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RESEARCH ARTICLE OPEN ACCESS

Chronic stress-induced neurodegenerative changes in Wistar rats: Hippocampal evidence and the mitigating effects of *Allium Sativum*

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Abstract
Objective: Stress triggers a biological response perceiving a threat, chemicals and hormones surge throughout the body, after long-term exposure, stress accelerates cell ageing, immune senescence, and some age-related diseases such as neurodegenerative disorders and osteoporosis. This study finds possible effects of *Allium sativum* ethanolic extract on the hippocampus of chronic stress-induced neurodegenerative changes in male Wistar rats.
Methods: Forty Wistar rats were grouped into eight of five (n=5). Group A - control group (no stress and treatment), Group B - 100mg/kg of *Allium sativum* (No stress), Group C - 250mg/kg of *Allium sativum* (No stress), Group D - 500 mg/kg *Allium sativum* (No stress), Group E - Stress alone + No treatment, Group F - 100mg/kg of *Allium sativum* (Stressed), Group G - 250mg/kg of *Allium sativum* (Stressed) and Group H - 500mg/kg of *Allium sativum* (Stressed), all for 14 days. Animals were euthanized humanely and the hippocampus was excised and fixed in 10% formalin solution for histological processing while the part used for biochemical assay was homogenized in phosphate buffer before centrifugation.
Results: Chronic exposure of rats to stress elicited reduced body and brain weights, altered neurotransmitter levels and overall neurobehavioural functions, while also resulting in marked histomorphological distortions in the hippocampus. Interestingly, supplementation with Allium sativum significantly ($p<0.05$) mitigated biochemical, neurobehavioural, and hippocampal aberrations.
Conclusion: Notably, data obtained from this study showed that *Allium sativum* may be a very effective adaptogen in curtailing the deleterious neurodegenerative changes associated with chronic stress.

Keywords: Chronic stress, *Allium sativum*, Hippocampus, Neurodegeneration, Adaptogen

Plain English Summary

The study investigated the effects of stress and Allium sativum (garlic) supplementation on rats. It found that stress led to changes in brain and body weight, neurotransmitter levels, oxidative stress markers, and behaviour. Allium sativum supplementation seemed to counteract some of these effects, showing

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potential benefits in reducing stress-induced alterations. It appeared to help maintain brain and organ health, regulate neurotransmitter levels, reduce oxidative stress, and alleviate stress-related behaviors like immobility and anxiety. However, excessive Allium sativum consumption may have negative effects on brain cells. Overall, the study suggests that Allium sativum could be a promising natural approach to mitigating the impacts of stress on health.

Introduction

Stress is the body's reaction to any change that requires an adjustment or response, the body reacts to these changes with physical, mental, and emotional responses and has many profound effects on the human biological systems (1). The central nervous system (brain and spinal cord) plays a crucial role in the body's stress-related mechanisms (2, 3). The sympathetic nervous system becomes primarily active during a stress response, regulating many of the body's physiological functions to make an organism more adaptive to its environment (4, 5). Stress, either severe, acute, or chronic low-grade may induce abnormalities in three principal regulatory systems in the body: serotonin systems, catecholamine systems, and the hypothalamic-pituitary-adrenocortical axis. Aggressive behaviour has also been associated with abnormalities in these systems (6). Stress causes reduced nerve branching and development and even causes nerve cell death in the hippocampus (7). Nerve cells are also less elongated in individuals with stressful lifestyles or who grew up in a stressful household and people suffering from an ongoing hypothalamic-pituitary-adrenal (HPA) axis stress response show shrinkage in the size of their hippocampus (6). The glucocorticoid receptors (GRs) on the hippocampus become over-activated with ongoing stress, which prevents nerve cell excitation (8). This explains why both short and long-term stress can cause memory lapses and poor focus. It is common for people to be incapable of recalling the details of a traumatic event and that is why it can be inconclusive to put victims of heinous crimes on trial or to ask a driver for the details of a car wreck i.e. meaning memories may be formed but their context is ambiguous (9, 10). It is possible to restore hippocampus function and improve memory through lifestyle modifications and various restorative therapies for HPA axis balance (11).

The hippocampus plays major roles in the normal functioning of the body, including regulating emotions, motivation, hormonal activity and memory formation. Meanwhile, the hippocampus is an area of the brain that appears to be implicated in the onset and maintenance of psychotic disorders and an increase in the experience of stress precedes the onset of a psychotic episode in individuals (12). Categorically, it contains two main interlocking parts: The hippocampus proper (also called *Cornu Ammonis* horn (CA); CA 1, CA2, CA3 and CA4) and the dentate gyrus. The CA1 field also known as the Sommer's sector and the hippocampal circuit, in contextual memory retrieval, contains longitudinally projecting synaptic network pyramidal cells, these neurons form a compact layer consisting of 5 to 8 superimposed rows of these neurons and it is important for representing space in the environment, by encoding for space and long-term memory (13). The CA2 is located between CA1 and CA3 to receive some input from the entorhinal cortex through the perforant path, it has pyramidal cells similar to those in CA3 (14). The CA3 is the pacemaker of the hippocampus and receives input from the mossy fibres of the granule cells, found in the dentate gyrus and from cells in the entorhinal cortex through the perforant path, it has pyramidal cells, axon collaterals that branch out extensively and make excitatory connections with local regions (15). CA4 is often considered to be part of the dentate gyrus, called the hilar region having mossy cells that mostly receive inputs from the granule cells in the dentate gyrus via mossy fibres likewise receiving a few connections from the CA3 pyramidal cells, and it projects into the dentate gyrus at septotemporal levels (16). The dentate gyrus consists of three distinct layers namely the molecular, granular and polymorphic, it is involved in the trisynaptic loop of the hippocampus and their cell distribution is random (17).

Adaptogens are herbs which can muffle the effects of both overactive and underactive stress response (18). Distinctively, *Allium sativum* (*Allium sativum*) contains a variety of effective compounds that exhibit anticoagulant (antithrombotic), antioxidant), antibiotics, hypocholesterolemic and hypoglycemic as well as hypotensive activities (19). Meanwhile, *Allium sativum* is well-reputed for its culinary use as a natural spice in different cuisines across the globe. It acts as an antioxidant by scavenging reactive oxygen species, enhancing cellular antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase, and inhibiting lipid peroxidation and activation of oxidant-induced transcription factors (20). *Allium sativum* improves cognition and increases plasma and brain concentrations of free tryptophan and serotonin while also suppressing amyloidogenesis (21). *Allium sativum* extract in the diet appeared to attenuate the accumulation

of both water-soluble amyloids and detergent-resistant amyloids in different strains of mice (21). Meanwhile, there is a dearth of empirical information on the roles of *Allium Sativum* in mitigating the deleterious effects of chronic stress exposure. As a result, the present study seeks to elucidate the possible neuromodulatory properties of *Allium sativum* in an animal model of chronic stress exposure using biochemical, neurobehavioral and histopathological approaches

Materials and methods

Materials

Plant Material

The *Allium sativum* cloves used for the study were procured from Ilisan Market Ilisan-Remo, Ogun State, Nigeria.

Experimental Animals

Forty (40) male Wistar rats of average weight 100±10g were purchased from the animal house of Babcock University, Ilisan-Remo, Ogun State, Nigeria. They were placed in plastic cages with net covers for ventilation. The rats were bred at the Department of Anatomy animal house, Babcock University, Ilisan-Remo, Ogun State, Nigeria. They were well fed with pelletized feed and water.

Methods

Preparation of *Allium sativum* cloves

Allium sativum was left to dry in the laboratory at ambient temperature (30 ± 2°C) for 10 days. They were thereafter pulverized with a laboratory mechanical grinder (Kenwood SHB-2088). The obtained fine powders were then stored until needed. 100 g of the powdered sample was subjected to crude extraction methods.

Animal Grouping and Treatment

After a two-week acclimatization period, the forty rats were divided into eight groups of five rats per group (n=5). Group A- control group (no stress and treatment), Group B- 100 mg/kg BW of *Allium sativum* (No stress), Group C- 250 mg/kg BW of *Allium sativum* (No stress), Group D- 500 mg/kg BW of *Allium sativum* (No stress), Group E- Stress alone + No treatment, Group F- 100mg/kg BW of *Allium sativum* (Stressed), Group G- 250 mg/kg BW of *Allium sativum* (Stressed) and Group H- 500 mg/kg BW of *Allium sativum* (Stressed). All the groups were treated for 14 days, thereafter they were sacrificed for biochemical and histological analysis. Table 1 and 2.

Table 1: Animal grouping and treatment

Groups	Day/Stress time (1- 14 days)	Type of stress	Treatment
A (Control)	No stress	No stress	Normal Saline
B (GarlicLD)	No stress	No stress	100mg/kg/BW of *Allium sativum*
C (GarlicMD)	No stress	No stress	250mg/kg/BW of *Allium sativum*
D (GarlicHD)	No stress	No stress	500mg/kg/BW of *Allium sativum*
E (Stress)	Stressed	Table 2	No Treatment
F (Stress+GarlicLD)	Stressed	Table 2	100mg/kg/BW of *Allium sativum*
G (Stress+GarlicMD)	Stressed	Table 2	250mg/kg/BW of *Allium sativum*
H (Stress+GarlicHD)	Stressed	Table 2	500mg/kg/BW of *Allium sativum*

Table 2: Stressors and exposure durations (for Group E to Group H)

Week1			Week2		
Day	**Stressor**	**Time**	**Day**	**Stressor**	**Time**
1	Bodyweight/Forced swimming	5 minutes	1	Bodyweight/Forced swimming	5 minutes
2	Restraint	5 minutes	2	Food deprivation	24 hours
3	Water deprivation	24 hours	3	Inverted light	1 day
4	Forced swimming	5 minutes	4	Overnight illumination	12 hours
5	Restraint	1 hour	5	Restraint	1 hour
6	Food deprivation	24 hours	6	Shaking	30 minutes
7	Shaking	30 minutes	7	Water deprivation	24 hours

Neurobehavioral tests

The neurobehavioral tests were carried out in a room with a quiet atmosphere between the hours of 10 am to 2 pm and all events were filmed and observed critically with a Canon camera.

Forced Swim Test

This is usually used to check depression in rats by forcing them to swim. The rats were placed in a cylinder; 40 cm tall in height and 20 cm wide in diameter. The animals were left for about 5 minutes each and how long each rat took to struggle to survive before it stopped to take the

survival method of putting its nose above water. Usually, it takes more than 2 minutes for non-depressed rats to take a survival method and less than 2 minutes for depressed rats (22).

Open Field Maze

This test is used for measuring anxiety and exploration as well as locomotion due to its large open area (breath and length of 50cm each and height of 38cm for each quadrant), a maximum of 4 individual rats could be tracked using each quadrant of the maze. If utilizing a multi-enclosure maze, after placing the first subject rat in its defined quadrant, place the remaining rats into their respective maze quadrant for tracking analysis. The rats are acclimated to the procedure room for a minimum of 30 min before starting the test. Remove a rat from the cage by gently grasping its tail and placing the rat in the middle of the open field maze while concurrently activating the SMART software by clicking on the Start button to begin tracking rat movement. It is normal for the rat to move immediately and the timing of release and tracking capture of the rat should coincide with the record of this movement. The open field maze was cleaned with 70% ethanol before the test began. The experimental rat was placed at the corner of one of the four corners of the box of the apparatus and allowed to explore the apparatus for 5 minutes. After 5 minutes, the apparatus was cleaned with 70% ethanol before the next test began. The behaviours were scored according to the number of lines crossed and rearing. The numbers of lines crossed and rearing were used to measure locomotion activity and also anxiety and exploration. Therefore, the higher the frequency of these behaviours, the higher the exploratory behaviour and the lower the anxiety behaviour (22).

Biochemical assay

The supernatants collected from the brain homogenate were used for malondialdehyde (MDA), glutamate, acetylcholine, and nitric oxide assay. The analyses were carried out using relevant Randox ELISA kits according to the manufacturer's instructions.

Data analysis

Data collected were analyzed using a two-way analysis of variance (ANOVA) followed by Tukey's (HSD) multiple comparison test with the aid of GraphPad Prism v.6 (GraphPad Software, Inc., La Jolla, CA, USA). Data were presented as means ± SEM (standard error of the mean). P value less than 0.05 ($p<0.05$) was considered statistically significant.

Results

Brain weight

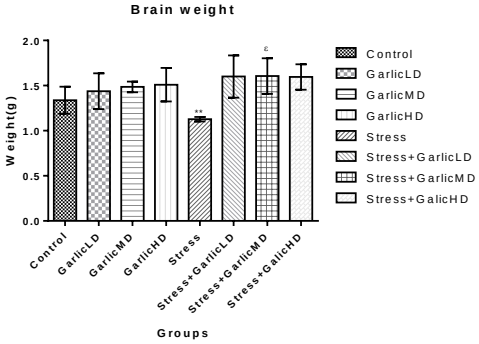
According to Figure 1A, there was a significant difference ($p<0.05$) in the organ weight of Group E when compared to Group A (1.334± 0.0749g). Group E had a significant decrease (1.128±0.0125g) when compared to other groups. Group F had a significant increase (1.600±0.1175g) when compared with group E.

Relative organ weight (row)

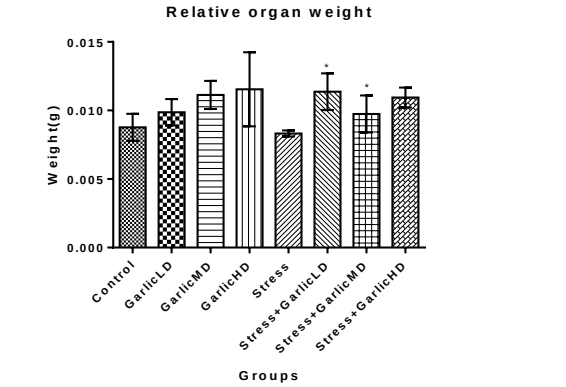
There was a statistically significant difference ($p<0.05$) in the relative organ weight (Figure 1B) of Group G (0.00974±0.0006760) when compared with Group E (0.01155±0.001349), showing an increase in the relative organ weight when compared with the stress group. Group E had the lowest ROW when compared to other groups.

Body weight change

Statistical significance was seen in Group E when compared with Groups A, B, C, and D. Statistical significance was also seen in Group G when compared to Group E. According to Figure 1C, the stress group seems to have had a significant weight reduction from the initial weight, while group F showed a significant increase in weight from the initial weight.



**:Indicates statistical significance when compared with group C at p<0.05
ε:Indicates statistical significance when compared with group E at p=0.0183



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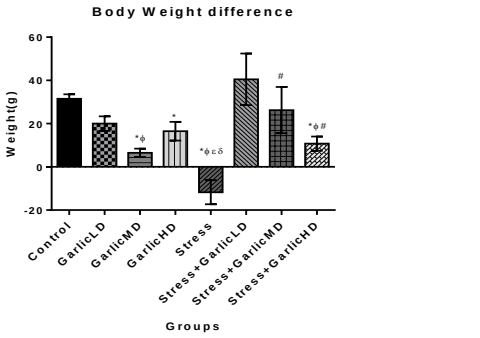

C

Figure 1 (A to C): Weight inference measured on Wistar Rats exposed to graded *Allium sativum* and stress. Figure 1A: Brain Weight, Figure 1B: Relative Organ Weight and Figure 1C: Body Weight difference

Glutamate

The level of glutamate (Figure 2A) in Group E was significantly higher (P<0.05) when compared with Groups A, B, C, D, F, G, and H. Glutamate levels of groups F, G, and H were relatively close when compared to group A. This could infer that the action of *Allium sativum* had a therapeutic effect on stress. Glutamate levels of Group C were also high when compared to Group A.

Acetylcholine

According to Figure 2B, there was an observable slight increase in the level of acetylcholine (Ach) in Group E when compared with Group A. There was also a significant increase in Ach level in Group C when compared with Group A.

Malondialdehyde concentration (MDA)

Figure 2C shows a statistically significant increase (0.2594±0.01127) in the serum levels of MDA in Group E when compared to Groups A, B, C, and D. There was a statistically significant decrease (p<0.05) in Group F when compared to Group E.

Nitric oxide

Figure 2D shows a statistically significant increase (p<0.05) in the nitric oxide level in Group E when compared with Group A, the nitric oxide levels of Groups F, G, and H are relatively close to Group A.

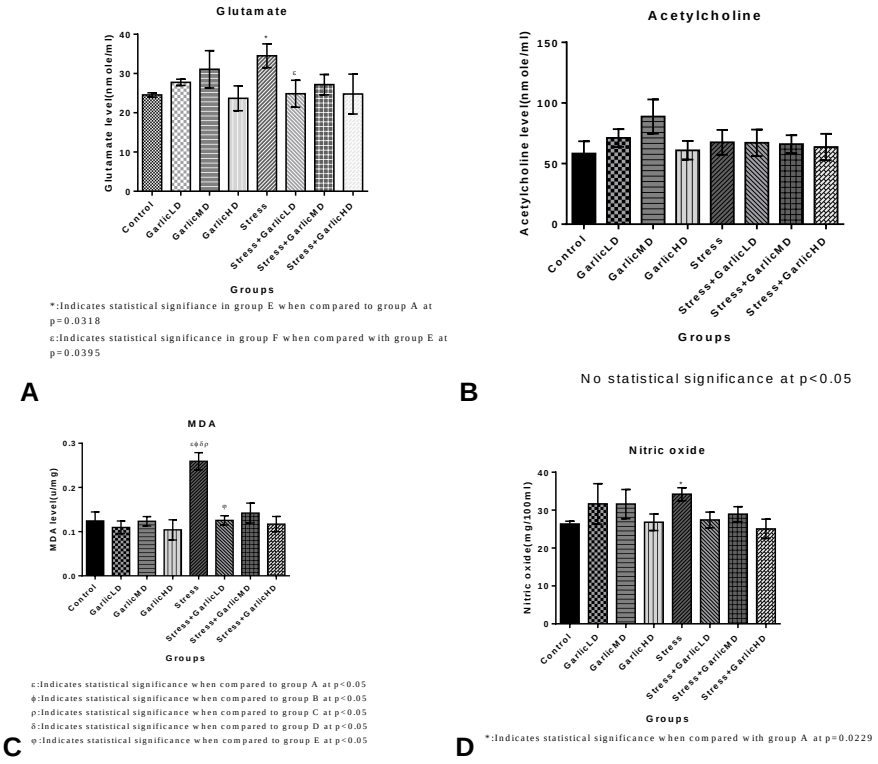

Figure 2 (A to D): Biochemical Concentrations in Wistar rats exposed to graded *Allium sativum* and stresses Figure 13A: Glutamate, Figure 13B: Acetylcholine, Figure 13C: MDA concentration and Figure 13D: Nitric Oxide

Open field test

The open-field test is used to measure locomotion and exploration activities. The number of lines crossed (Figure 3A) was statistically significant (11.25±1.750) in Group E when compared to Group A. There was also a statistical significance in Groups G and H when compared to group B (28.25±1.377). There was statistical significance (20.50±1.848) in group F when compared to groups B and E. There was also statistical significance (11.25±1.750; 18.75±1.109) in Group E and H when compared to Group D. Group E had the lowest mean value in the bar graph when compared to other groups. The rearing number (Figure 3B) of Group B was also moderately low. The number of times groomed (Figure 3C) was also statistically significant increase (8.750±0.4787) in Group E when compared to Groups A, B, and C. There was a statistically significant decrease (4.000±0.5774) in Group G when compared to Group E, while the centre square entries (Figure 3D) were statistically significantly decreased in all groups when compared to Group A (6.750±0.4787). Group E had no centre square entries as zero values were gotten, this indicates the stress levels and unwillingness to explore. Freezing times (Figure 3E) were significantly increased (147.3s±5.851s) in seconds related to Group E compared to Groups A, B, and C. There was also a significant decrease (85.00s±1.780s; 119.3s±8.199s) in Groups F and G when compared to Group E.

Forced swim test

The results of the forced swim test (Figure 3F) showed that there was a statistically significant in the immobility time across the groups. Figure 3F shows a statistically significant increase (201.0±2.739) in the immobility time of animals in Group E when compared to Groups A, B, and D. A statistically significant decrease (144.8±2.496; 144.5±2.102) was also seen in Group F and G when compared to group E.

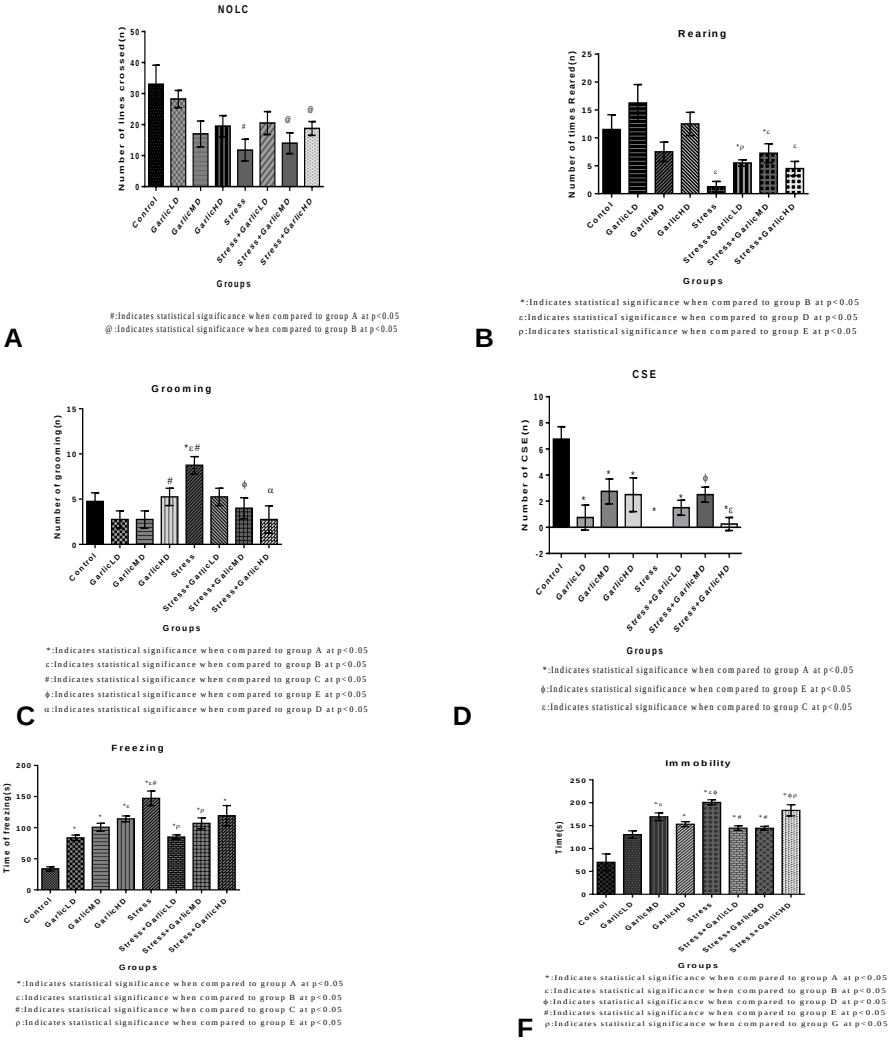

Figure 12 (A to F): Neurobehavioral Test (Open Field Test and Forced Swimming Test) on Wistar Rats exposed to graded *Allium sativum* and stress. Figure 12A: NOLC: Number of lines crossed, Figure 12B: Rearing, Figure 12C: Grooming, Figure 12D: CSE: Centre square entries, Figure 12E: Freezing, and Figure 12F: Immobility time in Forced Swimming Test

Histological report

Group E showed a decrease and sparsity in the cellular layer compared with Group A across the regions of the hippocampus (Figures 2 to 6), the red and yellow arrows show pyknosis and karyorrhexis of the pyramidal cell respectively. Increased pyknosis was also noticed in Group D, and Groups F and G showed relatively low pyknosis and degradation of cells in comparison with Group E. In Figure 3-6, there was an increase in the cellular layer of Groups C, E, F, and G when compared with Group A, but Group E had a sparse arrangement of granular cells in comparison with Groups C, F, and G.

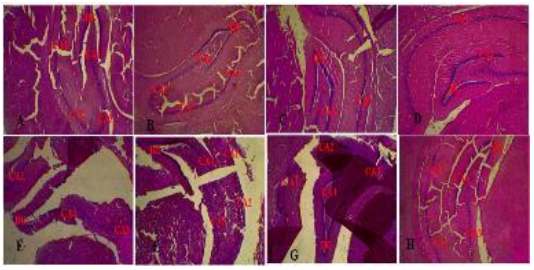

Figure 2: Hippocampus (Cornu Armonis) of Wistar rats exposed to graded *Allium sativum* and stresses showing the dorsolateral cortex formations (CA) 1-4. Mag. x40. Hematoxylin and Eosin Stain

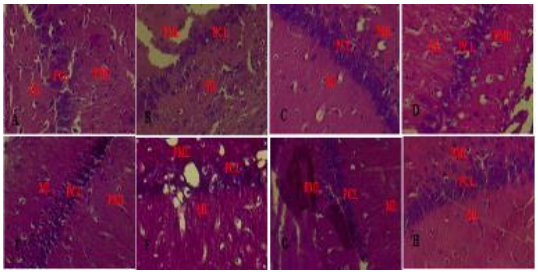

Figure 3: Dorsolateral cortex of Wistar rats exposed to grade *Allium sativum* and stresses showing the CA1 region of the hippocampus. ML= Molecular layer, PCL= Pyramidal cell layer, PML= polymorphic layer. Mag. x400. Hematoxylin and Eosin Stain

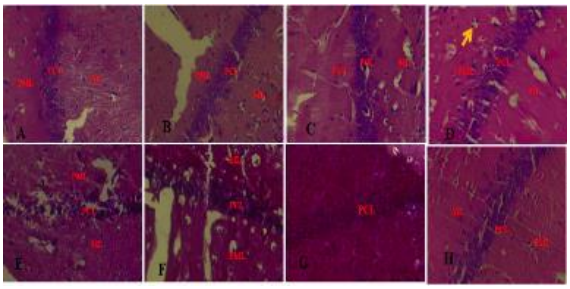

Figure 4: Dorsolateral cortex of Wistar rats exposed to grade *Allium sativum* and stresses showing the CA2 region of the hippocampus. ML= Molecular layer, PCL= Pyramidal cell layer, PML= polymorphic layer. Mag. x400. Stain; Hematoxylin and Eosin

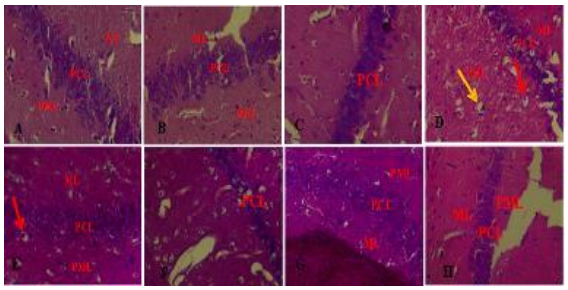

Figure 5: Dorsolateral cortex of Wistar rats exposed to grade *Allium sativum* and stresses showing the CA3 region of the hippocampus. ML= Molecular layer, PCL= Pyramidal cell layer, PML= polymorphic layer. Mag. x400. Stain; Hematoxylin and Eosin

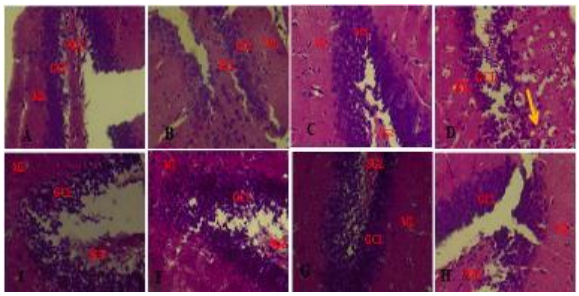

Figure 6: Dorsolateral cortex of Wistar rats exposed to grade *Allium sativum* and stresses showing the DG region of the hippocampus. ML= Molecular layer, GCL= Granular cell layer, SGZ= Subgranular zone. Mag. x400. Stain; Hematoxylin and Eosin

The nissl bodies (Figures 7 to 11) were lost and the Allium sativum was shown not to ameliorate the loss of nissl bodies induced by chronic stress likewise it leads to loss of more nissl bodies causing neurodegenerative changes around the cell.

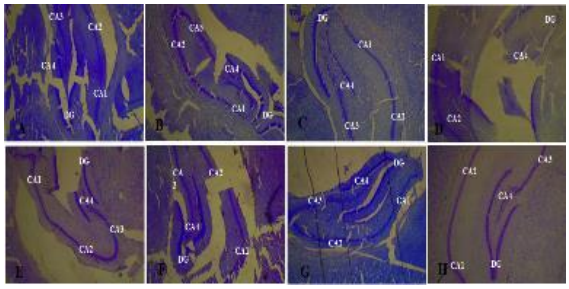

Figure 7: Dorsolateral cortex of Wistar rats exposed to grade *Allium sativum* and stresses, showing the hippocampal formation and its various parts including the dentate gyrus and Cornu Ammonis (CA) 1-4. Mag. x40. Stain; Cresyl fast Violet

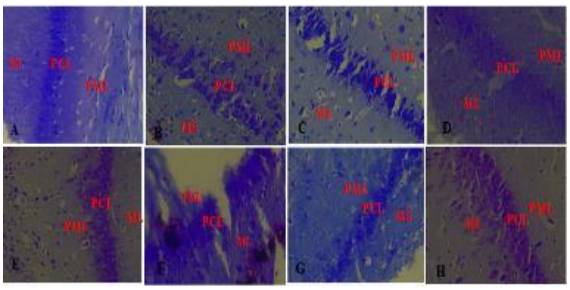

Figure 8: Dorsolateral cortex of Wistar rats exposed to grade *Allium sativum* and stresses showing the CA1 region of the hippocampus. ML= Molecular layer, PCL= Pyramidal cell layer, PML= polymorphic layer. Mag. x400. Cresyl fast Violet Stain

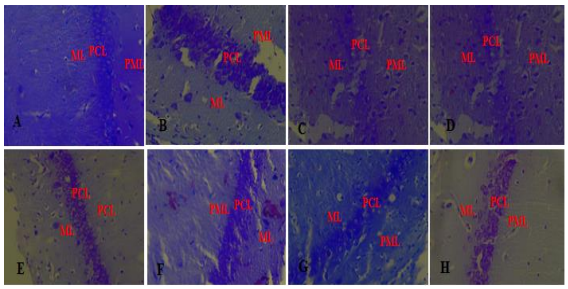

Figure 9: Dorsolateral cortex of Wistar rats exposed to grade *Allium sativum* and stresses showing the CA2 region of the hippocampus. ML= Molecular layer, PCL= Pyramidal cell layer, PML= polymorphic layer. Mag. x400. Cresyl fast Violet Stain

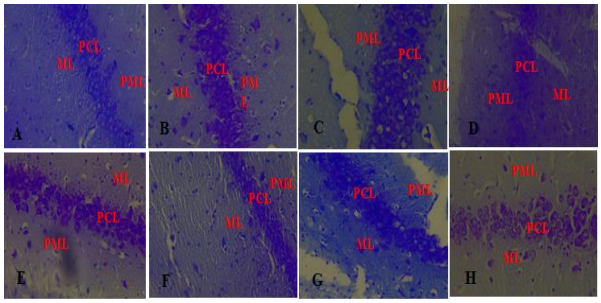

Figure 10: Dorsolateral cortex of Wistar rats exposed to grade *Allium sativum* and stresses showing the CA3 region of the hippocampus. ML= Molecular layer, PCL= Pyramidal cell layer, PML= polymorphic layer. Mag. x400. Cresyl fast Violet Stain

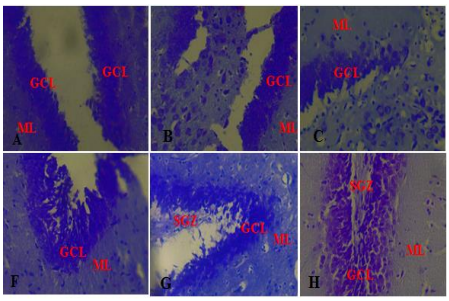

Figure 11: Dorsolateral cortex of Wistar rats exposed to grade *Allium sativum* and stresses showing the DG region of the hippocampus. ML= Molecular layer, GCL= Granular cell layer, SGZ= Subgranular zone. Mag. x400. Cresyl fast Violet Stain

Discussion

Medicinal plants are well-reputed as important repositories of bioactive compounds which can modulate downstream metabolic cascades in different pathological conditions (23, 24, 25, 26). The results of the present study revealed significant alterations in brain weight and relative organ weight (ROW) among different experimental groups subjected to stress and treated with *Allium sativum* supplementation. We observed significant changes in the body and organ weights of Wistar rats exposed to stress and treated with *Allium sativum* in our research. These findings offer an understanding of the effects of stress and *Allium sativum* on the brain and body.

Initially, it was noted that the brains of rats in Group E were notably lighter than those in Group A, indicating that stress can have a major impact on brain size. Continuous stress may lead to changes in the brain that could be linked to disorders like neurodegeneration, consistent with prior studies on the detrimental effects of persistent stress on brain health. Group F, which received *Allium sativum* supplementation, exhibited greater brain weight compared to Group E. *Allium sativum* may offer some defence against the detrimental effects of stress on the brain. *Allium sativum* is known for its ability to reduce inflammation and oxidative stress, which may help preserve brain mass (27). Upon analyzing the relative organ weight (ROW), it suggests that *Allium sativum* may improve or maintain organ health after experiencing stress. Group E had reduced organ weight in comparison to the other groups, demonstrating the effects of stress on organ health, perhaps leading to atrophy or impaired function.

The current study revealed significant variations in the impact of stress on the body and the potential benefits of *Allium sativum*, in mitigating these effects. It appears that stress plays a major role in the fluctuations of body weight. Consistent with findings from previous research, ongoing stress can disrupt the body's energy processing, affecting appetite, energy levels, and hormone levels, potentially leading to weight loss (28).

It is worth noting that Group G displayed a significant variation in body weight change in comparison to Group E, indicating that *Allium sativum* supplementation could potentially impact weight regulation following stress. The discrepancy suggests that *Allium sativum* may have contributed to preventing or reversing the weight loss observed in Group E, suggesting that *Allium sativum* could potentially mitigate the adverse impact of stress on body weight. Our data, illustrated in a bar graph depicting changes in body weight over time, provides additional support for these findings. Group E, known as the stress group, exhibited a noticeable decrease in weight since the beginning of the study. This weight loss aligns with the body's response to ongoing stress, which can disrupt metabolism and appetite, resulting in a decrease in body weight (29). However, group F displayed weight gain, indicating that *Allium sativum* could impact nutrient processing and metabolism, potentially assisting in weight maintenance or gain during stressful periods.

Alterations in neurotransmitter levels are associated with diverse neurological aberrations (30). Examining glutamate and acetylcholine levels provides insight into how stress affects brain chemistry and the possible advantages of *Allium sativum* in regulating neurotransmitters. Data obtained from the current study demonstrated the effects of chronic stress on the delicate balance of neurotransmitters, particularly in the hippocampus. Group E showed markedly higher amounts of glutamate compared to the other groups. This supports prior research indicating a correlation between glutamate imbalance and neurological symptoms associated with stress (31). Notably, stress can interfere with the release and function of glutamate, leading to an accumulation of glutamate in the brain (32). On the other hand, Groups F, G, and H exhibited glutamate levels closer to Group A, indicating that *Allium sativum* might help reduce the effects of stress on glutamate. By implication, this suggests that *Allium sativum* may be beneficial in controlling

glutamate signalling in the brain, thereby aiding in restoring equilibrium.

Nevertheless, group E showed a little increase in acetylcholine levels compared to Group A, potentially because the brain elevated acetylcholine synthesis in reaction to stress. This helps the brain adapt to challenges, suggesting that *Allium sativum* might boost acetylcholine transmission. *Allium sativum* can alter acetylcholine levels by altering cholinergic pathways or enhancing acetylcholine generation or release (33).

Oxidative stress is a key factor in various disease conditions (34) including neurological disorders (35). The results on malondialdehyde concentration (MDA) and nitric oxide levels provide important information about how the body reacts to oxidative stress and the possible therapeutic advantages of *Allium sativum*. Group E showed higher serum malonaldehyde levels compared to Groups A, B, C, and D, indicating an increase in lipid peroxidation, a direct sign of oxidative stress due to chronic stress (36). This is in tandem with previous investigations that persistent stress can result in increased oxidative damage, as shown by raised MDA levels (37). The notable reduction in MDA levels in Group F compared to Group E indicates that *Allium sativum* could provide defence against lipid peroxidation caused by chronic stress. *Allium sativum*, abundant in antioxidants, can potentially neutralize damaging reactive oxygen species (ROS) and inhibit lipid peroxidation (38).

The rise in nitric oxide levels in Group E compared to Group A indicates a link between chronic stress and an increase in nitric oxide synthesis, mostly caused by the brain enhancing the expression of inducible nitric oxide synthase (iNOS). Nitric oxide, a multifunctional signalling molecule, is intricately involved in stress reactions and brain inflammation (39). Our findings suggest that *Allium sativum* might boost nitric oxide synthesis by improving nitric oxide synthase (NOS) activity. The identical nitric oxide levels in Groups F, G, and H compared to Group A indicate that *Allium sativum* may help regulate nitric oxide levels, fostering equilibrium in difficult situations. The results highlight the advantages of utilizing *Allium sativum* supplements as a natural approach to combat oxidative stress and safeguard neurological well-being during periods of extended stress. *Allium sativum* has bioactive components that can boost NOS function, leading to higher nitric oxide generation (40). In a previous study, *Allium sativum* was shown to elicit neuroprotective effects by modulating biochemical pathways associated with increasing nitric oxide levels, thereby counteracting the negative effects of chronic stress (41).

The results of the forced swim test (FST) provide insight into the impact of stress on behaviour and the potential of *Allium sativum* to alleviate these effects. Group E displayed longer periods of immobility than Groups A, B, and D, indicating a more pronounced stress response. Extended immobility in the FST is a recognized indicator of stress and can suggest depressive-like conditions in animal research (42). It symbolizes surrender when confronted with an inevitable stressor, such as having to swim continuously without rest. The significant reduction in immobility time in Groups F and G compared to Group E suggests that *Allium sativum* may have a soothing impact on stress-induced behavioural alterations. The decrease in immobility indicates a reduction in the sensation of despair induced by stress, maybe due to alterations in neurotransmitters or stress-responsive circuits. The findings corroborate traditional medicine's assertion of *Allium sativum* 's capacity to alleviate stress and enhance mood. The decrease in immobility time seen in the *Allium sativum*-treated groups supports the conventional belief in *Allium sativum* 's stress management advantages. This highlights the potential of *Allium sativum* supplementation as a non-pharmaceutical approach to alleviate stress-induced behavioural alterations and enhance resilience. Suggestively, *Allium sativum* may be a promising approach for addressing mental health conditions associated with stress, such as depression and anxiety, by modifying the body's stress response and enhancing the coping mechanisms.

In the open field test, we gained valuable insights into how rats move, explore, deal with anxiety, groom themselves, and freeze under chronic stress and *Allium sativum* supplementation. The significant decrease in the number of lines traversed by rats in Group E compared to Group A suggests reduced exploration and potentially elevated anxiety levels in stressed rats. This aligns with existing knowledge that persistent stress can reduce animals' inclination to explore and increase their susceptibility to worry (43). The notable rise in lines crossed by rats in Groups G and H in comparison to Group B, and in Group F in comparison to Groups B and E, indicates that *Allium sativum* may have a soothing impact, decreasing anxiety and promoting exploration. These data suggest that *Allium sativum* may have anxiolytic effects, thus lowering stress-induced anxiety and promoting exploratory behaviour in rats.

The infrequent instances of rats in Group B standing on their hind legs indicate reduced anxiety levels, maybe due to the *Allium sativum* they were given. This study corroborates previous findings that *Allium sativum* has

anxiolytic properties and can decrease anxiety-related behaviours in animals (44). The significant rise in grooming activity in Group E, in contrast to Groups A, B, and C, supports the hypothesis that stressed rats tend to engage in increased self-grooming. Grooming is a recognized coping strategy in rats, used to manage stress and maintain equilibrium (22). The Centre Square Entries typically serve as a reliable gauge of anxiousness and investigation. Rats in Group E did not explore the centre square, indicating that stressed rats may exhibit increased anxiety and less exploration of the central area in the open field compared to Group A. This behaviour indicates nervousness and a proclivity to evade uncomfortable circumstances. The significant rise in freezing time in Group E in comparison to Groups A, B, and C suggests that stressed rats exhibit more freezing behaviour. Freezing is a typical reaction to fear in rodents, frequently observed in circumstances that induce anxiety or stress (44). The significant reduction in freezing time in Groups F and G compared to Group E indicates that *Allium sativum* may alleviate stress-induced freezing behaviour. This suggests that *Allium sativum* may alleviate stress-induced anxiety reactions and encourage rats to adopt more beneficial coping strategies.

The histopathological assessment of the hippocampus gives us vital clues about the cellular changes caused by long-term stress and *Allium sativum* supplementation. Additionally, pyknosis and karyorrhexis were observed in the pyramidal cells, indicating potential cellular damage caused by necrosis or apoptosis. The aforementioned histomorphological alterations indicate damage to the neurons and may likely result from the overstimulation of glutamate receptors due to stress-induced glucocorticoids. Excitotoxicity, caused by excessive glutamate levels, is a recognized outcome of prolonged stress and can lead to neuronal damage and cell death (45).

The heightened pyknosis recorded in Group D may be associated with the consumption of high dosages of *Allium sativum.* While *Allium sativum* is typically considered safe and beneficial for the brain, excessive use may be detrimental to cells. This emphasizes the importance of being careful with the dosage of *Allium sativum* and taking into account the potential hazards while using it as a medication.

Meanwhile, lower levels of pyknosis and cellular disintegration in Groups F and G compared to Group E indicate that *Allium sativum* supplements, particularly at moderate doses, may provide some protection. *Allium sativum* may protect neurons from stress-induced damage. The low density of granular cells in Group E, in comparison to Groups C, F, and G, provides additional evidence that *Allium sativum* supplements may mitigate the structural alterations induced by stress in the hippocampus. Furthermore, the absence of Nissl structures in many groups, including those exposed to *Allium sativum,* indicates extensive neuronal damage and symptoms of neurodegeneration due to prolonged stress. Nissl bodies are ribosome-rich structures located in the cytoplasm of neurons. Their absence is suggestive of defective protein synthesis and neuronal activity (46). The lack of evidence that *Allium sativum* supplements can prevent the loss of Nissl bodies due to stress, and the possibility that they may exacerbate the situation in certain instances, highlights the intricate nature of the connection between *Allium sativum* and stress-induced neuronal alterations.

Conclusion

The results obtained from the study, such as freezing time in the stressed group during the open field test and immobility time during the forced swimming test suggest that *Allium sativum* mitigated morphologically. One could infer that the rats were stressed, likewise from the ROW, a reduction in brain size as a result of the glucocorticoid-induced neurodegenerative changes in the brain. Pyknosis was also seen in groups C, D, E, and G, loss of Nissl bodies and reduction in cellular layer was also seen in groups E and H. The treatment group proved that *Allium sativum* has ameliorative effects on chronic stress-induced neurodegenerative changes, but graded doses of *Allium sativum* do not seem to have any significant differences in amelioration across the parameters studied except for the histological parameters which seem to be negatively affected by high dosage of *Allium sativum.* We therefore suggest that *Allium sativum* should be further studied as a natural herbal amelioration for stress as it seems to have stress mitigating effect.

List of Abbreviations

ANOVA:	Analysis of Variance
BUHREC:	Babcock University Health Research Ethics Committee
HPA:	Hypothalamic Pituitary Adrenal
CA:	Cornus Ammonis
GRs:	Glucocorticoid receptors
GarlicLD:	Garlic Lower Doses
GarlicMD:	Garlic Midian Doses
GarlicHD:	Garlic Higher Doses
BW:	Body Weight
Kg:	Kilogram
MDA:	Malondialdehyde
ROW:	Relative Organ Weight

Declarations

Ethics approval and consent to participate

This research was carried out following all rules and regulations in the guide for the care and use of animals in research and teaching approved by the Health Research Ethics Committee of Babcock University Ilishan-Remo, Ogun State, Nigeria. Approved number BUHREC 833/18) was issued.

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References

1. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A. The impact of stress on body function: A review. EXCLI journal. 2017;16:1057.
2. McEwen BS, Gianaros PJ. Central role of the brain in stress and adaptation: links to socioeconomic status, health, and disease. Annals of the New York Academy of Sciences. 2010 Feb;1186(1):190-222. https://doi.org/10.1111/j.1749-6632.2009.05331.x
3. Godoy LD, Rossignoli MT, Delfino-Pereira P, Garcia-Cairasco N, de Lima Umeoka EH. A comprehensive overview on stress neurobiology: basic concepts and clinical implications. Frontiers in behavioral neuroscience. 2018 Jul 3;12:127. https://doi.org/10.3389/fnbeh.2018.00127
4. Tsigos C, Kyrou I, Kassi E, et al. Stress: Endocrine Physiology and Pathophysiology. [Updated 2020 Oct 17]. In: Feingold KR, Anawalt B, Blackman MR, et al., editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK278995/
5. Chu B, Marwaha K, Sanvictores T, Ayers D. Physiology, Stress Reaction. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2024 [cited 2024 Feb 21]. Available from: http://www.ncbi.nlm.nih.gov/books/NBK541120/
6. Herman JP, McKlveen JM, Ghosal S, Kopp B, Wulsin A, Makinson R, Scheimann J, Myers B. Regulation of the hypothalamic-pituitary-adrenocortical stress response. Comprehensive physiology. 2016;6(2):603. https://doi.org/10.1002/cphy.c150015
7. Kim EJ, Pellman B, Kim JJ. Stress effects on the hippocampus: a critical review. Learning & memory. 2015 Sep 1;22(9):411-6. https://doi.org/10.1101/lm.037291.114
8. Saaltink DJ, Vreugdenhil E. Stress, glucocorticoid receptors, and adult neurogenesis: a balance between excitation and inhibition? Cellular and Molecular Life Sciences. 2014 Jul;71:2499-515. https://doi.org/10.1007/s00018-014-1568-5
9. Sandi C. 12 Memory Impairments Associated with Stress and Aging. Neural plasticity and memory: From genes to brain imaging. 2007 Apr 17;225. https://doi.org/10.1201/9781420008418.ch12
10. Samuelson KW. Post-traumatic stress disorder and declarative memory functioning: a review. Dialogues in clinical neuroscience. 2011 Sep 30;13(3):346-51.
11. Leschik J, Lutz B, Gentile A. Stress-Related Dysfunction of Adult Hippocampal Neurogenesis—An Attempt for Understanding Resilience? Int J Mol Sci. 2021 Jul 8;22(14):7339. https://doi.org/10.3390/ijms22147339
12. Phillips LJ, McGorry PD, Garner B, Thompson KN, Pantelis C, Wood SJ, Berger G. Stress, the hippocampus and the hypothalamic-pituitary-adrenal axis: implications for the development of psychotic disorders. Australian & New Zealand Journal of Psychiatry. 2006 Sep;40(9):725-41. https://doi.org/10.1080/j.1440-1614.2006.01877.x
13. Yang S, Yang S, Moreira T, Hoffman G, Carlson GC, Bender KJ, et al. Interlamellar CA1 network in the hippocampus. Proc Natl Acad Sci U S A. 2014 Sep 2;111(35):12919–24. https://doi.org/10.1073/pnas.1405468111

14. Tzakis N, Holahan MR. Social memory and the role of the hippocampal CA2 region. Frontiers in behavioral neuroscience. 2019 Oct 1;13:233. https://doi.org/10.3389/fnbeh.2019.00233
15. Prince LY, Bacon TJ, Tigaret CM, Mellor JR. Neuromodulation of the feedforward dentate gyrus-CA3 microcircuit. Frontiers in synaptic neuroscience. 2016 Oct 17;8:32. https://doi.org/10.3389/fnsyn.2016.00032
16. GoodSmith D, Lee H, Neunuebel JP, Song H, Knierim JJ. Dentate Gyrus Mossy Cells Share a Role in Pattern Separation with Dentate Granule Cells and Proximal CA3 Pyramidal Cells. J Neurosci. 2019 Nov 27;39(48):9570–84. https://doi.org/10.1523/JNEUROSCI.0940-19.2019
17. Haładaj R. Anatomical variations of the dentate gyrus in normal adult brain. Surgical and Radiologic Anatomy. 2020 Feb;42(2):193-9. https://doi.org/10.1007/s00276-019-02298-5
18. Panossian A, Wikman G. Effects of adaptogens on the central nervous system and the molecular mechanisms associated with their stress-protective activity. Pharmaceuticals. 2010 Jan;3(1):188-224. https://doi.org/10.3390/ph3010188
19. El-Saber Batiha G, Magdy Beshbishy A, G. Wasef L, Elewa YH, A. Al-Sagan A, Abd El-Hack ME, Taha AE, M. Abd-Elhakim Y, Prasad Devkota H. Chemical constituents and pharmacological activities of garlic (Allium sativum L.): A review. Nutrients. 2020 Mar 24;12(3):872. https://doi.org/10.3390/nu12030872
20. Capasso A. Antioxidant action and therapeutic efficacy of Allium sativum L. Molecules. 2013 Jan 4;18(1):690-700. https://doi.org/10.3390/molecules18010690
21. Nillert N, Pannangrong W, Welbat JU, Chaijaroonkhanarak W, Sripanidkulchai K, Sripanidkulchai B. Neuroprotective effects of aged garlic extract on cognitive dysfunction and neuroinflammation induced by β-amyloid in rats. Nutrients. 2017 Jan 3;9(1):24. https://doi.org/10.3390/nu9010024
22. David AA, Oladele AA, Philip AO, Zabdiel AA, Olufunso AB, Tolulope OO, Adebola AO. Neuroprotective Effects of Garcinia kola ethanol Seed Extract on Haloperidol-Induced Catalepsy in Mice. Tropical Journal of Natural Product Research (TJNPR). 2022 Feb 1;6(2):281-6.
23. Adeoye BO, Iyanda AA, Daniyan MO, Adeoye AD, Oyerinde AM, Olatinwo GO. Botanical and Bioactive Markers of Nigerian Bitter Honey. Tropical Journal of Natural Product Research. 2022 Nov 1;6(11).
24. Adeoye B.O Iyanda A, Daniyan M, Ayodeji A, Olajide L, Akinnawo O, et al. Anti-Dyslipidaemia And Cardio-Protective Effects Of Nigerian Bitter Honey In Streptozotocin-Induced Diabetic Rats. 2023 May 15;8:10–8. https://doi.org/10.22270/ujpr.v8i2.920
25. Adebola AO, Adegoke AO, Olufunso AB, Toyin AI, Linda NN, David AA. Ethanol and Benzene Induced Toxicity in Wistar Rats: Ameliorative Effects of Extra-Virgin Olive Oil on Haematological Indices and Spleen Damage. 2022 Aug 1;
26. Michael OA, Banji OM, Olufunso AB, Abiodun OO, Gbenga O, Adebola JB, Bori OO, Oluwamayowa AM, Damilola AS. Determination of Nutrients, Antinutrients and Antioxidants Concentrations in some edible Forest Vegetables in Ondo and Oyo State, South Western Nigeria. Nigerian Journal of Nutritional Sciences. 2023 Jul 1;44(2).
27. Hazzaa SM, Abdelaziz SA, Abd Eldaim MA, Abdel-Daim MM, Elgarawany GE. Neuroprotective potential of allium sativum against monosodium glutamate-induced excitotoxicity: impact on short-term memory, gliosis, and oxidative stress. Nutrients. 2020 Apr 9;12(4):1028. https://doi.org/10.3390/nu12041028
28. Yau YH, Potenza MN. Stress and eating behaviors. Minerva endocrinologica. 2013 Sep;38(3):255.
29. Sominsky L, Spencer SJ. Eating behavior and stress: a pathway to obesity. Frontiers in psychology. 2014 May 13;5:88770. https://doi.org/10.3389/fpsyg.2014.00434
30. Daniyan MO, Fisusi FA, Adeoye OB. Neurotransmitters and molecular chaperones interactions in cerebral malaria: Is there a missing link? Frontiers in Molecular Biosciences. 2022 Aug 24;9:965569. https://doi.org/10.3389/fmolb.2022.965569
31. Musazzi L, Treccani G, Popoli M. Functional and structural remodelling of glutamate synapses in prefrontal and frontal cortex induced by behavioral stress. Frontiers in Psychiatry. 2015 Apr 27;6:125121. https://doi.org/10.3389/fpsyt.2015.00060
32. Pal MM. Glutamate: The master neurotransmitter and its implications in chronic stress and mood disorders. Frontiers in Human Neuroscience. 2021 Oct 29;15:722323. https://doi.org/10.3389/fnhum.2021.722323
33. Akinyemi AJ, Faboya L, Awonegan A, Olayide I, Anadozie S, Oluwasola T. Antioxidant and anti-acetylcholinesterase activities of essential oils from garlic (Allium sativum) Bulbs. Int. J. Plant Res. 2018;31(2).
34. Abiola T.S., Akin-Akanbi B.A, Adeoye B.O. Differential Roles of Tannin -Rich Extract of

Chasmanthera Dependens in Modulating Piroxicam Induced Electrolyte Imbalance In Rats. 2022 Aug 4;9:474–82.
35. Opoggen T, Daniyan M, Asiyanbola I, Adeoye B.O, Oyemitan I. Effects of The Essential Oil of Dried Fruits of Piper Guineense (Piperaceae) on Neurological Syndromes Associated With Cerebral Malaria in Mice. Univers J Pharm Res. 2023 Mar 15; https://doi.org/10.22270/ujpr.v8i1.902
36. Olajide L, Adeoye B., Amadi N, Olajide O, Adetayo M, Ogunbiyi B. Costus Afer Ker Gawl Rhizome Ethyl Acetate Fraction Mitigated Diclofenac-Induced Renal Toxicity in Male Wistar Rats by Suppressing Oxidative Stress. 2023 Dec 1;
37. Juszczyk G, Mikulska J, Kasperek K, Pietrzak D, Mrozek W, Herbet M. Chronic stress and oxidative stress as common factors of the pathogenesis of depression and Alzheimer's disease: The role of antioxidants in prevention and treatment. Antioxidants. 2021 Sep;10(9):1439. https://doi.org/10.3390/antiox10091439
38. Nasr AY. Protective effect of aged garlic extract against the oxidative stress induced by cisplatin on blood cells parameters and hepatic antioxidant enzymes in rats. Toxicology reports. 2014 Jan 1;1:682-91. https://doi.org/10.1016/j.toxrep.2014.09.003
39. Andrabi SM, Sharma NS, Karan A, Shahriar SS, Cordon B, Ma B, Xie J. Nitric oxide: physiological functions, delivery, and Biomedical Applications. Advanced Science. 2023 Oct;10(30):2303259. https://doi.org/10.1002/advs.202303259
40. Shang A, Cao SY, Xu XY, Gan RY, Tang GY, Corke H, Mavumengwana V, Li HB. Bioactive compounds and biological functions of garlic (Allium sativum L.). Foods. 2019 Jul 5;8(7):246. https://doi.org/10.3390/foods8070246
41. Melguizo-Rodríguez L, García-Recio E, Ruiz C, De Luna-Bertos E, Illescas-Montes R, Costela-Ruiz VJ. Biological properties and therapeutic applications of garlic and its components. Food & Function. 2022;13(5):2415-26. https://doi.org/10.1039/D1FO03180E
42. Yankelevitch-Yahav R, Franko M, Huly A, Doron R. The forced swim test as a model of depressive-like behavior. JoVE (Journal of Visualized Experiments). 2015 Mar 2(97):e52587. https://doi.org/10.3791/52587-v
43. Atrooz F, Alkadhi KA, Salim S. Understanding stress: Insights from rodent models. Current Research in Neurobiology. 2021 Jan 1;2:100013. https://doi.org/10.1016/j.crneur.2021.100013
44. Rahmani G, Farajdokht F, Mohaddes G, Babri S, Ebrahimi V, Ebrahimi H. Garlic (Allium sativum) improves anxiety-and depressive-related behaviors and brain oxidative stress in diabetic rats. Archives of physiology and biochemistry. 2020 Mar 14;126(2):95-100. https://doi.org/10.1080/13813455.2018.1494746
45. Armada-Moreira A, Gomes JI, Pina CC, Savchak OK, Gonçalves-Ribeiro J, Rei N, Pinto S, Morais TP, Martins RS, Ribeiro FF, Sebastião AM. Going the extra (synaptic) mile: excitotoxicity as the road toward neurodegenerative diseases. Frontiers in cellular neuroscience. 2020 Apr 24;14:90. https://doi.org/10.3389/fncel.2020.00090
46. Adetunji OA, Oluwole BA, Afolayan S. Progesterone Improves Streptozotocin-Induced Prefrontal Nissl Substance Deficit. Austin J Anat. 2020; 7(1): 1092.