# Aged Garlic Extract Ameliorates Chronic Restraint Stress-Induced Depressive and Anxiety-like Behavior in Mice: *In Vivo* and *In Silico* Approaches

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**ABSTRACT:** Stress is a common factor that can lead to various health issues, including depression and anxiety. Aged garlic extract (AGE) has been suggested to have potential therapeutic benefits, including antidepressant and anxiolytic-like effects. This study aimed to investigate the effects of AGE on depressive and anxiety-like behavior induced by chronic restraint stress in mice using both *in vivo* and *in silico* approaches. *In vivo* experiments involved subjecting mice to chronic restraint stress to induce depressive-like behavior. The mice were then treated with AGE, and their behavioral outcomes were assessed using the forced swim test and tail suspension test, open field test, and elevated maze plus. Additionally, an *in silico* analysis was conducted to explore the potential mechanisms of action of AGE compounds, focusing on their interactions with the monoanime oxidase-A (MAO-A) enzyme. The *in vivo* experiments demonstrated that the administration of aged garlic extract significantly ameliorated behavioral deficits in mice subjected to chronic restraint stress. Specifically, AGE treatment led to reduced ($p<0.05$) immobility time in the forced swim test, with AGE125-treated mice showing (91.12±4.94) seconds and AGE250-treated mice (73.0±7.25) seconds, compared to (119.25±7.13) seconds for the CRS group. Similarly, in the tail suspension test, immobility time was reduced to AGE125 (69.25±5.65)s and AGE 250 (55.0±6.74)s compared with CRS group (102.75±6.84)s, thereby demonstrating antidepressant-like effects. Additionally, AGE-treated mice exhibited increased time spent in the periphery of the open field arena and spent more time in the open arms of the elevated plus maze, suggesting improved exploratory and anxiety-related behaviors compared to other groups. Additionally, *in silico* investigation suggested that compounds found in AGE may antagonize the MAO-A enzyme. This study provides evidence supporting the potential of AGE as a therapeutic agent for stress-related depressive disorders. AGE's ability to improve behavioral outcomes in stressed mice may be related to the modulation of neurotransmitter systems, reduction of oxidative stress, and anti-inflammatory effects of its compounds, including its potential interaction with the MAO-A enzyme. Further research is needed to fully elucidate the mechanisms of action and confirm the efficacy and safety of AGE in humans.

**Key words:** Aged garlic extract, depression, anxiety, chronic restraint stress, mice, *In vivo*, *In silico*.

## INTRODUCTION

Depression is a debilitating mental health condition that affects millions of individuals worldwide, and chronic stress is a significant contributing factor to its development. Major depressive disorder is a significant global health concern, with an estimated 3.8% of the world's population impacted by depression.[1] The World Health Organization has ranked depression as the fourth leading cause of global disease burden, and it is projected to become the leading contributor to the global disease burden by 2030. The high prevalence of depression has profound implications, as it is associated with a range of negative outcomes, including unemployment, poor physical health, impaired social functioning, and, in severe cases,

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suicide.[2] The economic impact of depression is also considerable, with the disorder resulting in substantial costs through medical treatment and diminished work productivity.[3] Given the widespread impact of depression, there is a critical need for effective interventions to alleviate the burden of this debilitating mental health condition.

Chronic stress is a well-established risk factor for the development of depression, and numerous studies have demonstrated the link between prolonged exposure to stressful life events and the onset of depressive symptoms. Chronic stress can lead to a range of adverse consequences for mental health, including the onset of depression, anxiety disorders, addiction, and even suicide.[4] Stress has been directly linked to almost every common disease, from heart disease to the flu, making it a major contributor to personal health.[5] Chronic stress can cause atrophy of the hippocampus, similar to what is observed in depression, and chronic stress paradigms in animals can recapitulate many of the core behavioral characteristics of depression, which are responsive to antidepressant treatment. Stress has been shown to have detrimental effects on the mechanisms of neuroplasticity, which are crucial for maintaining healthy brain function.[6]

*Allium sativum,* commonly known as garlic, is a perennial flowering plant that originates from Central Asia and has become ubiquitous in global cultivation and consumption. The process of aging fresh garlic cloves for an extended duration leads to the conversion of numerous volatile sulfur compounds into more stable and less pungent forms, such as S-allyl cysteine, during the production of aged garlic extract (AGE).[7,8] Numerous studies using in vitro and *in vivo* models have demonstrated the potential of aged garlic extract in the prevention and management of diverse conditions, such as cardiovascular disease, cancer, and neurodegenerative disorders, including Alzheimer's disease.[7] The antioxidant and anti-inflammatory properties of AGE have been attributed to its rich content of stable organosulfur compounds, such as S-allyl cysteine, which are known to exert multiple beneficial effects.[9-11] Previous studies have highlighted the potential of natural herbal sources, including garlic, to provide neuroprotective effects and alleviate the symptoms of neurodegenerative diseases. AGE has been shown to improve cognitive function, reduce neuroinflammation, and protect against neuronal damage in various animal models of neurodegenerative diseases.[11] The stable organosulfur compounds present in aged garlic extract, such as S-allyl cysteine, have been found to possess potent antioxidant and anti-inflammatory properties.[10-11] These compounds can help to reduce the production of reactive oxygen species, inhibit the activation of pro-inflammatory pathways, and attenuate the inflammatory response in the brain, thereby protecting against neuronal damage and cognitive impairment.[11,12] Additionally, AGE has been shown to influence the cholinergic, glutamatergic, and GABAergic neurotransmitter systems, which are known to be disrupted in Alzheimer's disease. This modulation of neurotransmitter systems may contribute to the cognitive-enhancing and neuroprotective effects of AGE. [13]

Chronic stress has been identified as a key driver of oxidative stress and neuroinflammatory processes, which are central to the pathogenesis of depressive disorders. Chronic stress activates the hypothalamic-pituitary-adrenal axis, leading to the release of glucocorticoids, such as cortisol, which can trigger a cascade of events that culminate in neuroinflammation and impaired neuroplasticity.[6] Chronic stress exposure leads to the overproduction of reactive oxygen species and reactive nitrogen species in the brain.[14] This excessive production of free radicals and oxidants can result in oxidative damage to lipids, proteins, and nucleic acids, contributing to neuronal dysfunction and cell death.[6, 14,15] The hippocampus, a brain region central to memory and emotion, is particularly vulnerable to the detrimental effects of oxidative stress. Chronic stress has been shown to induce atrophy and loss of hippocampal neurons, which can be prevented by antioxidant treatment, highlighting the role of oxidative stress in the pathogenesis of depressions.[6] The detrimental effects of chronic stress-induced oxidative stress and neuroinflammation can be

mitigated by therapies that target these pathways. For instance, AGE has been shown to possess potent antioxidant and anti-inflammatory properties.[6,14,16,17] By reducing oxidative stress and inflammation, AGE may help to alleviate the negative impact of chronic stress on the brain.

Emerging evidence suggests that the role of autophagy and adult hippocampal neurogenesis, brain-derived neurotrophic factor has been identified as a key molecule involved in the pathogenesis of depression. Variability in the regulation of BDNF expression, at the genetic, epigenetic, or environmental level, can lead to differential levels of mature BDNF in healthy or diseased subjects, which in turn may be associated with the development of depression and cognitive impairment.[18,19] Indeed, the consensus agrees that aging, the development of Alzheimer's disease, and exposure to chronic stress are related to reductions in BDNF levels.[19,20] Decreased levels of brain-derived neurotrophic factor, a crucial mediator of neuroplasticity, have been consistently reported in individuals with depression. Additionally, chronic stress and elevated glucocorticoid levels can lead to dendritic atrophy and neuronal loss in the hippocampus, further exacerbating the disruption in neuroplasticity.[21] Aged garlic extract has been shown to have neuroprotective effects, and it may help to promote neuroplasticity and neurogenesis, which are crucial for maintaining brain health and resilience to stress.[22-24]

Extensive research has demonstrated that one of the principal mechanisms linking chronic stress to the development of depression is the dysregulation of critical neurotransmitters, including serotonin, norepinephrine, and dopamine.[6,25] Prolonged exposure to stress has been shown to alter the synthesis, release, and reuptake of these neurotransmitters, leading to an imbalance that is characteristic of depressive disorders. AGE has been found to modulate neurotransmitter systems, such as the serotonergic and dopaminergic systems. Imbalances in these neurotransmitter systems have been linked to the development of depressive symptoms.[26,27]

Molecular docking has become an indispensable tool in the field of drug development, playing a crucial role in accelerating the process of identifying and optimizing potential drug candidates. As a computational technique, molecular docking aims to predict the binding mode and affinity between a drug molecule (ligand) and its target protein (receptor), providing valuable insights into the underlying molecular interactions.[28,29] This approach allows researchers to prioritize and focus on the most promising candidates, saving time and resources that would otherwise be spent on experimental screening.[30] In recent years, the application of molecular docking has expanded beyond its traditional use in drug discovery, with researchers exploring its potential in various other areas, such as understanding adverse effects, predicting polypharmacology, and even repurposing existing drugs for new therapeutic indications.[30] Monoamine oxidase-A is an enzyme involved in the metabolism of neurotransmitters, such as serotonin, dopamine, and norepinephrine, which play a crucial role in regulating mood and emotional states. Molecular docking analyses have elucidated the specific binding interactions between monoamine oxidase inhibitors and the target enzyme, enabling the development of more potent and selective inhibitors. This advancement has led to improved treatment strategies for depression, with enhanced therapeutic efficacy and reduced side effects.[30-32]

Chronic stress is a major risk factor for the development of depression and other mood disorders, as it can lead to various neurobiological changes, including oxidative stress, neuroinflammation, and neurotransmitter dysregulation. Given the potential antioxidant, anti-inflammatory, and neuroprotective properties of aged garlic extract, we hypothesized that this dietary supplement may offer therapeutic benefits in the context of stress-induced depressive and anxiety-like behaviors. Antidepressants can have undesirable side effects, and their onset of action can take several weeks. Moreover, a significant proportion of individuals do not respond adequately to these treatments. Psychotherapy, while valuable, is often resource-intensive and may not be readily

accessible to all those in need. Given the limitations of existing treatments, there is a need to explore alternative or adjunctive therapies that may offer additional benefits in the management of depression. Therefore, this study aimed to investigate the effects of aged garlic extract on depressive and anxiety-like behavior in a mouse model of chronic restraint stress.

## MATERIALS AND METHODS

### In vivo experimental procedures

**Drugs and reagents.** Fluoxetine was obtained from Sigma-Aldrich Corporation, while ethanol was procured from Merck. All other reagents used in this study were analytical grades.

**Aged garlic preparation.** Garlic bulbs were harvested, separated into individual cloves, and their outer layers were removed. The peeled cloves were then finely chopped and homogenized using a blender. The blended clover material was submerged in 1000 ml of 20% ethanol solution within a sealed glass container and allowed to naturally age at ambient temperature for 10 months. Afterward, a muslin cloth was utilized to decant the aged garlic extract. The filtered decanted extract was passed through Whatman No. 1 filter paper, and a vacuum suction filter was additionally employed for further filtration. The filtered solution was subsequently heated using a rotary evaporator to evaporate the liquid and obtain the dry AGE extract. The dry Aged Garlic Extract obtained represented a yield of 22.4% (based on the initial fresh garlic weight). The dried AGE then stored at 4°C in airtight, sealed containers to minimize degradation from light, air, and moisture. The study investigated the effects of fluoxetine as well as two dosages of AGE, specifically 125 mg/kg and 250 mg/kg.

**Animals.** All behavioral experiments were conducted using 7- to 8-week-old male Swiss albino mice obtained from International Centre for Diarrheal Disease Research, Bangladesh and acclimated in the laboratory for at least one week. The research rigorously adhered to the "Principles of Laboratory Animal Care" and applicable national regulations. The mice were maintained in a controlled environment with a constant temperature of 23°C, a relative humidity range of 40–60%, and a 12-hour light/dark cycle. Food and water were provided ad libitum. All behavioral tests were consistently performed during the light phase of the animals' 12-hour light/dark cycle, specifically between 8:00 AM and 2:00 PM, to minimize variability associated with circadian rhythms. All experiments were performed and analyzed in a blinded manner. The study design and execution were approved by the institutional ethical committee. (JnU/ERC/04/2023).

**Chronic restraint stress (CRS) procedure.** The study subjected mice to a 21-day confinement protocol, wherein they were housed in 50 mL tubes with sufficient ventilation from 8:00 AM to 11:00 AM, but without access to food or water. Despite the mice's inability to move freely or turn around, the conditions did not lead to suffocation. This protocol was designed to induce persistent stress without causing pain or physical harm.[33]

Figure 1. Chronic restraint stress procedure in mice.

**Experimental design.** Following a one-week habituation period, the 40 mice were randomly assigned to five experimental groups: Group 1, Unstressed + normal saline; Group 2, CRS (chronic restraint stress) + normal saline; Group 3, CRS + fluoxetine, a standard antidepressant; and Groups 4-5, CRS + AGE (aged garlic extract) in two different doses. Fluoxetine and AGE were administered once daily for 3 weeks, with treatment beginning on day zero of the experiment. Behavioral assessments, commencing in the fourth week of the research study, were chosen in the following sequence to minimize the influence of acute stress from one test on subsequent assessments, such as the open field test,

elevated plus maze, forced swim test, and tail suspension test. The detailed schedule of the experimental procedures is presented in figure 2.

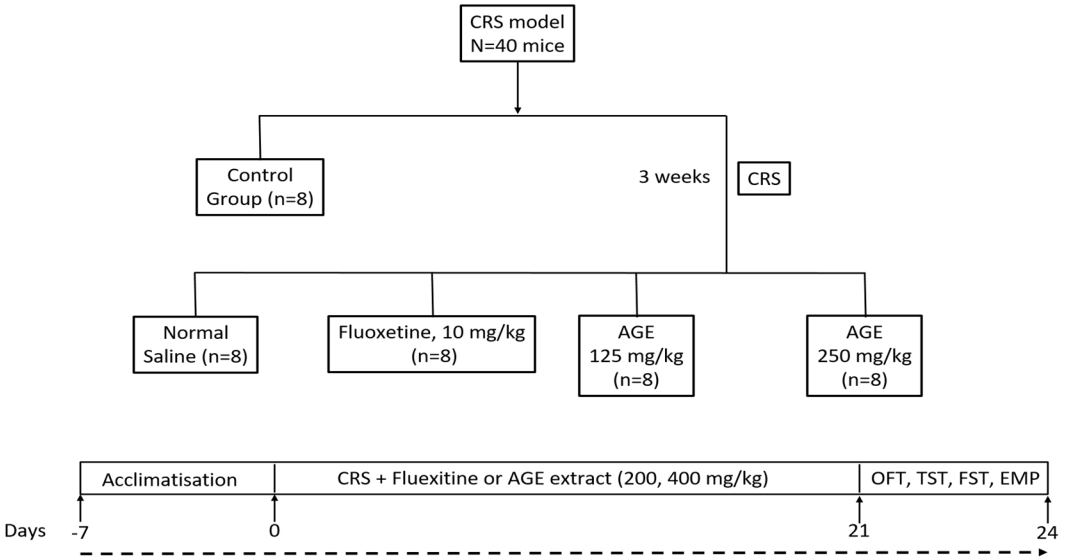


Figure 2. Experimental design for CRS induction, treatments, and behavioral tests. CRS: chronic restrain stress, Flx: Fluoxetine, AGE: Aged garlic extract, OFT: Open field test, TST: Tail suspension test, FST: Force swimming test, EMT: Elevated maze test.

***Behavioral Studies***

**Forced swimming test.** The forced swim test is one of the most widely used assessments for evaluating the antidepressant effects on depressive-like behaviors.[35] The FST involves observing the instinctive behaviors exhibited by mice when forced to swim in an inescapable cylindrical container. Before the test, the mice underwent a 15-minute pre-test acclimation session in a transparent cylinder filled with water to a depth of 30 cm. The same protocol was then repeated on the test day, with the mice being observed for a total of 5 minutes, and their activity being recorded for subsequent analysis. During the 5-minute test, the duration of immobility exhibited by the mice was measured. Immobility was defined as the absence of all movements, except for the small movements of the hind limbs necessary to keep the mouse's head above the water surface.

**Tail suspension test.** The tail suspension test is a widely used behavioral paradigm to assess antidepressant effects.[37] The test is conducted in a dark environment to minimize interference. Mice are suspended by their tails using tape, preventing them from escaping or grasping nearby objects. The test lasts approximately six minutes, and the duration of immobility, indicating escape-oriented behaviors, is measured. The mice are suspended individually in three-walled plastic compartments to prevent interaction. A video camera records the body movements, and only activities involving the hind legs are considered mobility. After each session, the suspension apparatus is thoroughly cleaned using a sterilizing solution.

**Open field test.** The locomotor activity of mice was evaluated using open field testing, as previously described.[34] The apparatus consisted of a Plexiglas square enclosure with an open top, measuring 50 cm in length, 50 cm in width, and 38 cm in height. The open field arena, representing the interior surface of the base, was subdivided into 25 smaller square units by demarcating lines. Mice were introduced to the device for 5 minutes the day prior to testing, allowing them to investigate and acclimate to the novel environment. On the test day, each mouse was individually placed in the center of the open field arena and permitted to explore for five minutes. Thigmotaxis, the degree of time spent in the peripheral zones, has been validated as a measure of

anxiety-related behavior in mice. The mice's movements were tracked and analyzed using a SMART software tool, which divided the arena into a set of 10 × 10 cm zones. As observed, the outer zone contained 16 blocks, while the inner zone had 9 blocks and was darkened. Increased thigmotaxis, reflected by spending more time in the maze's outer zones, indicates amplified anxiety-related behavior. A mouse was considered to have crossed the line when all four paws were within a new zone. To prevent the influence of odor cues, the device was cleaned with 70% ethanol in water and allowed to dry between trials.

**Elevated plus maze.** The study evaluated the level of anxiety in mice using the Elevated Plus-Maze test, which takes advantage of the innate tendency of mice to explore open environments. As previously described, the EPM apparatus was utilized for this investigation.[36] Each mouse was placed in the center of the device, and its exploratory behaviors were recorded for 5 minutes. The researchers examined various characteristics, such as the total number of entries into the open and closed arms of the apparatus, as well as the duration of time spent in each arm. The time the mice spent in the center of the device was not included in the analysis. After each trial, the maze was thoroughly cleaned with 70% ethanol and allowed to dry to eliminate any olfactory cues.

### *In silico procedures*

**Ligand and receptor preparation.** The three-dimensional crystal of the Monoamine Oxidase A enzyme was obtained from the RCSB Protein Data Bank (PDB ID: 2Z5X**).** The retrieved protein was prepared for further analysis by removing the co-factors, water molecules, and metal ions using Discovery Studio 2021 (https://www.3ds.com/products-services/biovia/ products/molecular-modeling-simulation/biovia-discovery-studio/).[38] The protein structure was stabilized through energy minimization using the SWISS PDB Viewer (https://spdbv.vital-it.ch/). Visualization of the protein and ligand 3D structures was performed using PyMOL. Concurrently, the 3D structures of the selected phytocompounds, along with their canonical SMILES, were obtained from the PubChem Compound database (https://pubchem.ncbi.nlm.nih.gov/) and converted into PDB format utilizing Open Babel software.

**Virtual screening and molecular docking.** PyRx was utilized, which provides a user-friendly interface to perform molecular docking, with AutoDockVina being one of the effective tools within PyRx. To determine the optimal binding interaction, the AutoDockVina wizard in PyRx was employed. Additionally, the BIOVIA visualizer of Discovery Studio 2021 was used to observe the binding pose of the protein-ligand complex.

**ADME analysis.** Computer-based characterization enables the observation of absorption, distribution, metabolism, and excretion properties. The *in silico* prediction of the ADME profile highlights parameters such as lipophilicity, hydrophobicity, and physicochemical properties. Following docking, our selected compounds were evaluated for their pharmacokinetic and ADME characteristics using SwissADME, an open-source web-based tool dedicated to predicting pharmacokinetics (PK) properties and the drug-likeness of small molecules.

**Toxicological properties.** Aside from evaluating absorption, distribution, metabolism, and excretion properties, it is vital to also assess the potential toxicity of a chemical compound on human organs or cells. The Protox-II online server (https://tox-new.charite.de/protox_II/index.php) was employed to determine the toxicity level of the selected compounds.[39]

**Statistical analysis.** The data were gathered and analyzed using one-way ANOVA with GraphPad Prism version 8 software. Tukey's post hoc test was employed where necessary to further examine the data; the results were presented as mean ± standard deviation, and a p-value less than 0.05 was deemed statistically significant.

## RESULTS AND DISCUSSION

The data presented in the figure shows the (mean ± SD) immobility time for the "forced swim test". The figure 3 indicates that the mean immobility time in the control group ($\mu_{Control}$ = 60) with SD = 6.14 was less than that observed in the CRS group ($\mu_{CRS}$ = 119.25) with SD = 7.13. A decrease in the mean immobility time has also been evident in the FLX ($\mu_{FLX}$ = 64), AGE 125($\mu_{AGE\ 125}$ = 91.12), AGE 250($\mu_{AGE\ 250}$ = 73.00) groups with SDs 7.09, 4.94 and 7.25 respectively compared to the CRS group ($\mu_{CRS}$ = 119.25).

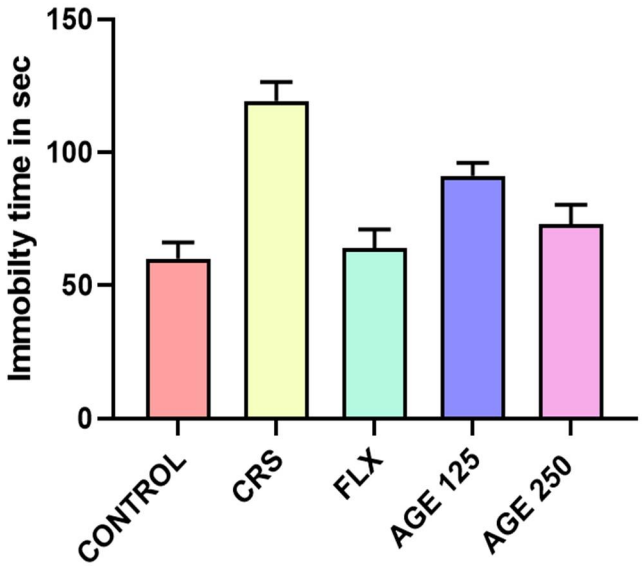


Figure 3. Average immobility time among the five groups of mice (n=8) for the forced swim test. CRS: chronic restrain stress, Flx: Fluoxetine, AGE: Aged garlic extract. Data were expressed as mean ± Standard Deviation (SD).

**Table 1. One-way ANOVA and pairwise post hoc comparisons among the five groups of mice of the forced swim test.**

| | F - statistic | P value | Multiple comparison | P value |
|---|---|---|---|---|
| One Way ANOVA | $F_{(4,35)}$ = 109.3 | 0.000* | $H_o: \mu_{Control} \geq \mu_{CRS}$<br>$H_1: \mu_{Control} < \mu_{CRS}$ | 0.000* |
| | | | $H_o: \mu_{FLX} \geq \mu_{CRS}$<br>$H_1: \mu_{FLX} < \mu_{CRS}$ | 0.000* |
| | | | $H_o: \mu_{AGE\ 125} \geq \mu_{CRS}$<br>$H_1: \mu_{AGE\ 125} < \mu_{CRS}$ | 0.000* |
| | | | $H_o: \mu_{AGE\ 250} \geq \mu_{CRS}$<br>$H_1: \mu_{AGE\ 250} < \mu_{CRS}$ | 0.000* |

*Indicates significant at 5% level of significance; $H_o$ and $H_1$ null and alternative hypothesis respectively

To determine whether there were any statistically significant differences in the mean immobility times among the five groups, a one-way ANOVA was conducted. Furthermore, post hoc Tukey's multiple comparison test was applied to examine the pairwise comparisons between the means of the CRS group and the Control, FLX, AGE 125 and AGE 250 groups. The following Table 1 presents the results of these analyses:

One-way ANOVA (F=109.3, P<0.05) analysis indicates a significant difference in the means among the five experimental groups. Furthermore, post-hoc Tukey's test confirms a significant increase in the mean mobility time for the CRS group compared to the control group (P<0.05). Additionally, a significant decrease in the mean immobility time has been observed for the FLX, AGE 125, and AGE 250 groups compared to the CRS group (P<0.05).

In the tail suspension test (TST), it has been experienced that, mean immobility time for the control group ($\mu_{\text{Control}}$ = 67.13) with SD = 7.88 was less then the CRS group ($\mu_{\text{CRS}}$ = 102.75) with SD = 6.84. Further, a plummet in the mean immobility time has been observed in FLX group ($\mu_{\text{FLX}}$ = 53.38), AGE 125 ($\mu_{\text{AGE 125}}$ = 69.25), and AGE 250 ($\mu_{\text{AGE 250}}$ = 55) with SDs 7.99, 5.65 and 6.74 respectively compared to CRS group, showed in figure 4.

To check whether the mean immobility time for TST differs significantly, one-way ANOVA has been implemented. Moreover, to determine whether the decrease in the mean immobility time was significant for the Control, FLX, AGE 125 and AGE 250 compared to CRS group, post hoc Tukey's has been implemented. The result of the One-way ANOVA and multiple comparison has been shown in the following table 2.

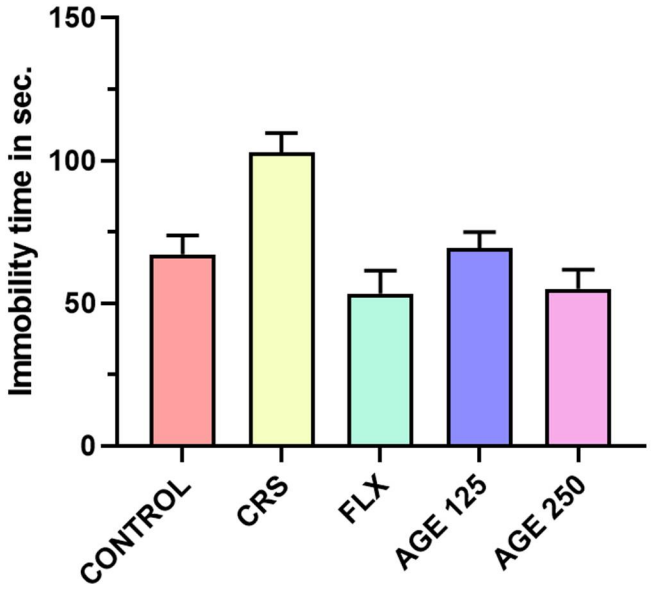


Figure 4. Average immobility time among the five groups of mice (n=8) for the tail suspension test. CRS: chronic restrain stress, Flx: Fluoxetine, AGE: Aged garlic extract. Data were expressed as mean ± Standard Deviation (SD).

**Table 2. One-way ANOVA and pairwise post hoc comparisons among the five groups of mice of the TST**

| F-statistic | | P value | Multiple comparison | P value |
|---|---|---|---|---|
| One Way ANOVA | $F_{(4,35)}$ = 63.20 | 0.000* | $H_o: \mu_{\text{Control}} \geq \mu_{\text{CRS}}$<br>$H_1: \mu_{\text{Control}} < \mu_{\text{CRS}}$ | 0.000* |
| | | | $H_o: \mu_{\text{FLX}} \geq \mu_{\text{CRS}}$<br>$H_1: \mu_{\text{FLX}} < \mu_{\text{CRS}}$ | 0.000* |
| | | | $H_o: \mu_{\text{AGE 125}} \geq \mu_{\text{CRS}}$<br>$H_1: \mu_{\text{AGE 125}} < \mu_{\text{CRS}}$ | 0.000* |
| | | | $H_o: \mu_{\text{AGE 250}} \geq \mu_{\text{CRS}}$<br>$H_1: \mu_{\text{AGE 250}} < \mu_{\text{CRS}}$ | 0.000* |

**Indicates significant at 5% level of significance; $H_o$ and $H_1$ null and alternative hypothesis respectively*

The results of the one-way ANOVA in table 2 have demonstrated a significant difference among the mean immobility time for the five group of mice ( $F_{(4,35)}$ = 63.20) at 5% level of significance (P<0.05). From the multiple comparisons, a significant decrease in the mean immobility time has been evident in the mice of Control group compared to CRS group (P<0.05). Further, a significant decrease in the mean immobility time in FLX, AGE 125 and AGE 250 group has been evident compared to the CRS group (P<0.05).

The mean time spent in the center during the OFT was calculated for the five different groups of mice. The data visualization presented in the Figure 5, indicated that the CRS group ($\mu_{CRS}$ = 40.75) with SD = 7.31 exhibited a lower mean time spent in the center compared to the other groups.

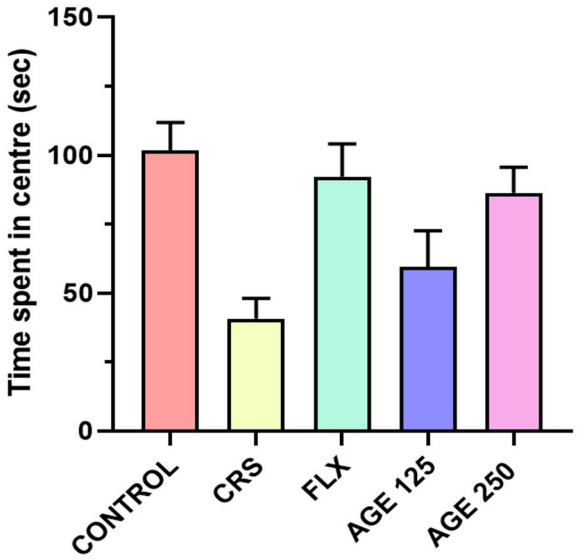


Figure 5. Average time spent in the centre of five groups of mice (n=8) for the open field test. CRS: chronic restrain stress, Flx: Fluoxetine, AGE: Aged garlic extract. Data were expressed as mean ± Standard Deviation (SD).

**Table 3. One-way ANOVA and pairwise post hoc comparisons among the five groups of mice of the open field test.**

| | F - statistic | P value | Multiple comparison | P value |
|---|---|---|---|---|
| One Way ANOVA | $F_{(4,35)}$ = 46.06 | 0.000* | $H_o: \mu_{Control} \leq \mu_{CRS}$<br>$H_1: \mu_{Control} > \mu_{CRS}$ | 0.000* |
| | | | $H_o: \mu_{FLX} \leq \mu_{CRS}$<br>$H_1: \mu_{FLX} > \mu_{CRS}$ | 0.000* |
| | | | $H_o: \mu_{AGE\ 125} \leq \mu_{CRS}$<br>$H_1: \mu_{AGE\ 125} > \mu_{CRS}$ | 0.000* |
| | | | $H_o: \mu_{AGE\ 250} \leq \mu_{CRS}$<br>$H_1: \mu_{AGE\ 250} > \mu_{CRS}$ | 0.000* |

*Indicates significant at 5% level of significance; $H_o$ and $H_1$ null and alternative hypothesis respectively

One-way ANOVA was performed to determine significant differences among the five groups, followed by Tukey's multiple comparison test to assess whether the mean time spent in the center for the CRS group was significantly lower than the Control Group ($\mu_{Control}$ = 101.75), FLX

group($\mu_{FLX}$ = 92.25), AGE 125 group ($\mu_{AGE\ 125}$ = *59.50*), and AGE 250 group ($\mu_{AGE\ 250}$ = *86.35*). The results of these statistical analyses are presented in the table 3.

The data presented in the preceding table indicates a statistically significant difference in the mean time spent in the center among the five mouse cohorts (F=46.06, P<0.05). Notably, the chronic restraint stress group exhibited a substantial reduction in mean center time relative to the control group (P <0.05). Furthermore, post-hoc analysis revealed a significant increase in the mean time spent in the center for the fluoxetine, age 125 and age 250 groups compared to the CRS group (P<0.05).

In the open field test, the average duration spent in the periphery was recorded, with the highest average time observed in the CRS group ($\mu_{CRS}$=199.25) with SD=10.11 compared to the control group ($\mu_{\text{Control}}$=138.25), FLX ($\mu_{\text{FLX}}$=147.75, AGE 125 ($\mu_{\text{AGE }125}$=180.50, AGE 250 ($\mu_{\text{AGE }250}$=153.62) with SDs 7.31, 11.9, 13.80 and 9.22 respectively as shown in Figure 6.

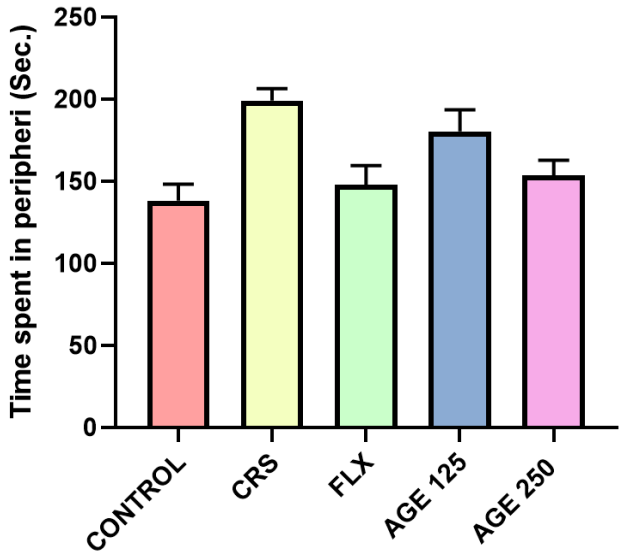


Figure 6. Average time (sec) spent in the peripheri of five groups of mice for (n=8) the open field test. CRS: chronic restrain stress, Flx: Fluoxetine, AGE: Aged garlic extract. Data were expressed as mean ± Standard Deviation (SD).

**Table 4. One-way ANOVA and pairwise post hoc comparisons among the five groups of mice of the open field test.**

| | F - statistic | P value | Multiple comparison | P value |
|---|---|---|---|---|
| One Way ANOVA | $F_{(4,35)}$ = 46.06 | 0.000* | $H_o$: $\mu_{\text{Control}} \geq \mu_{\text{CRS}}$<br>$H_1$: $\mu_{\text{Control}} < \mu_{\text{CRS}}$ | 0.000* |
| | | | $H_o$: $\mu_{\text{FLX}} \geq \mu_{\text{CRS}}$<br>$H_1$: $\mu_{\text{FLX}} < \mu_{\text{CRS}}$ | 0.000* |
| | | | $H_o$: $\mu_{\text{AGE 125}} \geq \mu_{\text{CRS}}$<br>$H_1$: $\mu_{\text{AGE 125}} < \mu_{\text{CRS}}$ | 0.017* |
| | | | $H_o$: $\mu_{\text{AGE 250}} \geq \mu_{\text{CRS}}$<br>$H_1$: $\mu_{\text{AGE 250}} < \mu_{\text{CRS}}$ | 0.000* |

*Indicates significant at 5% level of significance; $H_o$ and $H_1$ null and alternative hypothesis respectively

One-way ANOVA was implemented to determine if there were significant differences in the mean time spent in the periphery among the five groups of mice. Additionally, a multiple comparison test was applied to verify whether there were any significant decreases in the mean time spent in the

periphery for the Control, FLX, AGE 125, and AGE 250 groups compared to the CRS group. The results of these tests are presented in the following table 4.

The provided table indicates a significant difference in the average time spent in the periphery across the five mouse groups (F=48.26, p<.000). further multiple comparisons (Tukey's) analysis confirmed a substantial increase in the time spent in the periphery for the chronic restraint stress group compared to the control group (P<.000). Additionally, a significant decrease in the meantime was observed in the fluoxetine, aged 125 mg/kg, and aged 250 mg/kg groups relative to the CRS group (P<.000).

The Elevated Plus Maze test, Figure 7, demonstrated that the CRS group ($\mu_{CRS}$ = *41.25*) with SD 5.78 spent the least amount of time on the open arm compared to the Control ($\mu_{Control}$ = *99.87*), FLX ($\mu_{FLX}$ = *105.75*), AGE 125 ($\mu_{AGE\ 125}$ = *70.12*), AGE 250 ($\mu_{AGE\ 250}$ = *70.12*) with SDs 9.28, 10.61, 15.95 and 9.87 respective.

To determine if there were any significant differences in the average time spent on the open arm among the five groups, a one-way ANOVA was conducted along with multiple comparisons to assess whether the increase in average time on the open arm for the Control, FLX, AGE 125, and AGE 250 groups was significant relative to the CRS group. The findings are presented in the following Table 5.

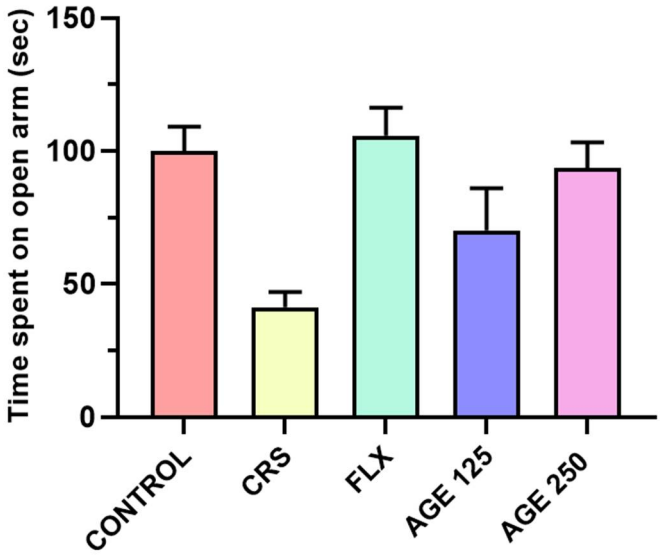


Figure 7. Average time spent on the open arm of five groups of mice (n=8) for the elevated plus maze test. CRS: chronic restrain stress, Flx: Fluoxetine, AGE: Aged garlic extract. Data were expressed as mean ± Standard Deviation (SD).

**Table 5. One-way ANOVA and pairwise post hoc comparisons among the five groups of mice of the elevated plus maze test.**

| | F - statistic | P value | Multiple comparison | P value |
|---|---|---|---|---|
| One Way ANOVA | $F_{(4,35)}$ = 48.26 | 0.000* | $H_o: \mu_{Control} \leq \mu_{CRS}$<br>$H_1: \mu_{Control} > \mu_{CRS}$ | 0.000* |
| | | | $H_o: \mu_{FLX} \leq \mu_{CRS}$<br>$H_1: \mu_{FLX} > \mu_{CRS}$ | 0.000* |
| | | | $H_o: \mu_{AGE\ 125} \leq \mu_{CRS}$<br>$H_1: \mu_{AGE\ 125} > \mu_{CRS}$ | 0.000* |
| | | | $H_o: \mu_{AGE\ 250} \leq \mu_{CRS}$<br>$H_1: \mu_{AGE\ 250} > \mu_{CRS}$ | 0.000* |

**Indicates significant at 5% level of significance; □□and □1 null and alternative hypothesis respectively*

From the above table, the one-way ANOVA analysis revealed a significant difference in the mean time spent on the open arm across the five mouse groups ( $_{(4,35)}$ = *48.26*). Furthermore, the post-hoc Tukey's test demonstrated that the chronic restraint stress group exhibited a significant reduction in open arm time compared to the control group ($p<0.05$). Conversely, the fluoxetine, aged garlic extract 125 mg/kg, and aged garlic extract 250 mg/kg groups displayed a significant increase in open arm time relative to the CRS group ($p<0.05$).

**Table 6. Molecular docking analysis of six compounds of age garlic extract with monoamine oxidase A.**

| Protein | Compounds | Binding affinity (kcal/mol) | Residues |
|---|---|---|---|
| Monoamine Oxidase A with Harmine (PDB ID: 2Z5X) | S-Allylcysteine | -5.0 | LEU A:277, TYR A:402, ILE A:273,ALA A:44, ALA A:272,ARG A:51, ARG A:45, GLY A:20, GLY A:22, GLY A:50, PRO A:274, SER A:203, GLU A:43 |
| | S-1 -propenylcysteine | -5.4 | GLY A:20, GLY A:22, GLY A:50,ALA A:44, ALA A:272, PRO A:274, SER A:403, TYR A:402,LEU A:277, ILE A:273, ARG A:45, ARG A:51, GLU A:43 |
| | s-Allylmercaptocysteine | -5.1 | ARG A:45, ARG A:51,GLY A:50, GLY A:20, GLY A:20, GLY A:434, TYR A:402,SER A:403, SER A:24, ALA A:448, ALA A:272, ILE A:273, THE A:435, TYR A:444 |
| | s-Allylmercaptoglutathione | -6.2 | ASN A:179,SER A:334, ILE A:326,ASP A:328,TYR A:175, PRO A:186, LYS A:357,GLU A:185,LEU A:176, LYS A:158, LYS A:102, GLU A:327, GLU A:329, VAL A:101, |
| | s-Allylglutathione | -6.1 | GLU A:492, GLY A:110, ASP A:132, THR A:204, THR A:205, ARG A:109, ARG A:129, TRP A:128, HIS A:488, ALA A:111, PRO A:113, PRO A:114, VAL A:115 |
| | gamma-GLUTAMYL-S-ALLYLCYSTEINE (GSAC) | -7.0 | GLY A:443, TYR A:444, MET A:350, MET A:445, GLY A:67, TYR A: 69, ALA A:68, GLN A:74,GLN A:215,VAL A:210, ILE A:207, ILE A:180, ILE A:335, LEU A:337, ASN A:181 |

**Table 7. Molecular docking analysis of moclobemide with monoamine oxidase A.**

| Compound (standard) | Protein | Binding affinity (kcal/mol) | Residues |
|---|---|---|---|
| Moclobemide | Monoamine Oxidase A with Harmine (PDB ID: 2Z5X) | -8.5 | GLY A:49, GLY A:67, GLY A:214, GLY A:22, GLY A:443, GLN A:215, SER A:24, ALA A:448, THR A:52, ARG A:51, ILE A:23, THR A:435, TYR A:407, MET A:445, ALA A:68, TYR A:69, TYR A:444 |

For the docking study, crystal structure of Monoamine Oxidase A with Harmine (PDB ID: 2Z5X) was used. The binding affinity for standard (moclobemide, a MAO-A inhibitor, is moderately effective antidepressant drug) inhibitor was -8.5 kcal/mol (Table 7). After observing the binding affinities of six compounds, three compounds were considered having maximum binding result. These results highlighted that gamma-GLUTAMYL-S-ALLYLCYSTEINE (GSAC), s-Allylmercaptoglutathione and s-Allylglutathione poses maximum docking score of -7.0 kcal/mol, -6.2 kcal/mol and -6.1 kcal/mol respectively showed in table 6. The 3D view and 2D view of protein-ligand interaction are showed in the figure 8 and figure 9. In this molecular docking study, GSAC poses the highest result. In addition, it has hydrophobic and electrostatic interactions with specific residues in the binding site of the MAO-A enzyme, namely, GLY A:443, TYR A:444, MET A:350, MET A:445, GLY A:67, TYR A: 69, ALA A:68, GLN A:74, GLN A:215, VAL A:210, ILE A:207, ILE A:180, ILE

A:335, LEU A:337, ASN A:181. On the other hand, the residues of standards (table 7) are GLY A:49, GLY A:67, GLY A:214, GLY A:22, GLY A:443, GLN A:215, SER A:24, ALA A:448, THR A:52, ARG A:51, ILE A:23, THR A:435, TYR A:407, MET A:445, ALA A:68, TYR A:69, TYR A:444. We can conclude that GSAC potentially inhibits MAO-A inhibitor from removing neurotransmitters from peripheral tissues.

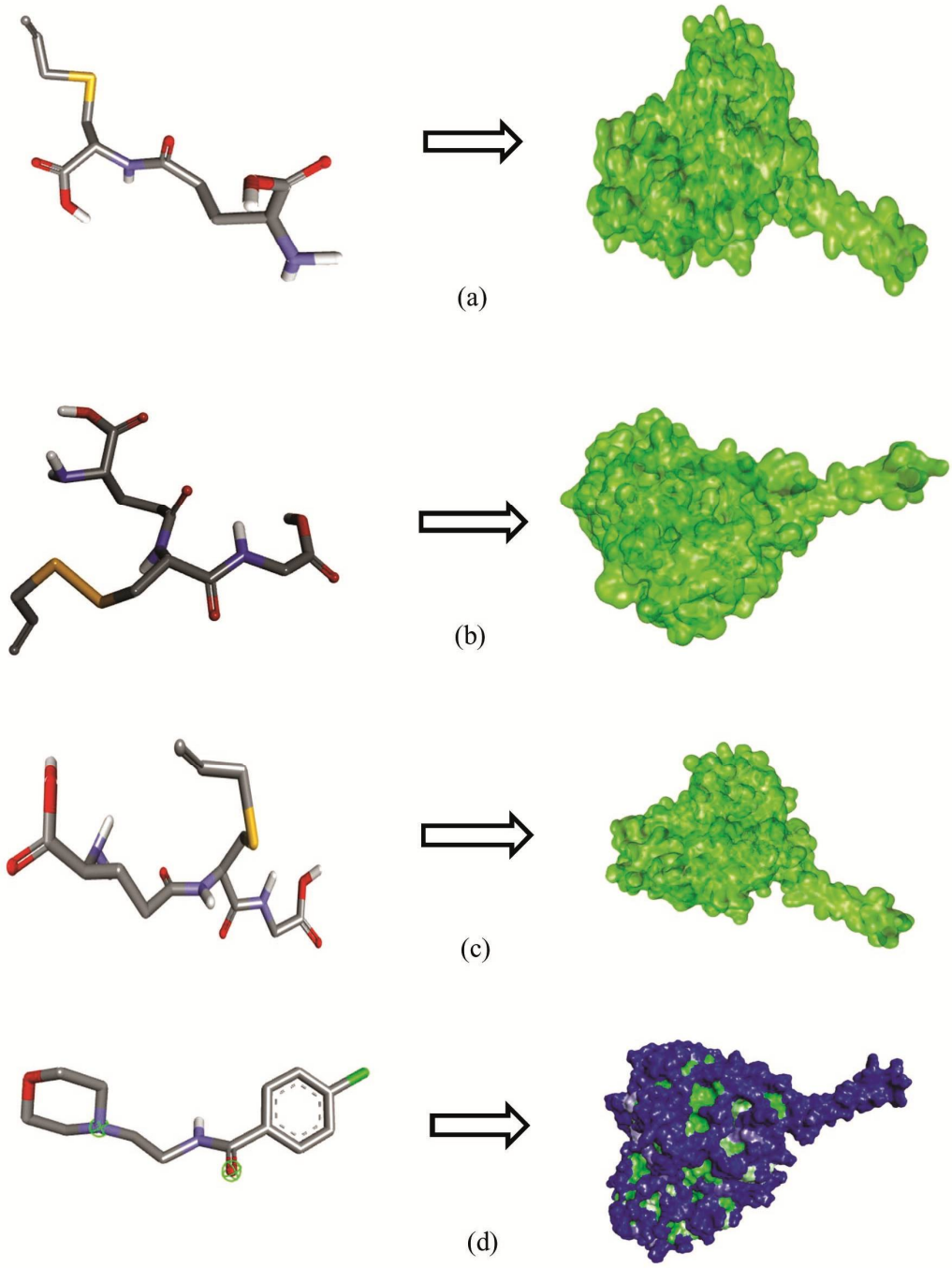


Figure 8. Surface view (3D view) of the Monoamine Oxidase A with Harmine (PDB ID: 2Z5X) showing the active site residue; a) gamma-GLUTAMYL-S-ALLYLCYSTEINE (GSAC), b) s-Allylmercaptoglutathione, c) s-Allylglutathione and d) Moclobemide (standard)

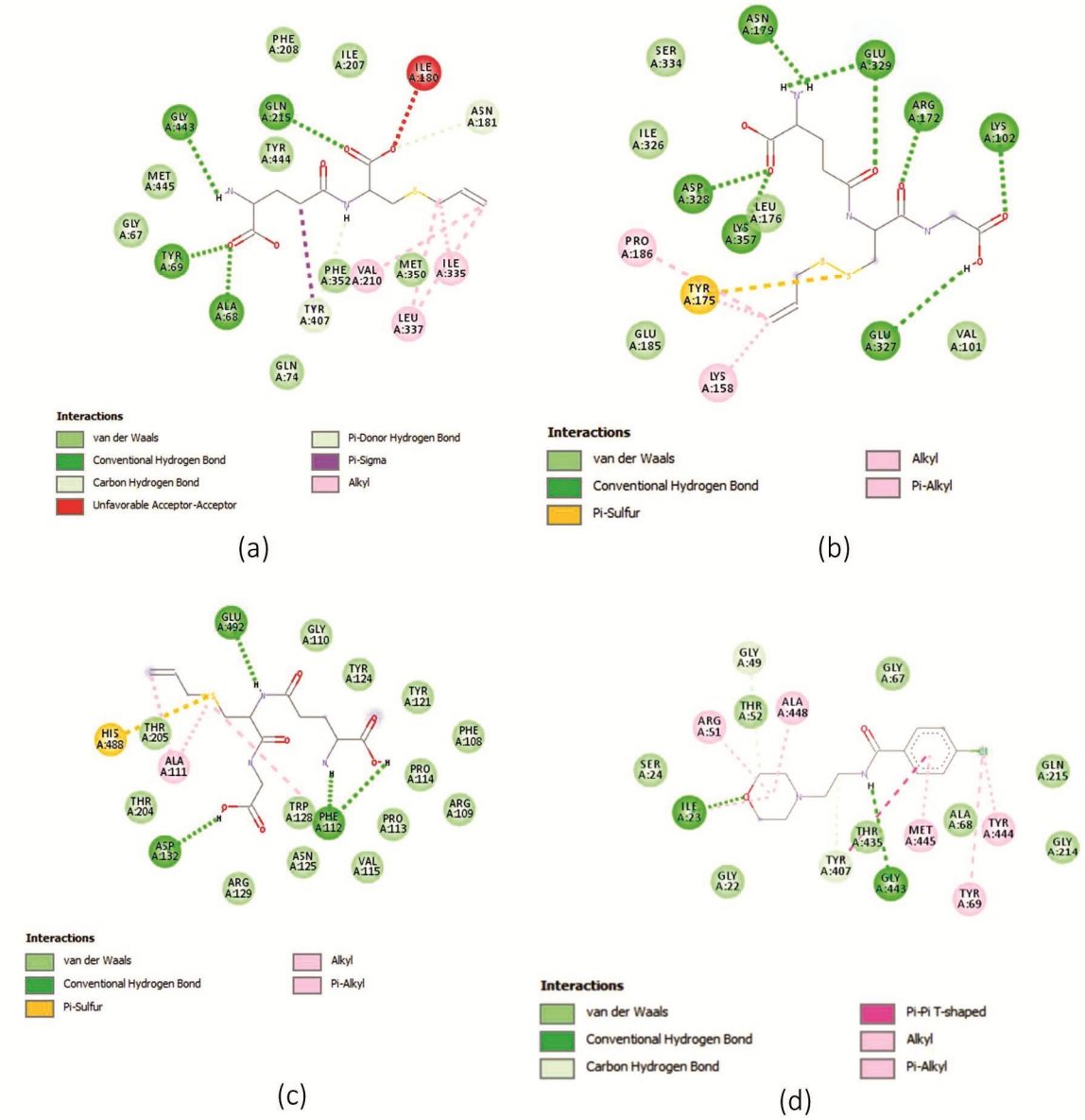


Figure 9. 2D view of the Monoamine Oxidase A with Harmine (PDB ID: 2Z5X) showing the active site residue; a) gamma-GLUTAMYL-S-ALLYLCYSTEINE (GSAC), b) s-Allylmercaptoglutathione, c) s-Allylglutathion and d) Moclobemide (standard).

**ADME properties of selected compounds of AGE.** Pharmacokinetic properties define whether the drug compound has been absorbed from the gastrointestinal tract or distributed it's target site properly. The Canonical SMILES of our compounds were used in the SwissADME website. It also checked the ADME properties including bioavailability score, some rules regarding drug likeness, BBB crossing predicted value etc shown in Tables 8 and 9.

**Toxicity predictions.** Another crucial element that must be considered while creating a novel medication to treat any illness is toxicity. Computer aided toxicity prediction is less time consuming in comparison. We have used Deep-PK server to predict toxicological properties of selected compounds include drug induced hERG (human ether-a-go-go-

related gene) inhibition. Here all of the compounds were shown to be safe for the hERG (Table 10). Here the most available compounds of aged garlic (S-allylcysteine (SAC) and S-allylmercaptocysteine (SAMC) showed no evidence of neurotoxicity though other compounds of aged garlic also showed no evidence of neurotoxicity using Protox-II Online sever (Table 11).

**Table 8. A drug likeness results of potential inhibitors of AGE**

| Ligand | Drug likeness | | |
|---|---|---|---|
| | Lipinski | Ghose | Veber |
| S-Allylcysteine | Yes; 0 Violation | Yes | Yes |
| S-1 –propenylcysteine | Yes; 0 Violation | Yes | Yes |
| s-Allylmercaptocysteine | Yes; 0 Violation | Yes | Yes |
| s-Allylmercaptoglutathione | Yes; 0 Violation | No ;1 violation: WLOGP<-0.4 | No; 2 violations: Rotors>10, TPSA>140 |
| s-Allylglutathione | Yes; 0 Violation | No; 1 violation: WLOGP<-0.4 | No; 2 violations: Rotors>10, TPSA>140 |
| gamma-GLUTAMYL-S-ALLYLCYSTEINE (GSAC) | Yes; 0 Violation | Yes | No; 2 violations: Rotors>10, TPSA>140 |

**Table 9. ADME results of the selected compounds of AGE**

| ADME Parameters | S-Allylcysteine | S-1 -propenyl-cysteine | S-Allylmercapto-cysteine | S-Allylmercapto-glutathione | S-Allylglu-tathione | Gamma-glutamyl-s-allylcysteine (GSAC) |
|---|---|---|---|---|---|---|
| Caco-2 (log paap) | -5.75 | -5.58 | -5.72 | -6.3 | -6.37 | -6.43 |
| Human Intestinal Absorption | 0.982 (Absorbed) | 0.984 (Absorbed) | 0.973 (Absorbed) | 0.475 (Non-absorbed) | 0.554 (Absorbed) | 0.925 (Absorbed) |
| Blood brain barrier (CNS) | -3.97 | -3.93 | -3.67 | -4.02 | -4.09 | -4.3 |
| Bioavailability Score | 0.55 | 0.55 | 0.55 | 0.11 | 0.11 | 0.11 |

**Table 10. Toxicological Properties of selected compounds include drug induced hERG inhibition**

| Compounds | hERG inhibition |
|---|---|
| S-Allylcysteine | Safe |
| S-1 –propenylcysteine | Safe |
| s-Allylmercaptocysteine | Safe |
| s-Allylmercaptoglutathione | Safe |
| s-Allylglutathione | Safe |
| gamma-glutamyl-s-allylcysteine (GSAC) | Safe |

**Table 11. Toxicological Profiling of the compounds through protox-II Online server**

| Compound name | Predicted LD50 | Neurotoxicity | BBB toxicity end points |
|---|---|---|---|
| S-Allylcysteine | 4000 mg/kg (Class 5) | Inactive | Active |
| S-1 -propenylcysteine | 3320 mg/kg (Class 4) | Inactive | Active |
| S-Allylmercaptocysteine | 3100 mg/kg (Class 4) | Inactive | Active |
| S-Allylmercaptoglutathione | 5000 mg/kg (Class 5) | Inactive | Active |
| S-Allylglutathione | 5000 mg/kg (Class 5) | Inactive | Active |
| gamma-glutamyl-s-allylcysteine (GSAC) | 4000 mg/kg (Class 5) | Inactive | Active |

In this study, we investigated the potential antidepressant and anxiety-like effects of aged garlic extract in a mouse model of chronic restraint stress-induced depression. Our results demonstrate that aged garlic extract significantly improved depressive-like behaviors, including immobility time in the forced swim test, tail suspension test, and time spent in the open arm in the EPM test, in mice subjected to repeated AGE treatment.

The forced swim test is a widely used behavioral assay to evaluate the efficacy of antidepressant drugs in rodents, and it has been extensively employed in the field of depression research. In the forced swim test, the rodents exposed to chronic restraint stress in this study spent significantly more time immobile in the water tank compared to the control mice. This increased immobility time is considered a surrogate marker of "behavioral despair" and is interpreted as a depression-like phenotype in these animals.[40] On the other hand, CRS-induced mice who were also given fluoxetine (10 mg/kg) or AGE (125 and 250 mg/kg) did not display depressive-like behaviors since they had a longer mobility time in the water. This suggests that these pharmacological interventions were able to rescue the detrimental behavioral effects of CRS, potentially through their antidepressant-like mechanisms of action.

The tail suspension test is considered a reliable test for evaluating the antidepressant potential of experimental drugs, as immobility in this test is reduced by a wide variety of antidepressant medications.[37,41] Rodents subjected to chronic restraint stress in this study exhibited a significantly increased duration of immobile behavior relative to the control group. This immobility has been interpreted as a state of behavioral despair or depression, which is reduced by antidepressant treatments.[41] Conversely, CRS-induced mice who were also given fluoxetine (10 mg/kg) or AGE (125 and 250 mg/kg) did not display depressive-like behaviors since they had a longer mobility time. The results indicate that the effects of AGE on depressive-like behaviors in mice were comparable to those of fluoxetine, suggesting that AGE may possess similar antidepressant-like properties.

The open field test is a well-established behavioral paradigm used to assess locomotor activity, exploratory behavior, and anxiety-like behaviors in rodents. Several studies have investigated the effects of various manipulations, such as chronic restraint stress, on open field activity in aging mice.[42,43] In the current investigation, mice exposed to 3 weeks of chronic restraint stress exhibited decreased locomotor activity compared to control mice. The results indicate that administering Flx 10 mg/kg or AGE to mice subjected to chronic restraint stress mitigated the locomotor deficits observed in these animals. Specifically, the CRS-exposed mice treated with either Flx or AGE exhibited greater time spent in the central area of the open field arena, suggesting the therapeutic interventions were effective in ameliorating the locomotor impairments induced by the chronic stress protocol.

The elevated plus maze is a widely used behavioral test for assessing anxiety-like behavior in rodents. Studies have shown that the time spent in the open arms of the maze is considered a measure of anxiety, with less time spent in the open arms indicating higher levels of anxiety.[44] Compared to the control group, mice subjected to chronic restraint stress exhibited heightened anxiety-like behavior, spending more time in the closed arms of the elevated plus maze rather than the open arms. However, the cognitive deficits observed in the chronic restraint stress-only group were mitigated in the chronic restraint stress-induced mice when co-administered with fluoxetine or with allium garlic extract at doses of 125 or 250 mg/kg.

Chronic stress and depression are two interconnected mental health conditions that have a significant impact on an individual's overall well-being and can lead to a wide range of adverse consequences for mental health, including the onset of major depression.[4,6,45] Chronic stress and depression share common underlying mechanisms, including the activation of inflammatory pathways

and the induction of oxidative stress. Oxidative stress, characterized by an imbalance between the production of reactive oxygen species and the body's ability to neutralize them, is another mechanism underlying the link between chronic stress and depression. Chronic stress can increase the production of free radicals and decrease the activity of antioxidant systems, leading to cellular damage and the disruption of normal brain function.[14] Studies have demonstrated that aged garlic extract possesses potent anti-inflammatory and antioxidant properties, which can help counteract the detrimental effects of chronic stress and depression on the brain.[16-17] The antioxidant properties of aged garlic extract, derived from its unique organosulfur compounds and flavonoids, have also been shown to be effective in reducing oxidative stress and protecting the brain from the damaging effects of free radicals. These findings suggest that aged garlic extract may be a promising therapeutic intervention for individuals suffering from chronic stress and depression, with the potential to ameliorate cognitive impairment and improve overall mental health.

Aged garlic extract has been shown to modulate the activity of the NLRP3 inflammasome, a key component of the innate immune system that is involved in the production of inflammatory cytokines. By reducing the activation of the NLRP3 inflammasome, aged garlic extract can help mitigate the neuroinflammatory response associated with chronic stress and depression.[14-15] Furthermore, aged garlic extract has been found to possess neuroprotective properties, with the ability to enhance neuroplasticity and neurogenesis, which are often impaired in individuals with depression.

The organosulfur compounds found in aged garlic extract, such as s-allyl cysteine, have been demonstrated to exert neuroprotective effects and promote the upregulation of neurotransmitters like serotonin, norepinephrine and dopamine.[11] This normalization of the neurotransmitter system may be a key mechanism by which aged garlic extract can alleviate the symptoms of depression and enhance cognitive function.[11]

Monoamine oxidase A is an enzyme responsible for the degradation of monoamine neurotransmitters, including serotonin, norepinephrine, and dopamine, within the brain. Inhibiting the activity of MAO-A can lead to increased levels of these neurotransmitters, which are recognized as playing a pivotal role in regulating mood. Many clinically validated antidepressant medications exert their therapeutic effects through this mechanism of action.

This *in silico* investigation serve primarily as predictive indicators of aged garlic extract compounds as potential inhibitors of Monoamine Oxidase A, an enzyme, involved in the degradation of neurotransmitters associated with mood regulation. The results suggest that aged garlic extract may be a promising candidate for further exploration in the development of antidepressant therapeutics. We have analyzed six compounds present in AGE and the results indicated that three of these compounds exhibited favorable binding properties. Specifically, gamma-glutamyl-s-allylcysteine (GSAC), S-Allylmercaptoglutathione and S-Allylglutathione demonstrated notable docking scores of -7.0 kcal/mol, -6.2 kcal/mol, and -6.1 kcal/mol, respectively (table 6), which is comparable to the binding affinity of a standard inhibitor was -8.5 kcal/mol. This suggests a potential interaction with biological targets relevant to antidepressant activity.

GSAC from AGE showed appreciable affinity, exhibiting binding energies of −7.0 kcal/mol. Further analysis revealed that GSAC's amino acid residues interact with specific residues in the binding site of the MAO-A enzyme (GLY A:443, TYR A:444, MET A:350, MET A:445, GLY A:67, TYR A: 69, ALA A:68, GLN A:74, GLN A:215, VAL A:210, ILE A:207, ILE A:180, ILE A:335, LEU A:337, ASN A:181) through hydrophobic and electrostatic interactions. Interestingly, the binding affinity of a standard inhibitor was -8.5 kcal/mol, with the residues being GLY A:49, GLY A:67, GLY A:214, GLY A:22, GLY A:443, GLN A:215, SER A:24, ALA A:448, THR A:52, ARG A:51, ILE A:23, THR A:435, TYR A:407, MET A:445, ALA A:68, TYR A:69, TYR A:444. This suggests that GSAC has the

potential to inhibit MAO-A, warranting further investigation.

The six compounds of aged garlic tested in this study showed no neurotoxicity activity and adhered to Lipinski's Rule of Five, indicating their potential for oral bioavailability. GSAC, which exhibited the maximum binding affinity, had a bioavailability score of 0.11 and a BBB (blood-brain barrier) value of -4.3. These values suggest that GSAC is suitable for human use and can potentially cross the blood-brain barrier to exert its effects in the brain.

In summary, the available evidence suggests that aged garlic extract and its bioactive constituents may hold promise as a therapeutic intervention for major depressive disorder by targeting the underlying pathological processes, such as dysfunctional autophagy, impaired adult hippocampal neurogenesis, and dysregulation of neurotroph.[13,46] Additionally, AGE demonstrated potential antioxidant, anti-inflammatory, and neuroprotective attributes. Further studies are needed to clarify the exact mechanisms through which aged garlic extract might influence these pathways, as well as its potential effectiveness in treating depression. Additionally, more sophisticated *in silico* analyses, including examination of hydrogen bonding, hydrophobic interactions, key active site residues, and RMSD validation, will be incorporated.

## CONCLUSION

This investigation presents compelling empirical and computational evidence bolstering the prospective application of aged garlic extract as a therapeutic modality for chronic restraint stress-induced depressive and anxiety-like phenotypes in murine subjects. The *in vivo* studies showed that the administration of aged garlic extract significantly enhanced the behavioral outcomes in chronically stressed murine subjects, as evidenced by reduced immobility durations in the forced swim test, tail suspension test and other relevant experimental paradigms. The observed behavioral enhancements may be facilitated by the antagonistic effects of compounds in aged garlic extract on monoamine oxidase, an enzyme, as demonstrated by *in silico* analysis. Furthermore, the modulation of neurotransmitter systems, the attenuation of oxidative stress and the anti-inflammatory effects of the compounds found in aged garlic extract may have contributed to the observed behavioral improvements. This study presents compelling evidence for the prospective therapeutic application of aged garlic extract in the treatment of stress-related depressive disorders. However, further research is required to fully elucidate the mechanisms underlying the observed beneficial effects and to establish the efficacy and safety of aged garlic extract in human populations.

## ACKNOWLEDGEMENTS

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## CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

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