



Nicotine-Impaired Cognitive and Psychosocial Behavior and Spatial Memory Deficit: The Combined Roles of Garlic Cloves and Honey in Wistar Rats

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Abstract

BACKGROUND AND AIM: Nicotine is an additive abused among youths, which gradually becomes behavioral. The aim of this study was to investigate the ameliorative roles of aqueous garlic cloves and honey extract on nicotine-induced cognitive, spatial memory, and psychosocial behavior impairments.

METHODOLOGY: Forty rats were divided into 5 groups of eight. Group A served as control, receiving food and water. All the groups except A received 50mg/kg of nicotine for 14 days. Group B served as the nicotine-untreated group; Group C received 200mg/kg of garlic and 1000mg/kg of honey; Group D received 400mg/kg body weight of garlic and 1500mg/kg of honey; and Group E received 600mg/kg of garlic and 2000mg/kg of honey. Garlic and honey treatment lasts 14 days. Spatial memory, anxiety, social behavior, and cognition were measured using the Morris Water Maze (MWM), elevated plus maze, sociability chamber, and novel object recognition test. Brain homogenate was used to determine biomarkers.

RESULTS: The acquisition latency was significantly decreased in the high-dose compared to the nicotine-untreated on day 4 of the MWM test at $p < 0.05$. The discrimination index reduced significantly in the high-dose group compared to the nicotine-untreated at $p < 0.05$. The time spent with both novel rats significantly increased in the nicotine-untreated group and was lowered by the garlic clove extract. Glutathion-S-Transferase (GST), glutathione oxaloacetate (GOT), and Thiobarbituric Acid Reacting Substances (TBARS) were significantly reduced in the garlic extract groups at $P < 0.05$.

CONCLUSION: Garlic cloves and honey enhanced brain cell regeneration, improved spatial memory, cognition, and social behaviors, and decreased anxiety.

Keywords:

Addiction; Behavioral; Cognitive; Psychosocial; Neurotoxicity; Nicotine

INTRODUCTION

Nicotine is a substance that is very addictive, and dependence on it is both physical and psychological, with habitual users always craving the drug and its effects (Brian *et al.*, 2006; Guerue and Kachrid, 2016). Nicotine is an alkaloid in coffee that readily enters the skin and is metabolized by the lungs, liver, and kidneys (Gould, 2006). Physiologically, it binds to nicotinic acetylcholine receptors (nAChRs), expressed by neuronal and non-neuronal cells throughout the body (Davis and Gould, 2008).

Nicotine addiction is the direct cause of smoking-induced disorders, even if nicotine has negligible, if any, involvement in their

development. The central nervous cholinergic system is associated with cognitive functions, including memory, selective attention, and emotional processing. Non-neuronal nAChRs are found in the respiratory tract, endothelial and immune cells, including proliferation, differentiation, migration, and apoptosis, with adverse effects manifesting as hyperglycemia and hypercholesterolemia (Kenny and Markou, 2001; Levin, 2002).

Garlic (*Allium sativum*) is highly regarded worldwide for its medicinal and culinary value, as early researchers like Hippocrates and Aristotle encouraged its therapeutic uses (Murray 2009). Today, it is the second most

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most utilized supplement with its high sulfur and traces of mineral content (Bongiorno *et al.*, 2008). It possesses various properties, including anti-viral, antibacterial, anti-fungal, and antioxidant, which helps it prevent Alzheimer's, cancer, cardiovascular diseases, atherosclerosis, stroke, thrombosis, hypertension, hyperlipidemias, children's conditions, dermatologic applications, stress, and infections (Bongiorno *et al.*, 2008). Honey is highly nutritious, with traces of minerals, vitamins, and antioxidants that destroy free radicals and delay aging; it is also safe and wholesome food for both old children and adults (Sampath *et al.*, 2010). Honey is also an energizer, helping workers and athletes overcome fatigue and regain energy (Yilmaz *et al.*, 2009). Honey is antibacterial, anti-viral, and anti-fungal, ideal for healing wounds (Sampath *et al.*, 2010). Honey is primarily made of water and carbohydrates, but traces of niacin, calcium, copper, riboflavin, Iron, magnesium, potassium, and zinc in honey are available. Honey also contains a blend of flavonoids and phenolic acids, antioxidants that eliminate destructive free radicals in the body (Elbanna *et al.*, 2014). Although some works have been done on the effects of nicotine and cognition, works are limited to the combined effects of garlic cloves extract and honey on nicotine-impaired cognitive and psychosocial behaviors. The study investigated the role of natural products (garlic and honey) in restoring impaired cognitive and psychosocial behaviors in male Wistar rats.

MATERIALS AND METHODS

Ethical Clearance

This work obeyed the Organization of Economic Co-operation Development (OECD) guidelines for testing chemicals usage in experimental animals (OECD, 2010). The ethical clearance was obtained from the Animal Use and Research Ethical Committee of the Faculty of Basic Medical Sciences, Alex-Ekwueme Federal University Ndufu Alike Ikwo, Ebonyi State, with the reference number AE-FUNAI-2023/003566.

Purchase of Chemicals

The nicotine tablet is made of nicotinic acid, code PB/DRUGS/1608-B, manufactured in India and marketed by Andez Drug LLP, 4 Lewis Kointe Street Brussels, and Belgium. The honey weighing 1.0 kg and containing Calories (64), Carbohydrates (17g), Protein (0.1g), Potassium (10.9mg), Iron (0.1mg), and Calcium (1.3mg) was obtained from Alex-Ekwueme Federal University Ndufu Alike Ikwo bee farm.

Collection and Extraction

The garlic cloves were obtained from Alex-Ekwueme Federal University Ndufu Alike, Ikwo crop science department. It was peeled and dried for three weeks at room temperature and ground into a fine powder in the University's Physiology department. The powder was soaked in distilled water for 72 hours in a ratio of 100g of powder to 500 ml of water to extract the active ingredients. The mixture was stirred every

3-6 hours during the soaking period, after which it was sieved using a cheesecloth and further filtered using Whatman filter paper. The filtrate was dried using a water bath 40°C to a 20g sticky paste substance preserved in the refrigerator at 4°C until required.

Animal Management and Experimental Design

The forty Wistar rats used for this study were obtained from the animal house of Alex Ekwueme Federal University Ndufu Alike, Ikwo, and housed in netted cages under standardized conditions and allowed feeds and water *ad-libitum*. The rats were assigned into five groups of eight, each preceded by seven days of acclimatization. Group A served as control and received feed and water *ad libitum*. Group B was only given nicotine (50 mg/kg body weight) for 14 days. Groups C, D, and E received 50 mg/kg body weight of nicotine for 14 days. Twenty-four hours after the last dose of nicotine, groups C, D, and E were treated with a low dose of garlic (300 mg/kg) and honey (1000 mg/kg); a medium dose of garlic (400 mg/kg) and honey (1500 mg/kg); and a high dose of garlic (600 mg/kg) and honey (2000mg/kg). All administration was done orally once daily and lasted twenty-eight days.

Assessment of Social Behavior

The sociability chamber was used to test for the social behavior of rodents, and testing was conducted in three sessions using a three-chambered triangular sociability box with doors between them and two cages in the two lateral chambers. The principle was based on rodents' freedom to spend time in any of the compartments during the sessions (Kaidanovich-Beilinet *et al.*, 2011). In the Habituation session, a rat was placed in the empty chamber to move around for five minutes to familiarize itself with its environment (Uchewa *et al.*, 2022). In the second Social Affiliation session, the rat encountered an empty cage in one lateral chamber and a first-intruder rat in the cage in the other lateral chamber for another ten minutes. In the last Social novelty session, the rat encountered a familiar and strange rat in the once-empty cage. The time spent in close contact with the cages, time spent in each chamber, and the number of entries into each chamber were presented. This experiment was conducted on the morning of the 27th day of the experiment.

Assessment of Anxiety

The elevated plus maze (EPM) is an ethologically based exploratory model of anxiety. It measures how animals, typically rats and mice, respond to a novel approach/avoidance situation by measuring their relative exploration of two distinct environments (Lister *et al.*, 1987). EPM assesses anxiety, emotion, and reactivity (Lister *et al.*, 2011; Komada *et al.*, 2008). The *Plus Maze* comprises two enclosed arms (25 x 5 x 16 cm), two open arms (25 x 5 x 0.5 cm), and a centre platform (5 x 5 x 0.5cm) equipped with rows of infrared photocells interfaced with a computer (Lister *et al.*, 1987; Komada *et al.*, 2008). This model is based on the

animals' aversion to open and elevated environments. The open arms have a minimal (0.5 cm) wall to decrease the number of falls, whereas the closed arms have a high (16 cm) wall to enclose the arm. The apparatus, 50 cm above the floor, was placed in an empty circular tank made of plastic to protect the rat from falls. The test took place between 9:00 AM and 6:00 PM on the 26th day of the experiment. All the experimental rats were transferred to the behavior testing room 30 min before the beginning of the first trial to habituate to the condition of the behavior testing room. The rats were allowed 10 minutes to move freely on the maze, and each received one trial test. The application for acquiring and analyzing behavioral data (Image EP) is based on the public domain Image J developed by the National Institute of Health, USA. After each trial, all arms and the centre area are cleaned with super hypochlorous water, an efficient odor removal agent with a relatively weak odor compared to other cleaning solutions. The Image EP program calculated the number of entries into each arm and the time spent in each arm.

Assessment of Spatial Memory

According to Vorhees and Williams (2006), the Morris Water Maze is used to study spatial memory. The rats were placed in a pool of water 150 cm in circumference and 35 cm deep with a platform (10cm diameter and 15cm height) at the centre. The rat was placed in one quadrant 20 cm away from the platform and allowed to swim and locate the platform within sixty seconds (Jarrard *et al.*, 1984). Before the test, the rats were trained to teach how to exit the water, and during this time, the platform was exposed at a height of 1 inch above the water. The animals underwent two experimental protocols, spatial acquisition, and spatial reversal, using standard procedures according to Cain *et al.* (1996). Eight locations were used for the acquisition and reversal learning to determine the animals' learning extent (Kesner *et al.* 1987). A probe trial in which the animals can swim randomly in the tank bowl is given to ascertain their retention ability 24 hours later (Vorhees and Williams 2006). The training of the animals was carried out during the acclimatization period, followed by a probe, while the acquisition and reversal test was conducted twice daily on the 25th, 26th, 27th, and 28th days.

Assessment of Recognition Memory

According to Silver *et al.* (2007) and Ennaceur (2010), the novel object recognition test is used in studying rodents' memory and measuring exploration of the environment or objects. It was also helpful in studying short-term, intermediate, and long-term memory by manipulating the retention interval (Angleton *et al.*, 2010). The procedure includes habituation, familiarization, and test phase. In habituation, the rat was allowed to explore the apparatus freely without objects for 5 minutes and then returned to its holding cage. In familiarization, the rat was placed in the arena with two identical sample objects for 5 minutes, called

the retention interval. The rats were released with their back to prevent coercion. During the test phase, the rat was returned to the apparatus with two familiar objects and another novel object (Gaskin *et al.* 2010). During the familiarization and the test phase, the objects are placed in opposite and symmetrical corners of the open-field arena, and the location of novel versus familiar objects is counterbalanced (Hammond *et al.* 2004). After the test phase, the following parameters are collected: Mean time spent sniffing familiar objects, Mean time spent exploring familiar objects, Mean time spent exploring novel objects, Percentage of object discrimination (%), and discrimination index.

Animal Sacrifice

At the end of 28 days, the animals were anesthetized and sacrificed by cervical dislocation. The brain was harvested by first detaching the head of the animal from the torso using sharp surgical scissors. The skin on the head was removed using a surgical blade and fixed in Bouin's fluid for 48 hours. Then, the skull was cut open along the left and right side (entering through the spinal cord extension) using blunt scissors (to preserve the integrity of the brain) while gently pushing the tip outwards. The brain was gently removed using a spatula. The prefrontal cortex (PFC) was homogenized to assay the biochemical parameters.

Estimation of Thiobarbituric Acid Reacting Substances (TBARS)

Thiobarbituric acid reactive substances (TBARS), as defined by Esterbauer and Cheeseman (1990), were created in this experiment to calculate the amount of lipid peroxidation. 25 ml of PFC homogenate combined with 15 ml of 1 phosphoric acid at a PH of 20 ml of TBA and chilled to room temperature in airtight tubes with 25 ml of butanol. The TBA was extracted by centrifuging at 4000xg for 10 minutes, allowing for measurements at 532 nm MDA and 99 of TBARS. The sample concentration of TBARS was determined using the mass extinction coefficient, which is 156 105 m1cm1, in terms of the expressed nmol TBARS mg lipid peroxidation.

Estimation of Glutathione - S- transferase (GST)

Glutathione-S-transferase (GST) was measured spectrophotometrically by the method of Habig *et al.* (1981) using CDNB (1-chloro-2, 4-dinitrobenzene) as an electrophilic substrate that binds to GSH (Glutathione) with the participation of the enzyme and forms a colored GSH-substrate complex, detected at 340 nm and expressed in $\mu\text{mol conjugate formed}/\text{min}/\text{mg protein}$. A 10-ml reaction master mix was prepared and used within 60 minutes. The spectrophotometer was set at 340 nm. It was read at an interval of 30 seconds in 5 minutes after a lag time of 1 minute. 1 ml of the substrate solution was transferred to a quartz cuvette while the Blank absorbance was read at 340 nm. 2-50 ml of GST sample was added directly to the quartz cuvette containing 1 ml of the substrate solution. The cuvette

was covered using a parafilm and mixed by inverting multiple times. The absorbance readings were recorded.

Estimation of Glutamic Oxaloacetic Transaminase (GOT)

The activity assay of GOT was measured at a total volume of 100 μ l containing 100 mM MES buffer pH 8.0, 20 mM α -KG, 20 mM PLP, and 0.015 μ M GOT in differing substrate concentrations (0–20 mM). After adding the substrate to start the reaction, it was incubated at 37 °C. The reaction was shaken vigorously using Eppendorf for proper oxygenation. The GOT-catalyzed reaction was terminated by adding 100 μ l of 5% sulfosalicylic acid, followed by centrifugation to remove precipitated proteins. The reaction product (glutamate) was then quantified via HPLC (Auza *et al.*, 2017). The total GOT activity was estimated using commercial serum glutathione oxaloacetate transaminase (SGOT) Colorimetric Assay Kit (BioVision).

Assessment of Antioxidant Activities

The catalase level was measured using Sinha's (1972) technique in which dichromate in acetic acid was reduced to chromic acetate by heating it in the presence of hydrogen peroxide, which formed chromic acid as an unstable intermediate. The formed chromic acetate was measured at 590 nm. CAT activity was allowed to split H₂O₂ for different periods. The reaction was stopped at various time intervals by adding a dichromate acetic acid, and H₂O₂ was determined by measuring chromic acetate colorimetrically immediately after heating. The serum was pipetted into a tube of 0.1 ml of plasma, 0.9 ml of phosphate, and 0.4 ml of H₂O₂. The reaction was paused at 15, 30, 45, and 60 seconds by adding 2 ml of dichromate acetic acid. The tubes were allowed to stay in boiling water for 10 minutes to cool, and the color developed was read at 530 nm. Standards in the concentration range of 20-100 micromoles were processed for the test. The catalase activity is expressed as U/ml for plasma (U-micro moles of H₂O₂ utilized/second). Superoxide dismutase (SOD) activity was estimated, and the principle was based on SOD's ability to inhibit the reduction of nitro-blue tetrazolium (NBT) (Li *et al.*, 2019). Adrenalin (0.01 g) was dissolved in 17 ml of distilled water, and 0.1 ml of serum and 0.9 ml of phosphate buffer (pH 7.8) were taken in triplicates in 2.5 ml buffer. 0.3 ml adrenaline solution was added and mixed inside the cuvette. The absorbance was taken at 480nm at 30-second intervals five (5) times. The changing absorbance rate was used to determine superoxide dismutase activity and is expressed as units/mg protein.

Statistical Analysis

The data from the experiment were analyzed using Statistical Package for Social Sciences (SPSS) version 20. The inferential statistics of Analysis of Variance (ANOVA) were adopted to check the significance level and presented as mean \pm standard error of the mean. At the same time, Dunnett's correction *Post Hoc Test* was used to compare the nicotine-

untreated group to the control group and the garlic and honey-treated groups to the nicotine-untreated group. The significance level was established at $P < 0.05$.

RESULTS

Social interactive behavior

There was a significant increase in the time spent in the central chamber compared to the control at $P < 0.05$ in the nicotine untreated group. In contrast to the nicotine group, the groups that received combined treatment of garlic and honey had the time they spent in the central chamber reduced significantly at $P < 0.05$ in all the dosages compared to the nicotine untreated group (Figure 1a). In Figure 1b, there was a significant reduction in the time spent in the social chamber by the nicotine-untreated group compared to the control group. At the same time, garlic and honey significantly increased the time spent in the social chamber compared to the nicotine-untreated group at $P < 0.05$. Nicotine significantly reduced ($p < 0.05$) the time spent in the novel chamber compared to the control group. At the same time, low and high-dose combined garlic and honey significantly increased ($p < 0.05$) the time spent in the central chamber, and the medium dose was significantly lowered at $p < 0.05$ (Figure 1c). In contrast, garlic and honey reduced the number of rearing compared to the nicotine group at $P < 0.05$ (Figures e).

Test for Anxiety

There was a significant decrease in the time spent in the open arm by the nicotine-untreated group compared to the control at $P < 0.05$, as seen in Figure 2a below. In contrast, the medium and low doses (600 and 1500mg) of the garlic and honey further reduced significantly, while the high dose increased but not significantly compared to the nicotine untreated group at $P < 0.05$ (Figure 2a). In Figure 2b, the time spent in the closed arm by the nicotine-untreated group increased significantly compared to the control group at $P < 0.05$.

Test for contextual fear

The nicotine-untreated group spent less time assessing their environment as the time spent in risk assessment and head dip by the nicotine-treated group significantly decreased compared to the control at $p < 0.05$ (Figures 3a and b). The administration of garlic and honey did neither significantly reduce nor increase the time spent in head dip and risk assessment compared to the nicotine untreated group at $p < 0.05$, as seen in Figures 3a and b, respectively.

Abnormal Verticality Behaviors

The time spent rearing by the nicotine untreated group significantly decreased compared to the control group at $P < 0.05$. At the same time, the garlic and honey caused the rearing time to significantly increase compared to the nicotine untreated group at $P < 0.05$ (Figure 4a). Still, in

contrast to Figure 4b, the nicotine caused a significant rise in the time spent freezing at $P < 0.05$. The treated groups significantly elevated ($P < 0.05$) the grooming time compared to the nicotine group (Figure 4c).

Spatial Memory and Learning

The latency period of the rats that received only nicotine significantly increased on days 1, 2, 3, and 4 of the Morris Water Maze test compared to the control at $P < 0.05$ (Figures 5a-d). In the same vane, the latency period in reversal memory on days 1, 2, 3, and 4 increased more than the control on the corresponding days. The latency period decreased in the garlic and honey-treated groups compared to the nicotine-untreated group at $p < 0.05$ (Figures 5a-d). There was also a significant decrease in the reversal memory compared to the nicotine untreated group at $p < 0.05$, as seen in Figures 5a-d.

Object Recognition Memory

The time spent with familiar objects (TSFO) significantly increased in the nicotine untreated group compared to the control at $p < 0.05$. In contrast, the treated groups spent significantly less time with the familiar objects than the nicotine untreated group at $p < 0.05$ (Figure 6a). The time spent with novel objects (TSENO) significantly decreased in the nicotine-untreated group compared to the control at $p <$

0.05 . In contrast, the treated groups spent significantly more time with the novel objects (TSENO) compared to the nicotine-untreated group at $p < 0.05$ (Figure 6a). The discrimination index of the nicotine group significantly increased compared to the control at $p < 0.05$. In contrast, the discrimination index of the low and medium-dose treated groups was significantly lowered compared to the nicotine untreated group at $p < 0.05$, as seen in Figure 6b below.

Biomarkers

The level at which the biomarkers are depleted signifies the extent of inflammations or insults caused in the brain. There was a significant increase in the level of TBARS in the untreated nicotine group. In contrast, garlic and honey-treated groups significantly decreased compared to the nicotine-untreated untreated group at $p < 0.05$ (Figures 7a). The level of GST in the untreated nicotine group was not significant at $p < 0.05$ compared to the control group. The GST level was only significantly reduced in the medium dose compared to the nicotine untreated at $p < 0.05$, as seen in Figure 7b below. In this research, the glutathione oxaloacetate transferase (GOT) was not significant in the untreated nicotine group at $p < 0.05$ (Figure 7c).

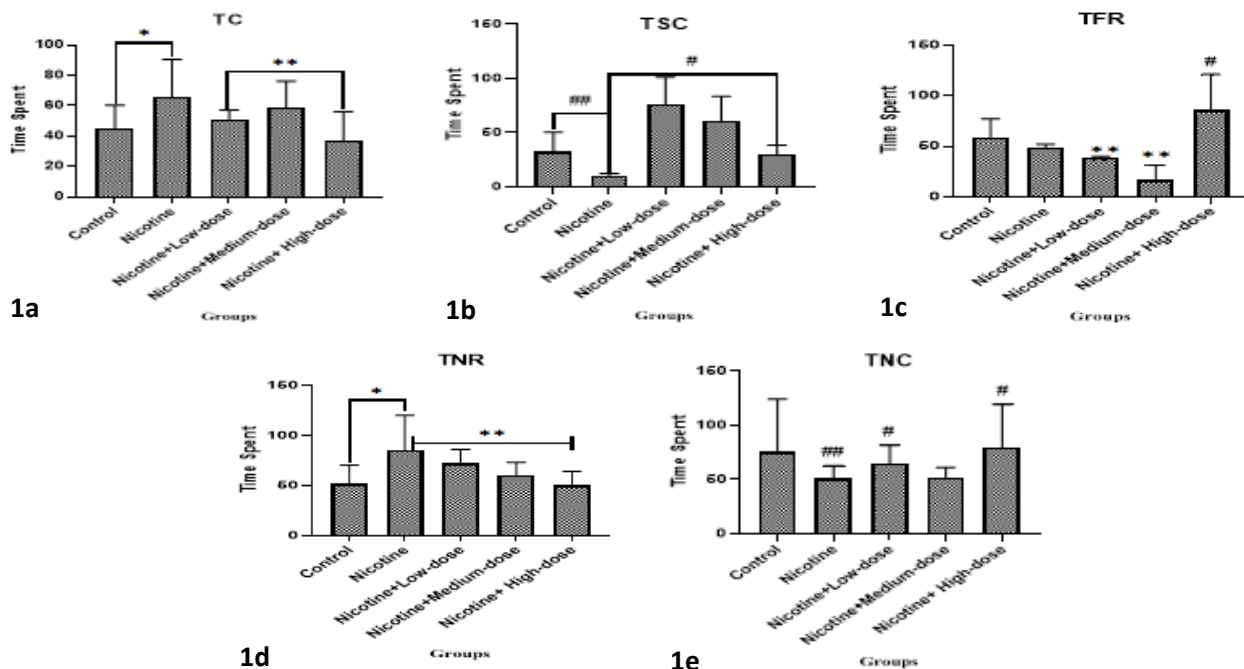


Fig. 1: The combined effect of garlic and honey on Nicotine-induced neurotoxicity on the social behavior of Wistar rats. *Significant increase compared to A at $P < 0.05$; *r Significant decrease compared to B at $P < 0.05$; ###Significant decrease compared to A at $P < 0.05$; #ignificant increase compared to B at $P < 0.05$, TC-time spent in the central chamber, TSC-time spent in the social chamber, TNC-time spent in the novel chamber, TFR-time spent with familiar rat, TNR-time spent with the novel rat.

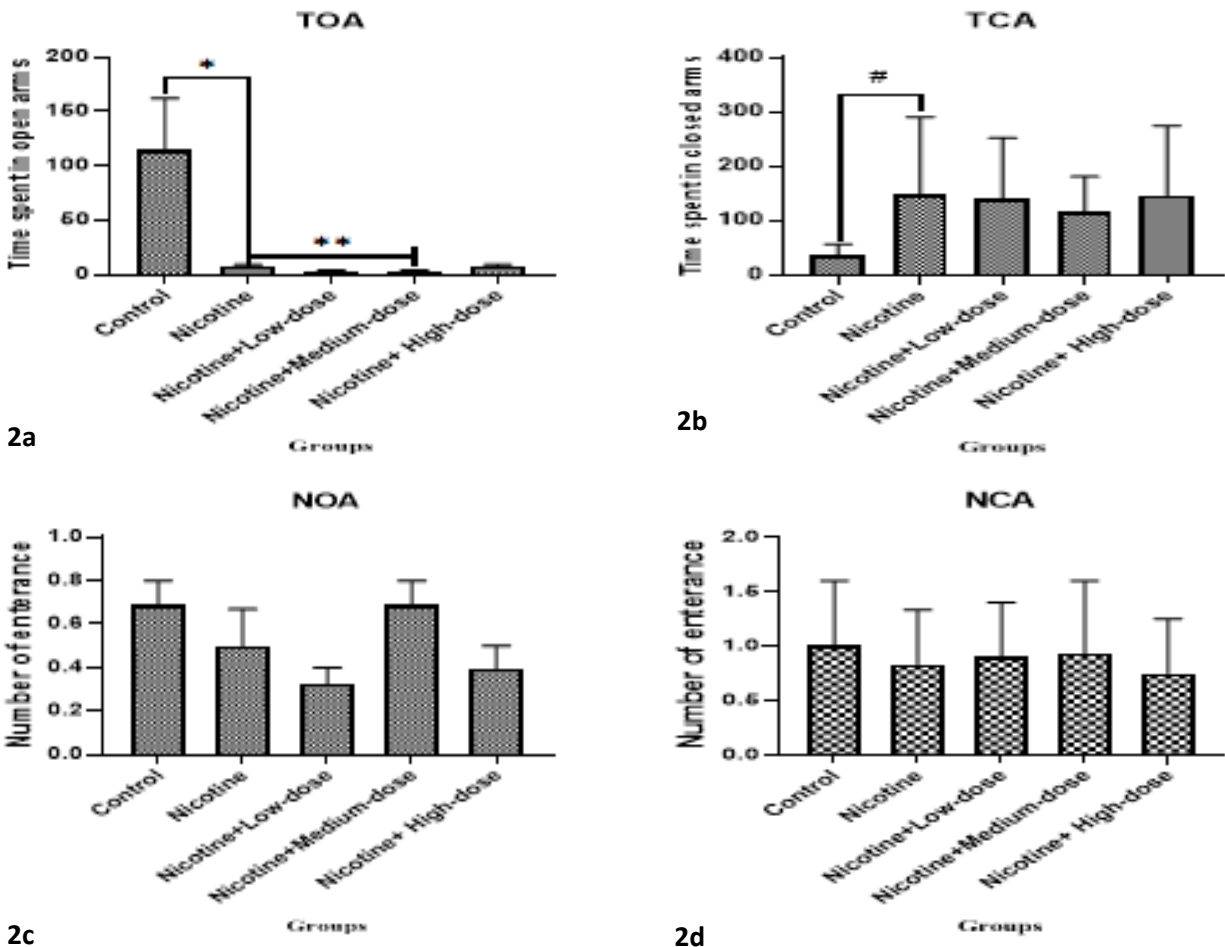


Fig. 2: The combined effect of garlic and honey on Nicotine-induced neurotoxicity on the anxiety level of adult Wistar rats. *Significant increase compared to A at $P < 0.05$; #Significant increase compared to A at $P < 0.05$; **Significant decrease compared to B at $P < 0.05$. NOA-Number of open arm entries, NCA-Number of close arm entries, TOA-Time spent in open arm, and TCA-Time spent in close arm.

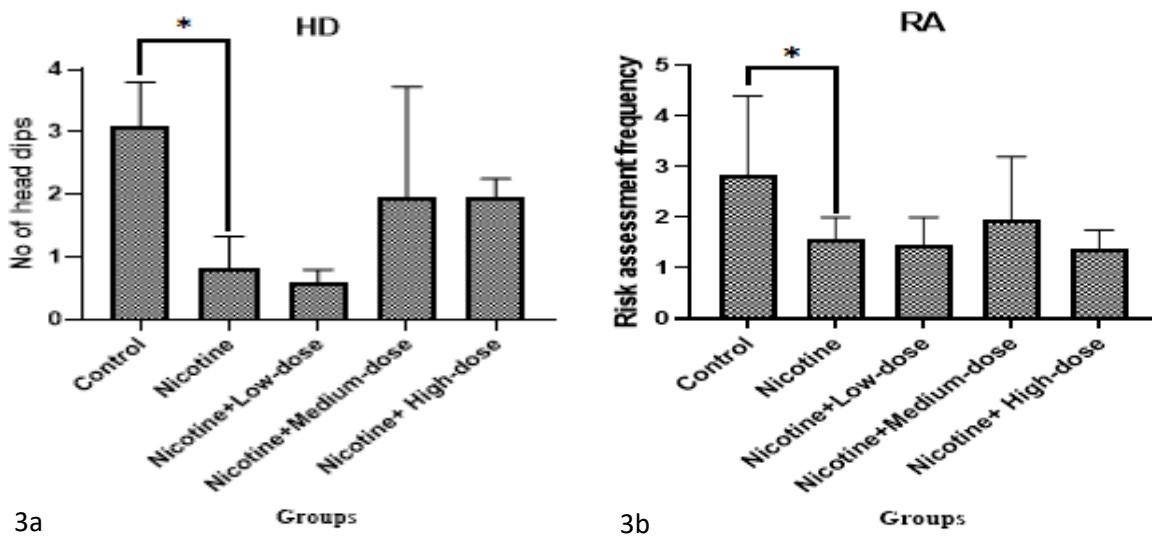


Fig. 3: The combined effect of garlic and honey on Nicotine-induced neurotoxicity on contextual fear and risk assessment behaviors in Wistar rats. *Significant decrease compared to the control at $P < 0.05$. HD-Head dip, RA-Risk assessment

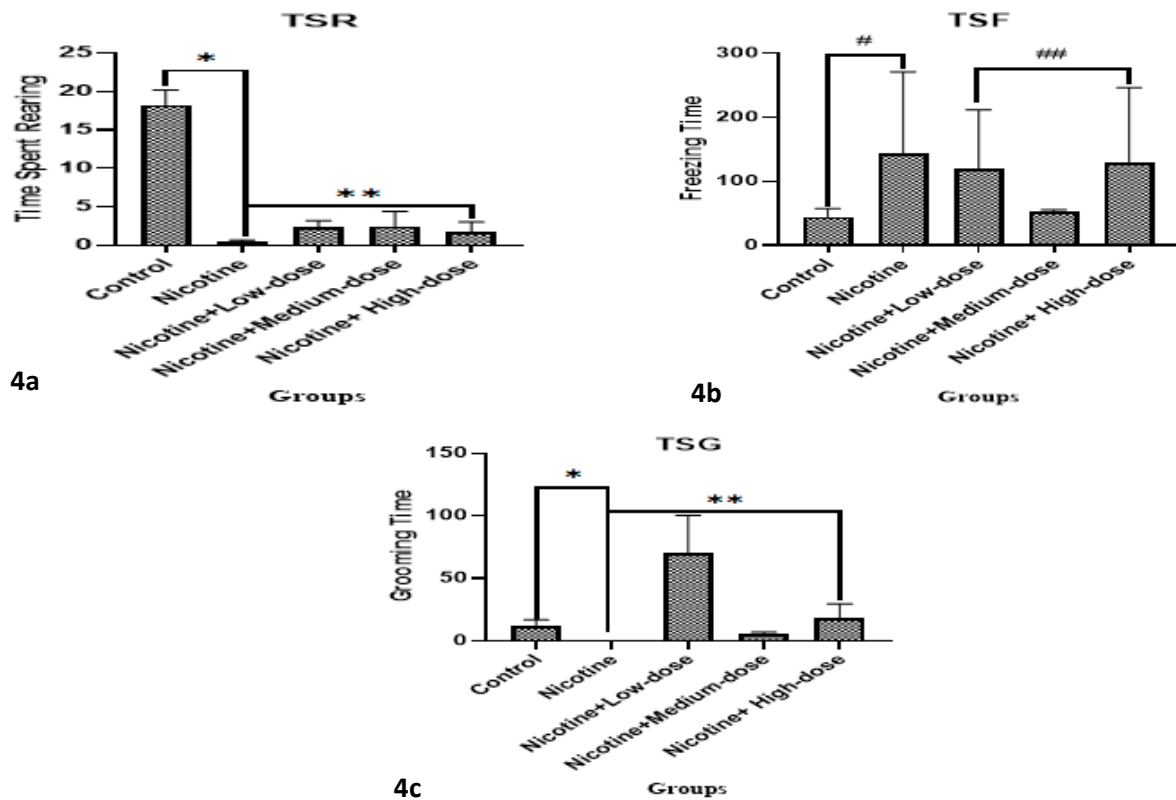


Fig. 4: The combined effects of garlic and honey on Nicotine-induced neurotoxicity on abnormal verticality behaviors in Wistar rats. *Significant decrease compared to the control at $P < 0.05$; #Significant increase compared to the control at $P < 0.05$; **Significant increase compared to B at $P < 0.05$; ###Significant decrease compared to B at $P < 0.05$. **TSR:** Time spent rearing, **TSF:** Time spent freezing, and **TSG:** Time spent grooming.

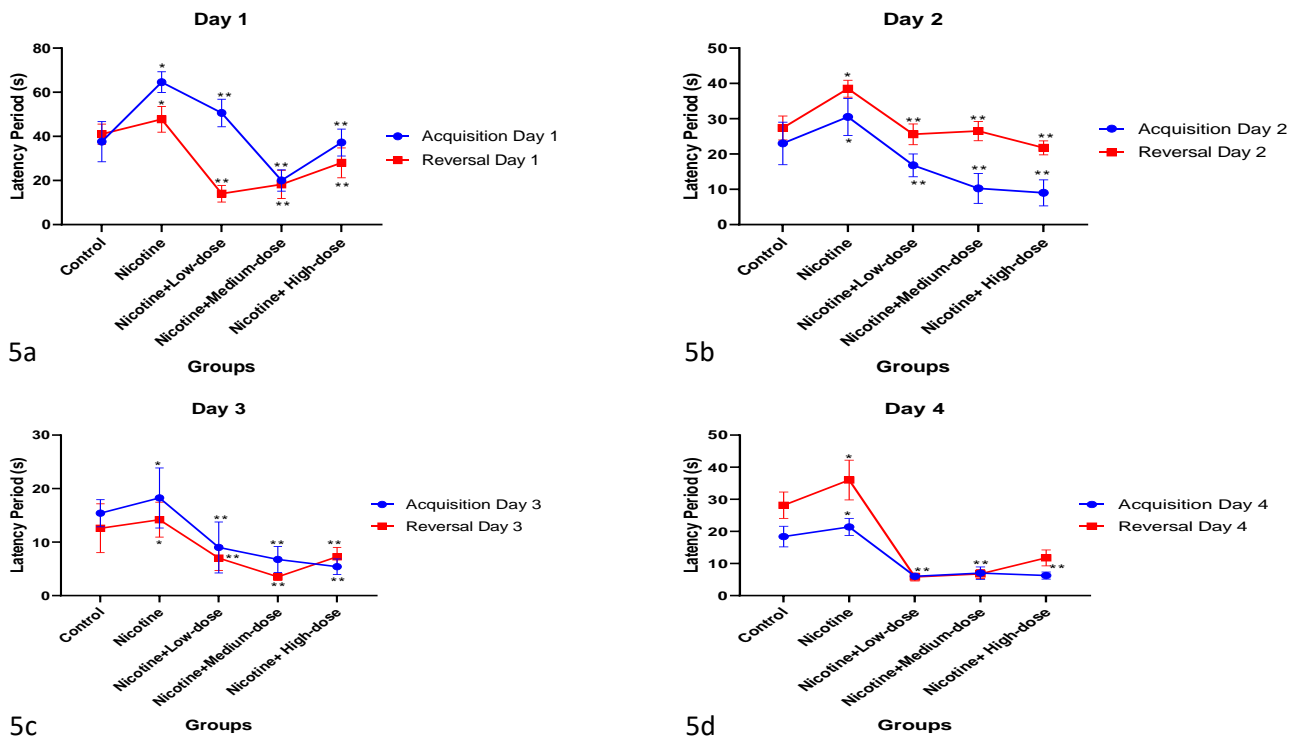
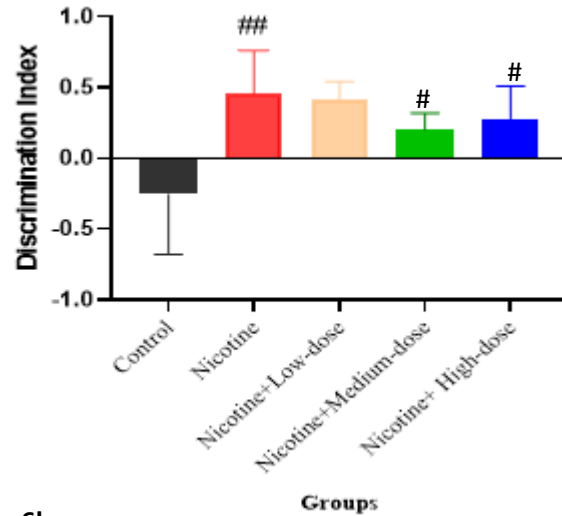
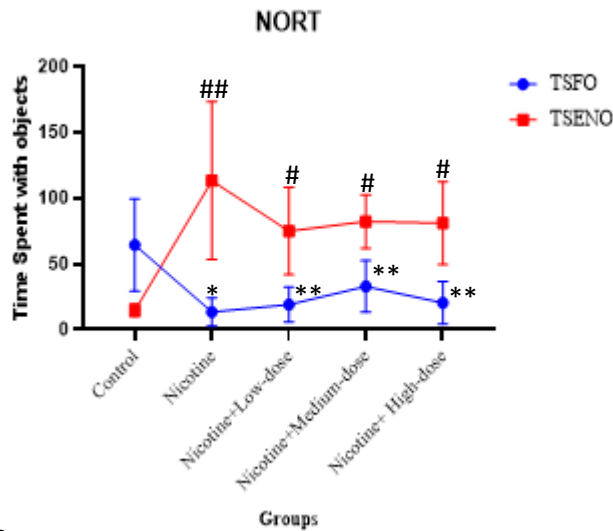


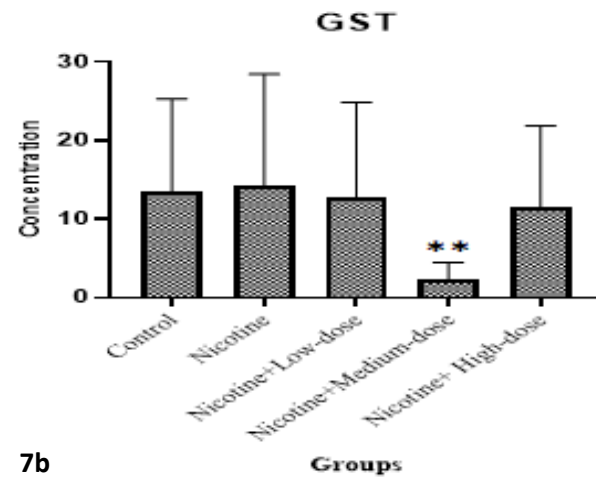
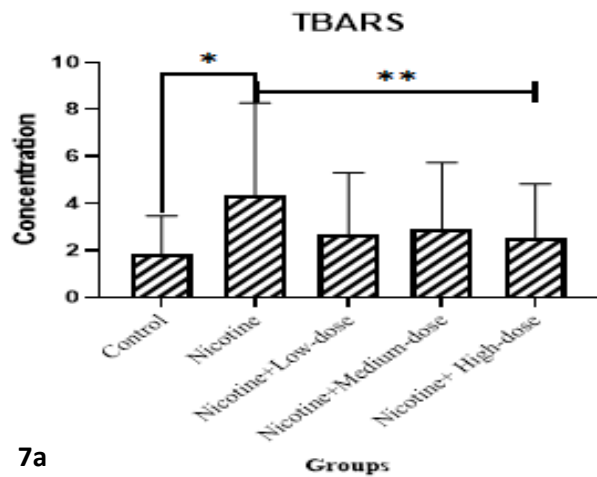
Fig. 5: The combined effects of garlic and honey on Nicotine-induced neurotoxicity on the spatial learning and memory of Wistar rats using MWM. *Significant increase compared to the control at $p < 0.05$; **Significant decrease compared to untreated at $p < 0.05$.



6a

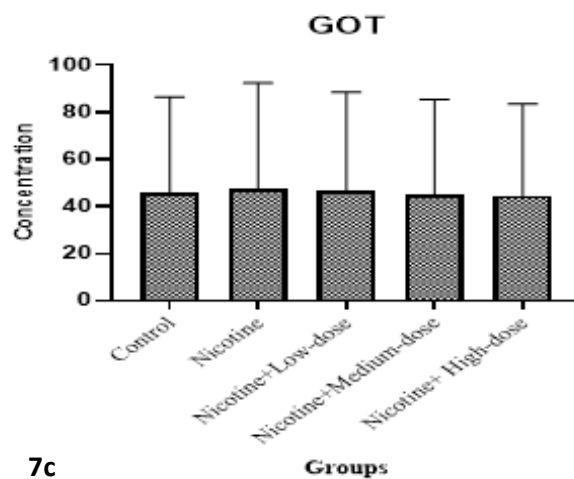
6b

Fig. 6: The combined effect of garlic and honey on Nicotine-induced neurotoxicity on Novel Object Recognition (NOR) in Wistar rats. ##Significant increase compared to the control at $p < 0.05$; #Significant reduction compared to the untreated at $p < 0.05$; *Significant reduction compared to the control at $p < 0.05$; **Significant increase compared to untreated at $p < 0.05$. **Note:** TSFO- Mean time spent exploring familiar object; TSENO- time spent exploring novel object; Discrimination index



7a

7b



7c

Fig. 7: The combined effect of garlic and honey on Nicotine-induced neurotoxicity on some brain biomarkers of Wistar rats. *Significant increase compared to the control at $p < 0.05$; **Significant reduction compared to nicotine untreated at $p < 0.05$

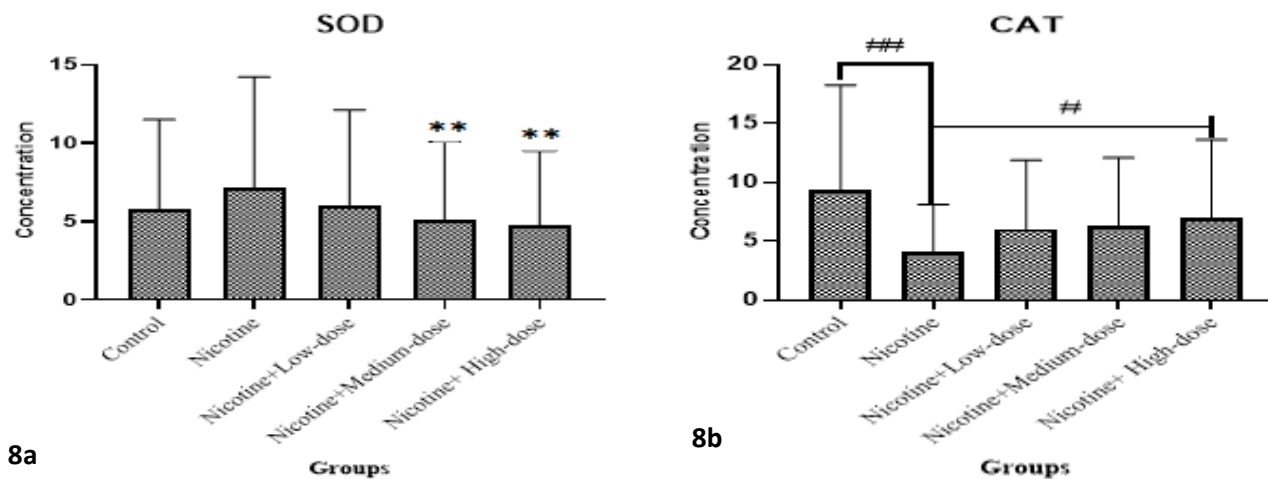


Fig. 8: The combined effect of garlic and honey on Nicotine-induced toxicity on antioxidants in Wistar rats. **Significant reduction compared to the nicotine-untreated at $p < 0.05$; #Significant reduction compared to control at $p < 0.05$; ##Significant reduction compared to untreated at $p < 0.05$

Antioxidant Concentration

The result of biochemical analyses of the Superoxide dismutase activity (SOD) and catalase (CAT) activity and the percentage estimation of these parameters are shown in Figure 8. The SOD analyses showed a significant decrease in concentration in the medium and high doses compared to the nicotine-untreated group at $p < 0.05$ (Figure 8a). The CAT activity level decreased significantly in the untreated nicotine group compared to the control at $P < 0.05$, as seen in Figure 8b. In contrast, the CAT activity levels increased in all the treated garlic and honey groups compared to the nicotine-untreated group in $P < 0.05$ (Figure 8b).

DISCUSSION

The rats' spatial learning cognitive abilities were demonstrated using MWM and NOR, as shown in Figures 5 and 6. The nicotine effect in the spatial memory was evidenced by the steady increase in the time it took the animals to discover the platform in both the acquisition and reversal phases across the groups from day 1 to day 4 (Figure 5). The poor learning ability in nicotine untreated might be due to neurodegeneration (Oyem and Odokuma, 2018). Treatment with honey and garlic improved spatial learning, as evidenced by the sharp decrease in latency during the acquisition and reversal test period (Figure 5). The garlic and honey used in treating nicotine toxicity were efficacious in neuronal genesis and may enhance memory and learning, especially in a combined state. The NOR test was used to investigate the influence of honey and garlic on animals' recognition memory (Tagliatela *et al.*, 2009). It has been established that toxicity affects recognition memory as seen in nicotine untreated, which shows positive value indicating more time exploring novel objects; thus, rats in the nicotine-untreated group showed a recognition memory deficit due to neurotoxicity in agreement with Denninger *et al.*, 2018. The high dose was negative, implying that honey and garlic can

impact memory recognition. The elevated plus maze model assesses rat anxiety (Shepherd *et al.*, 1994). Various researchers have discovered that neurotoxins are one of the major causes of anxiogenic effects (Moreira *et al.* 2001; Soeiro *et al.* 2007). Anxiolytic drugs increase, while anxiogenic drugs decrease the entries and time spent in open arms. In untreated, the decrease in the number of entries into and time spent in the arms suggests that neurotoxicity produces anxiogenic effects in rats, which agrees with Moreira *et al.* 2001; Soeiro *et al.* 2007. The treated groups seemed to have entered and spent more time in the closed arms, maybe due to the anxiolytic effects of those treatment agents (Ijomone *et al.*, 2015). Preference for social novelty is defined as the propensity to spend time with a novel rat rather than a familiar one (Moy *et al.*, 2004). The standard group showed more time spent with the familiar rat than the novel rat, indicating a favorable social but slow preference for a novel experience. The nicotine-untreated rats showed a significant increase in time spent with novel rats than in isolation, suggesting a propensity for novel experience but impaired social motivation and affiliation. The treated animals spent considerable time with familiar rats and less time with novel rats, indicating a slow predilection for a novel experience and spatial learning (Kaidanovich-Beilin *et al.*, 2011).

This research uses biochemical parameters such as TBARS, GST, and GOT to ascertain the effects of substances (nicotine, honey, and garlic) on the body's enzyme level. The result of TBARS agrees with the report of Gueroui and Kechrid (2016). As evidenced in Figure 4 above, there was a significant reduction in GST enzyme level in the medium group compared to the nicotine untreated group, in agreement with Gueroui and Kechrid, 2016. Oxidative stress is a common pathology due to disorders of homeostasis between the production and detoxification of reactive oxygen species (ROS), causing stimulation of lipid peroxidation and depletion of antioxidants (Patrick, 2006). It is a significant mechanism by which neurotoxins actuate poisonous substances.

According to Oyem *et al.* (2021), toxicity decreases the level of antioxidant enzymes which agrees with the decrease in CAT activity levels observed in the present study but disagrees with the SOD level, which increased in the untreated nicotine group. CAT activity decreased significantly ($p < 0.05$) in the nicotine untreated group, agreeing with Halegrahara *et al.* (2011). The treated groups showed increased CAT, while SOD levels only increased significantly in the medium and high-dose treated groups (Figures 6b and c). The increase in the antioxidant concentration might indicate that honey and garlic can serve as a clearing agent for system-accumulated ROS.

Conclusion

It was observed in this study that garlic clove extract and honey are capable of attenuating memory deficits induced by nicotine. The garlic clove extract and honey did this by increasing some of the antioxidants the nicotine lowered.

Declaration of Conflict of Interest

The authors of this manuscript declare no conflict of interest presently or in the future.

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