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Garlic Extract (*Allium sativum*) Enhances Spatial Working Memory in Wistar Rats: Involvement of Hippocampal Na^+/K^+ ATPase and Ca^{2+} ATPase Activities

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Abstract

Hippocampus plays a central role in the acquisition and recall of both episodic and spatial memory. Studies have shown that garlic has neuroprotective effects in various capacities and enhancement of different forms of memory. However, the effect of garlic spatial memory and direct effect of garlic extract on the activities of membrane bound enzymes Na^+/K^+ ATPase and Ca^{2+} ATPase in the hippocampus of rat still remain elusive. Therefore, we studied the effect of ethanolic extract of garlic on spatial working memory using object location memory OLM test and the hippocampal Na^+/K^+ ATPase and Ca^{2+} ATPase activities. Sixteen male wistar rats weighted 120 - 150 g were used and divided into two groups of eight rats each. The control and experimental groups were treated 1ml of normal saline and 500 mg/kg body weight of ethanolic extract of garlic respectively orally for three weeks. OLM test was carried out on the two groups. Animals were sacrificed and the brains were removed, and hippocampi were carefully excised and homogenate was obtained. Homogenate was analyzed for Na^+/K^+ ATPase and Ca^{2+} ATPase activities. There was significant increase in the exploration time in experimental group when compared with control group ($p < 0.001$). There was significant increase in both Na^+/K^+ ATPase and Ca^{2+} ATPase activities in experimental group when compared with control group ($p < 0.0001$). The results indicate that effects of garlic on improvement of hippocampal-dependent spatial memory could be mediated through activities of these membrane bound enzymes. The results showed that garlic enhancement of hippocampal-dependent spatial memory could be mediated by increasing the activities of Na^+/K^+

ATPase and Ca^{2+} ATPase. Our findings provide potential mechanism and therapeutic target for memory deficit neurological disorders.

Keywords

Ca^{2+} ATPase, Garlic, Hippocampus, Na^+/K^+ ATPase, Spatial Working Memory

1. Introduction

Crude herbal extracts of aromatic plants have been in use for myriad benefits such as drugs, food and perfumery since time immemorial [1]. Garlic (*Allium sativum*) is used throughout history for medicinal purposes such as antimicrobial, antithrombotic, antihypertensive, anti-hyperglycemic, antihyperlipemic and antiapoptosis [2] [3] [4] [5]. Its active components with well-known biological functions have been reported [6] [7]. Antioxidant effects of garlic extract by scavenging reactive oxygen species ROS, enhancing cellular antioxidant enzymes superoxide dismutase, Catalase, Glutathione peroxidase and inhibits lipid peroxidation and activation of oxidant induced transcription factors have been established [8] [9]. Fresh and cooked but not aged garlic extracts has been shown to improve both short and long term memory in both diabetic male and female rats [10]. Na^+/K^+ ATPase is a membrane bound enzyme involved in maintaining the Na^+ and K^+ gradient across the cell membrane. Inhibition of Na^+/K^+ ATPase activity produces edema and cell death at CNS level and also impairs learning and memory. Reduced Na^+/K^+ ATPase and Ca^{2+} ATPase activities were associated with Aluminum Chloride induced brain toxicity in various rat brain regions such as cortex, cerebellum and hippocampus [11]. Study has indicated that chronic deprenyl administration enhances basal electrical firing rate and the activities of Na^+/K^+ ATPase and PKC in CA1 and CA3 hippocampal areas, which are the sites for initial learning and memory processing [12]. Homocysteinthiolactone, anexcitotoxic compound, has been reported to inhibit activity of Na^+/K^+ ATPase in cortex, hippocampus and brain stem, suggesting that Na^+/K^+ ATPase and Ca^{2+} ATPase are essential for excitation of neurons [13]. In addition, Na^+/K^+ ATPase abnormality has been reported to be involved in several neurological diseases such as Alzheimer's disease [14], bipolar disorder [15] and haploinsufficiency of Na^+/K^+ ATPase $\alpha 2$ and $\alpha 3$ isoforms resulted in behavioral defects [16]. Calcium is an important signaling molecule in cells. Disturbances in Ca^{2+} homeostasis can lead to neuronal dysfunction and eventual neuronal death. Several neurological diseases are caused primarily by malfunctioning of Ca^{2+} channels or Ca^{2+}/Mg^{2+} ATPase [17]. Based on available literature, no study has assessed the effect of ethanolic extract of garlic on spatial memory using object location behavioural task. Also the direct effect of any forms of garlic on hippocampal membrane bound enzymes Na^+/K^+ ATPase and Ca^{2+} ATPase is not known. Therefore, we investigated the effect of ethanolic extract of garlic on

hippocampal-dependent cognitive function such as object location memory and then, evaluate the Na^+/K^+ ATPase and Ca^{2+} ATPase activities in hippocampus. Our findings show that garlic extract enhances spatial working which might be mediated through increase activity of Na^+/K^+ ATPase and Ca^{2+} ATPase.

2. Materials and Methods

2.1. Ethanol Extraction of Garlic

Extraction was done using cold maceration at the laboratory in the department of Pharmacology, Kampala International University Western Campus Uganda. Softneck type of garlic weighing 500 g was peeled cut into small pieces and homogenized in 70 ml of cold sterile 0.9% NaCl. The paste material was suspended in 80% ethanol for 48 hours in air tight glass jar using a rubber stopper, and the suspension was shaken periodically for three times a day at 5 minute interval. After 2 days, the suspension was filtered using Whitman filter paper to remove residue. Filtration was repeated 3 times and clear filtrate was obtained. The filtrate was concentrated using rotary evaporator at a bath temperature of 40˚C. The extract concentrate obtained was then transferred to a cornical flask and further evaporated in oven drier at 50˚C to obtain ultimately a gel like mass for the study [18].

2.2. Animals

Adult male wistar rats of age of 12 - 14 weeks weighing (120 - 150 g) were used in this experiment. The animals were obtained from the Animal House of College of Medicine, Mbarara University of Science and Technology, Uganda. The animals were housed in a well-ventilated room maintained under standard conditions of light, feeding and temperature of research laboratory of Kampala International University Western Campus Uganda. The study was conducted in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals.

2.3. Grouping

After one week of acclimatisation, the animals were randomly distributed into two groups of eight rats each in this order:

Control group: received 1 ml saline and fed a standard rat chow.

Experimental group: received 500 mg/kg body weight of ethanolic extract of garlic and fed a standard rat chow. [19]

Administration was carried out daily between 8 - 10 am for a period of three weeks.

2.4. Object Location Memory Test

The object location memory test was used to assess spatial working memory after the administration of garlic extract for 21 days. The protocol was modified from the method [20] and [21]. This test has three phases (trials) with one day

inter-trial interval. The first phase was habituation where rat was place in the center of an empty open field box of dimension [70 cm (L) × 40 cm (W) × 30 cm (H)] and allowing the rat to freely explore the box for two 5 min. Habituation was done for all the rats following the same arrangement. The second trial was conducted on the following day (same time) of the first trial and this involved placing the rat in the center of the same open field box having two identical objects of dimension [3.5 cm (L) × 3.5 cm (W) × 3 cm (H)] on opposite sides of the box 2.5 cm away from the wall of the box and was allowed to freely explore the objects for 10 min (*i.e.*, the training phase). In day 3, the testing phase was performed for 5 min by placing the rat in the center of the same open field box with one of the objects remaining in the same location as in training phase and the second object moved to a new location in the open field box. A rat is considered to be exploring an object when its nose is within 2 cm of the object. All the trials (experiments) were conducted at a very good illumination and recorded with VDO camcorder version-052, USA. The apparatus was cleaned with 70% ethanol prior to the commencement of each trial for every rat to reduce olfactory cues. All possible Data such as times spent in exploring the object moved to a novel place, the object remaining in the familiar place, and total time spent in the object exploration were measured. Also, the place discrimination index was calculated by using the formula, the time spent with the object moved to a novel place/the total time spent in exploring both the object moved to a novel place and the object remaining in the familiar place × 100. Then, the percentages of object exploration time spent with the object moved to a novel place and that of the object remaining in the familiar place were calculated. The preference of the rat to explore the object that has been moved to a new location reflects its ability for object location memory.

2.5. Measurement of Na^+/K^+ ATPase Activity

The hippocampal homogenates was analyzed for Na^+/K^+ ATPase according to the method of Tirri *et al.* 1973 [22]. Assay medium used consist of (in mM) 30 Tris-HCl buffer (pH 7.4), 50 NaCl, 6 $MgCl_2$, 5 KCl and 50 µg of protein in the presence and absence of ouabain, 0.1 EDTA, in a final volume of 350 µL. The reaction was started by the addition of ATP to a final concentration of 3 mM. After 30 min at 37˚C, the reaction was stopped by the addition of 50% (w/v) trichloroacetic acid (70 µL). The saturating substrate concentrations was used, and reaction was in linear with protein and time. Some controls was included in the assays for non-enzymatic hydrolysis of ATP. The Pi (amount of inorganic phosphate) released was quantified calorimetrically, as described [23], using 300 KH_2PO_4 as reference standard. Specific Na^+/K^+-ATPase activity was calculated from the overall activity (in the absence of ouabain) and was recorded as Pi/min/mg of protein in nmol.

2.6. Measurement of Ca^{2+} ATPase Activity

The method of Desaiah and Ho (1979) [24] was used to assay Ca^{2+} ATPase in

hippocampal homogenates. Pi (Inorganic phosphates) was estimated by the method [23]. The assay medium had a final volume of 200 µL. It consisted of (in mM), 30 Tris-HCl, 100 µg of protein in the presence or absence of 0.4 $CaCl_2$, buffer (pH 7.4), 3 $MgCl_2$ and 0.1 EGTA. The reaction was started by the addition of ATP to a final volume of 3 mM. 60 min after at 37˚C, the reaction was stopped by the addition of 50% (w/v), 70 µL of trichloroacetic acid. Substrate concentrations was used, and reaction was in linear with time and concentration of protein. Some controls were included in the assays to assess non-enzymatic ATP hydrolysis. The Pi (concentration of inorganic phosphate) released was quantified colorimetrically, as described [23], using KH_2PO_4 as a reference standard. The Ca^{2+} ATPase activity was determined by subtracting the activity measured from absence of Ca^{2+} (no added 0.1 mM EGTA and Ca^{2+}) and expressed as Pi/min/mg protein in nmol.

2.7. Statistical Analysis

All statistical analyses were performed using Microsoft Excel and SPSS version 20. All values were presented as means ± SEM (standard error of mean). Statistically significant differences were accepted at $p < 0.05$.

3. Results

3.1. Effect of Garlic Extract on Spatial Working Memory

In the OLM test, control group and experimental group showed no significant difference in time for each object in the training trial, suggesting that there was no preference for either of the object's location. In the testing trial, experimental group showed clear preference for the object moved to a novel location in comparison to the object remained in the familiar location, as these rats spent 70% of their object exploration time with the object that moved to a novel location (**Figure 1**). The preference for the object moved to novel location showed by experimental group (70% for object in novel location, 40% for object in familiar location) was more than that of control group (55% for object in novel location, 45% for object in familiar location) (**Figure 2(a)** and **Figure 2(b)**). Total times spent in object exploration during testing phase was significantly increased in experimental group (15.4 s ± 0.51) when compared with control group (12.4 s ± 0.51) ($p < 0.001$; **Figure 3**). A comparison of the location discrimination index between the two groups revealed enhanced location memory function in experimental group.

3.2. Na^+/K^+ ATPase Activity in the Hippocampus Following Administration of Garlic Extract

Figure 4(a) shows the Na^+/K^+ ATPase activity in the hippocampus of garlic treated rats and control group rats. The Na^+/K^+ ATPase activity (µ mol of pi liberated/min/mg protein) in the hippocampus of garlic treated group (0.53 ± 0.18) was significantly higher ($p < 0.0001$) as compared to control group (0.43 ± 0.11).

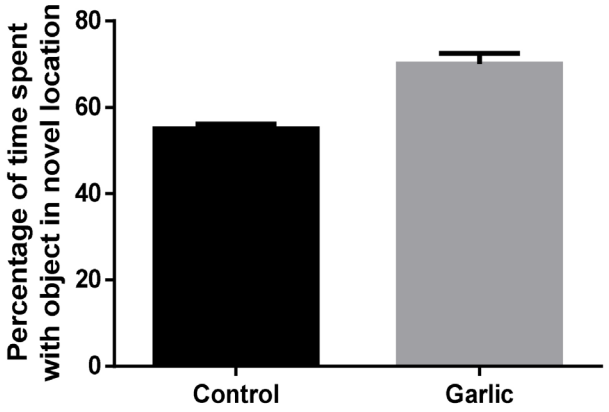

Figure 1. Comparison of the percentage of time spent with object in the novel location of the group treated with garlic and the control group in testing phase of the task. Significantly different at $p < 0.05$.

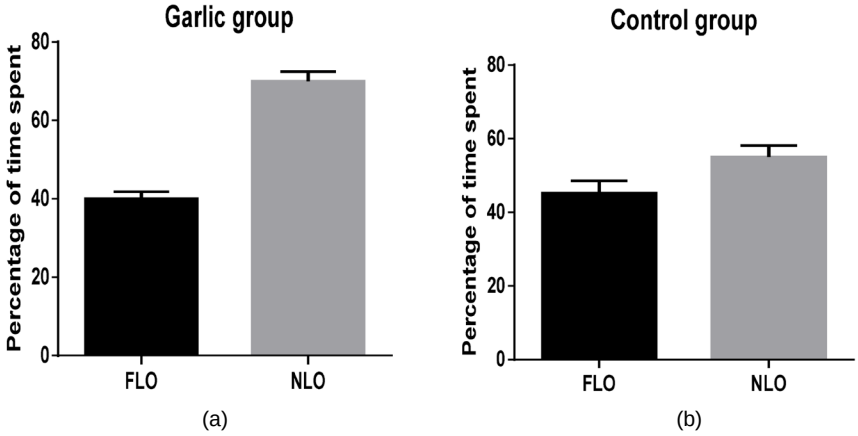

Figure 2. Shows the percentage of time spent with novel location object (NLO) and familiar location object (FLO) in testing phase for experimental group (a) and control group (b). Significantly different at $p < 0.05$.

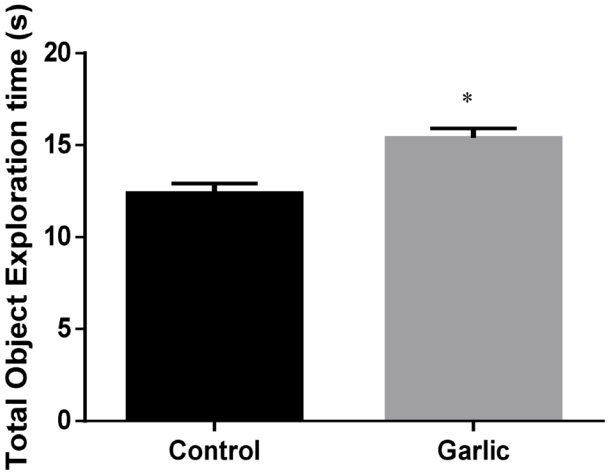

Figure 3. In testing trials, Total object exploration time was significantly increased in garlic treated group when compared with control group (*$P < 0.001$).

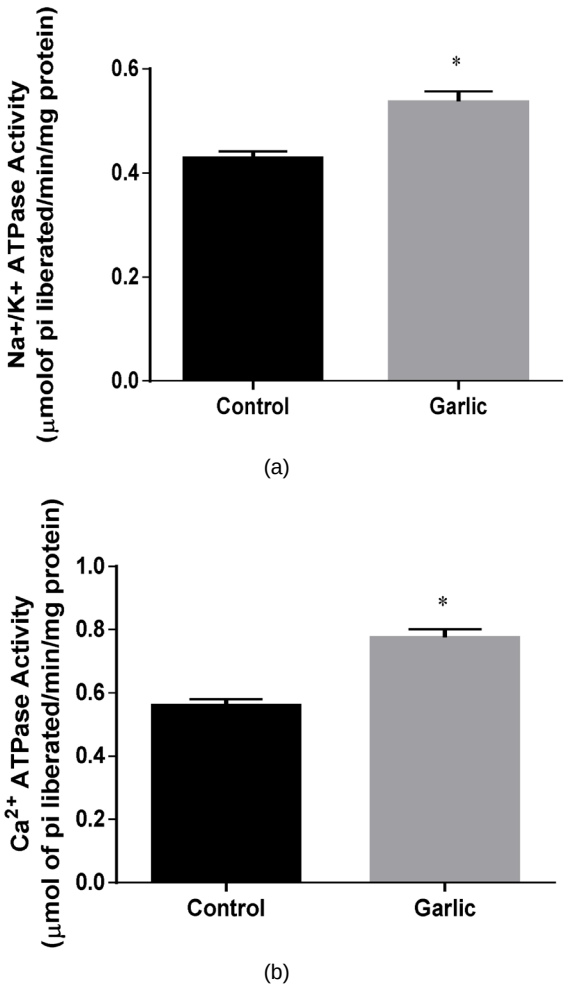

Figure 4. Na^+/K^+ ATPase activity in the hippocampus of garlic treated and control group rats. (a) Ca^{2+} ATPase activity in the hippocampus of garlic treated and control group rats. (b) * Denotes $P < 0.0001$, Garlic vs Control.

3.3. Ca^{2+} ATPase Activity in the Hippocampus Following Administration of Garlic Extract

Ca^{2+} ATPase activity in the hippocampus of garlic treated rats and control group rats is shown in **Figure 4(b)**. The Ca^{2+}ATPase activity (µ mol of pi liberated/min/mg protein) in the hippocampus of garlic treated group (0.78 ± 0.25) was significantly higher ($p < 0.0001$) as compared to control group (0.56 ± 0.18).

4. Discussion

Hippocampal-dependent memory can be influenced by many intervening factors which include pharmacological agents, physiological manipulations and environmental agents. Object location memory has been the test of choice for the spatial memory because its performance is hippocampus dependent [25]. K^+, Na^+ and Ca^{2+} play important roles in developing electrochemical gradient and in neuronal signaling. In fact, there balance is paramount for the proper excitability of the neurons. Altering the activities of Na^+/K^+ ATPase and Ca^{2+} ATPase would

have a significant impact on brain functions including hippocampal-dependent memory. Studies have investigated the effect of Garlic in various forms such as fresh, cooked, aged, aqueous and alcoholic extract on various forms of memory in both normal and disease (Diabetic, Alzheimer) animal models [10] [26] [27].

In the present study, our aim was to investigate the effect of ethanolic extract of garlic on spatial memory following garlic extract administration for 21 days. Animals treated with garlic and the control were able to discriminate between two identical objects in novel locations. However, rats administered garlic explored significantly the object in novel location more than the time rats in control group explored it. Thus, it indicated that garlic can enhance spatial memory. Studies have reported effects of garlic on memory. Sarkaki *et al*. reported increased memory (short and long term) effect of fresh and cooked but not aged garlic in both diabetic male and female rats [10].

Haider *et al*. (2008) reported an improvement in memory function of garlic treated rats using passive avoidance test [28]. Deficient spatial memory in senescence-accelerated mouse model was able to improve by aged garlic extract [27]. Our present result was not in disagreement with the above listed studies. In search of the probable mechanism to which garlic mediate enhancement of any forms of memory, we assessed and observed significant increase in Na^+/K^+ ATPase and Ca^{2+} ATPase activities in the hippocampus being the main brain structure memory depend on. Study has suggested memory-enhancing effect of garlic may be associated with increased brain serotonin (5HT) metabolism in rats [28]. The long-term administration of crude garlic extract may improve learning and memory in mice with the underlying mechanism been attributed to the anti-AchE activity and anti-oxidant property of garlic [29]. In conclusion, the present findings indicate that administration of ethanolic extract of garlic is effective in enhancing spatial working memory and the probable underlying mechanism of action is by increasing the activities of Na^+/K^+ ATPase and CA^{2+} ATPase in the hippocampus. Further research on the involvement of activities of these membrane bound enzymes of hippocampus in different forms of hippocampal-dependent memory and by extension learning will help to better understand the novel therapeutic approach to resolve the memory deficit patients.

References

[1] Cowan, M.M. (1999) Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*, **12**, 564-582.

[2] Mohsenipour, Z and Hassanshahian, M. (2015) The Effects of *Allium sativum* Extract on Biofilm Formation and Activities of Six Pathogenic Bacteria. *Jundishapur Journal of Microbiology*, **8**, e18971. https://doi.org/10.5812/jjm.18971v2

[3] Massadeh, A.M., Al-Safi, S.A., Momani, I.F., Alomary, A.A., Jaradat, Q.M and Al-Kofahi, A.S. (2007) Garlic (*Allium sativum* L.) as a Potential Antidote for Cadmium and Lead Intoxication: Cadmium and Lead Distribution and Analysis in Different Mice Organs. *Biological Trace Element Research*, **120**, 227-234. https://doi.org/10.1007/s12011-007-8017-3

[4] Mabrouk, M.A., Nnawodu, F.I., Tanko, Y., Dawud, F and Mohammed, A. (2009) Effect of Aqueous Garlic (Ag) Extract on Aspirin Induced Gastric Mucosal Lesion

in Albino Wistar Rat. *Current Research Journal of Biological Sciences*, 115-119.

[5] Orozco-Ibarra, M., Muñoz-Sánchez, J., Zavala-Medina, M.E., Pineda, B., Magaña-Maldonado, R., Vázquez-Contreras, E., Maldonado, P.D., Pedraza-Chaverri, J. and Chánez-Cárdenas, M.E. (2016) Aged Garlic Extract and S-Allylcysteine Prevent Apoptotic Cell Death in a Chemical Hypoxia Model. *Biological Research*, **49**, 7. https://doi.org/10.1186/s40659-016-0067-6

[6] Iciek, M., Kwiecien, I. and Włodek, L. (2009) Biological Properties of Garlic and Garlic-Derived Organosulfur Compounds. *Environ Mol Mutagen*, **50**, 247-265. https://doi.org/10.1002/em.20474

[7] Alnaqeeb, M.A., Thomson, M., Bordia, T. and Ali, M. (1996) Histopathological Effects of Garlic on Liver and Lung of Rats. *Toxicology Letters*, **85**, 157-164. https://doi.org/10.1016/0378-4274(96)03658-2

[8] Ji, H.J., Hee, R.J.Y., Hyeon, J.K., Jeong, H.S and Ho, J.H. (2013) Aged Garlic Extracts with Antioxidant Activities May Improve Cognitive Impairment against Aβ-Induced Neuronal Deficit. *BMC Complementary and Alternative Medicine*, **13**, 268.

[9] Gebreyohannes, G. and Gebreyohannes, M. (2013) Medicinal Values of Garlic: A Review. *International Journal of Medicine and Medical Science*, **5**, 401-408.

[10] Sarkaki, A., Valipour, S.C., Farbood, Y., Mohammad, S., Mansouri, T., Naghizadeh, B and Basirian, E. (2013) Effects of Fresh, Aged and Cooked Garlic Extracts on Short- and Long-Term Memory in Diabetic Rats. *Avicenna Journal of Phytomedicine*, **3**, 45-55.

[11] Sumathi, T., Shobana, C., Thangarajeswari, M. and Usha, R. (2015) Protective Effect of L-Theanine against Aluminum Induced Neurotoxicity in Cerebral Cortex, Hippocampus and Cerebellum of Rat Brain Histopathological and Biochemical Approach. *Drug and Chemical Toxicology*, **38**, 22-31. https://doi.org/10.3109/01480545.2014.900068

[12] Singh, R., Mishra, M., Singh, S. and Sharma, D. (2012) Effect of L-Deprenyl Treatment on Electrical Activity, Na^+, K^+ ATPase, and Protein Kinase C Activities in Hippocampal Subfields (CA1 and CA3) of Aged Rat Brain. *Indian Journal of Experimental Biology*, **50**, 101-109.

[13] Rasic-Markovic, A., Stanojlovic, O., Hrnic, D., Krstic, D., Colovic, M., Susic, V., Radosavljevic, T. and Djuric, D. (2009) The Activity of Erythrocyte and Brain Na^+/K^+ and Mg^{2+} ATPase in Rats Subjected to Acute Homocysteine and Homocysteine Thiolactone Administration. *Molecular and Cellular Biochemistry*, **327**, 39-45. https://doi.org/10.1007/s11010-009-0040-6

[14] Rose, A.M. and Valdes Jr., R. (1994) Understanding the Sodium Pump and Its Relevance to Disease. *Clinical Chemistry*, **40**, 1674-1685.

[15] Amiet, C., Gourfinkel-An, I., Bouzamondo, A., Tordjman, S., Baulac, M., Lechat, P., Mottron, L. and Cohen, D. (2008) Epilepsy in Autism Is Associated with Intellectual Disability and Gender, Evidence from a Meta-Analysis. *Biological Psychiatry*, **64**, 577-582. https://doi.org/10.1016/j.biopsych.2008.04.030

[16] Lingrel, J.B., Williams, M.T., Vorhees, C.V. and Moseley, A.E. (2007) Na, K-ATPase and the Role of Alpha Isoforms in Behavior. *Journal of Bioenergetics and Biomembranes*, **39**, 385-389. https://doi.org/10.1007/s10863-007-9107-9

[17] Cooper, E.C. and Jan, L.Y. (1999) Ion Channel Genes and Human Neurological Disease, Recent Progress, Prospects, and Challenges. *Proceedings of the National Academy of Sciences of the United States of America*, **96**, 4759-4766. https://doi.org/10.1073/pnas.96.9.4759

[18] Homayounfar, H., BaluchnejadMojarad, T., Roghani, M., Hosseini, M. and Kamalinejad, M. (2003) Effect of Aqueous Garlic (*Allium sativum* L.) Extract on Acetylcholine and Isosorbide-Induced Relaxation of Isolated Aorta in Rat. *Iranian Biomedical Journal*, **7**, 23-27.

[19] Hermawati, E., Sari, D.C.R. and Partadiredja, G. (2015) The Effects of Black Garlic Ethanol Extract on the Spatial Memory and Estimated Total Number of Pyramidal Cells of the Hippocampus of Monosodium Glutamate-Exposed Adolescent Male wistar Rats. *Anatomical Science International*, **90**, 275-286. https://doi.org/10.1007/s12565-014-0262-x

[20] Gerstein, H., Hullinger, R., Lindstrom, M.J. and Burger, C. (2013) A Behavioral Paradigm to Evaluate Hippocampal Performance in Aged Rodents for Pharmacological and Genetic Target Validation. *PLoS ONE*, **8**, e62360. https://doi.org/10.1371/journal.pone.0062360

[21] Bharathi, H., Vikas, M., Maheedhar, K., Bing, S., Xiolan, R. and Ashok, K.S. (2014) Object Location and Object Recognition Memory Impairments, Motivation Deficits and Depression in a Model of Gulf War Illness. *Frontiers in Behavioral Neuroscience*, **4**, 78.

[22] Tirri, R., Lagrspetz, K.Y.H. and Kohomen, J. (1973) Temperature Dependence of the ATPase Activity in Brain Homogenates during the Postnatal Development of Rat. *Comparative Biochemistry and Physiology Part B*: *Comparative Biochemistry*, **44**, 473. https://doi.org/10.1016/0305-0491(73)90021-7

[23] Fiske, C.H. and Subba Row, Y. (1925) The Colorimetric Determination of Phosphates. *Journal of Biological Chemistry*, **66**, 375-400.

[24] Desaiah, D. and Ho, I.K. (1979) Effect of Acute and Continuous Morphine Administration on Catecholamine-Sensitive Adenosine Triphosphatase in Mouse Brain. *Journal of Pharmacology and Experimental Therapeutics*, **208**, 80-85.

[25] Umka, J., Mustafa, S., ElBeltagy, M., Thorpe, A., Latif, L., Bennett, G. and Wigmore, P.M. (2010) Valproic Acid Reduces Spatial Working Memory and Cell Proliferation in the Hippocampus. *Neuroscience*, **166**, 15-22. https://doi.org/10.1016/j.neuroscience.2009.11.073

[26] Tasnim, S., Haque, P.S., Bari, S., Hossain, M., Mohd, S., Islam, A. and Sayeed, B. (2015) *Allium sativum* L. Improves Visual Memory and Attention in Healthy Human Volunteers. *Evidence-Based Complementary and Alternative Medicine*, 2015, Article ID: 103416. https://doi.org/10.1155/2015/103416

[27] Moriguchi, T., Saito, H. and Nishiyama, N. (1996) Aged Garlic Extract Prolongs Longevity and Improves Spatial Memory Deficit in Senescence-Accelerated Mouse. *Biological and Pharmaceutical Bulletin*, **19**, 305-307. https://doi.org/10.1248/bpb.19.305

[28] Haider, S., Naz, N., Khaliq, S., Perveen, T. and Haleem, D.J. (2008) Repeated Administration of Fresh Garlic Increases Memory Retention in Rats. *Journal of Medicinal Food*, **11**, 675-679. https://doi.org/10.1089/jmf.2006.0229

[29] Mukherjee, D. and Banerjee, S. (2013) Learning and Memory Promoting Effects of Crude Garlic Extract. *Indian Journal of Experimental Biology*, **51**, 1094-1100.