Original Article

IRANIAN JOURNAL OF PHARMACOLOGY & THERAPEUTICS

Iranian J Pharmacol Ther. 2017 (September);15:1-7.

β- glucan attenuated chronic unpredictable mild stress induced cognitive impairment in rodents via normalizing corticosterone levels

Saniya Hashim Khan[1], Ali Haider[1], Sheraz Khan[1], Ghulam Abbas[1, 2*]

[1] Department of Pharmacy, COMSATS Institute of Information Technology, Abbottabad, K.P.K., Pakistan
[2] H.E.J. Research Institute of Chemistry, International Centre for Chemical & Biological Sciences, University of Karachi, Karachi, Pakistan

Please cite this article as:
Hashim Khan S, Haider A, Khan Sh, Abbas Gh. β- glucan attenuated chronic unpredictable mild stress induced cognitive impairment in rodents via normalizing corticosterone levels. Iranian J Pharmacol Ther. 2017 (September);15: 1-7.

ABSTRACT

The current study was aimed at exploring the effect of β-glucan (a naturally occurring polysaccharide) on chronic unpredictable mild stress (CUMS) induced impairment in learning and memory. Briefly, the animal model of CUMS was generated (28 days) followed by assessment of the efficacy of β-glucan (2.5, 5 or 10 mg/kg) on learning and memory in Morris water maze (MWM). Moreover, the weight variation during the course of experiment and post-MWM corticosterone levels was also noted. Our data showed that the β-glucan provided dose dependent protection against deleterious effects of stress on spatial memory, which became statistically significant at 10 mg/kg. In similar manner, it has also antagonized the stress induced weight loss and corticosterone elevation. Taking everything into account, it can be deduced that β-glucan possesses therapeutic potential against stress induced cognitive impairment and this effect can be attributed to its normalizing effect on corticosterone levels.

Conflicts of Interest: Declared None
Funding: None

Keywords
β-glucan,
Chronic unpredictable mild Stress,
Corticosterone,
Morris water maze,
Spatial Memory

Corresponding to:
Ghulam Abbas,
H.E.J. Research Institute of Chemistry, International Centre for Chemical & Biological Sciences, University of Karachi, Karachi

Email:
ghulam.abbas@iccs.edu

Received: 15 Feb 2017
Revised: 15 Mar 2017,
Accepted: 24 May 2017

INTRODUCTION

Cognition is the dynamic cerebral process that aids to familiarize, amend and act according to the situation. Learning and memory are the important components of cognition. The former helps us in acquiring new information while the later store this information for potential imminent use [1]. Spatial memory is a sub-class of episodic memory which helps in manoeuvre and hoards information in the spatiotemporal frame. It has been proposed that encoding of spatial memory has a direct relation with hippocampus, a brain structure considered as cradle of cognition and necessary for organizing, encoding, and retrieval of stored information [2]. Chronic stress is one the most common cause of cognitive impairment. This injurious effect has been reported to be caused by hypercortisolemia-induced excitotoxicity in the hippocampus because it is heavily expressed with metabotropic glucocorticoid receptor (mGR). The chronic stress induced loss of hippocampal volume underlies the its cognition lowering effect [3, 4]. Thereof, the stress-cortisol-mGR axis presents itself as promising target to attain protection against harmful effects of the stress on cognition. Spatial memory is accountable for encoding and storing the information about one's milieu and ambiances, which helps in navigation. Innumerable approaches have been adopted to evaluate the spatial memory in animals.

Among all, Morris water maze (MWM), is frequently used behavioural tool to assess spatial memory in rodents. It is enormously subtle in assessing impairment in learning and memory due to deficits in motor, sensory and retrieval processes [5].

β-Glucan is a polysaccharide (chain of glucose molecule) found abundantly in the *Avena sativa* (Oat) which is used traditionally as neuro-tonic, anti-depressant and for treating insomnia [6]. β-Glucan has been reported to possess anti-oxidant, neuroprotective and cognition enhancing potential [7,8]. Keeping these neurological actions in mind, the current study was designed to explore the mnemonic effect of β-glucan in chronic unpredictable mild stress (CUMS) model.

MATERIALS & METHODS

Animals

Male Sprague-Dawley rats (150–200 g) were obtained from the Animal Care Facility of the COMSATS Institute of Information and Technology, Abbottabad and housed under the standard conditions of temperature (25±2 ˚C), controlled light and dark cycle (08:00 till 20:00) and *ad libitum* supply of food and water. All procedures adhered to the guidelines of the ethical committee of COMSAT Institute of Information and Technology Abbottabad, that follows the guidelines of Animal's Scientific Procedure Act 1986 (UK).

Treatment Groups

The rats were divided into 4 groups as follows: i) Vehicle control (CUMS plus normal saline), ii) CUMS plus β-glucan 2.5 mg/kg iii) CUMS plus β-glucan 5 mg/kg and iv) CUMS plus β-glucan 10 mg/kg. The test drug and normal saline

Table 1. Describes various types of stressors used in CUMS procedure: A.B, Air blow (hot or cold Air puff) [13,14], A.O, Aversive odour [15], C.R.I, Cold room isolation [16], D/W Bed, Wet bedding [14], F.D, Food deprivation [16], F & W.D, Food and Water deprivation [16], G.H, Grouped housing [14], Imm, Immobilization (limited area in the cage) [17], I L/D C, Inverted Light/Dark cycle [13], L & D, Light & dark transitions [15], L.S, Loud music/ sound [18], Osc, Oscillations (round & to-fro) [13], P.H, Paired housing [14], Res, Restraining [16], T. Cs, Tilted cages [14,17].

Days/ Week	*Stressor-1 (S-1)*	*Normal Saline/ Drug Administration*	*Stressor-2 (S-2)*	*Stressor-3 (S-3)*
WEEK 1	10:00am	12:00pm	02:00pm	05:30pm
DAY 1	C.R.I	+ Weighed	F & W D	F & W D (contd.)
DAY 2	Res		C.R.I	Osc,
DAY 3	D/W Bed		T. Cs	A.O
DAY 4	I. L/D C	+ Weighed	I. L/D C	I. L/D C
DAY 5	Res		D/W Bed	A.B
DAY 6	L.S		Res	G.H
DAY 7	D/W Bed		F & W D	A.O – F & W
WEEK 2	10:30am	12:30pm	02:30pm	05:30pm
DAY 8	T. Cs	+ Weighed	C.R.I	N.B
DAY 9	L.S		Imm	L & D
DAY 10	I. L/D C		I. L/D C	I. L/D C
DAY 11	D/W Bed	+ Weighed	Res	G.H + W.B
DAY 12	C.R.I		Res	N.B + G.H
DAY 13	F & W D		F & W D	A.O – F & W
DAY 14	T. Cs		D/W Bed	Osc
WEEK 3	10:00am	12:00pm	02:00pm	05:30pm
DAY 15	C.R.I	+ Weighed	N.B	T. Cs
DAY 16	Imm		L & D	L.S
DAY 17	I. L/D C		I. L/D C	I. L/D C
DAY 18	Res	+ Weighed	G.H + W.B	D/W Bed
DAY 19	Osc		C.R.I	N.B + G.H
DAY 20	F & W D		A.O – F & W	A.O – F & W
DAY 21	D/W Bed		Osc	T. Cs
WEEK 4	10:30am	12:30pm	02:30pm	05:30pm
DAY 22	F & W D	+ Weighed	F & W D (contd.)	C.R.I
DAY 23	C.R.I		Osc,	Res
DAY 24	T. Cs		A.O	L.S
DAY 25	I. L/D C	+ Weighed	I. L/D C	I. L/D C
DAY 26	D/W Bed		A.B	Res
DAY 27	Res		G.H	D/W Bed
DAY 28	F & W D		A.O – F & W	L.S
DAY 29		Weighed		

were administered intraperitoneally (*i.p.*), once a day for 5 weeks (four weeks of CUMS plus one week of MWM).

Body Weight Analysis

All rats were carefully weighed once a week, in order to assess the weight loss or gain during entire course of experiment.

Chronic Unpredictable Mild Stress

The procedure of CUMS was performed for 28 days. Various types of stressors were used as depicted in Table 1. At least three different stressors (morning, afternoon and evening) were applied each day with high degree of variability among days to avoid adaptation.

Morris Water Maze

The water maze consisted of a circular pool (black color with 180 cm diameter and 50 cm height), equally divided into 4 quadrants and filled with water (25±1.0 °C). A removable platform (black color with 10 cm diameter and 20 cm height) was placed into one quadrant of the pool. High contrast spatial cues were also fixed around the wall of tank for encoding of spatial data. In familiarization/acquisition sessions, three trials were conducted for each animal. On day1st, the rats were allowed to locate the visible platform i.e. water level 1 cm below the surface of the platform, in 60 sec. If the rat find the platform, it was allowed to stay for 5 sec. If the animal failed to find the platform within 60 s, then, it was gently guided to the platform and allowed to remain on it for 30 s (platform acquisition time; PAT). Inter trial interval (ITI) was 60 s.

The same protocol was adapted for Acquisition training from day-2nd till day-5th to find hidden platform i.e. platform was 1 cm below water surface in tank. The escape latency was measured each day. On 6th day (probe trial), the platform was removed to assess spatial memory. Each rat was subjected to pool for 60 s and allowed to locate the platform. The recorded swim sessions were observed later for measuring maximum time spent in the target quadrant, number of entrances into target quadrant and latency to enter the target quadrant.

Serum Corticosterone Measurement

All rats were rapidly decapitated after the probe trial of MWM and trunk blood was collected to obtain the serum. The Corticosterone levels were measured by using the ELISA Kit (AmgenixMicroLISA, CA, USA) as described by manufacturer.

Statistics

All results were expressed as mean ± standard error mean (S.E.M) of n= 6 rats/group. The difference between various means was evaluated using one-way ANOVA using SPSS. The criterion for statistical significance was set at $p<0.05$.

RESULTS & DISCUSSION

Learning & memory are the important cerebral processes. One of the major threats for these executive functions is chronic stress, which primarily affect the hippocampus (the key brain structure involved in mnemonic functions) through hypercortisolemia [3]. The rationale behind this selective damage is the major expression of mGR in the hippocampus, which act as mediator of excitotoxicity [4, 9]. Hence, the stress-cortisol-mGR axis presents itself as promising target to attain protection against harmful effects of the stress on cognition. Keeping this in view, the purpose of the present study was to explore the effectiveness of potential lead molecule i.e. β-glucan on stress induced cognitive impairment and its interaction with

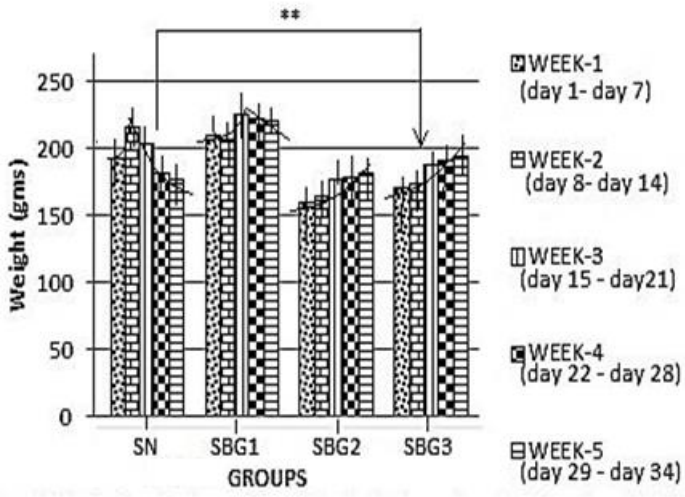

Figure 1. Effect of β- glucan treatment on weight variation in during Chronic Unpredictable Mild Stress (CUMS): The bar chart is exhibiting weight variation of various treated groups during the five weeks of CUMS. The treated groups include: SN (CUMS + normal saline; vehicle control), SBG1 (CUMS + β-glucan 2.5 mg/kg), SBG2 (CUMS + β-glucan 5 mg/kg) and SBG3 (CUMS + β-glucan 10 mg/kg). The trend line shows the increasing or decreasing levels of weight in various groups during the whole time. Error bars represent mean ± SEM (n=6) of animals' body weight. ** $p< 0.01$ treatment illustrates significant antagonizing effect of β- glucan (10 mg/kg) treatment on weight variation.

stress-glucocorticoid interplay.

Chronic stress has been shown to reduce the weight of the animals [10]. In similar lines, our data (vehicle control) exhibited prominent weight loss during the CUMS procedure (Fig. 1). However, this reduction remained unaffected by normal saline treatment, while it was found to be reversed by β-glucan in dose dependent manner. Thereby, the data strengthened the probability of its interaction with glucocorticoid system and suggestive of the protective effect of β-glucan in the presence of stress.

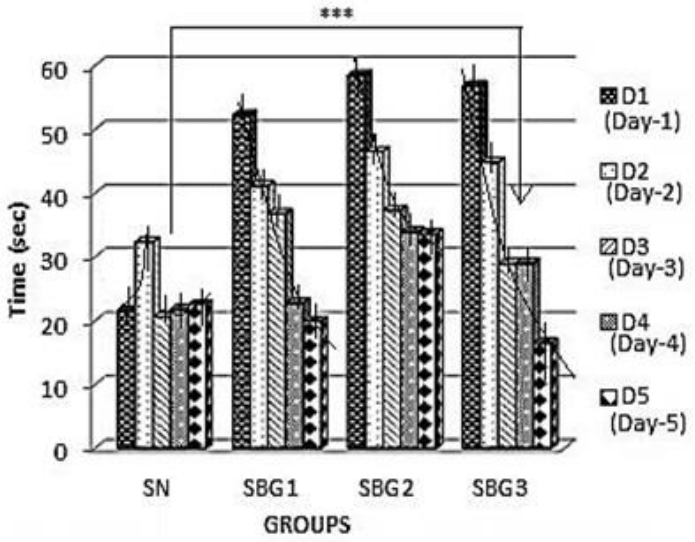

Figure 2. The effect of β- glucan treatment on escape latency in various treated groups during acquisition trials in Morris Water Maze (MWM): A multiple bar chart showing variations in the escape latency of animals throughout the 5 days of acquisition training of MWM test. The treated groups are: SN (CUMS + normal saline; vehicle control), SBG1 (CUMS + β-glucan 2.5 mg/kg), SBG2 (CUMS + β-glucan 5 mg/kg) and SBG3 (CUMS + β-glucan 10 mg/kg). The trend line shows the increasing or decreasing levels of escape latency in various groups during the whole time. Error bars represent mean ± SEM (n=6) of stay time in the target quadrant. *** p< 0.001 treatment illustrates significant reduction in escape latency with β- glucan (10 mg/kg) treatment.

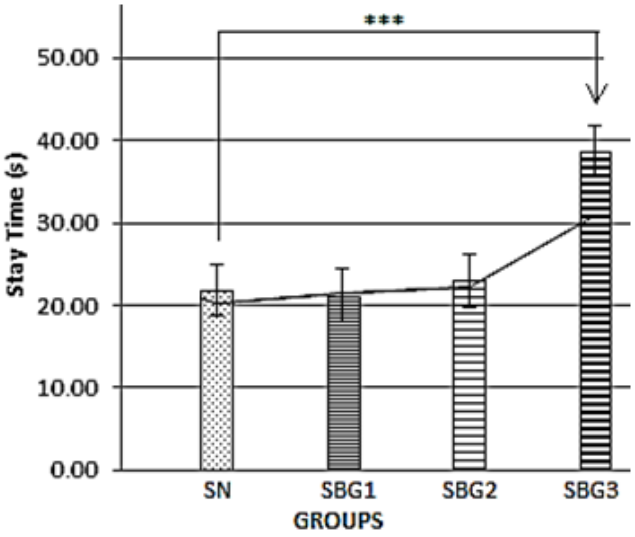

Figure 3. The effect of β- glucan treatment on maximum stay time in target quadrant in Morris Water Maze (MWM) during probe trials: A simple bar chart depicts the effect of β- glucan on stay time in target quadrant in various treated groups at dose of 2.5, 5 and 10 mg/kg. The groups are: SN (CUMS + normal saline; vehicle control), SBG1 (CUMS + β-glucan 2.5 mg/kg), SBG2 (CUMS + β-glucan 5 mg/kg) and SBG3 (CUMS + β-glucan 10 mg/kg). The trend line shows the increasing or decreasing levels of stay time (n=6). Error bars represent mean ± SEM of stay time in the target quadrant. *** p< 0.001 treatment exhibits significant increase in stay time as compared to control with β- glucan (10 mg/kg) treatment.

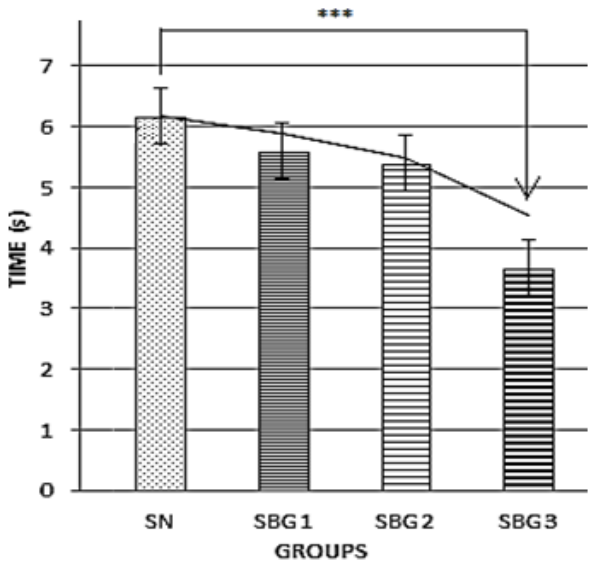

Figure 4. The β- glucan treatment effect (probe trial) on the latency to enter in the target quadrant in Morris Water Maze (MWM): The bar chart showing effects of different doses of β-glucan (2.5, 5 and 10 mg/kg) on latency to enter target quadrant using MWM. The groups are: SN (CUMS + normal saline; vehicle control), SBG1 (CUMS + β-glucan 2.5 mg/kg), SBG2 (CUMS + β-glucan 5 mg/kg) and SBG3 (CUMS + β-glucan 10 mg/kg). The trend line shows the variation in the time of entering into the quadrant. Error bars represent mean ± SEM of latency to enter in the target quadrant. (n=6). *** p< 0.001 treatment are significantly different as compared to vehicle control.

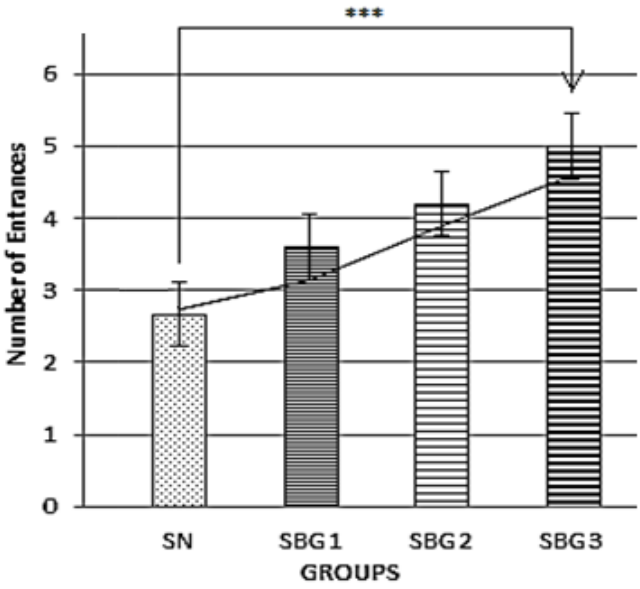

Figure 5. The β- glucan treatment effect on the number of entries in the target quadrant in Morris Water Maze (MWM) during probe trial: The bar chart exhibits the number of times rat crosses through the platform position in various treated groups in MWM. The groups are: SN (CUMS + normal saline; vehicle control), SBG1 (CUMS + β-glucan 2.5 mg/kg), SBG2 (CUMS + β-glucan 5 mg/kg) and SBG3 (CUMS + β-glucan 10 mg/kg). The trend line depicts variation number of crossings through platform location. Error bars represent mean ± SEM of number of crossings through platform position. *** p< 0.001 treatment are significantly different as compared to vehicle control (n=6).

Morris water maze is the commonly used behavioural paradigm used to assess the effect of test substances on spatial memory [11]. Our data of acquisition trials showed that the performance of chronically stressed rats (S) was comparably poor as reflected by higher escape latency values, which appeared to be significantly reduced by the treatment of β-glucan at dose of 10 mg/kg (p< 0.001) as shown in Fig. 2. In other words, the animals of this group have learned the specific location of the platform with the help of spatial cues, which has led to a notable decrease in

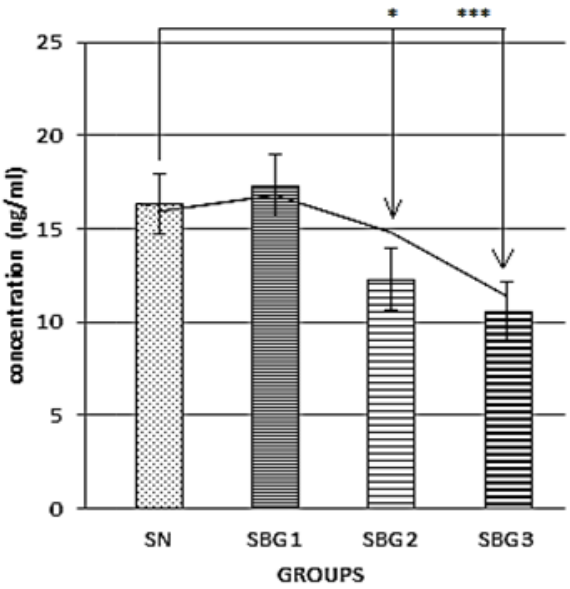

Figure 6. The effect of β- glucan treatment on the serum corticosterone levels (ng/ ml) in various groups: The bar chart illustrates the serum corticosterone levels in different groups after β- glucan treatment at dose of 2.5, 5 and 10 mg/kg in MWM. The groups are: SN (CUMS + normal saline; vehicle control), SBG1 (CUMS + β-glucan 2.5 mg/kg), SBG2 (CUMS + β-glucan 5 mg/kg) and SBG3 (CUMS + β-glucan 10 mg/kg). The trend line depicts decrease in the levels of serum corticosterone after β- glucan treatment. Error bars represent mean ± SEM of number of crossings through platform position. β-glucan treatment at dose of 5 mg/kg (*p< 0.05) and 10 mg/kg treatment (***p< 0.001) are significantly different as compared to vehicle control (n=6). Error bars signify mean ± SEM of corticosterone levels (n=6).

the time to find the platform in subsequent days. The aforesaid data suggested that β-glucan has beneficial effects in stress induced cognitive ailments. This favourable effect was also confirmed in the probe trial where the β-glucan treatment was found to reverse the deleterious effect of stress on various parameters (Stay time in target quadrant, latency to enter the target quadrant and number of entries in the target quadrant) observed in MWM as shown in Figs. 3, 4 and 5. β- glucan (10 mg/kg) treatment significantly ($p< 0.001$) enhanced the stay time in target quadrant, latency to enter the target quadrant and number of entries in the target quadrant as compared to control. Our results are also supported by earlier report, which suggest that carbohydrate rich dies enhances the cognitive abilities in stress induced memory impairment [12]. This strengthened our deduction that the β-glucan is endowed with the potential of antagonizing harmful effects of stress on learning and memory.

Stress and the resulting increase in glucocorticoid levels have been linked with cognitive impairment. It is well established that cortisol (corticosterone in rats), secreted by the adrenal cortex after a stressful event, negatively influence the cognitive performance [9]. Therefore, the effect of β-glucan on corticosterone levels was also assessed in animals that underwent aforesaid behavioural analysis. It is important to note that the β-glucan treatment reduced the corticosterone levels in dose dependent manner as compared to normal saline treated animals (Fig. 6) which is significant at dose of β-glucan 5 mg/ kg ($p< 0.01$) and 10 mg/kg ($p<0.001$), which is suggestive of the adaptogenic effect. In conformity with our results, carbohydrate rich diet was earlier shown to lower blood cortisol levels [12].

CONCLUSION

The current study revealed that β-glucan can ameliorate the stress induced memory impairment. This effect can be linked primarily with its corticosterone lowering potential. In the light of these findings, β-glucan (found abundantly in nature) can be considered as a potential candidate molecule for the alleviation of stress associated memory impairment.

ACKNOWLEDGEMENT

We thanks to Sheraz, Shahab Ali Khan, Mehreen Arif, Hifza, Hurmat and Wali Inam for their help and support during experimental work.

CONFLICT OF INTEREST

The authors declare that this research does not have any conflict of interest with anyone or any institute.

REFERENCES

1. Mullally SL, Maguire EA. Learning to remember: the early ontogeny of episodic memory. Dev Cogn Neurosci 2014;9:12–29.
2. O'Keefe J, Nadel L. Précis of O'Keefe & Nadel's. The hippocampus as a cognitive map. Behav Brain Sci 2011;2:487–94.
3. Küçük A, Gölgeli A, Saraymen R, Koç N. Effects of age and anxiety on learning and memory. Behav Brain Res 2008;195:147–52.
4. Lupien SJ, de Leon M, de Santi S, Convit A, Tarshish C, Nair NP, et al. Cortisol levels during human aging predict hippocampal atrophy

and memory deficits. Nat Neurosci 1998;1:69–73.
5. Hooge RD, Deyn PP De. Applications of the Morris water maze in the study of learning and memory. 2001;36.
6. Singh R, De S, Belkheir A. Avena sativa (Oat) A Potential Neutraceutical and Therapeutic Agent: An Overview. Crit Rev Food Sci Nutr 2013;53:126–44.
7. Nelson ED, Ramberg JE, Best T, Sinnott RA. Neurologic effects of exogenous saccharides: A review of controlled human, animal, and in vitro studies. Nutr Neurosci 2012;15:149–62.
8. Alp H, Varol S, Celik MM, Altas M, Evliyaoglu O, Tokgoz O, et al. Protective effects of beta glucan and gliclazide on brain tissue and sciatic nerve of diabetic rats induced by streptozosin. Exp Diabetes Res 2012;2012:230342.
9. Roozendaal B. Stress and memory: opposing effects of glucocorticoids on memory consolidation and memory retrieval. Neurobiol Learn Mem 2002;78:578–95.
10. Gouirand AM, Matuszewich L. The effects of chronic unpredictable stress on male rats in the water maze 2005;86:21–31.
11. Brandeis R, Brandys Y, Yehuda S. The use of the Morris Water Maze in the study of memory and learning. Int J Neurosci 1989;48:29–69.
12. Markus CR, Panhuysen G, Tuiten A, Koppeschaar H, Fekkes D, Peters ML. Does carbohydrate-rich, protein-poor food prevent a deterioration of mood and cognitive performance of stress-prone subjects when subjected to a stressful task? Appetite 1998;31:49–65.
13. Harris RB, Zhou J, Youngblood BD, Smagin GN, Ryan DH. Failure to change exploration or saccharin preference in rats exposed to chronic mild stress. Physiol Behav 1997;63:91–100.
14. Ducottet C, Griebel G, Belzung C. Effects of the selective nonpeptide corticotropin-releasing factor receptor 1 antagonist antalarmin in the chronic mild stress model of depression in mice. Prog Neuro-Psychopharmacology Biol Psychiatry 2003;27:625–31.
15. Willner P. Chronic Mild Stress (CMS) Revisited: Consistency and Behavioural-Neurobiological Concordance in the Effects of CMS. Neuropsychobiology 2005;52:90–110.
16. McFadden LM, Paris JJ, Mitzelfelt MS, McDonough S, Frye CA, Matuszewich L. Sex-dependent effects of chronic unpredictable stress in the water maze. Physiol Behav 2011;102:266–75.
17. Das A, Rai D, Dikshit M, Palit G, Nath C. Nature of stress: Differential effects on brain acetylcholinesterase activity and memory in rats. Life Sci 2005;77:2299–311.
18. Naqvi F, Haider S, Batool Z, Perveen T, Haleem DJ. Sub-chronic exposure to noise affects locomotor activity and produces anxiogenic and depressive like behavior in rats. Pharmacol Rep 2012;64:64–9.