

# Abelmoschus esculentus Ameliorates Stress-Induced Cognitive Dysfunction via Antioxidant and Neuroprotective Mechanisms in Mice

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## ABSTRACT

**Objective:** The objective of this study was to determine if there were any effect of Abelmoschus esculentus on chronic stress induced memory loss. **Methods:** The Swiss Albino Mice (n=30) were divided into five groups, namely control, chronic stress (90 days), chronic stress and ethanolic extract of Abelmoschus esculentus(200mg/kg body weight), and Chronic stress followed by diazepam (2mg/kg body weight), Chronic stress followed by diazepam (2mg/kg body weight) and ethanolic extract of abelmoschus esculentus (200mg/kg body weight). At the end of the experimental period (90 days), animal blood was collected retro-orbitally to analyze the cortisol, Brain tissue was homogenized, and antioxidants and Acetylcholine esterase level was estimated. **Results:** The Results of the data indicated that administering stress for a duration of 90 days led to a noticeable decline in spatial learning abilities in mice. Our findings indicate that combination of Abelmoschus esculentus and diazepam showed there was an significant improvement in retaining memory. **Conclusion:** Study indicated that Abelmoschus esculentus shows promise as a viable treatment option for conditions of stress related memory impairment.

**Keywords:** Abelmoschus esculentus, chronic stress, Acetylcholine esterase, superoxide dismutase, Malondialdehyde, Morris water maze.

## INTRODUCTION

Homeostasis is the body's self-regulating ability to maintain a stable internal environment, including hydration, temperature, pH, blood glucose and CO<sub>2</sub> levels, cardiac output, and waste concentration<sup>1</sup>. Various factors can disrupt this balance, such as diseases, disorders, stress, and environmental changes. Stress disrupts the body's normal physiological equilibrium by threatening homeostasis. Individuals under stress experience emotional, physiological, neuroendocrine, and psychological responses<sup>2</sup>.

Stress is becoming an increasingly common symptom as modern life speeds up. Surveys report that over half of the adult population experiences stress. The World Health Organization estimates the prevalence of anxiety at 3.6% and depression at 4.4%, with the highest rates found in adolescents, followed by females compared to males<sup>3,4</sup>.

Individuals experiencing stressful conditions often exhibit emotional, physiological, neuroendocrine, and psychological responses<sup>2</sup>. Regarding stress's impact on memory, it alters the structure and function of the hippocampus—the brain region responsible for converting short-term memory into long-term memory—leading to neurogenesis disorders and atrophy. This area also has the highest density of glucocorticoid receptors, making it particularly sensitive to stress. Chronic stress elevates plasma cortisol levels, reducing neuron numbers, dendritic branches, and neurogenesis in hippocampal tissue while altering synaptic terminal structures<sup>5</sup>. While stress can detrimentally affect memory and learning, moderate stress may

enhance the brain's information storage capacity. However, acute stress typically raises glucocorticoid levels, impairing working memory. Similarly, high cortisol from stress is linked to memory decline in men<sup>6</sup>.

The hippocampus has high levels of glucocorticoid receptors, rendering it more vulnerable to chronic stress than most other brain regions<sup>7,8</sup>. Stress-related steroids impact the hippocampus in at least three ways: first, by reducing the excitability of certain hippocampal neurons; second, by inhibiting neurogenesis in the dentate gyrus; and third, by inducing dendritic atrophy in pyramidal cells of the CA3 region. As demonstrated by Fu et al. individuals who have endured severe, prolonged traumatic stress exhibit hippocampal atrophy more prominently than in other brain areas. These changes may also contribute to hippocampal atrophy observed in schizophrenia and severe depression<sup>9</sup>. Notably, some of these effects appear reversible upon cessation of stress. Experimental studies in rats and mice by Sunanda et al. further reveal that stress adversely affects dendritic cytoarchitecture in various brain regions, including the hippocampus<sup>10</sup>.

Medicinal plants rich in flavonoids, polyphenols and polysaccharides have shown promise in mitigating cognitive deficits induced by stress or metabolic dysfunction, by restoring redox homeostasis, modulating neurotransmitter systems and improving synaptic integrity<sup>11</sup>. Among these, the edible vegetable Abelmoschus esculentus Moench (commonly "okra") is used in traditional medicine for its demulcent, anti-fatigue and digestive properties. Phytochemical investigations reveal that okra pods and seeds contain bioactive flavonoids like quercetin

derivatives, vitamins, and polysaccharides with strong antioxidant and anti-inflammatory effects<sup>12,13</sup>

It is very evident from the earlier Ayurvedic texts and clinical treatments with Ayurvedic medicines containing antioxidants improves memory. *Abelmoschous esculentus* is rich in antioxidants so it can be used as a nerve tonic in treating many of the nervous disorders. However not much experimental studies or clinical studies exist till date, that show the effect of its vegetable extract on amelioration of learning and memory impairments caused by stress. So to evaluate this the following aspects were studied neuroprotective effect in stress induced Swiss Albino Mice. The objective of our study was to observe and experimentally analyse the effect of oral intubation.

Despite this emerging evidence, direct investigation of *A. esculentus* in the context of chronic stress-induced cognitive dysfunction remains limited. Given that chronic stress triggers HPA activation, oxidative damage and cholinergic dysfunction, which are known to impair memory, it is imperative to explore whether okra extract can protect or reverse these changes. Therefore, the present study was designed to evaluate the neuroprotective and memory-restorative effects of ethanolic extract of *A. esculentus* pods in a mouse model of chronic stress, assessing behavioral performance, oxidative stress biomarkers and cholinergic enzyme activity. The aim is to clarify whether the antioxidant and neuro-protective properties of okra can mitigate stress-related cognitive decline<sup>14</sup>.

## MATERIALS AND METHODS

### Experimental Animals

This study utilized 6-week-old male Swiss albino mice weighing 18–20 g. The animals were housed in a well-ventilated facility under controlled conditions: 22°C temperature and a 12-hour light-dark cycle. They had ad libitum access to water and standard pellet diet. All experimental procedures were approved by the Institutional Animal Ethical Committee (reference no. VIMS/IAEC/2018/007).

### Preparation of Plant extract

Okra (*Abelmoschus esculentus* L.) pods were collected and only the edible ones were selected for the study. These chosen pods were then dried under shade and ground into a fine powder using a grinder. The powdered okra was subjected to solvent extraction using ethanol through the maceration technique. After extraction, the ethanol was removed by drying the extract in a rotary vacuum, resulting in dried extracts. These dried extracts were dissolved in water for further evaluation of their antioxidant activity in the brain of Swiss albino mice.

### Experimental Design

Mice were categorized into Five groups and each group contained six animals (n=6) (Figure 1).

Group 1 mice were maintained as a control group without inducing any stress and oral injection of ethanol extract.

Group 2 mice were only subjected to stress by forced swim test (FST) until animals got immobilized or sink.

Group 3 mice were orally given ethanol extract of ethanolic extract of *Abelmoschous esculentus* (200mg/kg body weight) by dissolved in sterile water followed by the induction of stress.

Group 4 mice were first subjected to stress followed by orally administered diazepam(2mg/kg body weight) prepared in sterile water.

Group 5 mice were orally administered with Diazepam (2 mg/kg body weight) and ethanolic extract of *Abelmoschous esculentus* (200mg/kg body weight) followed by induction of stress by FST.

In the study, both diazepam and the ethanol extract were orally administered to mice at a dose of 2mg and 200 mg/kg body weight daily for 90 days. Working memory and reference memory were evaluated in the experimental groups. Following the dosing period, blood was collected from all mice, which were then euthanized via cervical dislocation under anesthesia. Brains were harvested from all mice. Both blood and brain tissues were analyzed for stress biomarkers, including cortisol, acetylcholinesterase, malondialdehyde, and superoxide dismutase.

## RESULTS

### Chronic Stress Induced Memory Loss

#### *Latency to enter the target quadrant on probe trial test in MWM*

The mice in the chronic stressed group reached the target quadrant so late than the control group. However, the mice in the combination drug chronic stress reached the target quadrant quickly than all the other treated groups ( See Figure 2 to Figure 8).

### Biochemical Profile

#### *Cortisol*

After continuous exposure to stress and treatment with extract of Okra pods for 90days, blood and brains were collected from all mice i.e., Group 1 to 5, and analyzed for various biochemical parameters related to the stress and were correlated to the memory processes in mice. Organism being stressed is measured by the level of cortisol released in the brain which is important glucocorticoid in humans. Analysis of cortisol a potential biomarker for stress induction showed a significant increase in cortisol level i.e.,  $82.98\pm8.05\%$  in mice exposed to stress. However, when mice were treated with extract of *A. esculentus* L. pods followed by the exposure to stress the cortisol level was significantly reduced to  $24.63\pm1.52\%$  (group 3; p<0.001). The mice first exposed to stress and later treated with the diazepam the cortisol concentration was found to be  $34.11\pm2.32\%$  (group 4; p<0.01) (Figure 9). Diazepam and pod extract -treated mice showed a substantial decline in cortisol level ( $13.95\%$  group 5; p<0.001) that is equivalent to the control mice. The extract of *A. esculentus* L. pods effectively reduced the cortisol in mice that are treated with extract and exposed to mice and vice versa. Hence, Okra extract revealed significant anti-stress activity in stress-induced mice and would be the best choice for stress management

#### *Acetylcholine esterase*

Analysis of brain homogenate of indicated significant enzyme activity of AchE in stressed control mice i.e., 274.10 IU/L in compared to control. Mice treated with pods extract of Okra at 200mg/kg/bw and exposed to stress resulted in a substantial reduction in AchE activity (46.14 IU/L; p<0.001). A moderate level of reduction of AchE activity was observed in the mice treated with Diazepam after exposure to stress (IU/L; p<0.001). However, the highest decline in the AchE activity was noticed in mice treated with Diazepam standard followed by induction of stress (Figure 10).

#### *Superoxide dismutase*

SOD enzyme mainly detoxifies the superoxide radicals produced during oxidative stress. Hence the measurement of enzyme activity of SOD in mice indirectly signifies the exposure to stress conditions. Mice exposed to the stress showed less SOD activity in the brain homogenate in contrast to the control showing high activity of SOD. The mice treated with 200mg/kg/bw of *A. esculentus* L. extract and then exposed to stress by FST was showed excellent SOD activity (84%; p<0.01) when compared to the SOD activity of mice treated with the diazepam after induction of stress (74%; p<0.01). SOD activity of mice group treated

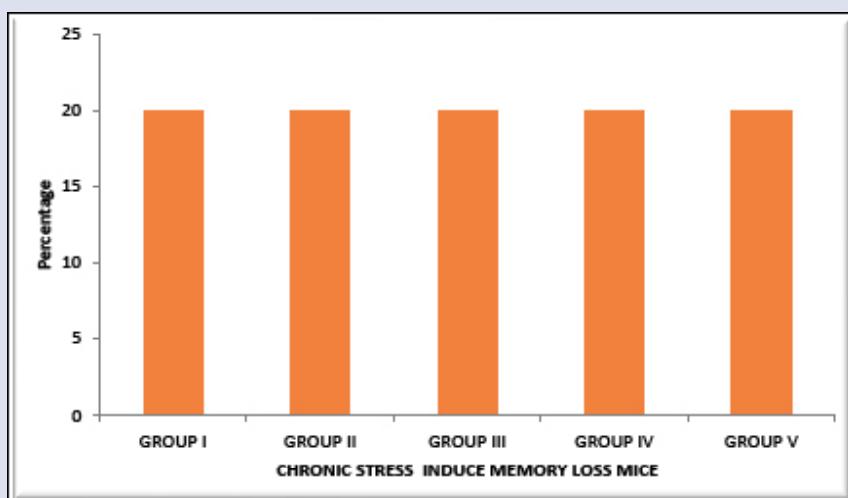


Figure 1. Chronic stress induced memory loss mice

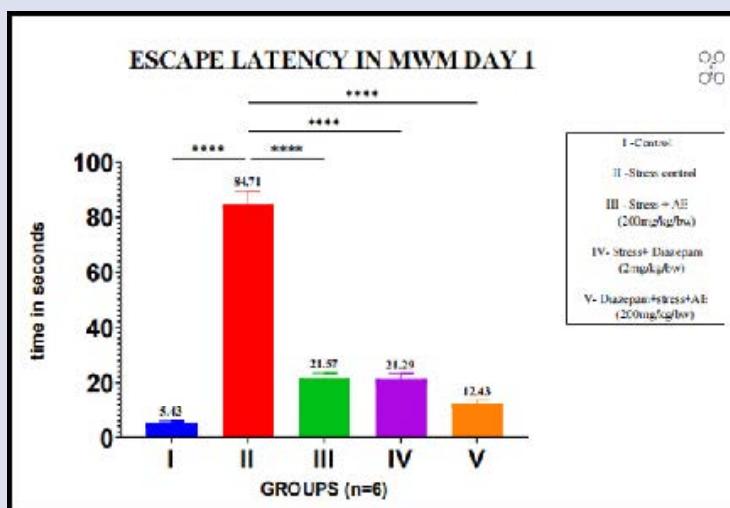


Figure 2. Chronic stress induced memory loss mice – Test for memory on day 1. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ , \*\*\*\* denotes  $P < 0.0001$ )

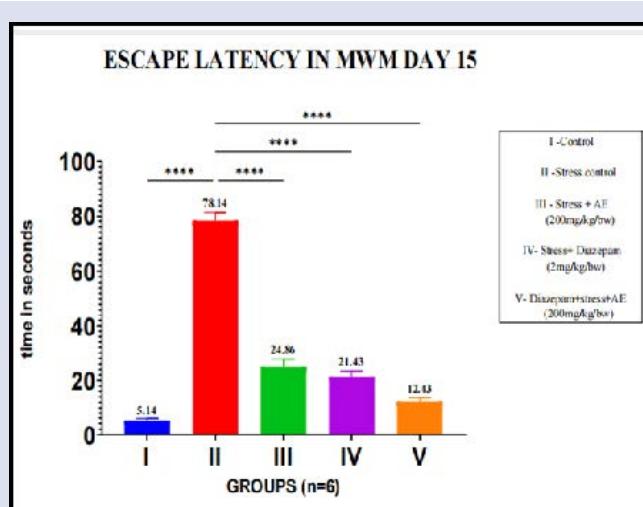
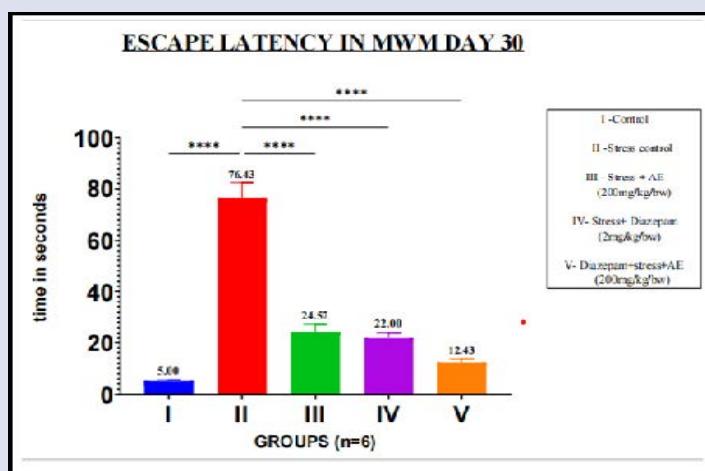
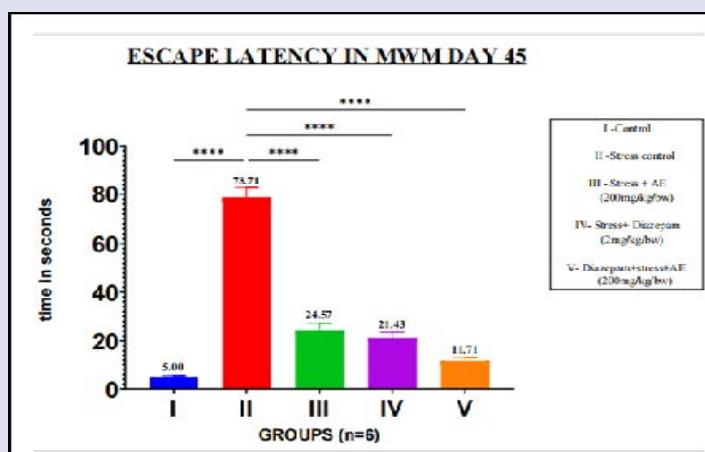


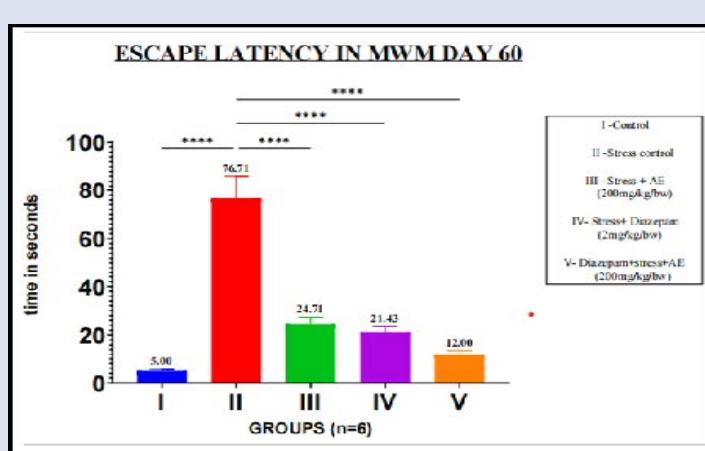
Figure 3. Chronic stress induced memory loss mice – Test for memory on day 15. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ , \*\*\*\* denotes  $P < 0.0001$ )



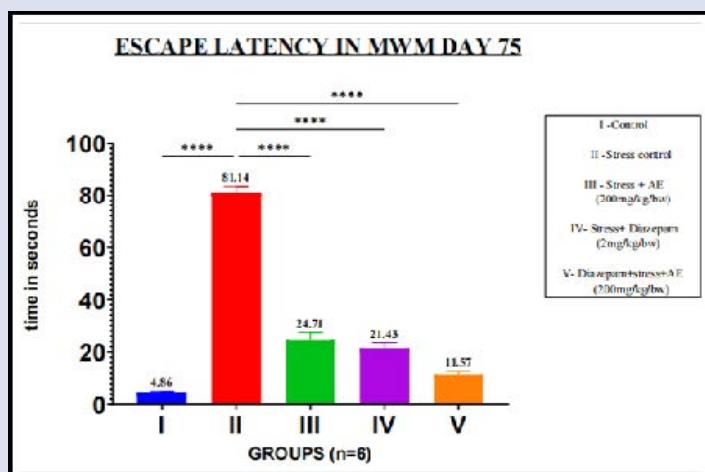
**Figure 4.** Chronic stress induced memory loss mice – Test for memory on day 30. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ , \*\*\*\* denotes  $P < 0.0001$ )



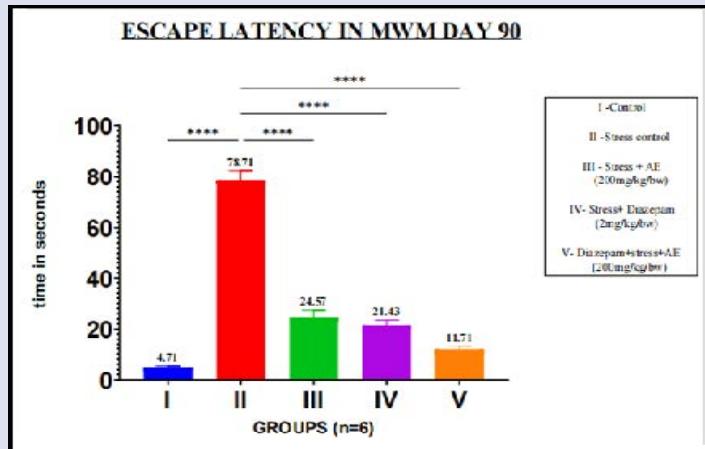
**Figure 5.** Chronic stress induced memory loss mice – Test for memory on day 45. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ , \*\*\*\* denotes  $P < 0.0001$ )



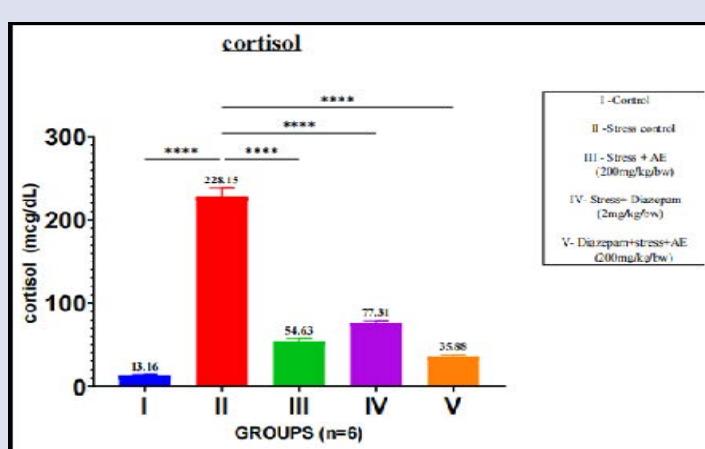
**Figure 6.** Chronic stress induced memory loss mice – Test for memory on day 60. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ , \*\*\*\* denotes  $P < 0.0001$ )



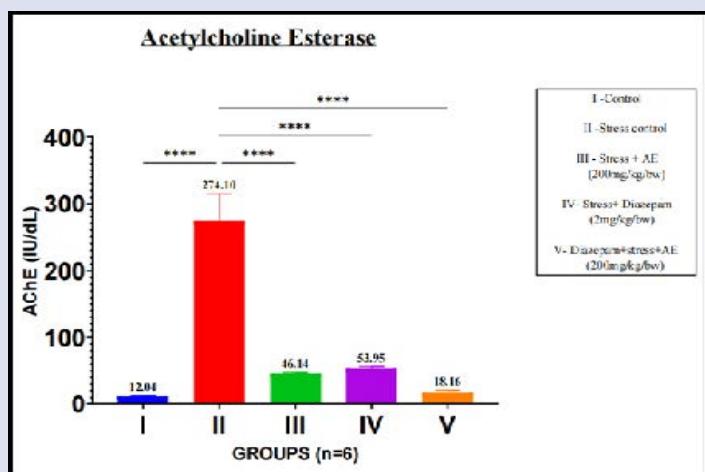
**Figure 7.** Chronic stress induced memory loss mice – Test for memory on day 75. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ , \*\*\*\* denotes  $P < 0.0001$ )



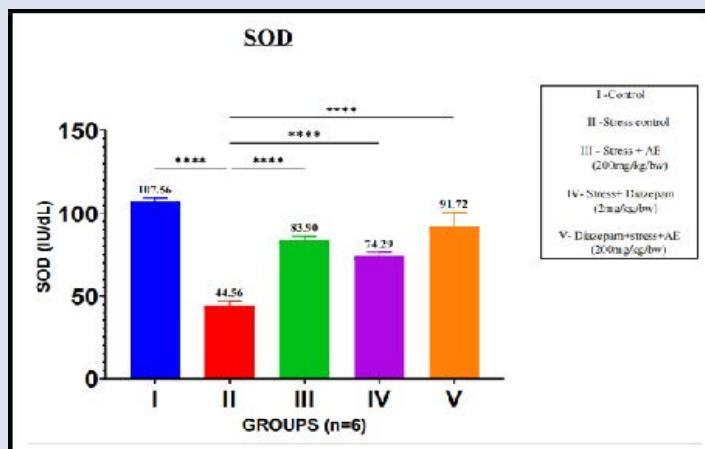
**Figure 8.** Chronic stress induced memory loss mice – Test for memory on day 90. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ , \*\*\*\* denotes  $P < 0.0001$ )



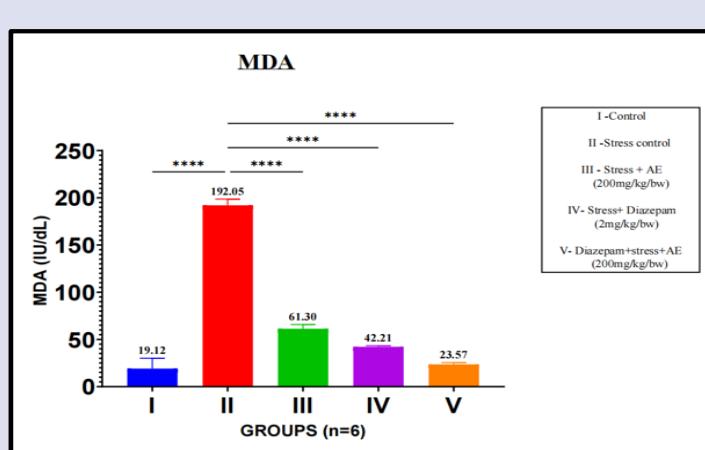
**Figure 9.** Cortisol level change in stress induced memory loss in Swiss albino mice. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ , \*\*\*\* denotes  $P < 0.0001$ )



**Figure 10.** Estimation of Acetylcholine esterase in stress induced mice. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ ,\*\*\*\* denotes  $P < 0.0001$ )



**Figure 11.** Estimation of super oxide dismutase in chronic stress induced mice. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ ,\*\*\*\* denotes  $P < 0.0001$ )



**Figure 12.** Estimation of melonyldehyde in stress induced Mice. Values are represented as + standard deviation (\* denotes  $P < 0.05$ , \*\*denotes  $P < 0.01$ , \*\*\* denotes  $P < 0.001$ ,\*\*\*\* denotes  $P < 0.0001$ )

with combination with standard drug Diazepam and extract after inducing stress was found to be normal (Figure 11).

#### *Malondialdehyde*

One of the oxidative stress biomarkers noticed in various psychological disorders and disease conditions is MDA. Therefore, the MDA concentration in tissue homogenate is an important indicator of stress. Estimation of MDA in stress control mice indicated an elevated level of MDA compared to its concentration in the vehicle control mice group. However, the mice group treated with *A. esculentus* L. extract showed the low concentration of MDA (30%;  $p<0.001$ ) specifically in animals treated before inducing stress. The concentration of MDA was slightly higher in the mice group that is treated with Standard drug, after exposure to stress. A significant reduction of MDA level was observed in mice treated with Diazepam and the extract and exposed to stress (Figure 12).

## DISCUSSION

This study provides scientific evidence supporting a close association between chronic stress and cognitive decline. Chronic exposure to stress is known to induce hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis and elevate circulating glucocorticoid levels, which can impair hippocampal structure and function<sup>15</sup>. In line with this, the stress-induced mice in our study exhibited marked deficits in learning and memory performance in the Morris Water Maze (MWM) test, confirming that persistent stress exposure leads to significant deterioration in spatial memory and learning ability.

Biochemically, the stress-induced group demonstrated a significant increase in acetylcholinesterase (AChE) and malondialdehyde (MDA) levels, together with a pronounced reduction in superoxide dismutase (SOD) activity. These changes indicate enhanced lipid peroxidation and impaired antioxidant defense, consistent with previous reports that stress elevates reactive oxygen species (ROS) generation and disrupts the redox balance in neuronal tissues<sup>16</sup>. The resulting oxidative damage compromises membrane integrity and contributes to neurodegeneration.

Treatment with alcoholic extract of *Abelmoschus esculentus* for 90 days significantly reversed these alterations by decreasing AChE and MDA levels and restoring SOD activity. The inhibition of AChE suggests enhanced cholinergic neurotransmission and improved synaptic function, thereby supporting learning and memory processes. This finding correlates with prior studies demonstrating that polyphenol-rich plant extracts enhance memory by modulating AChE activity and reducing oxidative stress<sup>17</sup>.

Stress is known to trigger ROS formation, which can attack cellular components, including membrane lipids, leading to lipid peroxidation (LPO). In the current study, increased LPO was accompanied by reduced SOD activity, an endogenous antioxidant enzyme responsible for dismutating superoxide radicals. Administration of *A. esculentus* extract significantly decreased LPO and normalized SOD activity, indicating a restoration of antioxidant homeostasis. The phytoconstituents of *A. esculentus*, such as quercetin, isoquercitrin, myricetin, and phenolic acids, have been shown to exert potent free-radical scavenging and neuroprotective properties<sup>18,19</sup>. These bioactives may stabilize neuronal membranes and improve mitochondrial efficiency, thereby mitigating oxidative injury.

The behavioral improvement observed in MWM performance further substantiates the biochemical findings. Chronic administration of *A. esculentus* enhanced spatial memory retention, comparable to the standard anxiolytic diazepam, although the underlying mechanisms differ. While diazepam exerts effects primarily through GABAergic modulation, *A. esculentus* appears to enhance cognition via antioxidant

defense and cholinergic enhancement. Similar observations have been made with other natural antioxidants that restore long-term potentiation (LTP), a neurophysiological correlate of learning, which is otherwise suppressed under chronic stress<sup>20</sup>.

Alzheimer's disease (AD), which accounts for the majority of global dementia cases, shares overlapping mechanisms with stress-induced memory loss, including oxidative stress, AChE overactivity, and neuroinflammation. Increased AChE activity and ROS production in AD lead to reduced acetylcholine availability and neuronal apoptosis. Therefore, the ability of *A. esculentus* to inhibit AChE and enhance SOD activity indicates its therapeutic potential as a natural neuroprotectant against both stress-related cognitive dysfunction and neurodegenerative diseases. The present findings provide biochemical and behavioral evidence supporting *A. esculentus* as a promising source of neuroprotective phytochemicals.

## CONCLUSION

The findings of this study demonstrate that chronic stress disrupts cognitive performance through oxidative damage and cholinergic dysfunction, as evidenced by increased acetylcholinesterase and malondialdehyde levels, reduced superoxide dismutase activity, and elevated cortisol. Treatment with the ethanolic extract of *Abelmoschus esculentus* effectively reversed these alterations, restoring redox balance and improving memory performance in mice.

The observed reduction in lipid peroxidation and enhancement of antioxidant enzyme activity indicate that the neuroprotective effects of *A. esculentus* are mediated, at least in part, by its antioxidant phytoconstituents such as flavonoids and phenolic compounds. Additionally, its anticholinesterase effect may contribute to improved neurotransmission and cognitive function under chronic stress conditions.

Overall, *Abelmoschus esculentus* shows promising potential as a natural, dietary-based therapeutic agent for preventing or managing stress-related cognitive impairment. Further mechanistic and clinical investigations are warranted to explore its molecular pathways, optimize dosage, and validate its efficacy in human subjects.

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