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MITIGATING EFFECTS OF MORINGA OLEIFERA ON STRESS INDUCED COGNITIVE DYSFUNCTION AND OXIDATIVE STRESS

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Abstract

Introduction: Chronic stress significantly impacts physiological and neurological health, causing hormonal imbalances and potentially contributing to neurodegenerative conditions. This study investigates the effects of Moringa oleifera (MO) leaves, recognized for their anti-diabetic and neuroprotective properties, on stress-induced cognitive and behavioral changes in an animal model. **Materials and Methods:** Rats were divided into five groups: control, stressed, positive control (standard treatment), Moringa-treated healthy rats (MTHR), and Moringa-treated stressed rats (MTSR). Over a 3-week treatment period, behavioral assessments and neurochemical analyses were conducted. Statistical analysis was performed to determine significance. **Results:** Stress altered acetylcholine (Ach) levels in the brain, indicating cholinergic dysfunction in stress-related behaviors. MO leaf extract supplementation normalized oxidative stress markers and Ach levels in both stressed and healthy rats. This normalization was associated with reduced anxiety and depression in MTSR and improved memory function. Chronic stress significantly increased anxiety, depression, and memory impairment in rats, accompanied by elevated malondialdehyde (MDA) levels and reduced activities of antioxidant enzymes (superoxide dismutase, catalase, glutathione). **Conclusion:** These findings highlight the therapeutic potential of MO leaves in mitigating stress-induced behavioral and neurochemical disruptions. By modulating acetylcholine levels, reducing oxidative stress, and enhancing memory function, MO supplementation shows promise for alleviating stress-related

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symptoms and preventing associated health complications. Further research in human trials is necessary to refine these insights and optimize clinical applications.

Keywords: Chronic Stress, Moringa Oleifera, Animal Model, Acetylcholine, Oxidative Stress.

INTRODUCTION

Chronic stress is a pervasive condition contributing to the onset and exacerbation of various physical and mental health disorders [1]. According to the National Center of Biotechnology Information (NCBI), the prevalence of stress is 36.5% globally [2]. In recent years, natural products have gained attention for their potential therapeutic properties in mitigating stress-related symptoms [3]. Curcumin for instance has been used as neuroprotective agent by exerting its effect on protein folding [4].

Moringa oleifera (MO), commonly known as the drumstick tree, has emerged as a promising candidate against many neurological diseases, including muscle spasm, epilepsy, nervousness, fatigue, memory impairment, and convulsion due to its rich phytochemical composition and reported adaptogenic properties [5, 6]. In recent years, MO leaves have been extensively studied due to its enormous potential as sources of functional food with therapeutic values [7, 8]. Previous studies have shown its various biological activities such as antioxidant, anti-inflammatory [9], and anxiolytic [5]. In addition, MO leaves were found useful in treating neuro-dysfunctional diseases such as Alzheimer's disease (AD), epilepsy and ischemic stroke [10-12] and also as shown in **Figure 1**. Moreover, the biological activities of MO are compiled below shown in **Figure 1**.

This figure shows the therapeutic potentials of MO leaves. Inner most circle denotes the botanical entity. The middle circle outlines various activities associated with MO. The outer most circle specifies the bioactive compound responsible for these activities. This visual representation aims to illustrate the relationship between biological activities of MO and its underlying phytochemical constituents.

In an earlier investigation, various leaf extracts were examined to evaluate the neuroprotective properties of various herbs and medicinal plants. The research found that MO leaf extract showed significant antioxidant properties with low cytotoxicity compared to other botanicals studied. The study suggested that MO extract's neuroprotective effects on SHSY5Y cells (neuroblastoma cells) may be due to its high levels of polyphenolic and other antioxidant compounds, which can effectively combat free radicals or enhance the cellular antioxidant defense system [13].

In addition to these findings, recent studies have shed light on the neuroprotective effects of MO in various experimental models. For example, MO leaf extract was found to protect against neurotoxicity induced by lead exposure in rats, suggesting its potential in mitigating environmental stressors that adversely affect brain function. Furthermore, MO extract has been shown to enhance cognitive function and alleviate oxidative stress in animal models of AD [14].

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Figure 1: Biological Effects of Moringa Oleifera

Previous research has demonstrated the diverse pharmacological activities of MO, including antioxidant, anti-inflammatory, and neuroprotective effects [15]. These properties suggest its potential efficacy in combating the deleterious effects of chronic stress on the central nervous system. It has been reported that hypoglycemic effects of MO leaves aqueous extract in hyperglycemic rats, indicating its potential in modulating metabolic pathways associated with stress response [16].

Moreover, the anti-cancer properties of MO against breast and colorectal cancer cell lines, highlighting its ability to modulate cellular processes implicated in stress-induced tumorigenesis [17]. Furthermore, researchers found that MO seeds exerted anti-inflammatory effects in an acute colitis rat model, suggesting its potential in attenuating stress-induced inflammatory responses [18].

These findings underscore the potential of MO as a multitargeted therapeutic agent against stress-related cognitive impairments and neurodegenerative disorders. Therefore, the present study aims to investigate the effect of MO extract on Chronic

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Unpredictable Mild Stress (CUMS) using a rat model. CUMS is a widely accepted experimental paradigm that simulates the unpredictable and chronic nature of stress experienced in human life, leading to behavioral and neurobiological alterations reminiscent of depressive-like symptoms [19].

Despite the growing interest in MO as a natural remedy for stress-related disorders, there is a paucity of studies specifically investigating its effects in the context of chronic unpredictable stress. Therefore, the proposed study aims to bridge this gap by elucidating the therapeutic potential of MO extract in mitigating the behavioral, biochemical and neurochemical alterations induced by CUMS.

However, this study aims to investigate the therapeutic potential of Moringa oleifera (MO) leaves in mitigating the negative effects of chronic stress on behavior and cognition. Given the physiological and neurological impacts of chronic stress, including contributions to neurodegenerative diseases, there is an urgent need for novel interventions.

With their established antidiabetic and neuroprotective properties, MO leaves present a promising natural remedy. This study examines the effects of MO leaf extract on stress-induced cognitive and behavioral alterations in an animal model, exploring its potential as a treatment for stress-related symptoms and health issues.

MATERIALS AND METHODS

Extract Preparation: First, we collected reasonable quantity of MO leaves from local plants in Karachi-Pakistan. Following evaluation, the leaves were washed, dried over several days, and subsequently powdered using an electric blender. Powdered of dried leaves were soaked in ethanol for 7 days in the room temperature. It was then filtered, with the help of Whatman's no.1 filter paper then filtrate was dried to form extract with the help of rotatory evaporator

Experimental Animal: Thirty locally bred Albino Wister male rats (150-250g) were purchased from local market which were used in study purpose. Animal were housed in cage under controlled room temperature with free access of standard rodent diet and tap water for 6 days. All experimentation was performed under the guideline procedure approved by Institutional Review Board (IRB) of Federal Urdu University, Karachi-Pakistan.

Research Protocol: The animals were grouped into five categories: control, stressed, positive control (fluoxetine), healthy rats treated with Moringa oleifera (MO), and stressed rats treated with MO. Stress in the rats was induced using unpredictable mild stressors, and MO extract was administered intraperitoneally at a dose of 250 mg/kg/ml to the MO-treated groups for three weeks, while the control groups received normal saline injections. The positive control group received fluoxetine at a dose of 20 mg/kg/ml, and the control group received normal saline.

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Throughout the study, weekly observations were made on body weight and food intake. Behavioral activities of the rats were monitored weekly post-administration of the drugs. At the end of the three-week period, the rats were euthanized, and their plasma and brain tissues were collected for biochemical and neurochemical analyses.

Behavioral Methods: After 3 weeks of drugs administration following behavior was monitored.

Open Field behavioral test: Locomotor, exploratory and ambulatory activity was monitored by open field test. In this test, we used an apparatus comprised of square zone (76 cm x 76 cm.). Length of wall is 42 cm high to prevent escape of rats from the apparatus. Square of equal sizes are drawn on the floor. To measure the activity rat was placed in the center square of the apparatus. Activity of rats was observed for five minutes by counting the numbers of square crossed.

Activity Cage Box test: Stimulatory activity was performed by using activity cage box test. Activity cage box is comprised of a square shape box. In this cage box, rats were placed for habituation and after habituation of 10 minutes the activity was evaluated for 5 min as the number of movement.

Novel Object Recognition Test (NORT): For assessment of recognition memory NORT was performed. The apparatus is made up of wooden square box and the objects discriminated are two similar solid shape squares of blue color (familiar) and a same size solid triangular shape of red color (novel object).

The test was conducted in 3 phases as habituation, training and test. On day first, animals are placed in an empty box for 10 min, this session referred as habituation. Next day rats are placed in box contained both similar objects then given ten min to rats, this is termed as training session.

On third day, in test session rats are exposed to one familiar object and other novel object, for 5 minutes. The time spends with both objects were measured on third day.

Light and Dark Box test: The light-dark box test was used to evaluate the anxiogenic and anxiolytic effects in rodents. This apparatus is partitioned into two compartments separated by a door. One compartment is brightly illuminated with transparent walls, while the other is darkened with opaque walls.

Typically, rats prefer spending more time in the dark compartment due to their natural preference for darkness. However, in novel environments, rats exhibit exploratory behavior, which can indicate anxiety levels. To initiate the experiment, each rat was placed in the light compartment, and the time spent in this area was recorded for a maximum of 5 minutes.

Forced Swim Test (FST): To observe the effects of drugs on depressive symptoms in animals, FST was used. This is a behavioral despair test. In this test, cylinder is used which is mainly made up of acrylic glass and is filled with water. Rats are introduced into the cylinder and forced to swim for 6 minutes.

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The rats are prevented from escape from the cylinder because of higher walls of the cylinder. Rats spend the time in water with swimming movement were measured for 6 minutes. The time in which rats stopped swimming considered as immobility time and with the use of antidepressant drugs this time will be decreased. To find out the immobility time, swimming time will be subtracted from the total cut off time (360 sec).

Passive Avoidance Test: The passive avoidance test was used to assess the effects of different drugs on memory in rats. This test involves a fear-conditioned response to evaluate learning and memory in rodent models of CNS disorders. The test chamber has a lit and a dark compartment separated by a gate. Animals explore both compartments initially.

The next day, they receive a mild foot shock in one compartment. Animals learn to associate the shock with that compartment. To assess memory, the animal is returned to the chamber without the shock. Rats with intact memory avoid the shocked compartment, which is measured by recording the time taken to cross the gate.

The passive avoidance test is valuable for studying the impact of new drugs on learning and memory and understanding cognitive mechanisms.

Elevated Plus Maze (EPM): To observe the anxiety like behavior in animals, EPM was used. The apparatus of EPM consists of "+" shaped maze which is elevated above the floor and consist of two open stressful, two close protecting arms and a center area. To start the experiment, placed the animals separately in the center and allowed them to explore the maze freely for 5 minutes then their behaviors are recorded. The preference of being in open arms over closed arms is calculated to measure anxiety-like behavior.

Decapitation and Blood Sample Collection: After decapitation, blood of the decapitated rodents was collected in heparinized tubes and centrifuged about 15 minutes to get plasma sample. Within 30 seconds brain was taken out and kept at low temperature (-70 °C) in Eppendorf's tubes.

Neurochemical Analysis:

Brain Acetylcholinesterase Activity: Acetylcholinesterase activity in the brain was estimated by Ellman's method using acetylthiocholine (ATC) as a substrate as previously described [20]. Reaction mixture containing brain homogenate (0.02 g/ml; 0.4 ml), 2.6 ml of phosphate buffer (0.1 M, pH 8.0), and 100 µl DTNB was mixed by bubbling air and placing it in a spectrophotometer.

Oxidative enzymes analysis: The whole brains were removed, rinsed in isotonic saline, and weighed. A 10% (w/v) tissue homogenate was prepared with 0.1 M phosphate buffer (pH 7.4) and centrifuged at 10,000×g for 10 min at 4 °C. The supernatant was used for the estimation of MDA, SOD, GSH and CAT activities as per protocol described [21].

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Statistical Analysis

Behavioral results were represented as means ± SD. Data analysis was done via one way ANOVA using SPSS. Tukey's HSD test was carried out for Post hoc evaluation P< 0.05 considered significant.

RESULTS

Effect of Moringa Oleifera on Locomotor activity in healthy and stressed rats:

Figure 2 shows the effect of *MO* extract on locomotor activity using open field test in healthy and stressed rats. Data investigated by one-way ANOVA revealed a non-significant treatment effect (F: 1.66; df:25,4; p>0.01) of MO extract on locomotor activity in healthy and stressed rats.

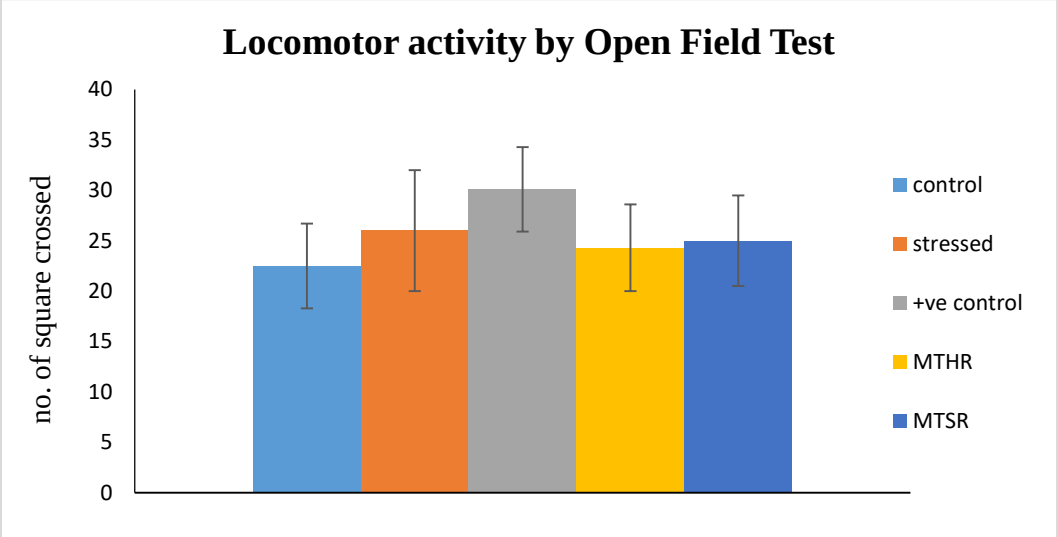

Figure 2: Locomotors activity: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a non-significant effect. (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats)

Effect of Moringa Oleifera on stimulatory activty in healthy and stressed rats

Figure 3 shows the effect of *MO* extract on stimulatory activity using activity cage apparatus in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F: 9.33, df: 25, 4, p<0.01) of MO extract on stimulatory activity in healthy and stressed rats.

Post hoc evaluation through Tukey's HSD test showed that exposure to stress rat's significantly decreased stimulatory activity in rats as compared to controls. MO administration significantly increased the stimulatory activity in MTHR and MTSR as compared to positive control and stressed rats respectively.

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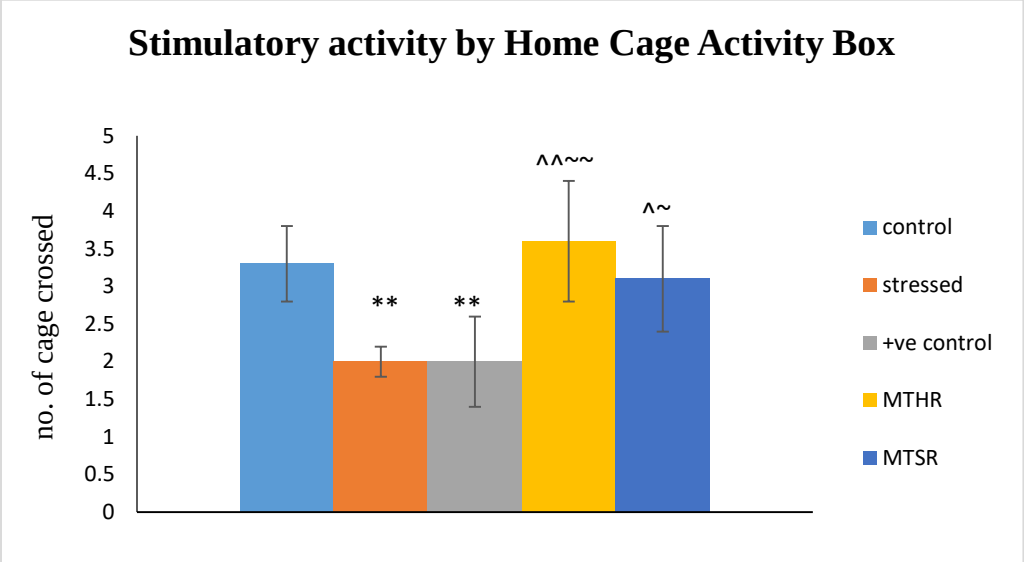

Figure 3: Stimulatory Activity: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect **p<0.01 vs Control; ^^p<0.01 ^p<0.05 vs stressed; ~~p<0.01 ~p<0.05 vs Positive control. (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats)

Effect of Moringa Oleiefera on recongnization memory in novel object recognition test in healthy and stressed rats

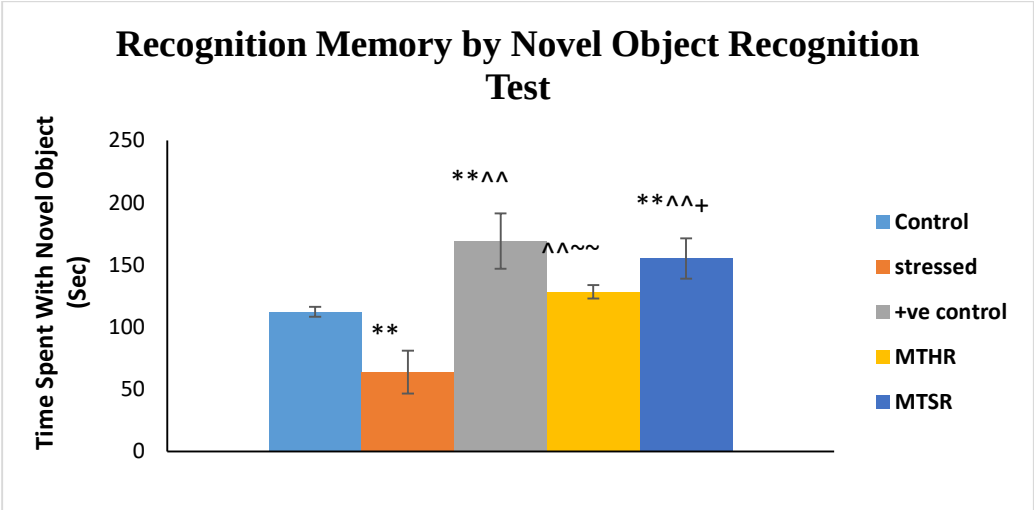

Figure 4: Memory analysis by NORT: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect, **p<0.01 vs Control; ^^p<0.01, vs stressed rats; ~~p<0.01 vs positive control; ++ p<0.01 vs MTHR (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats)

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Figure 4 shows the effect of *MO* extract on recognition memory using NORT in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F: 312.62, df: 25,4 $p<0.01$) of MO extract on recognition memory in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that MO treated healthy and stressed rats significantly enhanced recognition memory ($p<0.01$) as compared to control, stressed and positive control rats.

Effect of Moringa Oleifera on anxiety in healthy and stressed rats

Figure 5 shows the effect of *MO* extract on anxiety using EPM in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F:33.5 df:25,4 $p<0.01$) of MO extract on anxiety in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that exposure to stress significantly increased anxiety as compared to control rats ($p<0.01$). MO administration in stressed rats significantly decreased anxiety as compared to stressed and positive control ($p<0.01$). MO administration in MTHR group produced non-significant effect as compared to control.

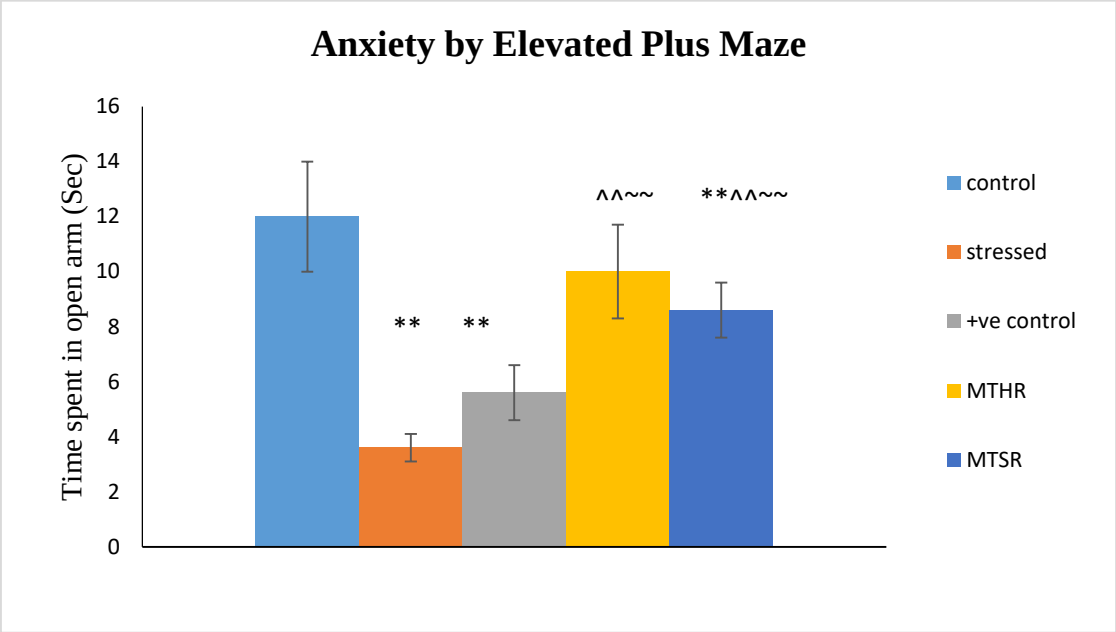

Figure 5: Anxiety analysis by EPM: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect **p<0.01 vs Control; ^^p<0.01 vs stressed rats; ~~p<0.01 vs positive control. (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats)

Effect of Moringa Oleifera on anxiety like symptoms in healthy and stressed rats

Figure 6 shows the effect of *MO* extract on anxiety like symptoms using light and dark box in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F: 74.69, df: 25, 4 $p<0.05$) of MO extract on anxiety in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that the

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exposure to stressed rats significantly increased anxiety as compared to control rats($p<0.01$). MO administration in healthy rats did not produce significant treatment effect. Whereas, MO administration significantly decreased anxiety ($p<0.01$) in MTSR as compared to stressed rats. Positive control also significantly decreased anxiety as compared to stressed rats.

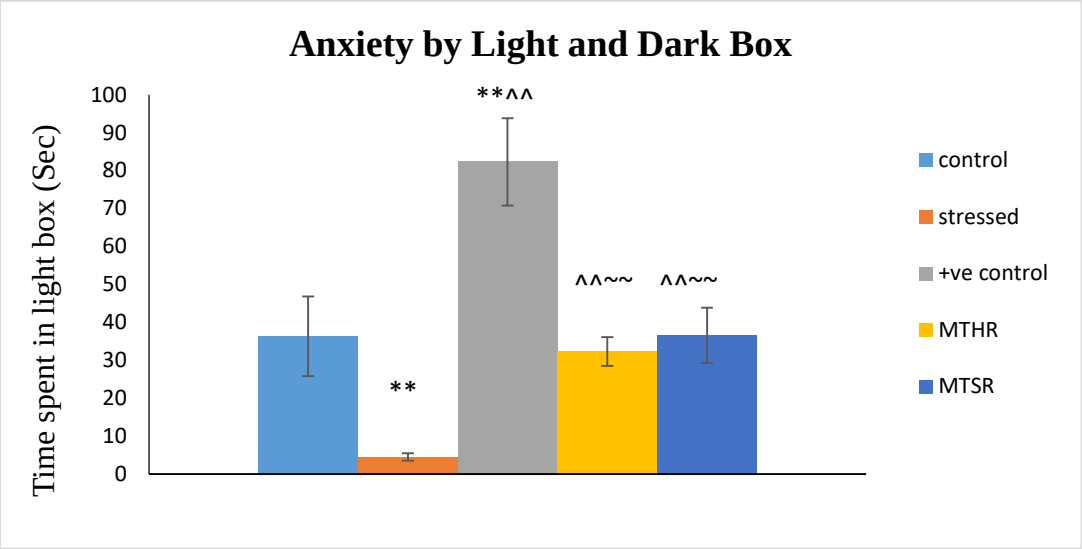

Figure 6: Anxiety analysis by Light and Dark Box: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect **p<0.01 vs ; ^^p<0.01 vs stressed rats; ~~p<0.01 vs positive control. (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats).

Effect of Moringa Oleifera on long term memory retention in healthy and stressed rats

Figure 7 shows the effect of *MO* extract on memory retention in passive avoidance apparatus in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect ($F: 68.46$ $df: 25,4$ $p<0.01$) of MO extract on memory in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that stressed rats significantly impaired memory functions as compared to controls. Positive control also significantly decreased memory function as compared to stressed rats. Moringa administration in MTHR and MTSR significantly enhanced memory functions as compared to stressed rats and positive control respectively.

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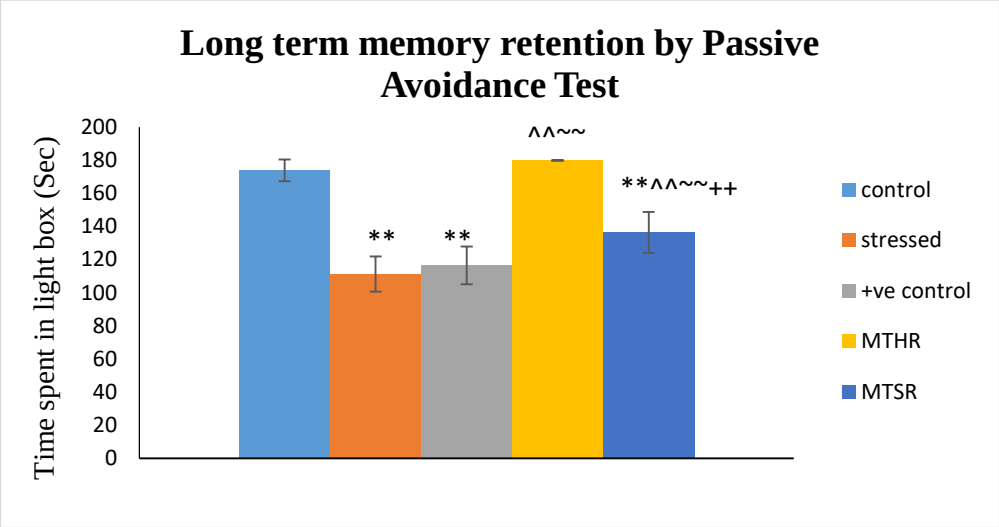

Figure 7: Long Term Memory analysis by Passive Avoidance Test: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect **p<0.01 vs Control; ^^p<0.01 vs stressed rats; ~~p<0.01 vs Positive control; ++ p<0.01 vs MTHR (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats).

Effects of Moringa Oleifera on depression like symptoms in healthy and stressed rats

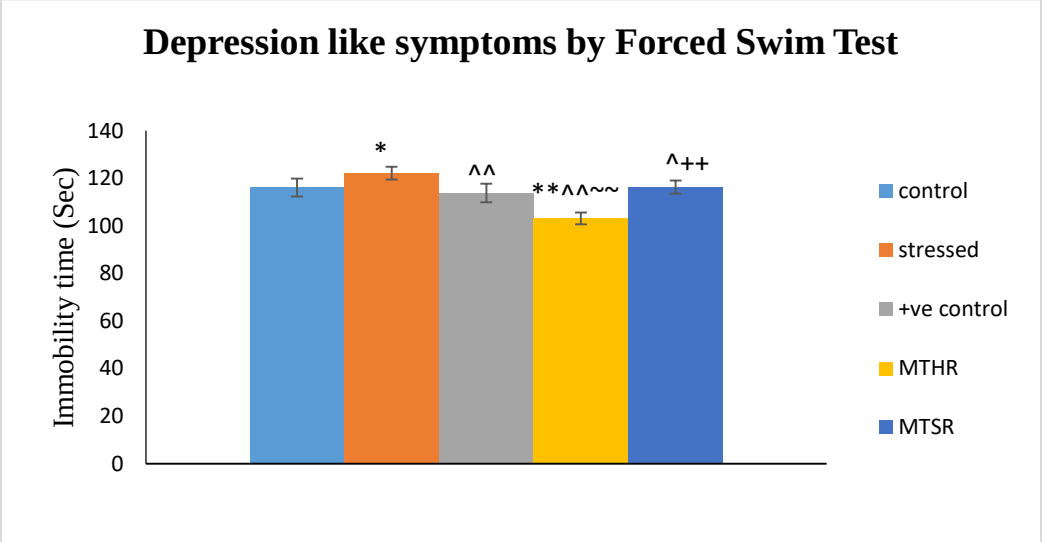

Figure 8: Depression analysis by FST: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect. *p<0.05, **p<0.01 vs Control; ^p<0.05, ^^p<0.01 vs stressed rats; ~~p<0.01 vs Positive control; ++ p<0.01 vs MTHR (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats).

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Figure 8 shows the effect of *MO* extract on depression like symptoms using forced swim test in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F: 28.08, df: 25, 4 $p<0.01$) of MO extract on depression like symptoms in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that exposure to stress significantly increased depression like symptoms. MO extract administration in stressed rats significantly decreased depression like symptoms as compared to stressed ($p<0.05$) and positive control ($p<0.01$). MO administration in MTHR also significantly decreased depression like symptoms ($p<0.01$) as compare to control, stressed and positive control.

Neurochemical Analyses

Effects of Moringa Oleifera on catalase activity in healthy and stressed rats

Figure 9 shows the effect of *MO* extract on catalase activity in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F: 44.55, df: 25, 4 $p<0.01$) of MO extract on catalase activity in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that exposure to stress significantly decreased catalase activity. MO extract administration in stressed and healthy rats significantly increased ($p<0.01$) catalase activity as compared to stress. Positive control also significantly increased ($p<0.01$) catalase activity as compared to stressed.

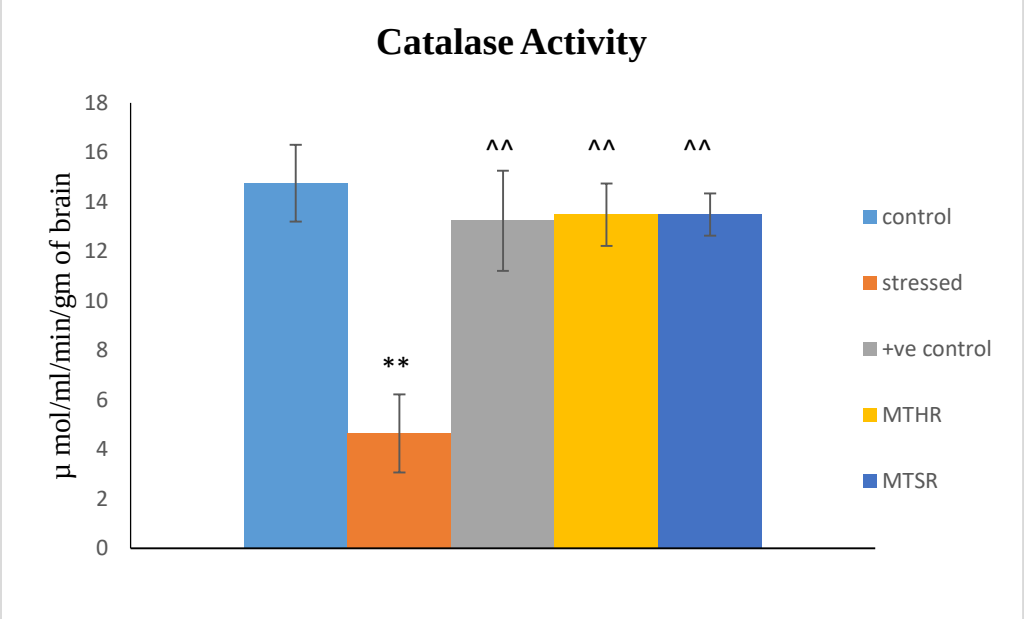

Figure 9: Neurochemical Analysis by Catalase Activity: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect. **p<0.01 vs Control; ^^p<0.01 vs stressed rats. (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats).

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Effects of Moringa Oleifera on glutathione reductase activity in healthy and stressed rats

Figure 10 shows the effect of *MO* extract on glutathione reductase activity in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F: 15.35, df: 25,4, p<0.01) of MO extract on glutathione reductase activity in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that exposure to stress significantly decreased glutathione reductase activity.

MO extract administration in stressed and healthy rats significantly increased (p<0.01) glutathione reductase activity as compared to stress. Whereas positive control did not produce any significant effect on glutathione reductase activity as compared to stressed.

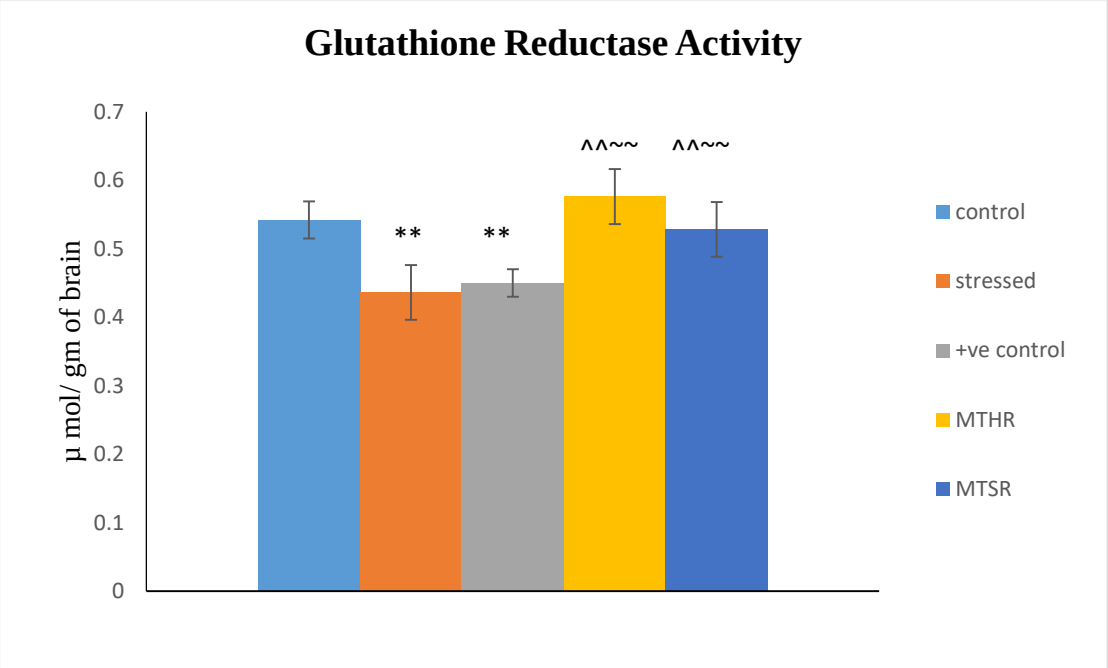

Figure 10: Neurochemical analysis by Glutathione Reductase Activity: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect. **p<0.01 vs Control; ^^p<0.01 vs stressed; ~~p<0.01 vs positive control. (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats).

Effects of Moringa Oleifera on sod (superoxide dismutase) inhibition in healthy and stressed rats

Figure 11 shows the effect of *MO* extract on SOD inhibition in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F: 8.85, df: 25,4, p<0.01) of MO extract on SOD inhibition in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that stress significantly increased SOD inhibition.

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MO extract administration in MTHR ($p<0.01$) and MTSR ($p<0.05$) group significantly decreased SOD inhibition as compared to stressed group respectively. Whereas positive control did not produce any significant effect on SOD inhibition as compared to stressed.

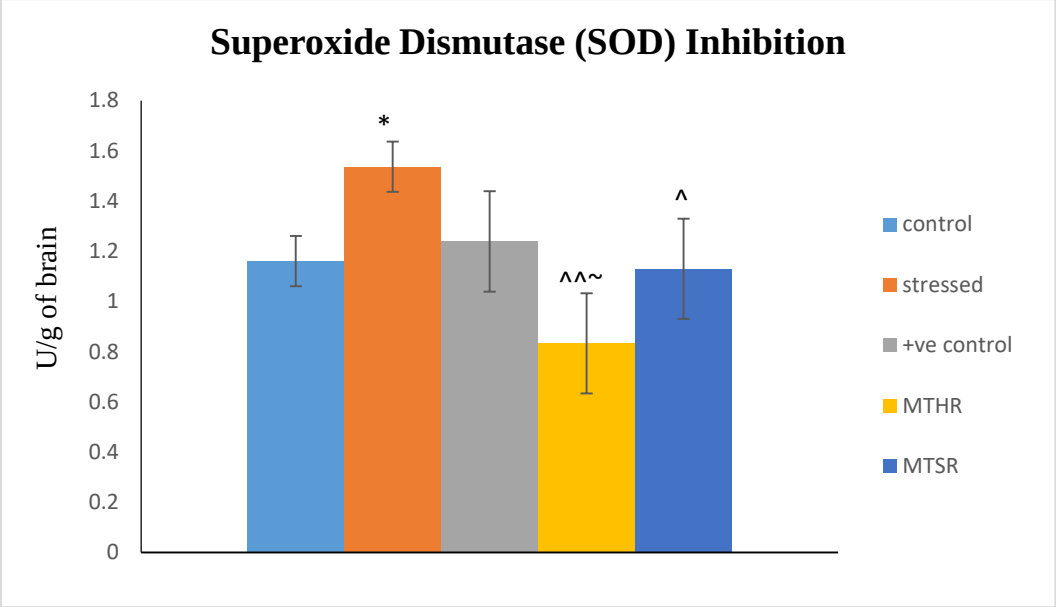

Figure 11: Neurochemical analysis by SOD Inhibition: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect. *p<0.05 vs Control; ^p<0.05, ^^p<0.01 vs stressed; ~p<0.05 vs positive control. (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats).

Effects of Moringa Oleifera on aectyl choline level in healthy and stressed rats

Figure 12 shows the effect of *MO* extract on acetyl choline level in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F: 21.55, df: 25,4, $p<0.01$) of MO extract on acetyl choline level in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that stressed rats significantly increased acetyl choline levels as compared to control.

MO extract administration in MTHR ($p<0.01$) significantly increased acetyl choline level as compared to control but decreased as compared to stressed. Whereas MO extract administration in MTSR group significantly decreased ($p<0.01$) acetyl choline level as compared to stressed and increased ($p<0.01$) as compared to positive control. Positive control also significantly ($p<0.01$) decreased acetyl choline level as compared to stressed.

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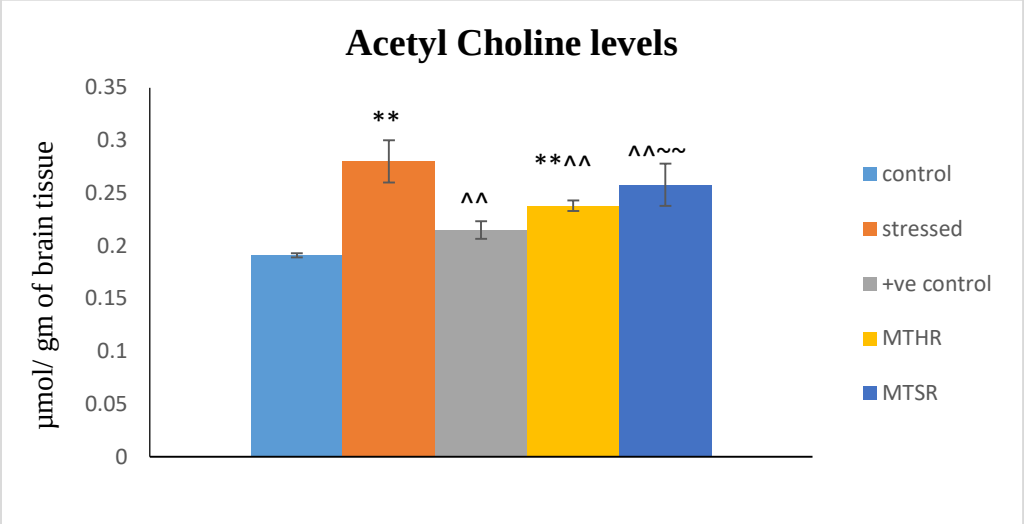

Figure 12: Neurochemical analysis by Acetyl Choline levels: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect. *p<0.05, **p<0.01 vs Control; ^^p<0.01 vs stressed; ~~p<0.01 vs positive control; +p<0.05 vs MTHR. (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats).

Effects of Moringa Oleifera on MDA (Malondialdehyde) concentration in healthy and stressed rats

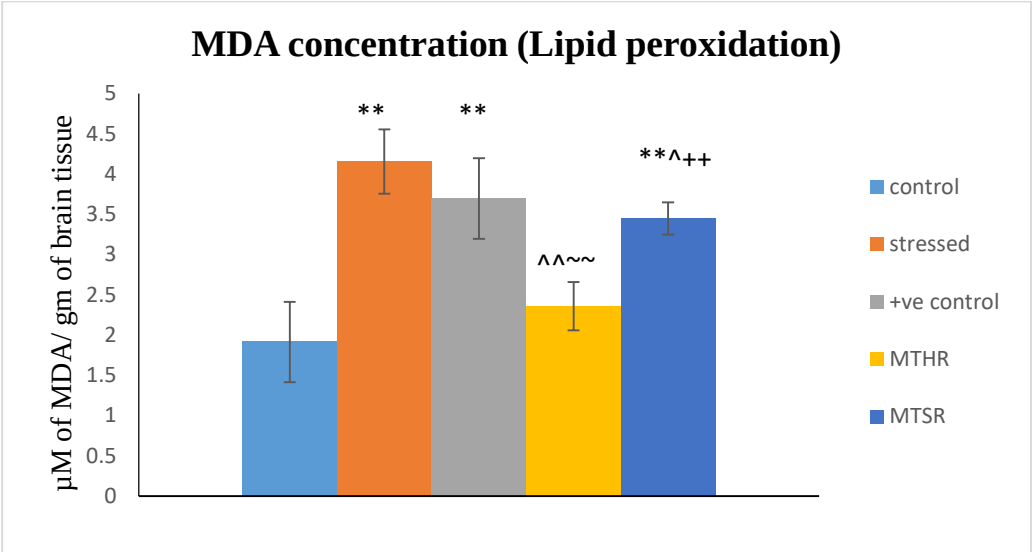

Figure 13: Neurochemical analysis by MDA: This figure shows all the values are presented in mean± SD. Data analyzed by one way ANOVA revealed a significant effect. **p<0.01 vs Control; ^^p<0.01, ^p<0.05 vs stressed; ~~p<0.01 vs +ve control; ++p<0.01 vs MTHR. (MTHR=moringa treated healthy rats, MTSR=moringa treated stressed rats).

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Figure 13 shows the effect of *MO* extract on MDA concentration in healthy and stressed rats. Data investigated by one way ANOVA revealed a significant treatment effect (F:2 4.64, df: 25,4, $p<0.01$) of MO extract on MDA concentration in healthy and stressed rats. Post hoc evaluation through Tukey's HSD test showed that stressed rats significantly increased MDA concentration as compared to control. MO extract administration in MTSR group significantly decreased ($p<0.05$) MDA concentration as compared stressed rats. Positive control didn't show any significant effect on MDA concentration in stressed rats.

DISCUSSION

Chronic stress impacts physiological and neurological health, leading to hormonal imbalances and potential neurodegenerative diseases. This underscores the need for new therapies. MO leaves, known for antidiabetic and neuroprotective benefits, show promise. Our study examined MO leaves extract on stress-induced cognitive and behavioral changes in an animal model. Acetylcholine emerges as a pivotal neurotransmitter implicated in stress-related behaviors, anxiety, depression, and memory functions [22, 23]. The intricate balance of acetylcholine signaling across different brain regions is essential for maintaining healthy behavior and proper functioning of the nervous system. In the context of stress, acetylcholine has been implicated in mediating anxiety- and depression-like behaviors. Our study observed elevated levels of acetylcholine in the brains of stressed rats, indicating dysregulation of cholinergic signaling in response to chronic stress. Treatment with MO leaves extract resulted in reduced acetylcholine levels in stressed rats, potentially alleviating symptoms of anxiety and depression associated with stress-induced alterations in acetylcholine levels.

Conversely, administration of MO leaves to healthy rats led to increased acetylcholine levels compared to untreated healthy rats. In the current study we also observed a significant reduction in anxiety and anxiety-like symptoms in Moringa Treated Stress Rats (MTSR) suggesting a potential anxiolytic and antidepressant effect of Moringa in stress. The similar study suggested that MO leaves extract has an anxiolytic-like effect in mice submitted to the elevated plus maze test. MO leaves also presents an antidepressant-like action in mice and is a promising herbal medicine for treating anxiety and depression disorders [24]. The methanolic and hexanic extracts (500 mg/kg) from MO leaves had an anxiolytic-like effect in mice [5]. In a separate study, Mahmoud et al. (2022) investigated the preventive administration of an ethanolic extract from MO leaves at a dose of 400 mg/kg/day orally for 14 days. They demonstrated neuroprotective effects against carbon tetrachloride-induced neurotoxicity, attributing these benefits to the extract's antioxidant and anti-inflammatory properties. Furthermore, they noted significant improvements in anxiety and depression conditions, indicating a broader therapeutic potential of MO leaf extracts beyond neuroprotection [25]. Acetylcholine, with basal forebrain cholinergic neurons (BFCNs) playing a key role in the hippocampus, is essential for memory formation [23]. Chronic stress disrupts cholinergic signaling, leading to impaired memory functions and potentially contributing to the development of AD [26]. In our study, stressed rats exhibited cognitive deficits and altered acetylcholine levels. In the current study

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supplementation with MO leaf extract significantly improved novel object recognition memory and long-term memory retention in stressed rats. This is possibly due the effect of MO on acetylcholine restoration mechanism. It was previously reported that MO leaves extract is used to treat dementia and improve spatial memory. The extracts have been shown to decrease the acetylcholine esterase (AChE) activity, thereby improving cholinergic function and cognition [27]. In addition, the richness of fatty acid, benzene, fatty aldehydes, terpenoids, tocopherol, and stigmasterol in MO could explain the neuroprotective effect of this herb [10]. Researchers suggested that MO leaf extract has beneficial effect on memory [28].

Chronic stress induces oxidative stress, characterized by elevated levels of reactive oxygen species (ROS) and impaired antioxidant defense mechanisms, contributing to depression and cognitive deficits such as poor memory and learning behavior [29, 30]. The current study results revealed a clear oxidative imbalance, evidenced by a significant increase in malondialdehyde (MDA) and inhibition of superoxide dismutase (SOD), alongside a decrease in the antioxidant activities of catalase (CAT) and glutathione reductase (GSH) in the stressed group. However, administration of Moringa oleifera (MO) leaf extract effectively mitigated these effects, restoring antioxidant enzyme activity to normal levels. Researchers confirmed the association between increased oxidative stress and depression, noting that chronic stress disrupts brain oxidative balance by elevating ROS production, significantly impacting the limbic system [31]. SOD is a crucial antioxidant enzyme that neutralizes harmful superoxide radicals by converting them into hydrogen peroxide and oxygen, thus preventing cell damage [32]. GSH is another vital antioxidant that eliminates hydrogen peroxide produced by SOD [33]. Reduced levels of SOD were observed in the hippocampus of stressed animals [31]. Furthermore, it has also been reported that chronic stress reduces overall antioxidant capacity, GSH levels, and SOD activity [34].

The brain's sensitivity to oxidative stress and the accumulation of damaged molecules can contribute to memory impairments, which are mitigated by antioxidants balancing ROS, superoxide ($O2^{-\bullet}$), and hydrogen peroxide ($H2O2$) [35, 36]. Consistent with previous research on transgenic mice, the overexpression of SOD and CAT significantly reduced markers of oxidative stress [37]. Our study found that in stressed rats treated with Moringa, MDA levels decreased while the activities of CAT and GSH increased compared to untreated stressed rats. This reduction in MDA and increase in antioxidant enzyme activity could be a contributing factor to improved memory and reduced depression. Additionally, the inhibition of SOD was lower in Moringa-treated stressed rats and healthy rats, which also aids in managing memory and depression. These findings suggest that Moringa reduces oxidative stress through regulation of antioxidant enzymes and assists in stress management. The antioxidant capacity of MO was also studied previously and it was shown that MO leave extract reduces the markers of oxidative stress and helps to maintain the inflammation in an animal model [38].

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CONCLUSION

The study concluded that Moringa leaves may be therapeutically beneficial in mitigating the adverse effects of chronic stress on behavioral and cognitive processes. Moringa supplementation has the potential to treat stress-related symptoms and prevent associated health issues by modulating acetylcholine levels, reducing oxidative stress, and enhancing memory functions. The study advocates for further investigation of these mechanisms in human trials to refine clinical applications and treatment strategies.

Data availability statement

The data that support the findings of this study are not openly available due to reasons of sensitivity and are available from the corresponding author upon reasonable request.

Funding Statement

No Funds have been received.

Conflict of interest disclosure

Authors declare no conflict of interest.

Ethics Approval Statement

This study has been approved by the ethical committee Federal Urdu University of Arts, Sciences and Technology, Karachi, Pakistan.

Permission to reproduce material from other sources

Not Applicable.

Author Contributions Statement

Conception and design of the study: SK, SA, and TR. Acquisition of data, analysis, and interpretation of data: TR, MW, and SA. Drafting the article: TR, and MW. Revising the article critically for important intellectual content: SK, SA, TR, MW, HSMS, and MS. Final approval of the version to be submitted: SK, SA, and MW. All authors contributed equally and have read and agreed to the published version of the manuscript.

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