COGNITIVE-ENHANCING AND ANTI-OXIDANT ACTIVITIES OF GARLIC AND GINGER MIXTURE IN WISTAR RATS

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Abstract - Cognitive-enhancing and anti-oxidant potentials of garlic and ginger were simultaneously investigated in the study. This came as a backdrop of the myriads of speculations that these fruits could possess the said properties. Fresh fruits of garlic and ginger were obtained and juiced using a standard fruit extractor and were mixed and administered to the animals as scheduled. Thirty albino wistar rats were randomly divided into five groups of various treatment: group 1 (control group), group 2 (garlic/ginger low dose), group 3 (garlic/ginger high dose), group 4 (Donepezil drug), group 5 (garlic/ginger/drug low dose), and group 6 (garlic/ginger/drug high dose) for 6 weeks. Various cognitive tasks were employed to all the groups during the treatment periods using Morris Water Maze and Elevated Plus Maze. A multi-trial approach was used to assess the cognitive behavior of the rats. Brain tissues of the rats were harvested and homogenized at negative degree and prepared for oxidative stress marker assays. The results showed a well improved cognitive modification in terms of memory consolidation and spatial learning especially in the groups served with the mixture at high dose. The memory re-enforcement increased exponentially as the trial tasks increased. The patterns of expression of the oxidative stress enzymes were up-regulated at high dose mixture group and high dose mixture plus drug group. The mixture of garlic and ginger blend prevented cognitive deficits in rats. In summary, the obtained results suggested that the blend of garlic and ginger could exert potent anti-amnesic and cognitive enhancing effects through modulation of the antioxidant activity in the hippocampus of the rat model.

Keywords: Garlic, Ginger, Cognitive, Anti-oxidant, Morris Water Maze, Elevated Plus Maze

INTRODUCTION

Garlic, Allium sativum L. is a member of the Alliaceae family, has been widely recognized as a Valuable spice and a popular remedy for various ailments and physiological disorders. (Rivlin , 2001, Banerjee et al., 2002). The name garlic may have originated from the Celtic word 'all' meaning pungent. Cultivated practically throughout the world, garlic appears to have originated in central Asia. Clinical indications of garlic include hemorrhoids, rheumatism, dermatitis, abdominal pain, and cough, loss of appetite and loss of weight (Banerjee & Maulik, 2002, Lau et al, 1990, Kyo et al, 2001, Wu et al, 2002, Kavutcu et al, 2004).

Ginger (Zingiber officinale, Roscoe Zingiberaceae) is one of the most widely consumed spices worldwide. From its origin in Southeast Asia and its spread to Europe, it has a long history of use as herbal medicine to treat a variety of ailments, including vomiting, pain, indigestion, and cold-induced syndromes [Borrelli et al, 2004, Prasanna et al, 2007]. Oxidative stress, resulting from an imbalance between oxidant molecules and antioxidant defence mechanisms, can damage proteins, lipids, and nucleic acids (Prasanna et al, 2008). Oxidative stress is involved in neurodegeneration (Kavoli & Tooliat 2002)) and in the pathophysiology of neuropsychiatric diseases, such as schizophrenia (Amin & Hamza, 2006: Mir, 1996: Zargari. 1999; Aimbire et al, 2007).

Material & Methods

Thirty healthy albino rats of 12 weeks old weighing between 80-200g were used in the study. The rats were obtained from the Experimental Animal Unit of Department of Human Physiology, University of Port Harcourt, Rivers State and were housed in conventional wire mesh cages under standard laboratory conditions. The animals were allowed free access to water and standard feed throughout the period of the experiment.

Acclimatization of animals

After the collection of the animals, they were weighted and identified and kept in a wire gauge cage floored with saw dust to maintain dryness, under favourable condition for two weeks.

MATERIALS

A measured quantities (5kg each) of Fresh garlic and ginger were purchased, washed, peeled and blended very finely and the pastes were squished to extract the juices. 2 litters of each were derived and mixed and preserved in a refrigerator to prevent fermentation and for use whenever needed.

Donepezil drug was manufactured by the company: Eisai Medical Research with Approval Date: 10/18/2004 was purchased

Experimental Design

groups	Treatment	Dosage/Administration
Group 1	Distilled water	1ml/day/4 weeks
Group 2	Garlic/ginger (low dose)	(100+100)mg/kg/4 weeks
Group 3	Garlic/ginger (high dose)	(300+300)mg/kg/4 weeks
Group 4	donepezil drug	50mg/kg/b.w./4 weeks
Group 5	Garlic/ginger+drug (low dose)	(100+100+50)mg/kg/4 weeks
Group 6	Garlic/ginger+drug (high dose)	(300+300+50)mg/kg/4 weeks

METHODS

The Morris water maze

The Morris water maze consisted of a circular pool with a white underside and black side surface, a white platform, a camera and a computer. The pool was made of circular galvanized steel pool (1.2 m diameter, 0.5 m height) filled with water (20-22°C). A white platform (8 cm diameter) was placed in the middle of one quadrant and submerged 1 cm below the surface of the water. A camera situated above the pool was used to capture the rats' swim trace. At the beginning of 0 day, rats was allowed to freely search platform with four trials. From the first day to the fourth day, the rat was given four trials, a trial lasted 60 s or until the rat reached the platform and remained a few seconds. If a rat didn't reach the platform in 60 s, it meant that its escape latency was 60 s, then the rat was allowed to rest for 30 s between trials. On the fifth day, the platform was removed and rats were tasked with a probe trial for 2 min.

Elevated Plus Maze (EPM)

Practical Steps in the Use EPM

Behavioral responses in the elevated plus maze were assessed and quantified. Briefly, rats were placed in the intersection of the four arms of the elevated plus maze and their behavior was typically recorded for 5 min. This was based upon the early studies by Montgomery (1958) that revealed that rats demonstrated the most robust avoidance responses in the first 5 min after placement in the elevated open alleys. The behaviors that were typically recorded when rats were in the elevated plus maze were the time spent and entries made on the open and closed arms. Behavior in this task (i.e., activity in the open arms) reflects a conflict between the rat's preference for protected areas (e.g., closed arms) and their innate motivation to explore novel environments.

BLOOD SAMPLE COLLECTION AND ANALYSIS

The Animals were sacrificed after the fourth week of administration.

Blood samples were collected via cardiac puncture for oxidative markers evaluation. And this Analysis took place at the Research Laboratory of the department of Biochemistry, University of Port Harcourt.

Measurement of Oxidative Stress Markers

Sodium Dismutase (SOD) and Malonhydehyde (MDA) in Brain Tissues

The activity of SOD in brain tissues of rat was detected by a total SOD assay kit according to the manufacturer's protocol. Briefly, brain extracts, nitroblue tetrazolium, and enzyme-working solutions were prepared and added into a 96-well plate. The mixtures were incubated at 37°C for 40 min, and then the absorbance was assayed at 550 nm using an MD-M5 microplate reader (Molecular Devices Corporation, Manlo Park, CA, USA). For MDA measurement, the brain extracts were added to the detection solution of MDA in eppendorf tubes and then boiled for 40 min. After centrifugation at 4000 rpm/min for 10 min, the supernatant was collected in a 96-well plate and then determined using an MD-M5 microplate reader at 532 nm [Misra & Fridovich, 1972].

Glutathiones and Catalase Estimations

Total glutathione (GSH) levels were measured colorimetrically using the Bioxytech GSH-420 assay kit (OXIS International Inc., Portland, OR). The method is based on the formation of a chromophoric thione with absorbance measured at 420 nm using a microplate reader (Synergy 2, Biotek). Catalase (CAT) activity was measured using a commercially available colorimetric assay kit (Bioxytech, Catalase 520, Oxis). (Beutler et al, 1963).

The method is based on a two-stage reaction where the rate of dismutation of hydrogen peroxide (H2O2) to water and molecular oxygen is proportional to the CAT concentration. First, a known amount of H2O2 is added to the sample with CAT and incubated for 1 min before stopping the reaction by adding sodium azide. The amount of H2O2 remaining in the mixture is then determined by the oxidative coupling reaction of 4-aminophenazone (AAP) and 3,5-dichloro-2-hydroxybenzenesulfonic acid (DHBS), catalyzed by horseradish peroxidase.

The resulting quinoneimine dye is measured by the absorbance at 520 nm (Synergy 2, Biotek). Glutathione peroxidase (GPx) activity was assessed using the Oxis Bioxytech® GPx-340 Assay (Oxis). The method is based on the principle that oxidized glutathione (GSSG) produced upon reduction of an organic peroxide by GPx is immediately recycled to its reduced form (GSH) with concomitant oxidation of NADPH to NADP+. The oxidation of NADPH is measured spectrophotometrically (Synergy 2, Biotek) as a decrease in absorbance at 340 nm. One GPx-340 unit is defined as 1 µmol of NADH oxidized per minute under the assay conditions.

Statistical Analysis

Data were analyzed using SPSS version 20 and results were presented as Mean ± SEM. Post Hoc test was done using LSD. Level of significance was set at $P \leq 0.05$.

RESULTS

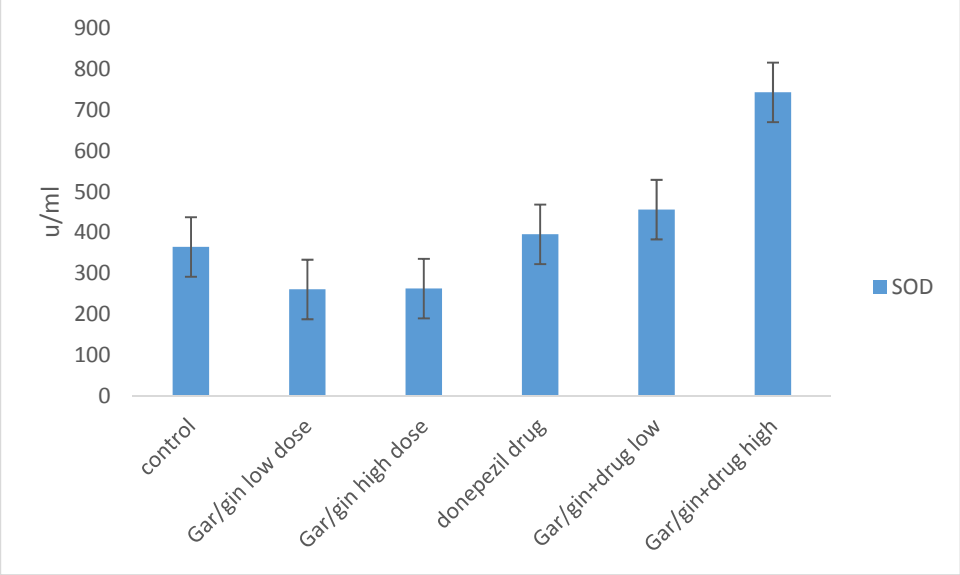

Fig. 1. Patterns of SOD on exposure to various doses of mixture of garlic and ginger in the brain homogenates of test groups

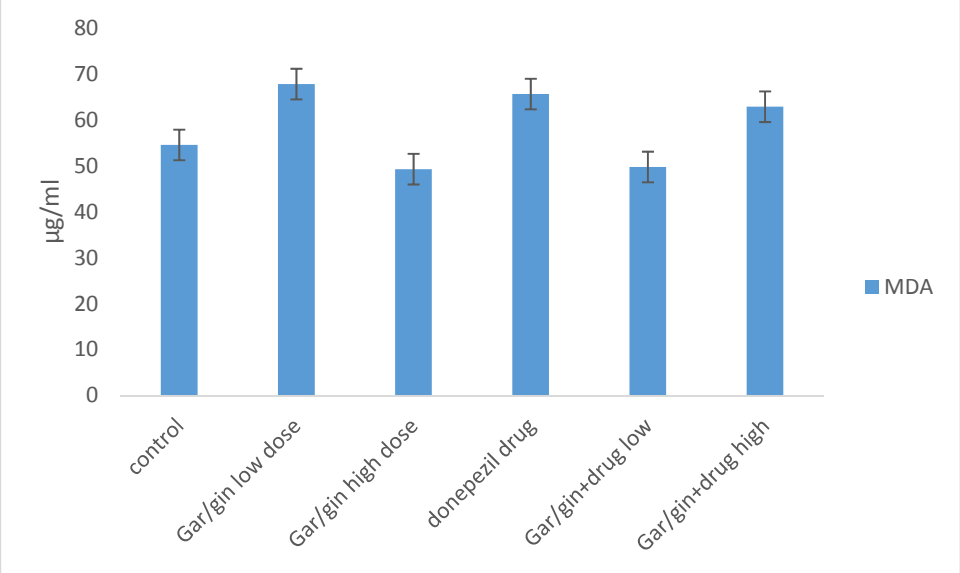

Fig.2. Patterns of MDA on exposure to various doses of mixture of garlic and ginger in the brain homogenates of test groups

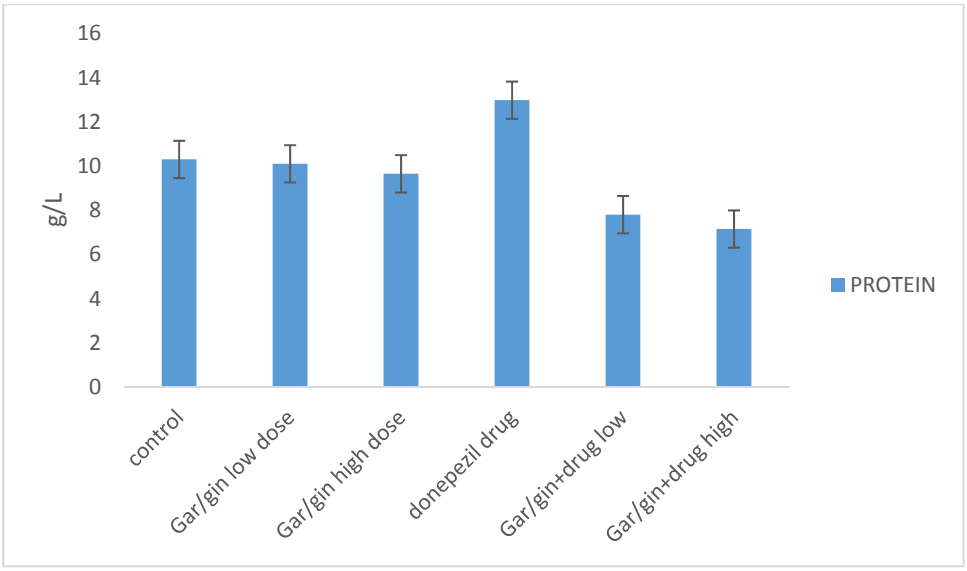

Fig.3. Patterns of protein on exposure to various doses of mixture of garlic and ginger in the brain homogenates of test groups

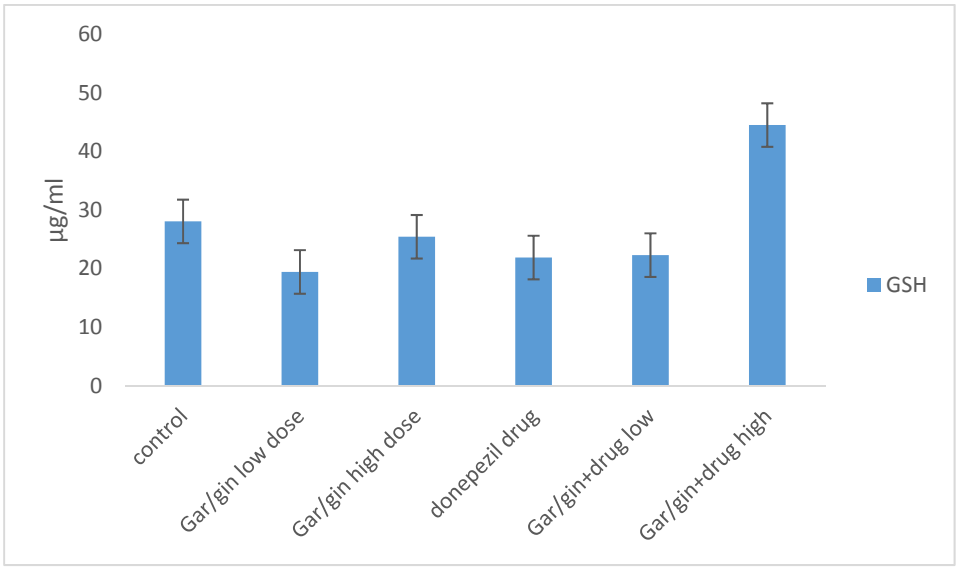

Fig.4. Patterns of GSH oxidase on exposure to various doses of mixture of garlic and ginger in the brain homogenates of test groups

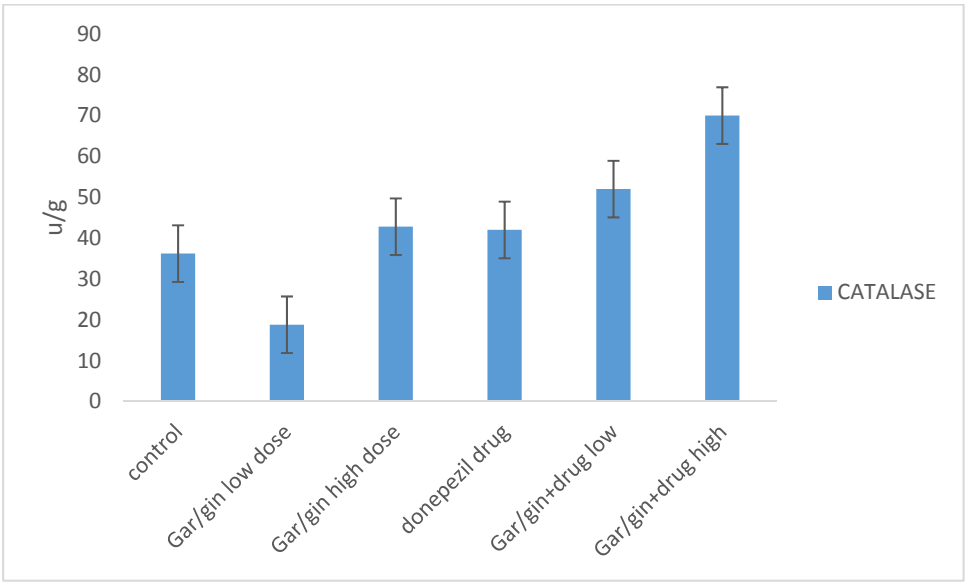

Fig.5. Patterns of catalase on exposure to various doses of mixture of garlic and ginger in the brain homogenates of test groups

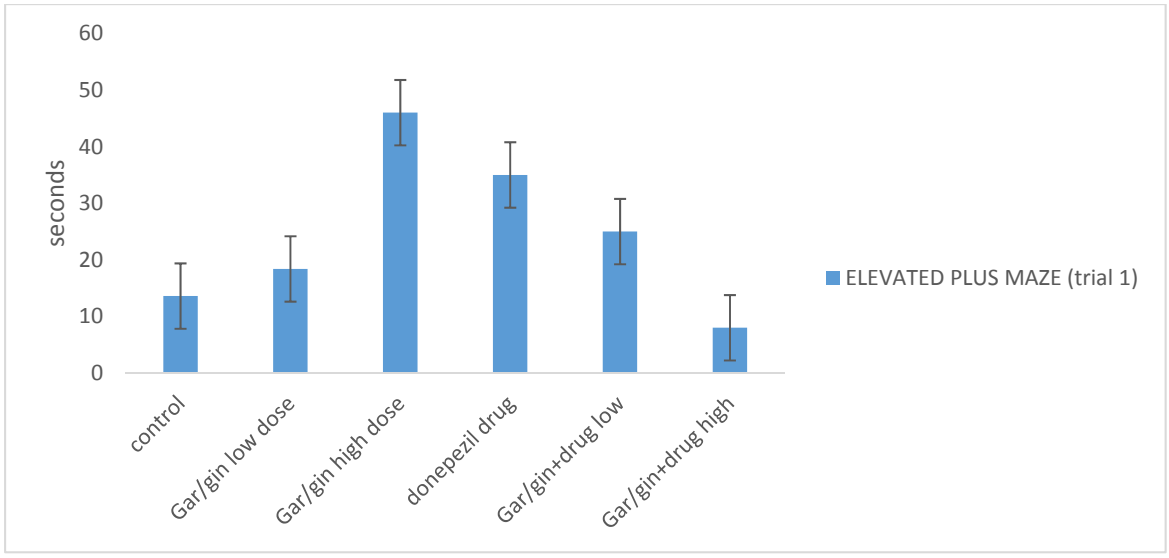

Fig.6. Pattern of time spent on the open arm of EPM at first trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

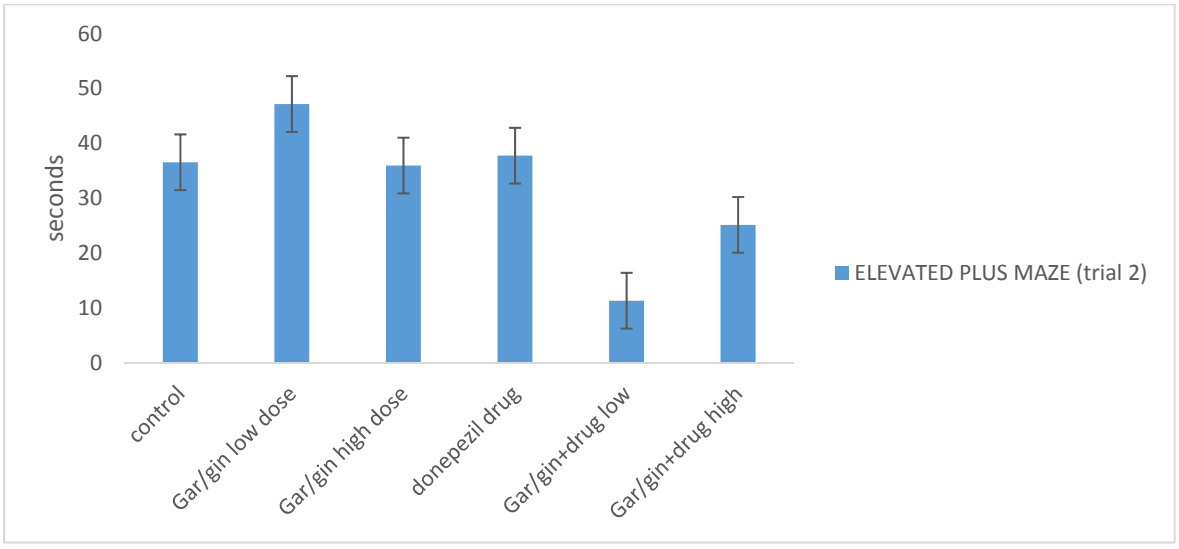

Fig.4.7. Pattern of time spent on the open arm of EPM at 2nd trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

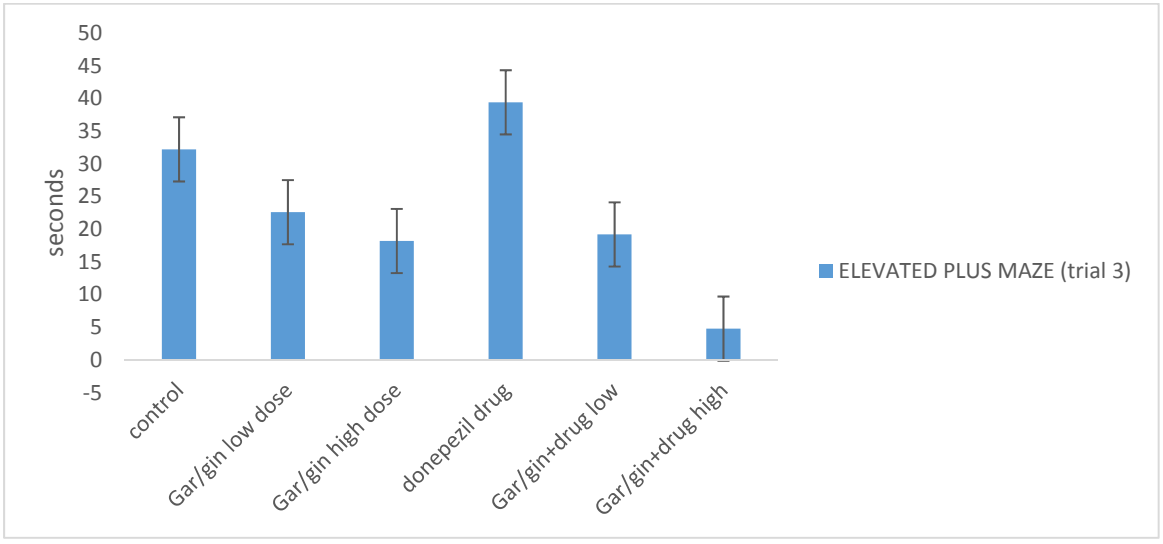

Fig.4.8. Pattern of time spent on the open arm of EPM at 3rd trial on exposure to various doses of mixture of garlic and ginger and Donepezil drugof test groups

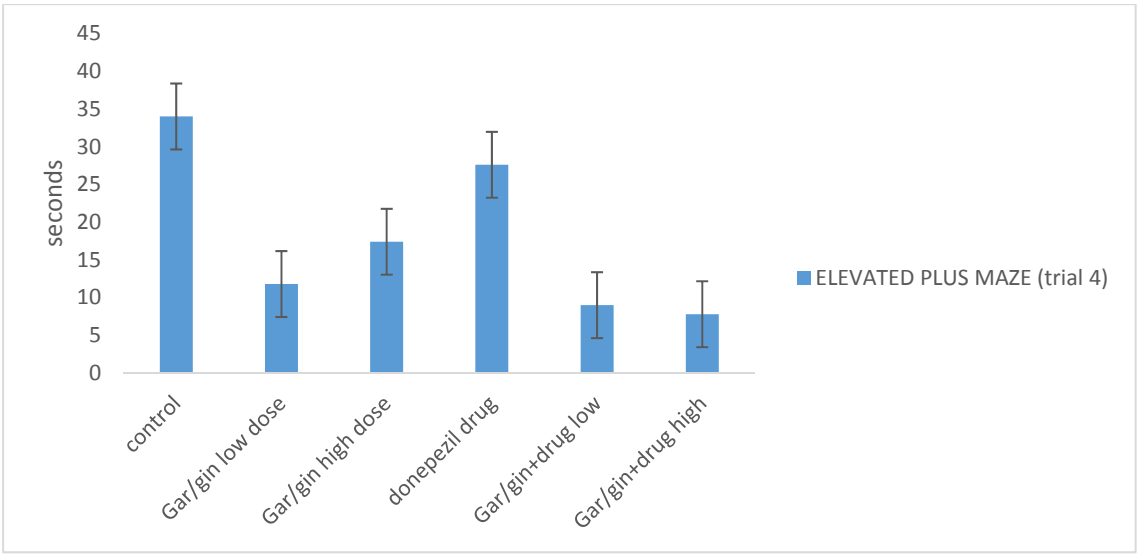

Fig.9. Pattern of time spent on the open arm of EPM at 4th trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

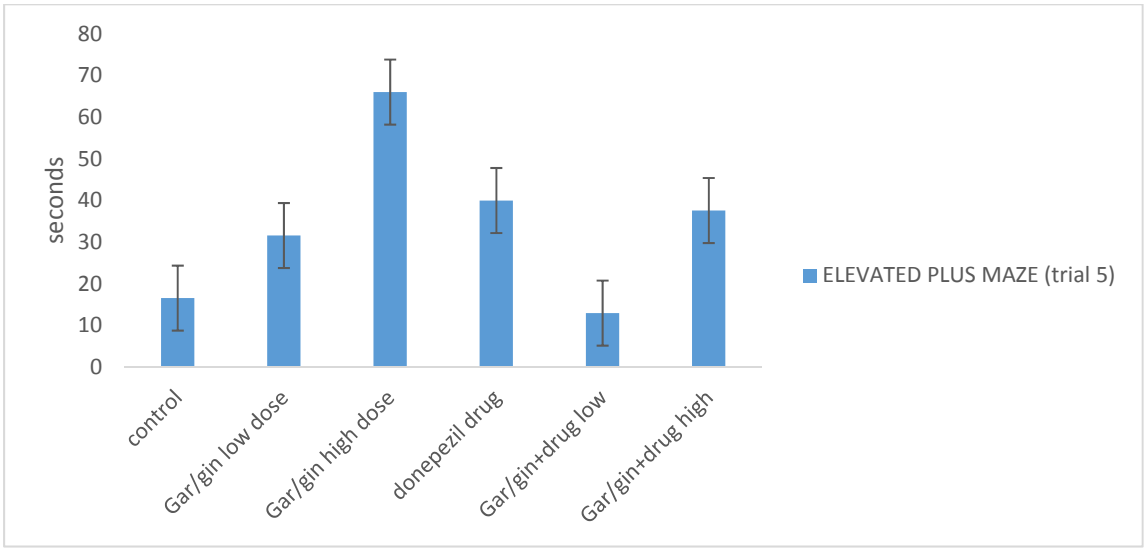

Fig.10. Pattern of time spent on the open arm of EPM at 5th trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

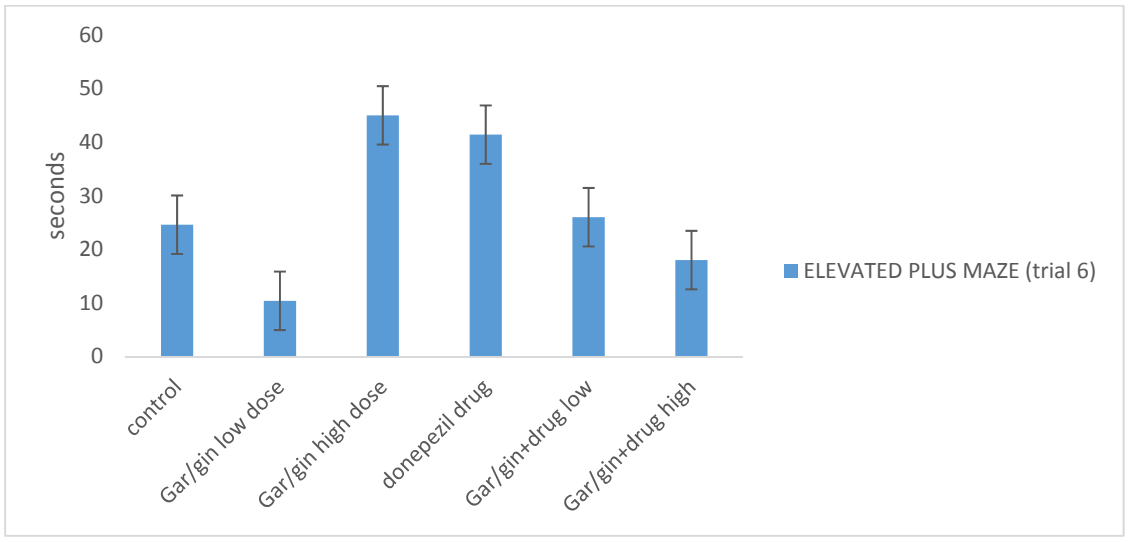

Fig.11. Pattern of time spent on the open arm of EPM at 6th trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

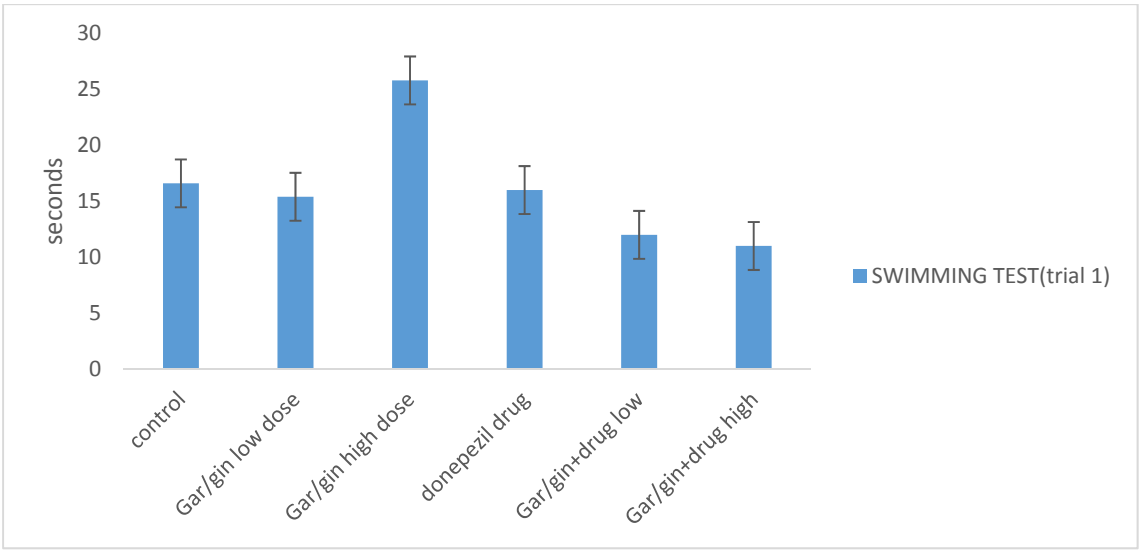

Fig.12. Pattern of time spent during Morris Water Maze (MWM) at 1st trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

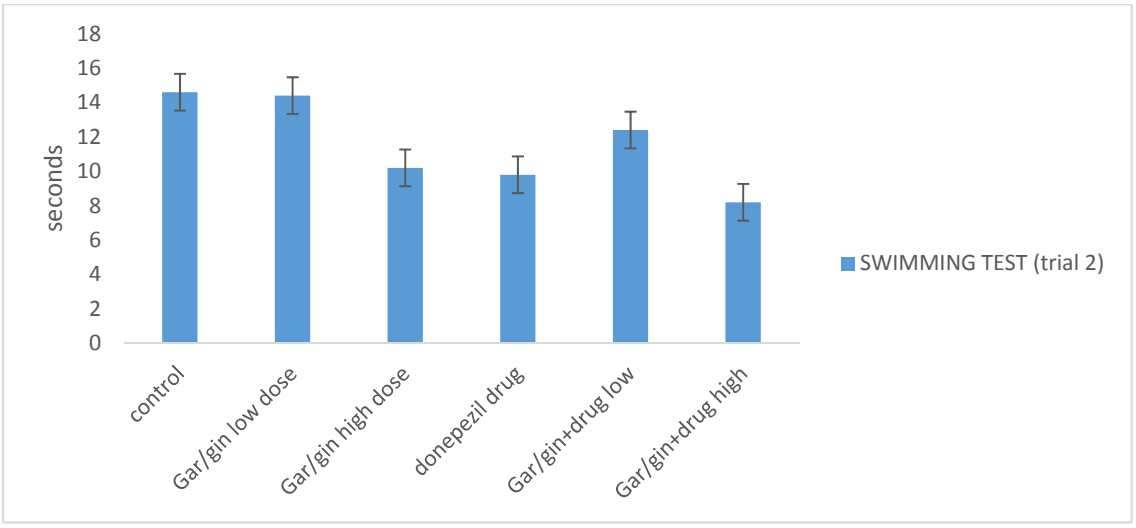

Fig.13. Pattern of time spent during Morris Water Maze (MWM) at 2nd trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

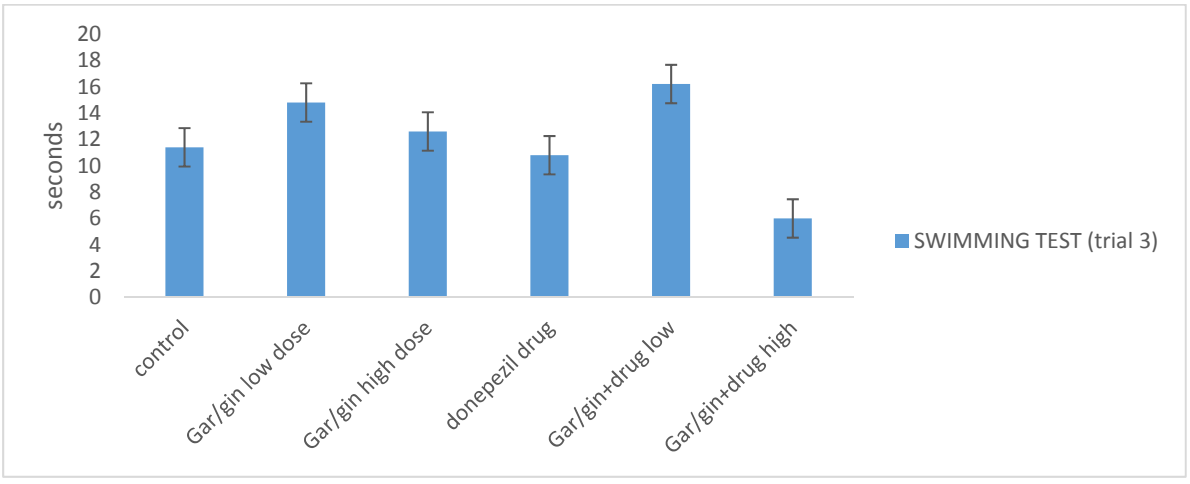

Fig.14. Pattern of time spent during Morris Water Maze (MWM) at 3rd trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

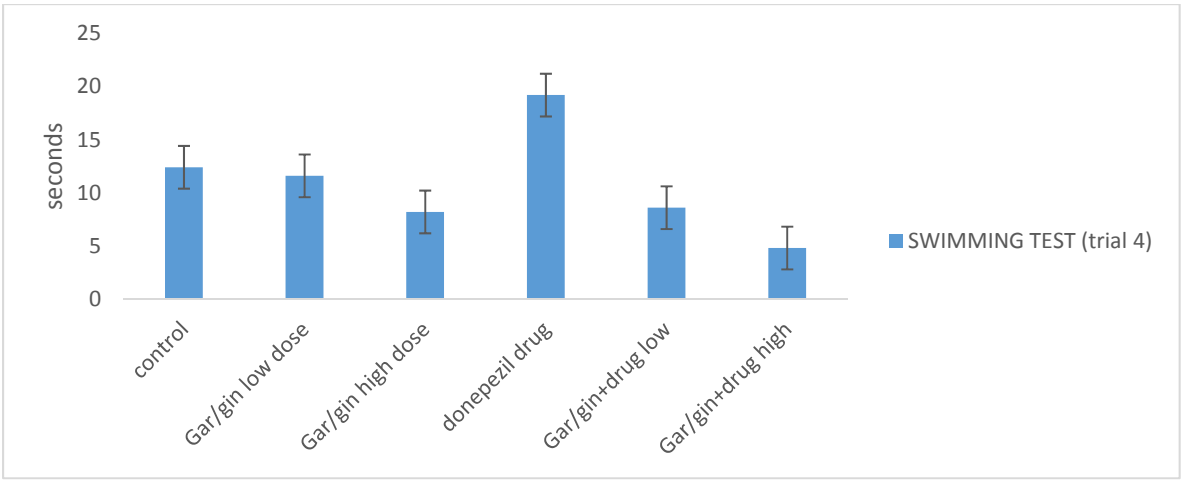

Fig.15. Pattern of time spent during Morris Water Maze (MWM) at 4th trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

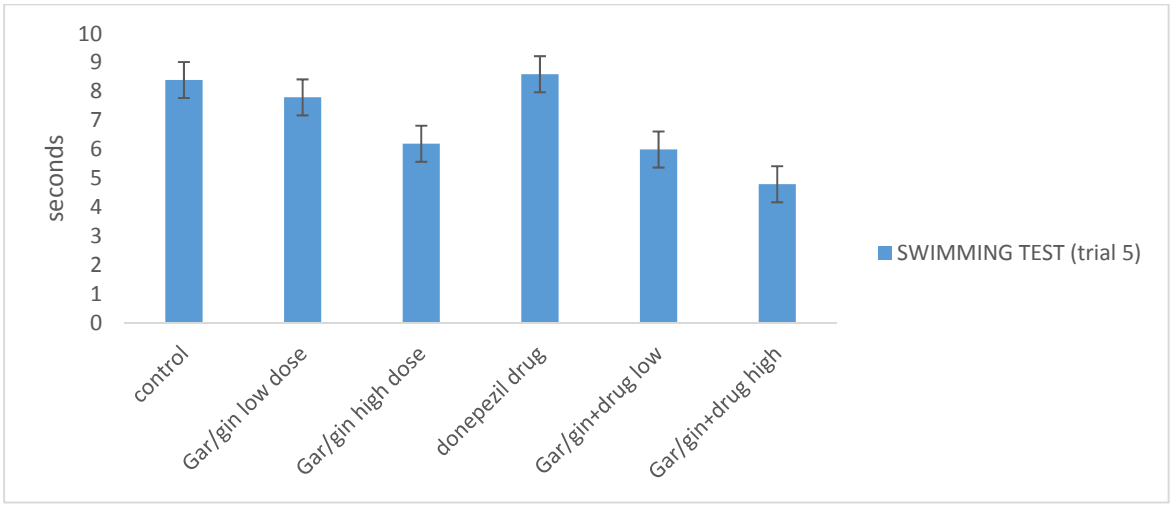

Fig.16. Pattern of time spent during Morris Water Maze (MWM) at 5th trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

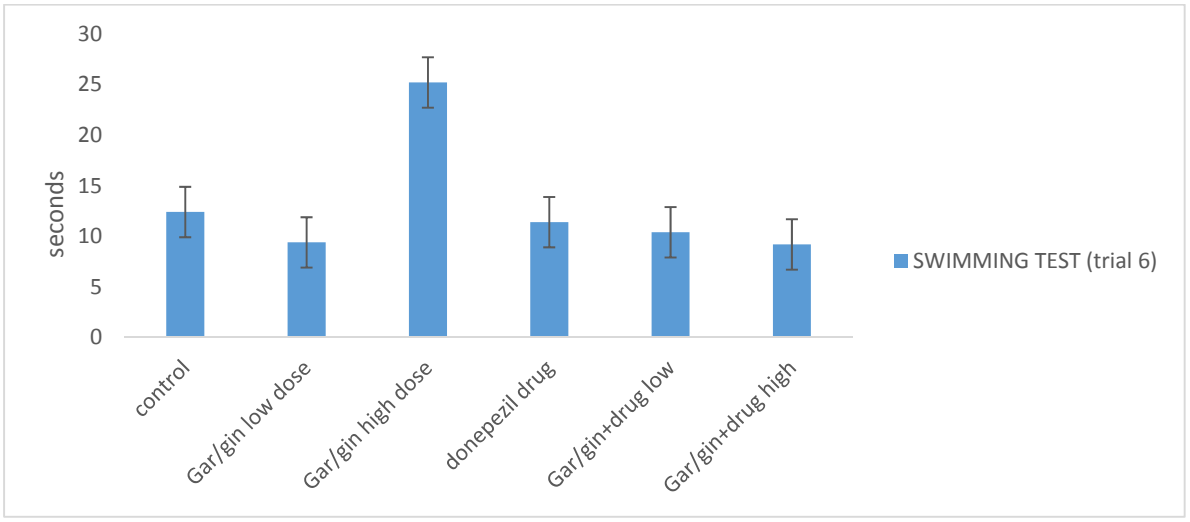

Fig.17. Pattern of time spent during Morris Water Maze (MWM) at 6th trial on exposure to various doses of mixture of garlic and ginger and Donepezil drug of test groups

DISCUSSIONS

The results of present work revealed that chronic treatment with a mixture of garlic and ginger blend could significantly prevent cognitive deficits in rats. Indeed, the in-vitro assays showed a clear antioxidant effect.

It was evidently shown and demonstrated in figures 12-17 that animals in the test groups swim more slowly throughout the experiments in all trials of the Morris Water Maze (MWM), when compared to controls especially at higher dose, with no difference from each other, no differences were observed as well between groups in relation to ambulatory activity in the open field and in the latency to enter in the dark compartment of the inhibitory avoidance apparatus during the training the opposite was the case when the drug Donepezil was introduced.

Probing further, the beneficial effect of GARLGING on the MWM and inhibitory avoidance paradigms are unlikely to be a result of differences in motor abilities. Indeed, because no significant difference was found in in the percentage of entries and time spent in the open arms of the elevated plus maze, alterations in the motivational and/or emotional state of the animal, which could have affected performance in the water maze learning, cannot be overlooked.

Pellow (Pellow et al, 1985) characterized elevated plus-maze behavior on the basis of levels of open arm exploration. Thus, test group 6 (high dose GARLGING PLUS DRUG group) and control group exhibiting low levels of open arm activity were classified as ``high reactive'', while those showing high levels of activity in the open arms (e.g., groups 2-5) were labeled ``low reactive.'' However, while time spent in the open arms was indeed relatively high, levels of exploratory behaviors and general locomotors activity in the rats were also substantially higher than those observed in laboratory/Swiss mice or those previously observed previously (Handley & Mithani 1984, Barnett, 1975, Gonzalez & File, 1997).

As such, rather than displaying low reactivity to the plus-maze, the activities of the test groups including the Donepezil drug group, (group 4), would be more accurately described as one of high reactivity. One of the important mechanism in the development and progression amnesia is oxidative stress and any substance that will inhibit or ameliorate oxidative stress would be highly beneficial to the organisms. In the present study, GARLING mixture at both high and low doses increased SOD, GPX, and GSH and decreased the MDA and protein carbonyl levels in the rat hippocampal homogenates opposite to what was obtained in the control group that was not treated.

The evidence from the study suggested that the GARLGING mixture could possess potent cognitive effects that may be mediated by improving the brain oxidative status [63–65]. Consequently, the mixture treatment restored the antioxidants status as evidenced by an increase of SOD, GPX, and GSH while the levels of MDA (lipid peroxidation) and protein carbonyl significantly decrease which supports its antioxidant property.

In summary, the obtained results suggest that the blend of garlic and ginger (GARLGING) (100 and 300 mg/kg) exerts potent anti-amnesic and cognitive enhancing effects through modulation of the antioxidant activity in the hippocampus of the rat model.

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