

# Psychoimmunomodulatory Effects of *Onosma bracteatum* Wall. (Gaozaban) on Stress Model in Sprague Dawley Rats

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## ABSTRACT

Gaozaban (*Onosma bracteatum* wall, Family-Boraginaceae) is a key ingredient in a number of Unani and Ayurvedic formulations (eg. Kameera gaozaban sada, Dawa-ul-misk Motadil Jawahardar, Shahi etc.) which is used as a memory and an immunity enhancer. The present study has evaluated the psychoimmunomodulatory effects of *O. bracteatum* on the stress model in SD rats. The psychoimmunomodulatory effects of *O. bracteatum* were measured by assessing the changes in the behavior and the immunity of the rats, as well as by assessing the biochemical changes. In stress control, the results indicated that the % alternation, retention transfer latency, total leukocytes counts (TLC), size/weight of spleen and liver decreased and the acquisition transfer latency, total paw oedema, size of the kidney, AChE activity and the blood glucose

level increased and agglutination disappeared with dilution significantly in comparison with normal control. In *O. bracteatum* treated rats, the % alternation, retention transfer latency, size of the spleen and liver, TLC, and the agglutination increased whereas the acquisition transfer latency, size of the kidney, total paw oedema, AChE activity and circulating glucose were significantly decreased in comparison with the stress control. This study therefore concluded that the extract of *O. bracteatum* showed a protective effect against the stress induced impaired immune functions and the psychological processes like memory and it also supported the traditional usage of *O. bracteatum* for the treatment of a variety of immune deficiency disorders (eg. asthma and rheumatoid arthritis) and abnormal memory disorders which included dementia.

**Key Words:** *Onosma bracteatum*, Acetylcholinesterase, Dementia, Agglutination, Immunity and Cytokines

## INTRODUCTION

*Onosma bracteatum* wall. (*O. bracteatum*), Family: Boraginaceae. It is known as Gaozaban in the Unani system of medicine and as Sedge in the Middle East [1]. The hydro alcoholic extract of *O. bracteatum* shows various chemical constituents like carbohydrates (52%), glycosides (13%), flavonoids (15%) and phenolic compounds (20%), which can be evaluated by using standard test methods [2]. *O. bracteatum* has been advocated in the use of a variety of ailments, which include asthma (it stabilizes the mast cell activity) [3] and in rheumatoid arthritis [4, 5]. A plethora of reports is available in the literature about its demulcent, diuretic, antileprotic, spasmolytic and tonic nature [6]. Sedge is traditionally used as a tonic that helps in building the body's immune resistance and in regulating the urine output. This plant is an alterative refrigerant and a tonic. It is considered to be very much useful in relieving excessive thirst and restlessness in febrile excitement, in relieving the functional palpitation of the heart and the irritation of the bladder and the stomach and strangury [7]. A study demonstrated its significant role in the marked reduction of bronchial hyper-responsiveness on decreasing the infiltration of the eosinophils and the neutrophils in rodents [8]. Psycho Immunology (PI) is the study of the interaction between the psychological processes and the immune systems of the human body. Stress is a very common health problem which is associated with the disturbances of the body homeostasis or with the disturbances of the normal body physiology like psychological (behavioral changes), immunological and hormonal imbalances. It

causes the pathogenesis of certain chronic diseases like Alzheimer's disease, Parkinson's disease, and hypertension, weakness of the immune system of the human body, asthma, diabetes, heart ailments and even cancer. *O. bracteatum* is generally used in the Unani system of medicine for some of the above said diseases. Therefore, this research was aimed at scientifically proving the psychoimmunomodulatory effects of the said drug.

## MATERIALS AND METHODS

### Chemicals and Reagents

The Folin Ciocalteu's Phenol Reagent and bovine serum albumin were obtained from Merck Specialties Pvt. Limited, India, Acetylthiocholine iodide was obtained from Across Organic, USA, and 5'5,-bisdithionitrobenzoic acid (DTNB) was obtained from Himedia Laboratories Pvt. Ltd. India. The glucose estimating kits were obtained from Span Diagnostics, India.

### Animals

Sprague Dawley (SD) rats (Body weight: 150-250 g; Age: 8-10 weeks old) were procured from the Central Animal House Facility, Central Drug Research Institute, Lucknow, India. The animal experiments were performed according to the guidelines of the CPCSEA and the proposed work was approved by the Integral University's Institutional Animal Ethics Committee (IAEC), Lucknow, India (Approval No: IU/Pharm/PhD/CPCSEA/02).

## Plant Specimen

The best quality of *O. bracteatum* (Gaozaban) was purchased from Hamdard Dawakhana, Aminabad, Lucknow, India and the specimens were authenticated by the National Botanical Research Institute, Lucknow, India (Voucher specimen: NAB 191253).

## Extraction and Sample Preparation

The plant material was chopped into small pieces and it was gently dried at 50±5°C in a tray dryer (Decibel, Model No: DB-5017). Thereafter, the specimen was pulverized into fine powder and the powdered material (250 g) was suspended in a hydroalcoholic solution (50% v/v) for 24 hour. The obtained mixture was then boiled for 1 hour and it was kept undisturbed for another 1 hour before its filtration. The filtrate was then concentrated to a semi-solid mass under reduced pressure and it was dried at 60°C under vacuum (Remi Scientific, India). The yield of the extract was 21.2% (w/w) of the dried plant material. The extract was stored at 4°C (Whirlpool Refrigeration, USA) until it was needed for use and it was then freshly prepared in distilled water immediately before its administration to the animals.

## Acute Toxicity Study

The SD rats (150-250 g weight) of either sex were divided into 4 groups of 5 rats each. The groups I to III received intragastric 5, 10 and 20 mg/kg of the *O. bracteatum* extract, respectively, while the control (group IV), received distilled water (10 mL/kg) by the same route. The general symptoms of toxicity and mortality in each group were observed within 24 hours. The animals that survived after 24 hours were observed for any signs of delayed toxicity for two weeks.

## Animal Study Protocol

The pathogen free SD rats were maintained on a standard pelleted diet (Dayal Laboratory, Barabanki, U.P.) and water *ad libitum* under the environmental conditions of controlled temperature (25±2°C), humidity (60±5%) and illumination (12 h light-dark cycle), for 1 week before the experiment. The animals were divided randomly into five groups of 6 rats each (n=6). The rats of the normal control group (NC)/non-stress group (Gr. 1) were housed and fed under normal conditions. Stress was induced on the experimental rats by allowing them to swim in cold water (temperature-10±5°C) for 120 min/day, for three consecutive weeks. The stressed groups were further divided into two groups: (i) the non drug-treated group or the stress controlled (SC or Gr. II); and (ii) the drug (*O. bracteatum*) treated groups (Gr. III, Gr. IV and Gr. V).

## Drug Administration

The *O. bracteatum* extract at doses of 5 mg/kg, 10 mg/kg and 20 mg/kg and with distilled water as a vehicle, were administered intragastrically by using animal feeding intubation needles at the same time of the day, for a total of 21 days, and the control animals received an appropriate amount of vehicle for the same number of treatment days. 30 minutes after the treatment of the drug or the vehicle, the rats were subjected to stress once a day, for a period of 21 days, except for the non-stress group. All the behavioral and the biochemical tests were assessed 2 1/2 hours after the treatment with the drug/vehicle.

## Passive Avoidance Test

The behavior of the rats on the avoidance chamber (passive paradigm) is used for the passive avoidance test, which is used as

a short term memory task. The transfer latency of the animal is noted, which is related to the retention of the memory task. The step-through passive avoidance apparatus (a shuttle box) was used for the evaluation of the memory retention deficit in rodents. The apparatus consists of equal sized (30cm long x 30cm wide x 40cm high) light and dark chambers which are separated by a central wall. This wall has an opening, through which the animal can pass to both the chambers. The floor consists of a metal grid which is connected to a shock scrambler. The test consists of an acquisition and a retention trial. On day 20 after the treatment, the rat was placed in the light chamber. A trap-door which separated the chamber was open and the latency in entering the dark chamber was measured in seconds. Immediately after the rat entered the dark chamber, the trap-door was closed and an electric shock (1mA) was delivered for three secs. 5 seconds later, the rat was removed from the dark chamber and returned to its home cage. The retention test was performed 24 hours later in the same way, as in the acquisition trial and it was termed as the retention latency. Without applying the foot shock, the latency time was recorded to a maximum of 3 minutes [9,10].

## Behaviour on the Maze (Spontaneous Alteration Behavior)

A plus maze is used to evaluate the spontaneous alternation behavior by measuring the percentage alternation. The nootropics (memory enhancer drugs) increase the percentage alternation, whereas the amnesia drugs reduce it. The assessment was made on a plus maze which was made of poly wood which was painted blue/grey. It consisted of a symmetrical arm (50cm long x 10cm wide) with 15cm high side walls. The arms extended from a central platform (10 x 10cm) at a height of 50cm above the floor and they were labeled as A, B, C and D. The rat was placed in the centre and allowed to travel on the maze freely for 6 minutes. The sequence of the arm entries into the different arms was recorded. 4/5 alternations were defined as the entry into 4 different arms, overlapping the quintuplet sets of five consecutive arm entries/choices within the total set of arm entries. A B C A C was not considered as alternation. By using this procedure, the possible alternation sequences were found to be equal to the number of arm entries minus 4 [11].

Therefore, the percent alternation was scored as:

$$\frac{\text{Actual number of alternations}}{\text{Number of arm entries}-4} \times 100$$

## Agglutination Method

Blood samples were collected from the tail veins of the rats and the measurement of the antibody titer by haemagglutination was performed by using the Micro technique, which employed 96 well, micro test, flat bottom plates (TARSONS, CAT NO. 941196). Briefly, each well of the plate received 25 µl of a serial two-fold dilution of the serum in normal saline and an additional 25 micro liter volume of 1 % (V/V) Sheep Erythrocyte Suspension (SES) in normal saline (5x10<sup>8</sup> cell/mL). An hour after the incubation of the mixture at room temperature, the haemagglutination capacity was read. A positive haemagglutination reaction was visualized as a mat formation at the bottom, whereas a button formation indicated a negative haemagglutination reaction. The titers of the sera were determined as the reciprocals of the maximum dilutions which presented the positive hemagglutinations [12].

## TLC (Total Laeukocytes Count)

The blood samples were collected from the tail veins, and the TLCs were assessed by the routine haematological method by using a Neubauer's Chamber with a haemocytometer.

## Footpad Reaction Test

The cell mediated immune response was assessed by the footpad reaction test in the rats. The increase in the paw volume which was induced by an injection of sheep RBCs ( $5 \times 10^8$  cells/ml in normal saline), in the sub-plantar region of the right hind paw. The mean percent increase in paw volume was considered as an index of the cell mediated immunity [13].

## Brain Acetylcholinesterase (AChE) Activity

The rate of formation of thiocholine from acetylthiocholine iodide in the presence of tissue cholinesterase was measured by first treating with DTNB and by then measuring the Optical Density (OD) of the yellow colored compound which was formed during the reaction, at 412 nm, by using a UV-visible spectrophotometer (UV Pharmaspec-1700, SHIMADZU), every minute for a period of 3 minutes. The rat was sacrificed by using ether anesthesia. The brain was quickly removed and kept in an ice bath. The whole brain was used to measure the acetyl cholinesterase activity. A known weight of the brain tissue was homogenized in 0.32M sucrose solution to get a 10% homogenate. The homogenate was centrifuged at 3,000 rpm for 15 minutes, followed by centrifugation at 10,000 rpm for 10 minutes at a constant temperature of 4°C. Following the centrifugation, 1 ml of the supernatant was mixed with 9 ml of sucrose solution to get a 1% post mitochondrial supernatant (PMS). The acetyl cholinesterase estimation was done in the above 1% PMS by Ellman's method [14, 15].

## Blood Glucose

Blood samples were collected from the tail vein, and the blood glucose levels were estimated by using the Glucose estimating Kit (Span Diagnostics, India). The procedure and the time which were indicated for the kit were carefully followed. Dual measurements were performed and the average value was used as the measured value. The absorbance was measured with a UV-visible spectrophotometer (UV Pharmaspec-1700, SHIMADZU) at a wavelength of 505 nm.

## Relative Organ Weight

The rats of all the groups were weighed and sacrificed on the last day of the treatment. The relative organ weight (organ weight/100g body of weight) of the kidney, liver and the spleen were determined for each animal.

## STATISTICAL ANALYSIS

The data were expressed as means  $\pm$  SEM (Standard Error of the Mean) and the results were analyzed by ANOVA, followed by the Dunnett's t-multicomparison test. The p values which were  $<0.05$  were considered as significant.

## RESULTS

The acute toxicity study showed that all the doses (5, 10, and 20 mg/kg) of the *O. bracteatum* extract were non-toxic.

### Effect on the Spontaneous Alteration Behavior

In [Table/Fig-1], the % alternation on a plus maze of Stress Control (SC) was found to be significantly decreased ( $p < 0.05$ ), as compared

to the NC, it was found to be significantly increased ( $p < 0.05$ ) with a median dose (MD = 10mg/kg i.e., Gr. IV) and it was found to be non-significant ( $p > 0.05$ ) with the lower and higher doses (LD = 5mg and HD = 20mg i.e., Gr. III and Gr. V) of *O. bracteatum* which were treated on the days 10 and 20 respectively, as compared to the SC.

Groups	Treatment Schedule	Dose per kg (p.o.)	Percentage alternations	
			10 <sup>th</sup> day	20 <sup>th</sup> day
I	Vehicle + <i>O. bracteatum</i>	10 mL+0 mg	65.83 $\pm$ 13.72	67.08 $\pm$ 12.10
II	Vehicle + <i>O. bracteatum</i> + Stress	10 mL+0mg	30.83 $\pm$ 3.63*	23.33 $\pm$ 4.86*
III	<i>O. bracteatum</i> + Stress	5 mg	56.94 $\pm$ 3.49 <sup>n.s.</sup>	45.83 $\pm$ 10.51 <sup>n.s.</sup>
IV		10 mg	63.88 $\pm$ 5.77 <sup>#</sup>	65.00 $\pm$ 9.57 <sup>#</sup>
V		20 mg	55.14 $\pm$ 7.36 <sup>n.s.</sup>	56.02 $\pm$ 10.95 <sup>n.s.</sup>

**[Table/Fig-1]:** Effect of *O. bracteatum* on spontaneous alternation behavior in rats.

Values are expressed as Mean $\pm$ SEM, (n=6), \* $p < 0.05$ =Significant, when compared with normal control (i.e. group I), n.s.=Non-significant, <sup>#</sup> $p < 0.05$ ; Significant, when compared with stress control (i.e. group II).

### Effect on the Acquisition and the Retention Latency

In [Table/Fig-2], the acquisition transfer latency of the SC was found to be increased ( $p < 0.01$ ) and the retention transfer latency was found to be decreased ( $p < 0.01$ ) significantly, as compared to the NC. The acquisition transfer latency was significantly decreased ( $p < 0.05$ ) with MD, it was no significant with LD and HD, and the retention transfer latency was increased ( $p < 0.01$ ) in both LD and MD significantly, non significant HD of the *O. bracteatum* treated rats, as compared to the SC.

Groups	Treatment Schedule	Dose per kg (p.o.)	Acquisition transfer latency (sec)	Retention transfer latency (sec)
I	Vehicle + <i>O. bracteatum</i>	10 mL+0 mg	9.50 $\pm$ 1.44	177.50 $\pm$ 2.50
II	Vehicle + <i>O. bracteatum</i> + Stress	10 mL+0 mg	17.00 $\pm$ 0.91**	90.25 $\pm$ 14.59**
III	<i>O. bracteatum</i> + Stress	5 mg	13.50 $\pm$ 0.65 <sup>n.s.</sup>	127.50 $\pm$ 8.54 <sup>#</sup>
IV		10 mg	11.75 $\pm$ 1.32 <sup>#</sup>	155.00 $\pm$ 6.46 <sup>#</sup>
V		20 mg	13.50 $\pm$ 1.19 <sup>n.s.</sup>	125.55 $\pm$ 14.29 <sup>n.s.</sup>

**[Table/Fig-2]:** Effect of *O. bracteatum* on acquisition and retention transfer latency of passive avoidance paradigm in rats.

Values are expressed as Mean $\pm$ SEM, (n=6), \*\* $p < 0.01$ =Significant, when compared with normal control (i.e. group I), n.s.=Non significant, <sup>#</sup> $p < 0.05$ , <sup>#</sup> $p < 0.01$ =Significant, when compared with stress control (i.e. group II).

### Effect on the Cellular Immunity

In [Table/Fig-3], the Total Leukocytes Counts (TLCs) of the SC were found to be decreased ( $p < 0.01$ ) and the total paw oedema was found to be increased ( $p < 0.01$ ) significantly, as compared to the NC. On the other hand, the TLC was found to be significantly increased with all the doses (highly significant ( $p < 0.01$ ) with MD), the total paw oedema was found to be significantly decreased ( $p < 0.05$ ) with MD and it was no significant with the LD and HD of the *O. bracteatum* treated rats, as compared to the SC.

### Effect on the Humoral Immunity

In [Table/Fig-3], the agglutination (means the net formation in the cup number) of the SC significantly disappeared ( $p < 0.01$ ) with dilution, as compared to the NC, and it was significantly increased

Groups	Treatment	Dose per kg (p.o.)	Cellular immunity		Humoral Immunity
			Total Leukocytes Count (Cell/mm <sup>3</sup> )	Paw edema (%)	Agglutination (in cup number)
I	Vehicle + <i>O. bracteatum</i>	10 mL+0 mg	6224±497.19	31.12±3.82	11.00 ± 0.41
II	Vehicle + <i>O. bracteatum</i> + Stress	10 mL+0 mg	2627±177.78 <sup>**</sup>	67.02±3.84 <sup>**</sup>	3.50 ± 0.65 <sup>**</sup>
III	<i>O. bracteatum</i> + Stress	5 mg	3870±134.23 <sup>#</sup>	51.04±4.30 <sup>n.s.</sup>	6.50 ± 0.65 <sup>#</sup>
IV		10 mg	4325±238.94 <sup>##</sup>	40.97±3.84 <sup>#</sup>	9.25 ± 0.49 <sup>##</sup>
V		20 mg	3820±142.65 <sup>#</sup>	53.82±4.97 <sup>n.s.</sup>	6.25 ± 0.85 <sup>#</sup>

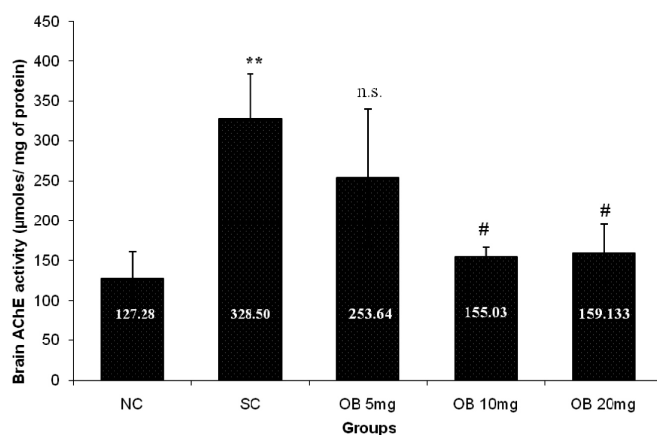
**[Table/Fig-3]:** Effect of *O. bracteatum* on cellular and humoral immunity in rats.

Values are expressed as Mean±SEM, (n=6), <sup>\*\*</sup>p<0.01=Significant, when compared with normal control (i.e. group I), n.s.=Non significant, <sup>#</sup>p<0.05, <sup>##</sup>p<0.01=Significant, when compared with stress control (i.e. group II).

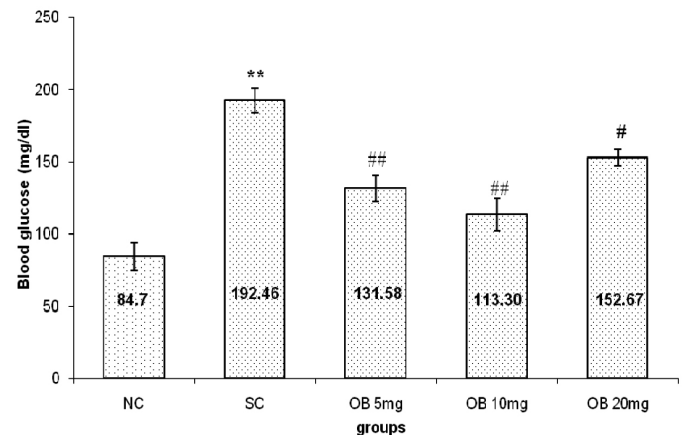
Groups	Treatment	Dose per kg (p.o.)	Organ weight (gm/100g body weight)		
			Spleen	Liver	Kidney
I	Vehicle + <i>O. bracteatum</i>	10 mL+0 mg	0.443±0.07	3.29±0.14	0.67±0.04
II	Vehicle + <i>O. bracteatum</i> + Stress	10 mL+0 mg	0.212±0.01 <sup>**</sup>	2.05±0.02 <sup>**</sup>	0.95 ± 0.03 <sup>*</sup>
III	<i>O. bracteatum</i> + Stress	5 mg	0.39±0.032 <sup>#</sup>	3.06±0.30 <sup>#</sup>	0.76±0.05 <sup>n.s.</sup>
IV		10 mg	0.426±0.011 <sup>##</sup>	2.99±0.17 <sup>#</sup>	0.71±0.08 <sup>#</sup>
V		20 mg	0.387±0.022 <sup>#</sup>	2.82 ±0.25 <sup>#</sup>	0.77 ± 0.41 <sup>n.s.</sup>

**[Table/Fig-4]:** Effect of *O. bracteatum* on relative organ weight in rats.

Values are expressed as Mean±SEM, (n=6), <sup>\*\*</sup>p<0.01=Significant, when compared with normal control (i.e. group I), n.s.=Non significant, <sup>\*</sup>p<0.05, <sup>##</sup>p<0.01=Significant, when compared with stress control (i.e. group II).



**[Table/Fig-5]:** Effect of *O. bracteatum* on brain acetylcholinesterase activity in rats. All values are expressed as Mean±SEM, (n=6 for each group), OB=*O. bracteatum*, <sup>\*\*</sup>p<0.01= significant, when compared with normal control group=NC, n.s.=Non significant, <sup>#</sup>p<0.05=significant, when compared with SC=stress control group.



**[Table/Fig-6]:** Effect of *O. bracteatum* on blood glucose level in rats. All values are expressed as Mean±SEM, (n=6 for each group), OB=*O. bracteatum*, <sup>\*\*</sup>p<0.01= significant, when compared with normal control group=NC, <sup>#</sup>p<0.05, <sup>##</sup>p<0.01= significant, when compared with SC=stress control group.

with all the doses (highly significant (p<0.01) with MD) of *O. bracteatum* treated, as compared to the SC.

### Effect on the Organ Weight/100g Body Weight

In [Table/Fig-4], the sizes of the spleen and the liver of the SC were found to be decreased (p<0.01) and the size of the kidney was found to be increased (p<0.05) significantly, as compared to those of the NC. The sizes of the spleen and the liver were increased (p<0.05) with all the doses, the size of the kidney decreased (p<0.05) significantly with MD and it was no significant with the LD and the HD of *O. bracteatum* treated, as compared to the SC.

### Effect on the Brain Acetyl Cholinesterase (AChE) Activity

In [Table/Fig-5], the AChE activity in SC was found to be significantly increased (p<0.01), as compared to the NC, it was found to be significantly decreased (p<0.05) with MD and it was no significant with the LD and the HD of *O. bracteatum* treated, as compared to the SC.

### Effect on the Blood Glucose Level

In [Table/Fig-6], the blood glucose level of the SC was found to be significantly increased (p<0.01), as compared to the NC and it was found to be significantly decreased with all the doses (highly significant (p<0.01) with LD and MD) of the *O. bracteatum* treated, as compared to the SC.

## DISCUSSION

Stress is thought to affect the immune functions through emotional and/or behavioral manifestations such as anxiety, fear, tension, cognition, anger and sadness and through physiological changes such as the heart rate, blood pressure, glucose metabolism and sweating. The naturalistic stressors are associated with an increase in the number of the circulating neutrophils, a decrease in the number and the percentages of the total T cells, an increase in the pro-inflammatory plasma cytokine production which indicates a weak immune function [16], a decrease in the size of the spleen and the liver, an increase in the size of the kidney, altered cognitive functions

(eg. loss of memory) and an increase in the circulating glucose levels. In the present study, the *O. bracteatum* extract showed an increase in the number of the total T cells (with all the doses, but it was highly significant with 10mg/kg), an increase in the antibody production (with all the doses, but it was highly significant with 10mg/kg), a significant decrease in the total paw oedema (with the dose of 10mg/kg and it was no significant with 5mg/kg and 20mg/kg), an increase in the size of the spleen and the liver (with all the doses) and a significant decrease in the size of the kidney (with the dose of 10mg/kg and it was no significant with 5mg/kg and 20mg/kg), in comparison to SC. The circulating glucose concentrations regulate many brain functions which include learning and memory [17]. A study suggested that glucose enhances the cognitive performance [18]. In another report, glucose was suggested to be critical for the production of acetyl-CoA, a precursor of acetylcholine [19]. Thus, one strong possibility is that glucose enhances the memory processes by increasing the acetylcholine synthesis and its release [20]. The brain acetylcholine is associated with the consolidation of memory, and it is hydrolyzed by the brain cholinesterase. In the pathogenesis of Alzheimer's disease (the major symptom is loss of memory), acetylcholine deficiency is one of the causes and therefore, cholinesterase inhibitors are beneficial in improving the memory. In the present study, the *O. bracteatum* extract (10mg/kg) showed an inhibitory effect on the cholinesterase activity and it improved the behavior significantly in comparison with SC. The regular raised blood glucose levels like in the conditions of chronic stress or diabetes depressed the cognitive functions and the immune functions. In this study, the *O. bracteatum* extract (doses 5, 10 and 20mg/kg) showed a regulatory effect on the circulating glucose levels significantly in comparison with SC.

## CONCLUSION

This study concluded that *O. bracteatum* showed a protective effect against the stress induced impaired immune function, cognitive functions and the circulating blood glucose levels. These findings also supported the traditional use of *O. bracteatum* for the treatment of immunity ailments and dementia as well.

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