ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 16, Issue 5, 2023

Online - 2455-3891
Print - 0974-2441

Research Article

NEUROPROTECTIVE EFFECT OF *ABELMOSCHUS ESCULENTUS* ON CHRONIC STRESS AND EVALUATION OF LOSS OF MEMORY, ADAPTOGENIC EFFECT

SHABINA KOMATH CHENOLY[1], SHANKARAPPA C[1], VENKATA BHARATKUMAR PINNELLI[2]

[1]Department of Physiology, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru, Karnataka, India. [2]Department of Biochemistry, Vydehi Institute of Medical Sciences and Research Centre, Bengaluru, Karnataka, India. Email: shabina.chenoly@gmail.com

Received: 23 November 2022, Revised and Accepted: 20 January 2023

ABSTRACT

Objective: Objective of this study was to determine if there was any neuroprotective effect of *Abelmoschus esculentus* L and its role in preventing memory loss during stressful conditions.

Methods: The powder of *A. esculentus* L. pods was extracted with methanol and was used for evaluating anti-stress activity in experimental mice groups. The five experimental mice groups, *namely,* control, stress control, animals treated with extract followed by exposure to stress, animals exposed to stress followed by extract treatment, and mice groups treated with diazepam was evaluated. Biomarkers included were cortisol, brain homogenate acetylcholine esterase (AchE), superoxide dismutase (SOD), and malondialdehyde (MDA). In conjugation, working memory and reference memory were also studied in all animal groups by radial arm maze test, and results were recorded as the percentage of alteration score (PAS).

Results: The concentration of stress indicators such as cortisol, MDA, and AchE activity was significantly elevated in stress control animals and associated with deficit working and reference memory. However, SOD was reduced in stressed mice and increased in treatment groups compared to the control mice. The anti-stress activity of *A. esculentus* L. pods was significantly correlated with higher working memory and reference memory with 1.33±0.51 and 1.17±0.40 PAS in pre-stress and post-stress treated mice groups, respectively.

Conclusion: Methanolic extract of *A. esculentus* L. pods revealed the excellent anti-stress potential and also played a significant role in enhancing both working memory and reference memory in mice.

Keywords: *Abelmoschus esculentus* L. pods, Anti-stress activity, Cortisol, Acetylcholine esterase, Radial arm maze, Working memory, Reference memory.

 DOI: http://dx.doi.org/10.22159/ajpcr.2023v16i5.46886. Journal homepage: https://innovareacademics.in/journals/index.php/ajpcr

INTRODUCTION

The self-regulated propensity of the body to retain the constant internal environment concerning to hydration, temperature, pH, and the concentration of glucose, CO_2 in blood, cardiac output, waste concentration, etc. is known as homeostasis [1]. The factors disturbing homeostasis are immense, *namely,* disease, disorders, stress, and fluctuation of environment. On contrary, stress is an experience of an individual perceiving the demand more than a person's capacity to mobilize. Stress disturbs the normal physiological equilibrium of the body as consequence of threatened homeostasis. Individual who encounter stressful conditions is associated with emotional, physiological, neuroendocrine, and psychological responses [2].

The impacts of stress on the body are varying from homeostasis changes to life-threatening events and stressful situation is the main aggravating factor for many disease, disorders, and pathological conditions [3]. In humans, stress system activation is associated with approximately 75–90% of diseases, and some of the chronic stress-related diseases include depression, non-alcoholic fatty liver diseases, and atherosclerosis [4]. The World Health Organization estimates 3.6% and 4.4% of anxiety and depression, respectively, in which the highest prevalence rate was observed in adolescents followed by females compared to males [5,6]. As far as the effect of stress on memory is concerned, stress causes the changes in structure and function of the hippocampus (part of the brain responsible for conversion of short-term memory to long-term memory) resulting in neurogenesis disorders and atrophy. In addition, this is an area where the highest density of glucocorticosteroid receptors is located have a greater response to stress. Chronic stress and thus, increased cortisol in plasma leads to a decrease in the number of neurons, dendritic branches, and neurogenesis in hippocampus tissue and a change in the structure of the synoptic terminal [3].

Stress as a potent modulator of memory and learning is not always associated with the detrimental effects of memory, moderate stress is enhancing the information storage ability of the mind. However, it is expected that acute stress induces an increased concentration of glucocorticoids resulting in working memory impairment. Similarly, high cortisol mediated stress is associated with decreasing memory in men [7].

It is well known that medicinal plants are the immense resource of drugs for the treatment of various ailments. Plant-derived bioactive molecules have been reported for their excellent biological activities including anti-psychotic, anti-antioxidant, antidepressant, neuroprotective, and hypnotic properties [8]. In addition, many plants are also reported for anti-stress activity, namely, *Morus alba*, *Sparagus racemosus*, and *Withania somnifera* [9-11]. *Abelmoschus esculentus* L. (*Malvaceae*) is generally known as Okra, bhindi, Lady's finger, etc., is an annual shrub majorly grown in the tropical and subtropical areas all over the world. The plant is cultivated as a food crop, regularly used vegetables, pods are eaten raw, and they are also used as thickening agents in soups and sauces [12].

Conventionally, pods of Okra are used in the treatment of urinary complications, gonorrhea, and dysentery. They are also used as an aphrodisiac, astringents, and appetite boosters [13]. *A. esculentus* L. also reported for antidiabetic, hypolipidemic, antioxidant, anti-stress, nootropic, and anti-Alzheimer's activities [2,14,15]. In the present investigation, the extract of *A. esculentus* L. pods was prepared by

solvent extraction method. The extract was evaluated for anti-stress activity on Swiss albino mice keeping Diazepam as a positive control. The effects of the extract on working memory and reference memory of treated mice were also evaluated. Hence, in the present study, we evaluated the effect of *A. esculentus* L. in chronic stress induced memory loss in swiss albino mice.

METHODS

Reagents and chemicals

NBT

Animals

A 6-week-old male Swiss albino mice with body weight 18–20 g of were selected. Animals were quarantined in a well-ventilated house with the controlled environmental conditions of temperature 22°C with 12 h cycle of light and dark. Mice were nourished with water and a pellet diet of standard quality *ad libitum.*

Ethical clearance

Ethical clearance obtained from Institutional Animal ethical e VIMS/IAEC/2018/007.

Plant material

A. esculentus L. commonly called Okra.

Preparation of plant extract

A. esculentus L. or Okra collected and edible pods plucked were dried under shade and pulverized into powder in a grinder which is further used for solvent extraction. The extraction of phytoconstituents was performed by the maceration extraction technique using ethanol as solvent. After extraction, the extract was dried in a rotary vacuum to evaporate ethanol, and dried extracts dissolving in water were used for anti-stress activity study in Swiss albino mice.

Experimental design

Mice were categorized into five groups and each group contained six animals (n=6). 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 force swim test (FST) until animals got immobilized or sink. Group 3 mice were orally given ethanol extract of Okra pods by dissolved in sterile water followed by the induction of stress. Group 4 mice were first subjected to stress followed by orally administered Okra extract prepared in sterile water. Group 5 mice were orally administered with Diazepam followed by induction of stress by FST. Both Diazepam and ethanol extract were orally administered to mice at 200 mg/kg/bw regularly for 90 days and working memory and reference memory were also evaluated in experimental mice groups. After completion of dosing, blood was collected from all mice, and mice were anesthetized through cervical dislocation, and brains of all mice were collected. Both blood and brain were used for the estimation of stress biomarkers such as cortisol, acetylcholine esterase (AchE), malondialdehyde (MDA), and superoxide dismutase (SOD).

FST

FST is extensively followed *in vivo* pharmacological model to evaluate anti-stress activity [16]. The test was conducted in Plexiglas-made transparent cylinder measuring 20 cm diameter and 30 cm height. The cylinder was filled with 15 cm of water and marked and the temperature of the water in the tank was consistently maintained at 23–25°C. It was assured that mice's tail and feet do not touch the bottom of the cylinder while swimming and the height of the cylinder were chosen in a such way that mice cannot escape from the tank. The mice were brought to the waiting room and kept for 2 min, mice were immersed into the tank by holding the tail and allowed for swimming until they get tired or immobilized and mice were recorded for the time taken for immobilization.

Tissue homogenization

Soon after collection of the brain, it was washed with chilled water and blotted with filter paper and weighed. Tissue homogenate was prepared in 0.05 M phosphate buffer of pH 7.0 in a homogenizer at 4°C. Brian homogenate was centrifuged for 20 min at 10,000 rpm and supernatant was collected and used for the estimation of different biochemical parameters.

Estimation of cortisol

Estimation of cortisol in mice was performed using Electrochemiluminescence immunoassay (ECLIA) method. Blood collected in vacutainer was centrifuged at 1300 g for 10 min at room temperature. Followed by this, 1.5 mL of serum was transferred a sample tube and used for analysis of circulating cortisol by ECLIA method [17].

Estimation of AchE

The brain supernatant obtained after homogenization was used for the estimation of AchE within 1 h [18]. In a container, 0.2 mL of brain homogenate was allowed to react with the solution of 100 µL of 0.075 M acetylthiocholine iodide (ATCh iodide) and 0.01M 5, 5,-dithio-bis-(2-nitrobenzoic) acid. The container was incubated at 25°C for 5 min and the formation of yellow color was observed after incubation. The absorbance of yellow color was recorded at 412 nm using a UV spectrophotometer. The activity of AchE was expressed as an international unit, that is, IU/L. The total protein concentration was measured by Lowry's method [19].

Estimation of SOD

SOD activity of brain homogenate was estimated using McCord and Fridovich (1969) method. In a container, 0.2 mL of brain homogenate was taken and was added to the reaction mixtures containing 0.1M EDTA, 1.5 mM NBT, and 67 mM phosphate buffer pH 7.0 were dissolved. The tubes were incubated at room temperature for 15 min. Followed by the incubation, a blue color formation was observed. The absorbance was recorded at 560 nm. The activity of SOD was calculated and expressed as IU/g protein [20].

Estimation of MDA

In 4 mL of reaction solution containing 1.5 mL of 20% acetic acid (pH 3.5), 0.8% TBA, and distilled water each 0.4 mL of Brian homogenate was added. The reaction mixture was incubated at 95°C for 1 h in a boiling water bath and followed by incubation the reaction mixture was cooled down and 1 mL of distilled water was added. To the reaction mixture 5 mL of pyridine and butanol at 1:15 ratio was added and mixed well. The solution was centrifuged for 10 min at 3000 rpm and supernatant was collected. The absorbance of clear supernatant was recorded at 532 nm using pyridine and butanol solution as a blank [21].

Radial arm maze (RAM) test

The working memory and reference memory were evaluated using this study **[**22]. The wooden platform apparatus containing 1–8 arms of 12 cm wide and 48 cm long raised 50 cm above the floor. Eight arms radiating from 32 cm diameters central area. The food cups with Kelloggs (choco chips) were kept at the end of three arms permanently. Throughout experiment, the visual cue was positioned around the maze and maintained. Before the RAM test, mice were restricted to food but free access to water for a week to maintain their free-feeding rate at 85%. In trail training, mice were left at the center of the RAM and allowed to eat food kept at the end of the arm platform and they were allowed to explore entire radial arms freely and sequences of the number of alterations were recorded. Gradually, mice were restricted to arms and foods. Mice were allowed eat food. The mice were trained twice like morning and evening. The mice failed to achieve 80% correct choice after the end of the training periods were not taken for the study. Mice selected for the study were rested for a week before commencing the 8-day test.

In the 8-day test, working memory and reference memory were assessed by placing mice at the center of the RAM facing the arm. During training, mice have baited four arms, that is, two, three, six, and eight out of eight arms. The entry of mice in an arm which is not baited is counted as reference memory and the mice's entry of an arm containing food that is already baited is counted as working memory. Mice entry was counted only when

all four limbs were within the arm. The residual odor was eliminated after each passage of animals by wiping the maze with 70% ethanol. Using the below formula, the percentage of alternation score (PAS) was calculated.

PAS=total alternation number/total number of entries – 2×100.

Statistical analysis

The results of all the experiments conducted (n=6) were expressed as mean±standard deviation. The data were analyzed using one-way Analysis of variance and Tukey's *post hoc* test at the 95%confidence limit. The results were considered statistically significant if p<0.05.

RESULTS

The therapeutic potential of *A. esculentus* L. or Okra pods against stress-induced mice was evaluated in the present investigation. Mice were treated with Okra pods extract and exposed to stress and another group of mice was induced stress followed by the treatment with the extract. The stress-related biochemical parameters were analyzed in both types the treatment. The standard drug Diazepam was used as a positive control. The effect of stress and Okra pods extract on working memory and reference memory was also evaluated and the results obtained are discussed here.

After continuous exposure to stress and treatment with extract of Okra pods for 90 days, blood and brains were collected from all mice *i.e.,* Group 1–5, and analyzed for various biochemical parameters related to the stress and were correlated to the memory processes in mice (Tables 1 and 2). 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, that is, 82.98±8.05% 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±1.52% (group 3; p<0.001). The mice first exposed to stress and later treated with the extract the cortisol concentration was found to be 34.11±2.32% (group 4; p<0.01) (Fig. 1). Diazepam-treated mice showed a substantial decline in cortisol level (13.95%l 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.

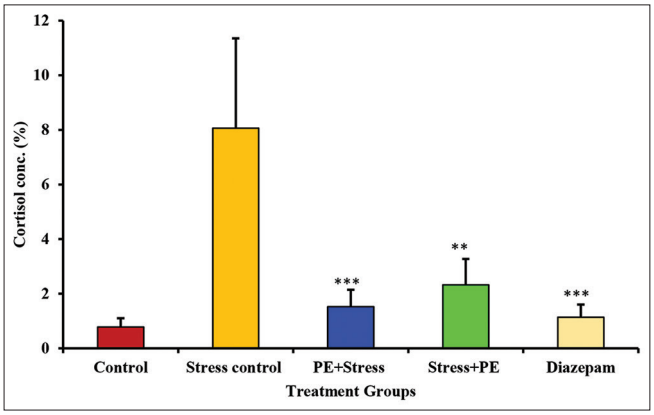

Fig. 1: Cortisol level in brain homogenates of mice groups treated with *Abelmoschus esculentus* L. extract

Exposure to chronic stress is associated with the development of various neuropsychiatric diseases. Acetylcholine release from ventral hippocampus elevated during restraining chronic stress resulted in the defective cognitive process, anxiety-like behavior, and social avoidance [23]. Hence, AchE is another indicator of stress in animals, and analysis of brain homogenate of indicated significant enzyme activity of AchE in stressed control mice, that is, 228.15 IU/L in compared to control. Mice treated with pods extract of Okra at 200 mg/kg/bw and exposed to stress resulted in a substantial reduction in AchE activity (53IU/L; p<0.001). A moderate level of reduction of AchE activity was observed in the mice treated with pods extract of Okra after exposure to stress (77IU/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 (Fig. 2).

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 200 mg/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 same extract after induction of tress (74%; p<0.01). SOD activity of mice group treated with standard Diazepam was found to be normal (Fig. 3).

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 very low concentration of MDA (30%; p<0.001) specifically in animals treated before inducting stress. The concentration of MDA was slightly higher in the mice group that is treated with *A. esculentus* L. after exposure to stress (42%; p<0.01). A signification reduction of MDA level was observed in mice treated with Diazepam standard drug and exposed to stress (Fig. 4).

In general, it was observed that in each experimental mice group stressed control mice indicated elevated concentrations of biomarkers. It is evident; stress stimulates production of these biomarkers. On the treatment of test groups with extract of *A. esculentus* L. pods after and before exposure to stress, these stress-induced biochemical parameters significantly declined. Hence, the methanolic extract of *A. esculentus* L. pods was successfully showed anti-stress activity in Swiss albino mice.

The behavior of mice in all groups was observed after 90 days completion of treatment with Okra extract and result is tabulated in Table 2. The correlation study of memory in mice groups indicated highest

Table 1: Biochemical profile of stress induced and okra treated mice groups

Animal groups	Estimation of biochemical parameter (Mean±SD)			
	Cortisol (%)	AchE (IU/L)	SOD (IU/mg protein)	MDA (nM/mg protein)
Control	13.55±0.78	19.21±2.8	105.58±6.09	55.76±4.78
Stress control	82.98±8.05	228.15±10.4**	44.07±2.20***	184.18±9.88***
Pods extract+stress (200 mg/kg/bw)	24.63±1.52***	52.96±3.12***	83.81±2.40**	29.69±5.02***
Stress+Pods extract (200 mg/kg/bw)	34.11±2.32**	77.30±2.05***	74.45±1.87**	42.07±1.47**
Diazepam+stress (200 mg/kg/bw)	13.95±1.13***	35.71±2.19**	74.51±24.1*	23.50±1.92***

Results were expressed as Mean±SD. One-way ANOVA followed by Tukey's post hoc test. ***p<0.001 against control, **p<0.01 against control, *NS: Non-Significant, MDA: Malondialdehyde, SOD: Superoxide Dismutase, AchE: Estimation of Acetylcholine Esterase

Table 2: Evaluation of memory process in stress induced and okra treated mice

Animal groups	Assessment of memory (PAS)	
	Working	Reference
Control	0.83±0.40	0.67±0.51
Stress control	4.00±0.63**	4.00±0.63***
Pods extract+stress (200 mg/kg/bw)	1.33±0.51**	1.00±0.63***
Stress+Pods extract (200 mg/kg/bw)	1.17±0.40***	1.17±0.40**
Diazepam+stress (200 mg/kg/bw)	0.83±0.75***	1.00±0.63***

Results were expressed as Mean±SD. One-way ANOVA followed by Tukey's post hoc test. ***p<0.001 against control, *p<0.01 against control, * NS: Non-Significant, PAS: Percentage of alteration score

memory error (4 PAS; group 2; p<0.01) expressed as PAS in stress control. However, the memory error made by the mice was declines on treatment with pods extract in both before and after induction of stress. This indicated a substantial increase in short-term memory or working memory and long-term memory or reference memory of extract treated mice. The improvement in memory errors especially in short memory (1.33PAS; group 3; p<0.01) was notice in pre-stress treated mice compared to post-stress treated group (1.17PAS; group 4; p<0.001). As far as, long-term memory enhancement is concerned, mice groups treated with extract followed by induction of stress showed highest memory increase and showed less memory errors (1 PAS; group 3; p<0.001) encountered by mice. Post-stress mice were also showing considerable long-memory mice with PAS value of 1.17±0.40 with p<0.01. The pods extract of *A. esculentus* L. showed significant anti-stress activity along with excellent memory improvement potential in stress induced mice groups.

DISCUSSION

Human encounters stress in everyday life like a work deadlines, home bills, unpleasant jobs, automobile accidents, and other threatening situations. Stress is the mechanism of the body that responds to stressors through psychological and physical barriers. Although stress enhances the learning ability and works performance to some extent, long-term exposure to stress is associated with various long-term defects such as depression, anxiety, and cognitive deficits. [24]. The chronic stress associated with significant elevation of cortisol level in plasma resulted in decreased memory in healthy subjects [25]. Among the anti-stress therapies, herbal-based treatments are gaining more attention due to their natural-based cure without any severe side effects. Although anti-anxiety medications are commonly prescribed to subside stress still on long-term usage, the major population are worried about the side effects of these medicines. Hence, alternative medications such as herbal medicines, yoga, and exercise are the best choice for stress management today as they have been used thousands of years ago. Today, with advancements in scientific techniques, these medications are also proven for their therapeutics potential like allopathic medications. Herbal medications are highly accessible for the wide population in a reasonable cost or freely. Nevertheless, herbal medicines have potential therapeutic value the scientific basis underlying their therapy is need to be well established.

Thus, in the present investigation, methanol extract of *A. esculentus* L. pods was evaluated for anti-stress activity in Swiss albino mice exposed to stress by FST. Analysis of biochemical stress biomarkers in stress-induced mice indicated up-regulation of AchE and down-regulation of SOD and increased concentration of cortisol and MDA. The treatment of stressed mice with methanol extract at 200 mg/kg/bw significantly reversed the expression of AchE and SOD. The concentration of stress indicators, namely, cortisol and MDA was effectively reduced in mice treated with ethanol extract of *A. esculentus* L. pods at 200 mg/kg/bw. The anti-stress activity of *A. esculentus* L. pods extract was relatively higher in mice groups treated prior exposing to stress by FST. The

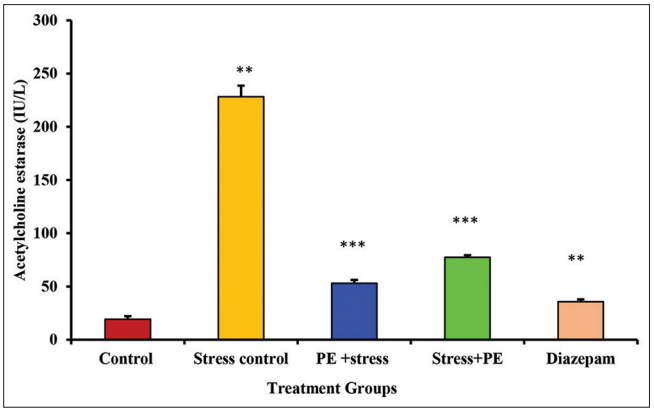

Fig. 2: Effect of *Abelmoschus esculentus* L. extracts on AchE activity in stress-induced mice groups

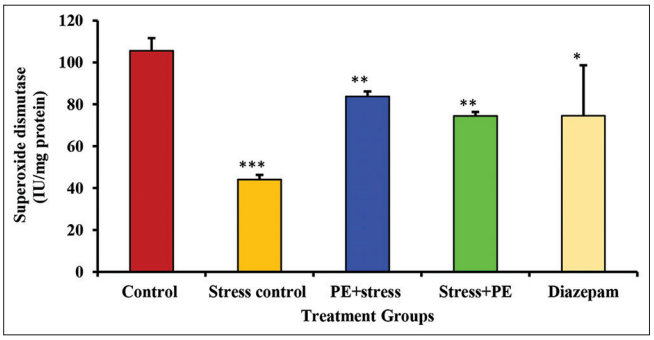

Fig. 3: SOD activity in brain homogenate mice groups treated with *Abelmoschus esculentus* L. extract

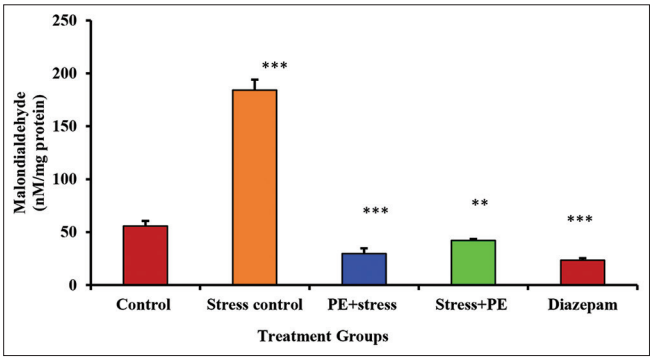

Fig. 4: MDA concentration in brain homogenate of mice treated with ethanol extract of *Abelmoschus esculentus* L

correlated study of memory process in extract-treated mice indicated a considerable recovery in both working memory and reference memory as compared to the memory process in stressed control. Hence, the methanol extract of *A. esculentus* L. pods showed significant anti-stress activity and improved both working and reference memory. Therefore, in the present study, Okra has been shown for its potent anti-stress activity as well as its enhancing the memory in mice.

CONCLUSION

Due to busy life, stress management has become the greatest challenge all over the world in modern society. The study plays a significant role in stress management in today's competitive life and with the growing population as Okra is easily accessible to the wide population. Okra is frequently used as a vegetable and eaten raw in most tropical and subtropical nations. In addition to its anti-stress activity, Okra is also known to improve memory which is essential for the learning population of the society.

A. esculentus L. pods extract based treatment of stressed mice revealed significant decreased in the stress-induced biochemical markers. In addition, mice treated with the pods extract also showed improvement in short-term memory and long-term memory compared to the stress control mice which indicated significant loss in memory. Therefore, the anti-stress potential of *A. esculentus* L. pods was successfully confirmed in present investigation. The study plays an important role in stress management in modern society that frequently encountering stress as plants are easily accessible for all categories of society. Further investigation of Okra extract for isolation of potent anti-stress molecules may be helpful in the drug discovery process and to study the mechanism underlying its anti-stress activity.

ACKNOWLEDGMENTS

The authors would like to thank the management of Vydehi Institute of Medical Sciences and Research Centre, Bengaluru, India, for their support during the research process.

AUTHORS CONTRIBUTION

Shabina Komath Chenoly conceived the study, collected data, analyzed the data, interpreted the results, and authored the manuscript. Shankarappa C provided guidance throughout the study and played a key role in manuscript editing. The manuscript was revised by Venkata Bharath Kumar.

CONFLICTS OF INTEREST

The authors declare that there was no conflicts of interest in this research.

AUTHORS FUNDING

Self-funding.

REFERENCES

1. Caon M. Homeostasis. In: Examination Questions and Answers in Basic Anatomy and Physiology. Cham: Springer; 2020. [DOI. ORG/10.1007/978-3-030-47314-36]
2. Doreddula SK, Bonam SR, Gaddam DP, Desu BS, Ramarao N, Pandy V. Phytochemical analysis, antioxidant, antistress, and nootropic activities of aqueous and methanolic seed extracts of ladies finger (*Abelmoschus esculentus* L.) in mice. ScientificWorldJournal 2014;2014:519848. [DOI: 10.1155/2014/519848]
3. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A. The impact of stress on body function: A review. EXCLI J 2017;21:1057-72. [DOI: 10.17179/EXCLI2017-480]
4. Liu YZ, Wang YX, Jiang CL. Inflammation: The common pathway of stress-related diseases. Front Hum Neurosci 2017;20316. [DOI: 10.3389/FNHUM.2017.00316]
5. World Health Organization. Depression and Other Common Mental Disorders: Global Health Estimates. Geneva, Switzerland: World Health Organization; 2017.
6. Kessler RC, Avenevoli S, Costello EJ, Georgiades K, Green JG, Gruber MJ, *et al*. Prevalence, persistence, and sociodemographic correlates of DSM-IV disorders in the National Comorbidity Survey Replication Adolescent Supplement. Arch Gen Psychiatry 2012;69:372-80. [DOI: 10.1001/ARCHGENPSYCHIATRY.2011.160]
7. Luers P, Schloeffel M, Prüssner JC. Working memory performance under stress. Exp Psychol 2020;67:132-9. [DOI: 10.1027/1618-3169/A000484]
8. Sarris J, Sinclair J, Karamacoska D, Davidson M, Firth J. Medicinal *Cannabis* for psychiatric disorders: A clinically-focused systematic review. BMC Psychiatry 2020;20:24. [DOI: 10.1186/S12888-019-2409-8]
9. Nade VS, Yadav AV. Anti-stress effect of ethyl acetate soluble fraction of *Morus alba* in chronic restraint stress. Pharm Biol 2010;48:1038-46. [DOI:10.3109/13880200903473741]
10. Joshi T, Sah SP, Singh A. Antistress activity of ethanolic extract of *Asparagus racemosus* Willd roots in mice. Indian J Exp Biol 2012;50:419-24.
11. Speers AB, Cabey KA, Soumyanath A, Wright KM. Effects of *Withania somnifera* (Ashwagandha) on stress and the stress- related neuropsychiatric disorders anxiety, depression, and Insomnia. Curr Neuropharmacol 2021;19:1468-95. [DOI:10.2174/1570159X19666210712151556]
12. Elkhalifa AE, Alshammari E, Adnan M, Alcantara JC, Awadelkareem AM, Eltoum NE, *et al*. Okra (*Abelmoschus Esculentus*) as a potential dietary medicine with nutraceutical importance for sustainable health applications. Molecules 2021;26:696. [DOI: 10.3390/MOLECULES26030696]
13. Islam MT. Phytochemical information and pharmacological activities of Okra (*Abelmoschus esculentus*): A literature-based review. Phytother Res 2019;33:72-80. [DOI: 10.1002/PTR.6212]
14. Fan S, Zhang Y, Sun Q, Yu L, Li M, Zheng B, *et al*. Extract of okra lowers blood glucose and serum lipids in high-fat diet-induced obese C57BL/6 mice. J Nutr Biochem 2014;25:702-9. [DOI: 10.1016/J.JNUTBIO.2014.02.010]
15. Mairuae N, Connor JR, Lee SY, Cheepsunthorn P, Tongjaroenbuangam W. The effects of okra (*Abelmoschus esculentus* Linn.) on the cellular events associated with Alzheimer's disease in a stably expressed HFE neuroblastoma SH-SY5Y cell line. Neurosci Lett 2015;603:6-11. [DOI: 10.1016/J.NEULET.2015.07.011]
16. Yankelevitch-Yahav R, Franko M, Huly A, Doron R. The forced swim test as a model of depressive-like behavior. J Vis Exp 2015;97:52587. [DOI: 10.3791/52587]
17. Manguso F, Bennato R, Lombardi G, Viola A, Riccio E, Cipolletta L. Electrochemiluminescence immunoassay method underestimates cortisol suppression in ulcerative colitis patients treated with oral prednisone. World J Gastroenterol 2014;20:10895-9. [DOI:10.3748/WJG.V20.I31.10895]
18. Ellman GL, Courtney KD, Andres V Jr., Feather-Stone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961;7:88-95. [DOI: 10.1016/0006-2952(61)90145-9]
19. Lowry OH, Rosebrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951;193:265-75.
20. McCord JM, Fridovich I. Superoxide dismutase. An enzymic function for erythrocuprein (hemocuprein). J Biol Chem 1969;244:6049-55.
21. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8. [DOI: 10.1016/0003-2697(79)90738-3]
22. Zingue S, Foyet HS, Djiogue S, Ezo'o Y, Abaïssou HH, Fachagbo P, *et al*. Effects of *Ficus umbellata* (*Moraceae*) aqueous extract and 7-methoxycoumarin on scopolamine-induced spatial memory impairment in ovariectomized wistar rats. Behav Neurol 2018;2018:5751864. [DOI: 10.1155/2018/5751864]
23. Mei L, Zhou Y, Sun Y, Liu H, Zhang D, Liu P, *et al*. Acetylcholine muscarinic receptors in ventral hippocampus modulate stress-induced anxiety-like behaviors in mice. Front Mol Neurosci 2020;13:598811. [DOI: 10.3389/FNMOL.2020.598811]
24. Sha P, Dong X. Research on adolescents regarding the indirect effect of depression, anxiety, and stress between tiktok use disorder and memory loss. Int J Environ Res Public Health 2021;18:8820. [DOI: 10.3390/IJERPH18168820]
25. Peavy GM, Salmon DP, Jacobson MW, Hervey A, Gamst AC, Wolfson T, *et al*. Effects of chronic stress on memory decline in cognitively normal and mildly impaired older adults. Am J Psychiatry 2009;166:1384-91. [DOI: 10.1176/APPI.AJP.2009.09040461]