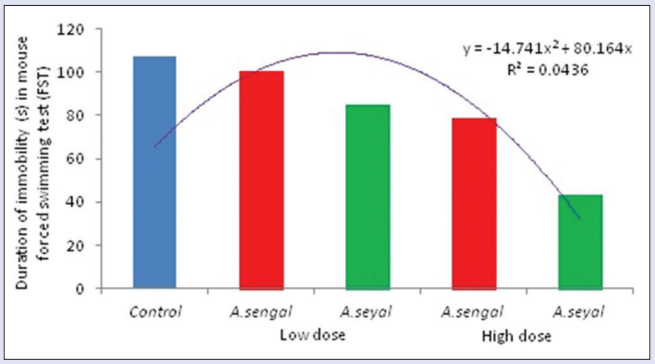


Figure 1: The effects of *Acacia senegal* and *Acacia seyal* on the duration of immobility in the mouse swimming test (forced swimming test)

Anxiety reflex by elevated plus maze

The EPM was employed to assess the anxiety reflex. The oral administration of both low and high doses of *A. senegal* and *A. seyal* showed a significant increase in the percentage of time spent in the open arms of the maze, compared to the control group [Figure 2]. The treated mice showed an increase in the latency time on their first entry to the closed arm. This finding is consistent with previous results reported by Deole *et al.*[11] The increased time spent by the treated mice in the open arms suggested an anxiolytic effect compared to the control group. Moreover, the reduction in time spent on the central platform is indicative of decreased decision-making behavior. Both parameters are reliable indicators of anxiety and fearfulness.[35]

Motor coordination

The acceleration Rotarod system was used to assess motor coordination and balance. The treatment with *A. senegal* and *A. seyal* showed significant decrease in motion balance and function at both low (108.3 and 54.2) and high (97.6 and 43.1) concentrations of doses, respectively [Figure 3]. The decline in motor coordination was statistically significant compared to the control group. Similar results were described by Hosseinzadeh and Noraei.[36] The researchers suggested that these effects could involve facilitation of some inhibitory systems, like the GABAergic. Some flavonoids isolated from plants have shown to hold an affinity for $GABA_A$/BDZ receptors similar to a partial agonistic action.

Active avoidance test

In active avoidance test, the treated mice showed a dose-dependent reduction in avoidance compared to the control group. The total number of avoidance behaviors was found statistically significant with respect to the control group. Both *A. senegal* and *A. seyal* exhibited a dose-dependent

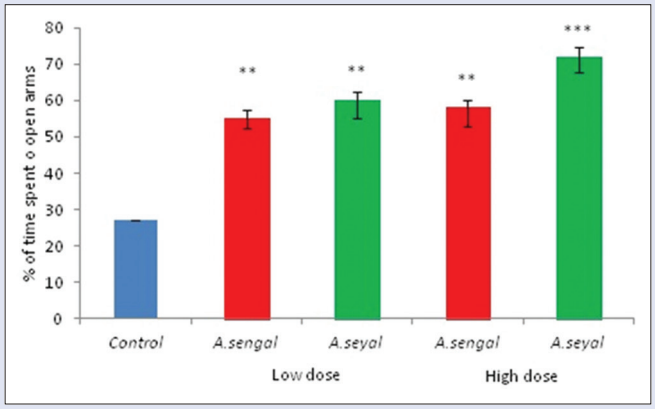


Figure 2: Effects of *Acacia senegal* and *Acacia seyal* on the percentage of time spent in the open-arm entries of the elevated plus maze test. Data are expressed as mean ± standard error of the mean (n = 8) in the open arm. $^{**}P < 0.05$, $^{***}P < 0.001$, compared with control, Tukey–Kramer test

Table 2: Effects of *Acacia senegal or Acacia seyal* on various measures of locomotor activity in mice after 21 days of treatment (values are mean±standard error of mean, *n*=8)

Parameter	Control	Low dose				High dose			
		Acacia senegal	*P*	*Acacia seyal*	*P*	*Acacia senegal*	*P*	*Acacia seyal*	*P*
Number of squares crossed	155.33±9.82	150.00±9.90	>0.05	140.67±5.06	<0.05	139.83±8.76	<0.05	121.83±7.88	<0.001
Locomotion duration(s)	180.00±3.20	168.83±9.0	>0.05	105.33±4.18	>0.05	118.6±5.00	>0.05	83.00±8.27	>0.05
Immobility duration(s)	107.83±3.80	116.67±7.71	>0.05	184.00±6.22	<0.05	178.50±11.85	>0.05	4202.00±6.94	<0.001

P<0.05 and <0.001 are considered statistically significant as compared to control

decrease in the number of avoidance behaviors in the treated mice in response to the external stimuli [Figure 4a]. The inter-trial crossing in the absence of shock also reduced upon treatment with two different doses [Figure 4b]. The abolishment of inter-compartmental movement or delay in the movements upon application of extracts was indicative of anxiolytic-like action in the treated mice. The variation in doses had a profound effect on memory and learning behavior, as exhibited by an increase in the latency time(s) compared to the control group [Figure 4c]. Similar findings were earlier reported on dose-dependent decrease in the number of avoidance behaviors upon treatment with aluminum.[20] An increase in the latency period may also be attributed to the disinhibitory behavior. Another possible factor may include sedation, making an animal unable to move quickly to the other compartment.[37]

Determination of plasma cholesterol, glucose and hemoglobin

The estimation of cholesterol level in the treated mice revealed a significant decrease in the cholesterol level in response to an increase in the dose [Table 3]. The reduction in cholesterol level by *A. seyal* was statistically significant compared to that of the control group. Previous studies reported the effectiveness of GA in reducing total plasma cholesterol, triglyceride, and low-density lipoprotein.[38] The findings of the current study included an insignificant decrease in the plasma glucose concentration after treatment with *A. senegal* or *A. seyal.* However, *A. senegal* at high dose significantly decreased glucose level. This result conforms with the findings of Nasir *et al.*[26] *Acacia* reduces blood glucose by inhibiting intestinal absorption through its interaction with sodium-glucose transporter-1.[39] Another possible explanation for this finding is that GA protects β-cells, resulting in a decline of blood glucose levels. Even though no marked variations in hemoglobin content have been observed in the present study for a low dose of the extracts, a significant decrease was observed for high doses. This observation implies that *A. senegal* or *A. seyal* could disrupt hemoglobin synthesis at high doses. Failure to synthesize hemoglobin occurs in many ailments, including iron deficiency anemia and anemia associated with chronic infection or ailment. Iron is an essential component of enzymes in cells and is also part of the heme group in hemoglobin. Much of the body's iron stores are within red blood cells. Iron shortage can be caused by inadequate intake or absorption of iron, excessive loss with external hemorrhage, or interference with iron metabolism.[40]

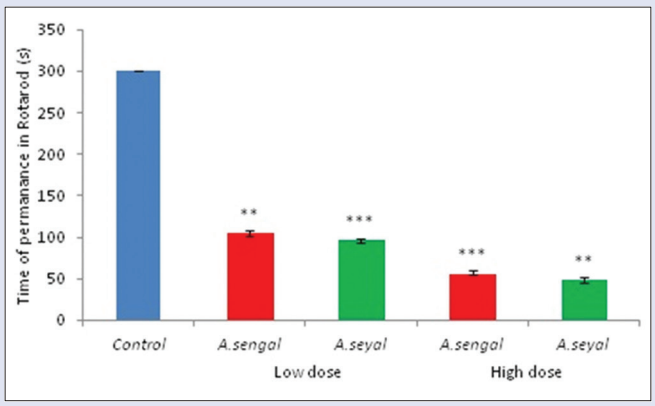


Figure 3: Effects of *Acacia senegal* and *Acacia seyal* on motor function in rotarod test. Data are expressed as mean ± standard error of the mean ($n = 8$). $^{**}P < 0.05$, $^{***}P < 0.001$, compared with control, Tukey–Kramer test

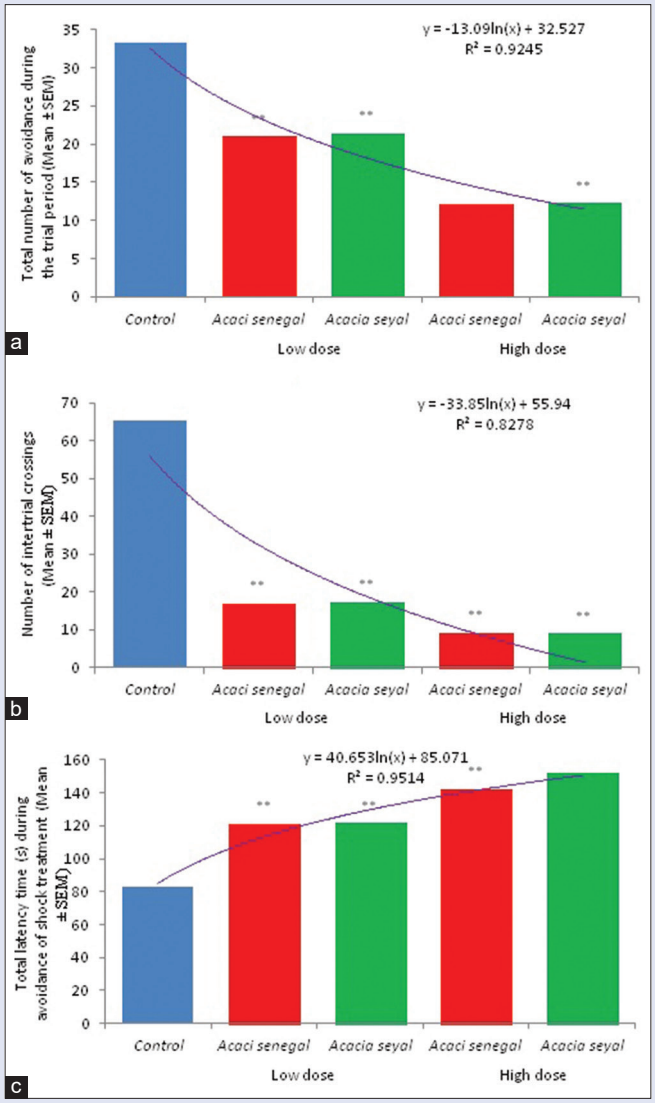


Figure 4: Mean active avoidance response of mice in shuttle box experiment, (a) total number of avoidance during shock treatment as exhibited by inter-trial movements between the chambers, (b) inter-trial movement in the absence of shock, (c) latency time(s) to avoid shock. **Statistically significant at $P < 0.05$ with respect to the control group

Table 3: Biochemical analysis of blood plasma of control and treated mice with gum Arabic (*Acacia senegal or Acacia seyal*) after 35 days of treatment

Parameter	Control	Low dose				High dose			
		Acacia senegal	*P*	*Acacia seyal*	*P*	*Acacia senegal*	*P*	*Acacia seyal*	*P*
Cholesterol (mg/dL)	219.50±4.39	213.50±4.66	>0.05	196.50±6.09	<0.05	203.83±2.24	>0.05	112.50±5.30	>0.05
Glucose (mmol/L)	6.38±0.21	6.33±0.30	>0.05	6.25±0.56	>0.05	5.08±0.21	<0.05	5.23±0.22	>0.05
Hemoglobin (mg/dL)	7.25±0.18	6.97±0.22	>0.05	6.77±0.19	>0.05	5.65±0.08	<0.05	6.35±0.33	<0.05

Arithmetic means±SEM of n=8 measurements. P<0.05 considered statistically significant as compared to control. SEM: Standard error of mean

Acetylcholinesterase activity

The breakdown of neurotransmitter acetylcholine is mediated by enzyme AChE. Estimation of AChE activity in the whole brain homogenate revealed a minor decline in the activity for the treated group [Figure 5]. The decrease in the activity was more pronounced upon treatment with *A. senegal,* as evidenced by the increasing concentration of acetylcholine. Although previous studies noted the relationship between changes in the behavioral pattern with the level of acetylcholine, no such relationship could be established in the present study.[41]

Lipid peroxidation level

Table 4 shows the increased level of lipid peroxidation (µmol of MDA) in all brain parts of the mice treated with high doses. Measurement of MDA levels in tissues provides an index of lipid peroxidation process. Increased content of MDA was more manifested in the cerebral cortex and cortex structures. The mean contents were much higher compared to the control group. This alteration reflects considerable changes in the regulatory activities, including behaviors of the treated mice. The increased peroxidation in the brain suggests the outcome of stress responses in mice.[42] A high lipid peroxidation product is also an indirect indicator of free radical production.[43,44]

Histopathological study

Histology of the liver of the mice in the treated and control groups showed normal morphology with slight alterations. The microscopic structure revealed hexagonal lobules in the central vein (CV). The hepatocytes and portal areas appeared normal in both *A. senegal*- and *A. seyal*-treated mice but with significant alteration in CV at high doses. The congested CV was prominent after high-dose treatment. A significant alteration in hepatocytes lobular structure was also displayed. These observations may be attributed to transportation and the accumulation of fatty acids in these areas. The administration of *A. senegal* and *A. seyal* led to lobular degeneration, dilation of interlobular passage, inflammatory cell infiltrations, wide vacuolar hepatocytes, dilation, and congestion of CV with disturbed epithelium. However, the severity of hepatic alterations was lower in *A. seyal*-treated mice compared to *A. senegal*-treated mice [Figure 6a and b]. The present results contradict with some earlier findings on rats.[45]

Histological evaluation of the brain structures of the treated mice revealed minor lesions in the cortex. Some neuronal cells were seen with shrunken cytoplasm and nucleus. The morphology of neurons was normal for the control group. The pyramidal cells were normal in the treated and control groups. Neurocytechromatolysis and moderate pyknosis cells were visible in the medulla region. Some fissures were seen in the cerebellum cortex, and the Purkinje cells appeared normal [Figure 7a and b]. Structural disorganization alteration in motor behavior was evident. These regions are in oxidative stress and vulnerable to injury, as indicated by higher lipid peroxidation.

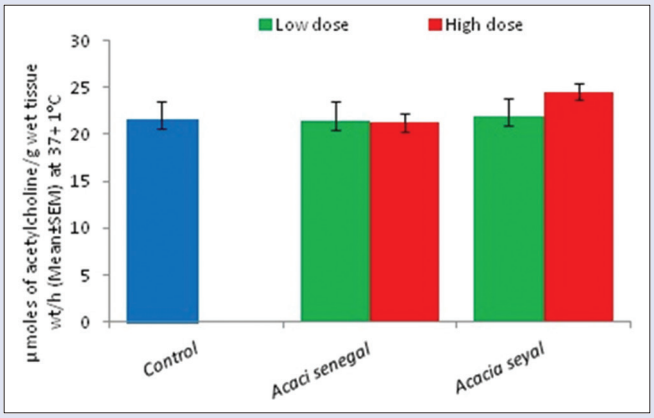


Figure 5: Effect of *Acacia senegal* and *Acacia seyal* on acetylcholine esterase activity on the treated mice

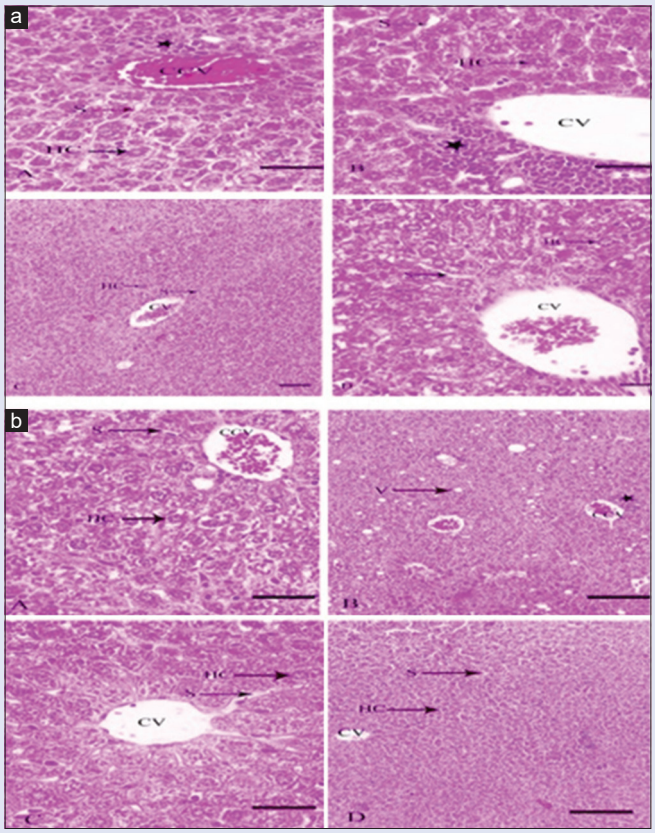


Figure 6: Section of liver showing central vein, congested central vein, hepatocyte, hepatic sinusoid, and inflammatory cells from the treated mice with (a) *Acacia senegal* and (b) *Acacia seyal*. (A and B) high dose. (C and D) low dose

Table 4: Level of lipid peroxide (µmol/g wet wt.) in the brain of postnatal day 21 mice treated with *Acacia seyal* and *Acacia seyal*

Brain parts	Controls	Low dose		High dose	
		Acacia senegal	*Acacia seyal*	*Acacia senegal*	*Acacia seyal*
Cortex	10.67±0.39	12.38±0.88*	11.98±0.44*	14.45±0.26**	13.10±0.62*
Subcortical structures	11.13±0.51	11.86±0.65	11.90±0.72	13.23±0.27	14.38±0.88*
Medulla oblongata	12.34±0.78	12.52±0.34	12.10±0.22	13.16±1.04*	13.00±0.29
Cerebellum	10.72±0.35	11.33±0.52	11.45±0.32	12.69±0.39**	12.50±0.77**

Results were calculated as means±SEM, n=8. *P<0.05, **P<0.001. SEM: Standard error of mean

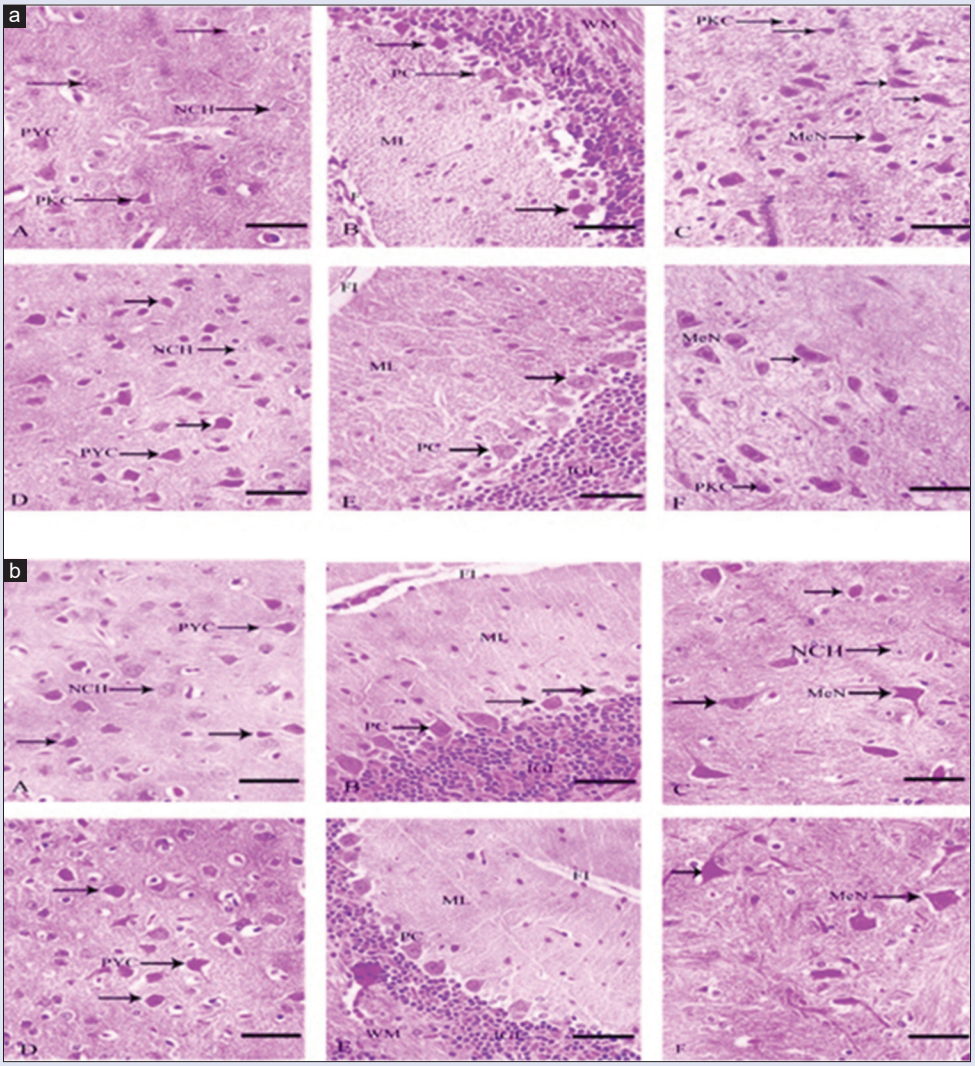


Figure 7: Sections of the cerebral cortex (AD), cerebellum (BE), medulla oblongata (CF) in pyramidal cell (PYC), neurocyte chromatolysis (NCH), pyknosis (PKC), Purkinje cell (PC), fissure (F), granular layer (GL), molecular layer (ML), white matter (WM), and medulla neurons (MeN) from the treated mice with (a) *Acacia senegal* and (b) *Acacia seyal*. (A-C) high dose. (D-F) low dose

CONCLUSIONS

The present study demonstrated that the extracts of *A. senegal* and *A. seyal* have anxiolytic and antidepressant effects. This observation can be attributed to the presence of pharmacologically active ingredients in *A. senegal* and *A. seyal*, which makes their extracts potentially suitable for the treatment of depression and anxiety. At both low and high concentration of doses, the treated mice spent most time in the open-sided arms, indicating anxiety and the sedative effect of the extracts. The sedative property of these plants may be successfully exploited in the treatment of agitated and anxious patients. However, the experiments revealed a decrease in motor activity and impairment of learning and memory. The findings confirmed a toxic effect on body weight upon treatment with *A. seyal* at different levels of concentration. The locomotion and behavioral pattern also decreased in a dose-dependent manner in the open-field test. The results of rotarod test confirmed motor imbalance and hypnotic effect at high doses. Estimation of AChE activity showed a minor decrease in the activity in the treated group compared to the control group mice. Histological analyses uncovered marked changes in liver and brain tissues upon treatment with *A. senegal* and *A. seyal* at their high concentrations. Future studies should explore the exact mechanism of action of *A. senegal* and *A. seyal*. Further investigation on the neurological characteristics of *A. senegal* is recommended to explore the potential application of this plant to the treatment of CNS disorders.

Financial support and sponsorship

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding this work through research group (no. RG-1439-60).

Conflict of interest

There are no conflicts of interest.

REFERENCES

1. Al-Yahya AA, Asad M. Antiulcer activity of gum Arabic and its interaction with antiulcer effect of ranitidine in rats. Biomed Res 2016;27:1102-6.
2. Nasr MM, Gondal MA, Ahmed MM, Yousif MM, Al-Muslet NA. Direct spectral analysis of different gum Arabic samples using laser induced breakdown spectroscopy. AIP Conf Proc 2018;1976:20025.

3. Babiker R, Merghani TH, Elmusharaf K, Badi RM, Lang F, Saeed AM. Effects of Gum Arabic ingestion on body mass index and body fat percentage in healthy adult females: Two-arm randomized, placebo controlled, double-blind trial. Nutr J 2012;11:111.
4. Maurice MI. Pharmacognostical profile of selected medicinal plants. Handbook of African Medicinal Plants. U.K., London: Rutledge; 2014.
5. Musa HH, Ahmed AA, Musa TH. Chemistry, biological, and pharmacological properties of gum Arabic. In: Mérillon JM, Ramawat KG, editors. Bioactive Molecules in Food. Cham: Springer International Publishing; 2017. p. 1-18.
6. Sharma AK, Kumar A, Yadav SK, Rahal A. Studies on antimicrobial and immunomodulatory effects of hot aqueous extract of *Acacia nilotica* L. leaves against common veterinary pathogens. Vet Med Int 2014;2014:747042.
7. Hamid M, Abdulrahim Y, Liu D, Qian G, Khan A, Huang K. The hepatoprotective effect of selenium-enriched yeast and gum Arabic combination on carbon tetrachloride-induced chronic liver injury in rats. J Food Sci 2018;83:525-34.
8. Kaddam L, Fadl-Elmula I, Eisawi OA, Abdelrazig HA, Saeed AM. *Acacia senegal* (Gum Arabic) supplementation modulate lipid profile and ameliorated dyslipidemia among sickle cell anemia patients. J Lipids 2019;2019:3129461.
9. Nasir O, Artunc F, Saeed A, Kambal MA, Kalbacher H, Sandulache D, Boini KM, *et al.* Effects of Gum Arabic *Acacia senegal* on water and electrolyte balance in healthy mice. J Renal Nutr 2008;18:230-8.
10. Beyer M, Reichert J, Heurich E, Jandt KD, Sigusch BW. Pectin, alginate and gum Arabic polymers reduce citric acid erosion effects on human enamel. Dent Mater 2010;26:831-9.
11. Deole YS, Chavan SS, Ashok BK, Ravishankar B, Thakar AB, Chandola HM. Evaluation of anti-depressant and anxiolytic activity of Rasayana Ghana Tablet (A compound Ayurvedic formulation) in albino mice. Ayu 2011;32:375-9.
12. Abdel Moneim AE. Citrus peel extract attenuates acute cyanide poisoning-induced seizures and oxidative stress in rats. CNS Neurol Disord Drug Targets 2014;13:638-46.
13. Muller CP, Kalinichenko LS, Tiesel J, Witt M, Stockl T, Sprenger E, *et al.* Paradoxical antidepressant effects of alcohol are related to acid sphingomyelinase and its control of sphingolipid homeostasis. Acta Neuropathol 2017;133:463-83.
14. Shimizu T, Minami C, Mitani A. Effect of electrical stimulation of the infralimbic and prelimbic cortices on anxiolytic-like behavior of rats during the elevated plus-maze test, with particular reference to multiunit recording of the behavior-associated neural activity. Behav Brain Res 2018;353:168-75.
15. Ajarem JS, Al-Basher G, Allam AA, Mahmoud AM. *Camellia sinensis* prevents perinatal nicotine-induced neurobehavioral alterations, tissue injury, and oxidative stress in male and female mice newborns. Oxid Med Cell Longev 2017;2017:5985219.
16. Abu-Taweel GM. Effects of perinatal exposure to Zamzam water on the teratological studies of the mice offspring. Saudi J Biol Sci 2017;24:892-900.
17. Porsolt RD, Bertin A, Jalfre M. "Behavioural despair" in rats and mice: Strain differences and the effects of imipramine. Eur J Pharmacol 1978;51:291-4.
18. Rodgers RJ, Cutler MG, Jackson JE. Behavioural effects in mice of subchronic chlordiazepoxide, maprotiline and fluvoxamine. II. The elevated plus-maze. Pharmacol Biochem Behav 1997;57:127-36.
19. McIlwain KL, Merriweather MY, Yuva-Paylor LA, Paylor R. The use of behavioral test batteries: Effects of training history. Physiol Behav 2001;73:705-17.
20. Abu-Taweel GM, Ajarem JS, Ahmad M. Neurobehavioral toxic effects of perinatal oral exposure to aluminum on the developmental motor reflexes, learning, memory and brain neurotransmitters of mice offspring. Pharmacol Biochem Behav 2012;101:49-56.
21. Zlatkis A, Zak B, Boyle AJ. A new method for the direct determination of serum cholesterol. J Lab Clin Med 1953;41:486-92.
22. Sadaki T, Tanemura K, Maki R, Nagai T. Effect of the quality of glacial acetic acid on the determination of blood glucose by the O-toluidine reaction. Rinsho Byori 1969;17:482-4.
23. Wroblewski F, Weiner M, Shapiro S. A simplified procedure for blood cell counts and haemoglobin determination. J Clin Pathol 1949;2:138-40.
24. Ellman GL, Courtney KD, Andres V Jr., Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95.
25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
26. Nasir O, Umbach AT, Rexhepaj R, Ackermann TF, Bhandaru M, Ebrahim A, *et al.* Effects of gum Arabic (*Acacia senegal*) on renal function in diabetic mice. Kidney Blood Press Res 2012;35:365-72.
27. Dauqan EM, Aminah A. Blood lipid profile and liver lipid peroxidation in normal rat fed with different concentrations of *Acacia senegal* and *Acacia seyal*. Int J Biol Biomol Agricult Food Biotechnol Eng 2014;8:1291-7.
28. Assaf SA, Phillips GO, Williams PA. Studies on acacia exudate gums. Part I: The molecular weight of *Acacia senegal* gum exudate. Food Hydrocoll 2005;19:647-60.
29. Rodriguez-Landa JF, Cueto-Escobedo J, Flores-Aguilar LA, Rosas-Sanchez GU, Rovirosa-Hernandez MJ, Garcia-Orduna F, *et al.* The aqueous crude extracts of *Montanoa frutescens* and *Montanoa grandiflora* reduce immobility faster than fluoxetine through GABAA receptors in rats forced to swim. J Evid Based Integr Med 2018;23:2515690X18762953.
30. Al Omairi NE, Al-Brakati AY, Kassab RB, Lokman MS, Elmahallawy EK, Amin HK, *et al.* Soursop fruit extract mitigates scopolamine-induced amnesia and oxidative stress via activating cholinergic and Nrf2/HO-1 pathways. Metab Brain Dis 2019;34:853-64.
31. Khatoon MM, Khatun MH, Islam ME, Parvin MS. Analgesic, antibacterial and central nervous system depressant activities of *Albizia procera* leaves. Asian Pac J Trop Biomed 2014;4:279-84.
32. Sultana T, Mannan MA, Ahmed T. Evaluation of central nervous system (CNS) depressant activity of methanolic extract of *Commelina diffusa* Burm. in mice. Clin Phytoscience 2018;4:5.
33. Borrelli F, Izzo AA. Herb-drug interactions with St John's wort (*Hypericum perforatum*): An update on clinical observations. AAPS J 2009;11:710-27.
34. Xu Y, Ku BS, Yao HY, Lin YH, Ma X, Zhang YH, *et al.* The effects of curcumin on depressive-like behaviors in mice. Eur J Pharmacol 2005;518:40-6.
35. Saeed MA, Sabir AW. Antibacterial activity of *Caesalpinia bonducella* seeds. Fitoterapia 2001;72:807-9.
36. Hosseinzadeh H, Noraei NB. Anxiolytic and hypnotic effect of *Crocus sativus* aqueous extract and its constituents, crocin and safranal, in mice. Phytother Res PTR 2009;23:768-74.
37. Bourin M, Hascoët M. The mouse light/dark box test. Eur J Pharmacol 2003;463:55-65.
38. Ahmed AA, Fedail JS, Musa HH, Kamboh AA, Sifaldin AZ, Musa TH. Gum Arabic extracts protect against hepatic oxidative stress in alloxan induced diabetes in rats. Pathophysiology 2015;22:189-94.
39. Nasir O, Babiker S, Salim AM. Protective effect of gum Arabic supplementation for type 2 diabetes mellitus and its complications. Int J Multidiscip Curr Res 2016;4:288-94.
40. Ladokun O, Ojezele M, Arojojoye O. Comparative study on the effects of aqueous extracts of viscum album (mistletoe) from three host plants on hematological parameters in albino rats. Afr Health Sci 2015;15:606-12.
41. Abdel Moneim AE. Oxidant/antioxidant imbalance and the risk of Alzheimer's disease. Curr Alzheimer Res 2015;12:335-49.
42. Elkhadragy MF, Kassab RB, Metwally DM, Almeer R, Abdel-Gaber R, Al-Olayan EM, *et al.* Protective effects of *Fragaria ananassa* methanolic extract in a rat model of cadmium chloride-induced neurotoxicity. Biosci Rep 2018;38:BSR20180861.
43. Abdel Moneim AE. *Indigofera oblongifolia* prevents lead acetate-induced hepatotoxicity, oxidative stress, fibrosis and apoptosis in rats. PLoS One 2016;11:e0158965.
44. Al Olayan EM, Aloufi AS, AlAmri OD, El-Habit OH, Abdel Moneim AE. Protocatechuic acid mitigates cadmium-induced neurotoxicity in rats: Role of oxidative stress, inflammation and apoptosis. Sci Total Environ 2020;723:137969.
45. Afsar T, Razak S, Almajwal A, Rashid Khan M. Modulatory influence of *Acacia hydaspica* R. Parker ethyl acetate extract against cisplatin inveigled hepatic injury and dyslipidemia in rats. BMC Complement Altern Med 2017;17:307.