Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 13 [2] January 2024 : 121-132

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD

ORIGINAL ARTICLE **OPEN ACCESS**

Impact of β-Sitosterol on Stress-Induced Neurobehavioral Alteration in Experimental Animal

Yashashwi Shrivastav, Neelam Balekar*, Gaurav Parihar
IPS Academy College of Pharmacy, Rajendra Nagar A.B. Road Indore 452012(M.P.), India
Corresponding Author's E-mail: neelambalekar@ipsacademy.org

ABSTRACT
Stress significantly impacts mental health, prompting a focus on stress-induced neurobehavioral alterations. β-Sitosterol, a plant-derived phytosterol, emerges as a potential neuroprotective agent. This study aimed to evaluate β-Sitosterol's therapeutic potential in mitigating neurobehavioral alterations induced by stress, shedding light on its adaptogenic or neuroprotective capacity. Animals were categorised into distinct groups, including Negative Control, Control Socially Isolated (SI), Control Group Housed (GH), Standard SI, Standard GH, Test SI, and Test GH. Stress, induced by environmental deprivation and social isolation, led to neurobehavioral alterations. The study employed a comprehensive design, including Negative Control group with no stress or treatment. Control SI and Control GH groups underwent social isolation and vehicle dosing. Standard SI and Standard GH received Diazepam (5 mg/kg). Test SI and Test GH received β-sitosterol (10 mg/kg) dosing. In the Elevated Plus Maze Test (EPM), Control GH and Control SI groups exhibited anxiety-related behaviours, mitigated by Diazepam in Standard GH and β-Sitosterol in Test GH. In the Open Field Test (OFT), β-Sitosterol enhanced exploratory behaviour and locomotor activity in the Test SI and Test GH groups. Social Interaction Test in Novel Environment (SIT) results showed reduced social interaction in Control GH and Control SI, countered by increased interaction, diminished social anxiety, and augmented behaviour in Test GH and Test SI with β-Sitosterol. β-Sitosterol showed adaptogenic and anxiolytic properties, mitigating depression-like behaviours and enhancing social interaction and locomotor activity. Further research is essential to unveiling its mechanisms and therapeutic potential in stress-related neurobehavioral alterations.
Keywords: *β-Sitosterol, Stress, Neurobehavioral Alteration, Social Isolation, Adaptogenic Activity*

Received 14.09.2023 Revised 01.11.2023 Accepted 29.12.2023

INTRODUCTION

Stress responses refer to the body's adaptive reactions to different forms of pressure. Depending on the type, timing, and intensity of the encountered stressor, stress can induce a wide range of physiological alterations, spanning from disruptions in internal balance (homeostasis) to potentially life-threatening outcomes, including fatalities. Stress has the potential to exacerbate or initiate numerous illnesses and pathological conditions. It is well-known that stress in mammals including mice provokes a series of physiological and psychological reactions. Chronic stress can cause a variety of neurobehavioral changes, including decreased locomotor function, anxiety-like behaviours, and changes in social behaviours. For the purpose of creating efficient strategies to treat stress-related diseases, it is essential to comprehend the underlying mechanisms of these changes [1,2].

The chronic stress has been shown to negatively impact people's mental health and wellbeing, stress-related neurobehavioral alterations are a serious problem in contemporary culture. The biological causes of chronic pain are still not fully understood, despite its significant negative effects on people's physical, mental, and financial health [3,4].

Neurobehavioral Alterations refer to modifications or disturbances in a person's actions, cognition, or emotional functioning that result from underlying neurological or brain-related issues. Changes in mood, memory, attention, motor skills, linguistic proficiency, or social interactions could result from these modifications. They may be brought on by a number of things, such as neurological diseases, brain injuries, or exposure to particular chemicals or environmental influences [5-7].

Social withdrawal refers to withdrawing from friends, family, and society, which causes individuals with depression to experience a state of Social Isolation (SI), which exacerbates their depressive symptoms. As

an independent risk factor, SI is linked to a higher incidence of depression, anxiety, and substance abuse [8].

Environmental deprivation in rodents involves controlled restriction of vital stimuli, impacting their well-being and natural behaviours, including limited social interaction, reduced physical activity, and disrupted light-dark cycles. Researchers use this in labs to induce physiological or behavioural changes, studying environmental factors' effects on health and Behavior ethically. Light significantly affects mammalian physiology, with common eco-friendly lighting conditions harming rodents. Dim light at night and constant darkness disrupts circadian rhythms, damaging neural health and inducing depression-like Behavior. A study on mice exposed to 3 and 5 weeks of constant darkness revealed gender-specific effects, reversible upon returning to a normal light-dark cycle, supported by metabolome analysis [9,10].

The rising incidence of stress-related disorders, such as anxiety and depression, has given research in this area more dynamism. Natural substances like β-sitosterol have gained attention in recent years due to their possible neuroprotective attributes, making them enticing options for reducing the damaging effects of stress on the brain [11].

β-sitosterol is one such phytosterol, which resembles cholesterol in structure and is found in a variety of plants. It is a natural micronutrient that is present in considerably lower concentrations than cholesterol in healthy people. Sitosterolin, a glycoside of β-sitosterol, is regarded as a safe and reliable dietary supplement. Various Research Studies have demonstrated that β-Sitosterol offers a variety of potential advantages, including anti-inflammatory, anticancer, antibacterial, angiogenic, immunomodulatory, antidiabetic, and antioxidant characteristics [12-14].

The Molecular Docking Study conducted by author Khan *et al.,* Consequently, was found that β-sitosterol can be employed to boost defences against SARS-CoV-2 infection as well as to limit viral invasion into host cells via ACE-2 by suppressing spike glycoprotein. Increased consumption of β-sitosterol and other phytosterols can help to modify immunity, which is necessary nowadays to combat COVID-19 [15].

According to studies, Environmental Deprivation, social isolation and loneliness have an impact on one's mental health and general wellbeing. β-sitosterol, a potent phytosterol, possesses a wide array of pharmacological properties. Additionally, studies have discovered that phytosterol has adaptogenic effects, which may affect how your body responds to stress, anxiety, and exhaustion. β-sitosterol can be useful for treatment of Neurobehavioral Alterations.

MATERIAL AND METHODS

Drugs and Chemicals:

All the chemicals and drugs required for the experiment were of analytical grade. β-Sitosterol was procured as a gift sample from Pharmed Ltd. Solan, Himachal Pradesh (India). Marketed Preparation of Diazepam Tablets I.P. were used. Sodium Carboxymethyl Cellulose (CMC) was used as a vehicle and was procured from Labogens Fine Chem Industry Ludhiana Punjab.

Animals:

Swiss Albino Mice weighing between 20-30 g of either sex was used. The group of animals were housed in polypropylene cage, paddy husk bed covered with stainless steel wire mesh. The Animals were housed in group of 5 mice /cage and maintained at 24˚C±1˚C, with relative humidity of 45-55%, and 12:12 hour day/light cycle. The animals had free access to food standard chew pellets, and water *ad libitum*. Normal conditions of environmental, food and water were maintained until the commencement of experiment.

All experimental procedures and protocols used in the study was prior approved by the Institutional Animal Ethics Committee (IAEC), IPS Academy College of Pharmacy, Indore, constituted under the norms given by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Ministry of environment and Forest, Government of India. Ethical guidelines were strictly followed during the experiment. The Protocol Approval number was CPCSEA/218/2023.

Evaluation of Neurobehavioral Parameters

Experimental Design

Induction of Stress in Experimental Mice:

For the present study stress was induced with social isolation and environmental deprivation factor. The mice were placed individually in opaque walled cages, thus depriving of environmental conditions. Experimental Room was lit by dim light. Neurobehavioral Alterations such as anxiety and depression-like Behavior were observed after 6 weeks of social isolation [16].

The animals were categorized into different groups (n=6 animal in each group), including Negative Control, Control Socially Isolated (SI), Control Group Housed (GH), Standard SI, Standard GH, Test SI, and Test GH. The study employed a comprehensive experimental design where Negative Control Animals were not subjected to stress or treatment. In the Control SI group, mice experienced 6 weeks of SI followed with 1

week of vehicle (0.5% Sodium CMC suspension p.o.) dosing and the same vehicle dosing in Control GH. The Standard SI and Standard GH groups underwent for 6 weeks of stress induction and received a single dose of Diazepam (5 mg/kg p.o.) before 30 mins of Behavioural Assessment. The Test SI group went through 6 weeks of SI followed with 1 week of β-sitosterol dosing (10 mg/kg p.o.), and same β-sitosterol dosing in Test GH.

Pharmacological Methods:

The Evaluation of Neurobehavioral Parameters took place upon the completion of the treatment schedule. The animal models used for the evaluation of neurobehavioral parameter were as follow:

Elevated Plus Maze Test:

The apparatus was with two lit open arms and two closed arms covered with cardboard to block out the light; all four arms radiated from a central platform. The apparatus was mounted on a base raising the arms to a height of 38.5 cm above the floor. To initiate the test session, the mouse was placed on the central platform, facing an open arm, and was observed for 5 min. The mouse was considered to be on the central platform whenever two paws were on it and in one of the arms when all four paws were inside. The following behavioural variables were recorded, counting both number and duration: entries into an open arm, entry into a closed arm and unprotected head-dipping that is animal extending its head into the open, below the open arm [17].

Open Field Test:

The open field test was conducted in an open field chamber measuring 50 cm (length) ×50 cm (width) ×38 cm (height) made up of white acrylic plastic sheet.4×4 grid lines was drawn to divide the floor into 10×10 square, and an additional 20×20 cm square zone was drawn in centre. Mice were individually placed at the centre of an arena and activity was assessed for 10 minutes. The following parameters was summed for each animal during the 10-minute test: The time spend in the central zone, the time spent in the 4 corners square grid, path-length (cm) travelled in the apparatus (determine by measuring the distance of the nose of the mice relative to the 10×10 cm square grid lines on the floor of the open field chamber), and the number of times the animal reared. All the scoring was conducted manually in a double-blind manner, with each recording being observed three times to minimize the error [16].

Social Interaction Test in a Novel Environment:

Two mice that were previously housed in different cages, were placed into a box together (40 × 40 × 30 cm) and were allowed to explore freely for 10 min. Animal behaviour was analysed. The total duration of contacts (s), number of contacts, total duration of active contacts (s), mean duration per contact, and total distance travelled (cm) were measured. The active contact was defined as follows: images were captured at three frames per second, and distance travelled between two successive frames was calculated for each mouse. If the two mice contacted each other and the distance travelled by either mouse was 5 cm and more, the behaviour was considered an "active contact" [18].

Statistical Analysis

Data were expressed as the Mean ± Standard Error of Mean (S.E.M.) and statistical analysis was carried out employing Ordinary One-Way Analysis of Variance (ANOVA) followed by a "Tukey-Kramer" multiple comparison test of $p<0.05$ significance level using "GraphPad Prism" version 10.0.2 for windows, GraphPad Software, San Diego, California, USA (www.graphpad.com).

RESULT

Elevated Plus Maze

Effect of the Treatment on Group Housed (GH) Animals: The Table 1 and Figure 1(A) presents the extensive results, which outline how different groups performed in terms of the number of entries they made and the amount of time they spent in the closed and open arms of the elevated plus maze test:

- Control Group showed decreased frequency of entries into the closed arm, along with increased time spent in closed arm. On the other hand, they had a decreased propensity to enter the open arm and a shorter time spent there.
- Control GH mice showed higher no. of entries in closed arm and also spent most of the time in closed arm and on the other hand the no. of entries in open arm is lowest and time spent is also less.
- Standard GH Group which received Diazepam increased the preference of mice in open arm and have highest no. of entries in open arm and spend more time in open arm and decreased no. of entries in closed arm and less time spent in closed arm and had significant effect when compared to Control and Control GH.
- Test GH Group which received β-Sitosterol showed significant effects. Specifically, there was a significant decrease in the number of entries into the closed arm compared to the control group, along with a marked reduction in the time spent within the closed arm when contrasted with the control GH

and standard GH groups. Conversely, there was a significant increase in the number of entries into the open arm and the time spent within the open arm when compared to the Control GH group.

Effect of the Treatment on Socially Isolated (SI) Animals: The Table 2 and Figure 1(B) presents the parameters of the different groups where it was found that there was a significant effect of β-Sitosterol over Diazepam on the number of entries and time spent in closed arm and open arm which attained study-wide significance.

- Control Group showed decreased frequency of no. of entries into the closed arm, along with increased time spent in closed arm. On the other hand, they had a decreased propensity to enter the open arm and a shorter time spent there.
- Control SI Group mice showed highest no. of entries in closed arm and also spent most of the time in closed arm and on the other hand the no. of entries in open arm is lowest and time spent is also less.
- Standard SI Group which received Diazepam increased the preference of mice in open arm and have highest no. of entries in open arm and spend more time in open arm and lowest no. of entries in closed arm and less time spent in closed arm and had significant effect when compared to Control and Control SI.
- Test SI Group which received β-Sitosterol showed significant effects. Specifically, there was a significant decrease in the number of entries into the closed arm compared to the control group and standard, along with a marked reduction in the time spent within the closed arm when contrasted with the control SI and standard groups. Conversely, there was a significant increase in the number of entries into the open arm on comparison to Standard and the increase in time spent within the open arm when compared to the Control, Control SI, Standard SI group.

Open Field Test

Effect of the Treatment on Group Housed (GH) Animals: The Table 3 and Figure 2(A) outlines the outcomes of β-Sitosterol over the Diazepam and Sodium CMC. The result outcomes show or depicts that Specific patterns, trends, or effects relating to the behaviour of the study animals have been found in the data gathered from the test. Changes in locomotion, time spent in various open-field zones, grooming behaviour, or other pertinent behaviours were observed.

- Control Group travelled shorter pathlength distance than the other groups and also tended to spent more time in 4 corner square grid line rather than in central zone. There is also reduction in vertical activity that is rearing and increased frequency of defecation.
- Control GH travelled shorter pathlength distance than the Standard GH and Test GH groups and also tended to spent longer duration of time in 4 corner square grid line rather than in central zone. There is also reduction in rearing and increased frequency of defecation as compared to Standard GH and Test GH.
- Standard GH travelled the highest pathlength distance compared to the other groups and there was observed that the mice spend more time in centre zone than 4 corner square grid lines. The mice also exhibit highest no. of rearing's when compared to other groups. The least frequency of defecation was also observed.
- Test GH shows significant results for the travelled pathlength distance as compared to Control, Control GH, Standard as suggesting that the β-Sitosterol increased the motor activity. There is also increased time spend in centre zone while decreased staying in 4 corner square grid line. The increased no. of rearing's that suggested less anxiety behaviour in mice where the values are significant to Control and Standard GH but insignificant to Control GH. The frequency to defecate was also low and significant to other groups.

Effect of the Treatment on Socially Isolated Animals: The data displayed in Table 4 and Figure 2(B) offers an extensive insight into the impact of β-Sitosterol when contrasted with the effects of Diazepam and Sodium CMC. The results for the Socially Isolated group reveal distinct behavioural patterns and trends in the mice.

- Control Group travelled shorter pathlength distance than the other groups and also tended to spent more time in 4 corner square grid line rather than in central zone. There is also reduction in vertical activity that is rearing and increased frequency of defecation.
- Control SI traversed shorter pathlength distance than the Standard SI and Test SI groups and also tended to spent highest duration of time in 4 corner square grid line rather than in central zone as compared to Control. Additionally, compared to Standard SI and Test SI, there was decrease in rearing and an upsurge in defecation frequency.

- Standard SI group showed the most extensive pathlength distance travelled, surpassing the other groups. Additionally, notable observations included an increased amount of time spent within the central zone of the testing area as opposed to the four corner square grid lines. Moreover, this group exhibited the highest frequency of rearing behaviours in comparison to the other groups. Conversely, defecation events were the least frequent, a noteworthy observation in this context.
- The Test SI group yielded notable and statistically significant findings concerning pathlength distance travelled when compared to the Control, Control SI, and Standard SI groups. These results suggest that β-Sitosterol administration led to an enhancement in motor activity in mice. Furthermore, there was a marked increase in the time spent within the central zone of the testing environment, accompanied by reduced occupancy of the four corner square grid lines. Additionally, the Test SI group displayed an elevated frequency of rearing behaviours, indicative of reduced anxiety levels in the mice. This increase in rearing behaviours was statistically significant when compared to the Standard GH group, while differences observed in comparison to the Control and Control SI groups were not statistically significant. Moreover, the frequency of defecation events was notably low in the Test SI group, and this observation achieved statistical significance when compared to the other groups. Collectively, these results find suggested that the Isolation decreased the exploratory and locomotor activity in mice and that β-Sitosterol treatment ameliorates these behavioural responses in socially isolated mice.

Social Interaction Test in a Novel Environment

Effect of the Treatment on Group Housed Animals: The Table 5 and Figure 3(A) serve as comprehensive visual representations and data summaries that encapsulate the findings derived from the social interaction test conducted within a unique and unfamiliar environment.

- Control group showed less total duration of contact, total duration of active contact, mean duration per contact, distance travelled and lower no. of contacts than the Test GH and Standard GH.
- Control GH showed less total duration of contact, total duration of active contact, mean duration per contact, distance travelled and lower no. of contacts than the Control, Test GH and Standard GH.
- Standard GH showed highest total duration of contacts as compared to the other groups. The no. of contacts was also observed to be significantly higher in Standard GH group when compared to Control and Control GH. Total duration of active contact, Mean duration per contact and Distance Travelled was also found to be highest from other groups.
- Test GH showed significant values for the total duration of contacts when compared to Control, Control GH and Standard GH which shows increased social interaction in mice. The increased no. of contacts also shows less social anxiety to unfamiliar mice when compared to the other groups in significant way. The total duration of active contact and mean duration per contact also falls significant when compare to other groups and shows increased social Behavior. The increased total distance travelled values signifies increased locomotor activity.

Effect of the Treatment on Socially Isolated Animals: The Table 6 and Figure 3(B) shows the detailed results which were obtained.

- Control group showed less total duration of contact, total duration of active contact, mean duration per contact, distance travelled and lower no. of contacts than the Test SI and Standard SI.
- Control SI group exhibited lower values in terms of total contact duration, active contact duration, average contact duration, distance travelled, and the number of contacts when compared to the Control, Test SI, and Standard SI groups.
- Standard SI showed highest total duration of contacts as compared to the other groups. The no. of contacts was also observed to be significantly higher in Standard SI group when compared to Control and Control SI. Total duration of active contact, Mean duration per contact and Distance Travelled was also found to be highest from other groups.
- Test SI showed significant values for the total duration of contacts when compared to Control, Control SI and Standard SI which shows increased social interaction in mice. The increased number of contacts suggests reduced social anxiety toward unfamiliar mice compared to other groups. Additionally, both the total duration of active contact and mean duration per contact showed significant increases, signifying enhanced social Behavior. Elevated total distance travelled values also indicate increased locomotor activity. The Control group displayed reduced social interaction and locomotor activity compared to Test GH and Standard GH. Control SI exhibited the lowest values in various interaction parameters. Standard SI demonstrated the highest social interaction levels. Test SI displayed significantly increased social interaction, reduced social anxiety, enhanced social Behavior, and greater locomotor activity compared to other groups.

DISCUSSION

Stress is a generalized bodily reaction that has been shown to change the physiological balance of the organism and cause different visceral, endocrine, and neurological dysfunctions. Survival depends on one's capacity to build and maintain resistance to the various stresses that can arise in daily life. If stress levels rise, an organism may develop a disease. According to physiologists, stress is the body's response to a stressor, whether it be actual or imagined, or a stimulus that induces stress. Because the body and immune system are worn down and their function is compromised when tiredness is prolonged, decompensation may occur. The outcome can show up as various mental disorders as well as outward illnesses like ulcers, depression, diabetes, digestive system problems, or even cardiovascular issues [19].

Adaptogens are molecules that aid organisms in adapting to stressful, unfavourable environments. These factors might be physical, chemical, biological, or mental. The ability to produce a nonspecific response is one of the criteria put forth by some of the field's researchers. Other requirements include being innocuous, which means that it must not adversely affect normal bodily functions more than is necessary. Adaptogens can be either artificial or organic molecules. However, the majority of research on adaptogens has been on naturally occurring chemicals, particularly plants, and the name "Phyto- adaptogens" is now frequently used for adaptogens derived from plants [20].

β-Sitosterol and Adaptogens exhibit overlapping physiological properties with potential implications for the body's response to stress and overall well-being. These shared characteristics encompass their effects on stress modulation, promotion of homeostasis, and potential anti-inflammatory and antioxidant activities. While intriguing, it's vital to note distinctions: Adaptogens are well-established for adaptogenic traits, while β-Sitosterol is primarily a phytosterol. Thorough research is needed to find whether β-Sitosterol possess adaptogenic activity. These areas hold significance for stress resilience and overall well-being.

Environmental lighting profoundly influences physiological processes. Artificial lighting, prevalent in modern society, disrupts circadian rhythms, inducing anxiety-like behaviours in laboratory animals and posing health risks for humans. Conversely, dim light at night disturbs metabolism, immunity, and circadian timing. Understanding these effects can uncover the molecular mechanisms underpinning behavioural issues. In a previous study, it was found that subjecting Swiss Albino mice to either dim light at night (dLAN) or complete darkness (DD) for three weeks led to cognitive and non-cognitive Behavior impairment. This was linked to changes in hippocampal protein expression and key genes (BDNF, CREB, DCX, SYN, and SIRT1) associated with neurodegeneration [21].

Social isolation (SI) is a stressor that leads to various alterations in stress responses, social Behavior, neurochemistry, and more, in both animals and humans. During early life, acute or chronic SI can manifest as psychiatric and neurological disorders like anxiety, depression, schizophrenia, epilepsy, and memory issues. SI triggers changes in the endocrine system, including the activation of the hypothalamic–pituitary–adrenal (HPA) axis, resulting in the release of stress hormones like glucocorticoids. It also affects neurotransmitter levels and receptor sensitivity in the central nervous system, influencing systems like dopamine, serotonin, GABA, and glutamate. SI's impact extends to oxidative and nitrative stress, mitochondrial function, inflammation, neurotrophins, and genes associated with neurological and psychiatric disorders [22].

Prolonged stress exposure during critical developmental periods increases sensitivity to psychiatric or mental diseases, which might have gender-specific implications. In this research, researchers look at the neural pathways that mediate behavioural alterations in the mice after persistent social isolation stress in adolescent. Stressed men exhibit escalating hostility, but stressed females exhibit social withdrawal. In stressed males, pyramidal neurons in the prefrontal cortex (PFC) shows or exhibit reduced spike activity during aggressive attacks, whereas stressed females present or shows a diminished increased in discharge rates during the sociability tests, based on multichannel recordings of freely moving animals. Both Chemo genetic and electrophysiological findings suggest that the decreased PFC activity and heightened activity of main neurons in the BLA contribute to increased aggression in males which were stressed, while decreased PFC activity and reduced activity of VTA dopamine neurons leads to decreased sociability in stressed females. These findings provide an explanation to comprehend the neural and physiological mechanisms behind stress's sex-specific differential effects [23].

EPM results indicated anxiolytic properties of β-Sitosterol, evidenced by reduced anxiety-like behaviours. In the OFT, β-Sitosterol-treated groups exhibited increased motor activity, reduced anxiety-like behaviours, and enhanced exploratory activity, suggesting an anxiolytic effect. Social Interaction Test results highlighted the modulation of social behaviours by β-Sitosterol, with increased social interaction, reduced anxiety, augmented social behaviour, and heightened locomotor activity observed in the Test SI group. These findings emphasize the potential anxiolytic and social behaviour-modulating effects of β-

Sitosterol in mice.

The biological system undergoes extensive alterations as a result of stressful situations, which are frequently encountered in our daily lives. The neuroendocrine, immunological, and brain are all impacted by such stress reactions, which work together to either increase or diminish the body's capacity to deal with such stressors. There is a reciprocal relationship in how stress impacts and is influenced by both the brain and peripheral responses. This intricate dynamic involves the brain's regulation of peripheral stress responses through the manifestation of complex behavioural patterns. Numerous stressors can cause anxiety, which has a common neurobehavioral correlation. The immune system and the brain interact in psychoneuroimmunology, and it is becoming increasingly understood that the immune system may have a role in the neurobehavioral reactions to stress. According to studies, the brain's intricate neurotransmitter networks may have an impact on immunological response, and there may be a connection between the development of anxiety and immunomodulation under stress conditions [4].

This study explores the therapeutic potential of β-Sitosterol which shares physiological properties with adaptogens, suggesting its role in enhancing stress resilience. β-Sitosterol proves effective in reducing stress-related behaviours and symptoms in mice, further research may explore its applicability in clinical settings, offering new avenues for stress management and improved mental health for individuals facing the challenges of modern life.

Future research should prioritize unravelling the precise mechanisms by which β-Sitosterol exerts its effects, exploring a dose-response relationship, and assessing its long-term impact. Clinical trials in humans are a pivotal next step to determine its safety and efficacy, potentially opening doors for its application in managing stress-related disorders. Combination therapies, safety assessments, investigations into neuroprotective properties, and consideration of gender and age-related differences are all crucial areas for future exploration.

Table 1: Effect of β-Sitosterol on Group Housed Animals in Elevated Plus Maze

Groups	Closed Arm		Open Arm	
	No. of Entries	Average Time Spent (sec/5 min)	No. of Entries	Average Time Spent (sec/5 min)
Control	8.6 ± 0.57	15.6 ± 0.38	3.9 ± 0.30	5.0 ± 0.24
Control GH	11.8 ± 0.5	16.5 ± 0.27	3.5 ± 0.18	4.7 ± 0.10
Test GH	10.8 ± 0.14^{a}	15.0 ± 0.18^{bc}	3.8 ± 0.14^{c}	5.5 ± 0.13^{bc}
Standard GH	9.8 ± 0.30^{ab}	13.0 ± 0.29^{ab}	5.8 ± 0.18^{ab}	6.7 ± 0.13^{ab}

Group Housed (GH), Negative Control (Control)

Values are expressed in Mean ± SEM, (n=6 animal in each group); Data were analysed using one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where $p^{a}<0.05$ when compared with Control, $p^{b}<0.05$ when compared with Control GH, $p^{c}<0.05$ when compared with Standard GH.

Table 2: Effect of β-Sitosterol on Socially Isolated Animals in Elevated Plus Maze

Groups	Closed Arm		Open Arm	
	No. of Entries	Average Time Spent (sec/5 min)	No. of Entries	Average Time Spent (sec/5 min)
Control	8.6 ± 0.57	15.6 ± 0.38	3.9 ± 0.30	5.0 ± 0.24
Control SI	11.6 ± 0.18	16.5 ± 0.42	3.1 ± 0.14	4.8 ± 0.03
Test SI	11.3 ± 0.12^{ac}	14.9 ± 0.14^{bc}	4.1 ± 0.21^{c}	6.0 ± 0.13^{abc}
Standard SI	9.2 ± 0.62^{b}	12.5 ± 0.15^{ab}	5.6 ± 0.35^{ab}	7.1 ± 0.36^{ab}

Social Isolation (SI), Negative Control (Control)

Values are expressed in Mean ± SEM, (n=6 animal in each group); Data were analysed using one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where $p^{a}<0.05$ when compared with Control, $p^{b}<0.05$ when compared with Control GH, $p^{c}<0.05$ when compared with Standard SI.

Table 3: Effect of β-sitosterol on Group Housed Animals in Open Field Test

Groups	Pathlength Travelled(cm)	Time Spent (sec)		No. of Rearing	No. of times Defecated
		4-Corner Square Grid	Central Zone Grid		
Control	3310 ± 237	347.8 ± 33.0	186.8 ± 7.9	16.17 ± 0.9	2.66 ± 0.2
Control GH	3810 ± 59	414.2 ± 8.0	208.3 ± 13.2	17.67 ± 0.5	2.55 ± 0.2
Test GH	4689 ± 137^{abc}	325.5 ± 6.3^{bc}	287 ± 17^{abc}	20.7 ± 0.3^{ac}	1.5 ± 0.2^{abc}
Standard GH	5624 ± 67^{ab}	231.7 ± 23^{ab}	335.3 ± 5.8^{ab}	24.8 ± 1.1^{ab}	0.5 ± 0.2^{ab}

Group Housed (GH), Negative Control (Control)

Values are expressed in Mean ± SEM, (n=6 animal in each group); the Data were analyzed using one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where $p^a<0.05$ when compared with Control, $p^b<0.05$ when compared with Control GH, $p^c<0.05$ when compared with Standard GH.

Table 4: Effect of β-sitosterol on Socially Isolated Animals in Open Field Test

Groups	Pathlength Travelled(cm)	Time Spent (sec)		No. of Rearing	No. of times Defecated
		4-Corner Square Grid	Central Zone Grid		
Control	3310 ± 237	348 ± 33.3	187 ± 7.9	16.2 ± 0.9	2.7 ± 0.2
Control SI	3370 ± 168	410 ± 20	207 ± 19.4	14.3 ± 0.8	2.3 ± 0.1
Test SI	4575 ± 119^{abc}	262 ± 0.6^{abc}	279 ± 19.7^{abc}	17.2 ± 2.0^{c}	1.7 ± 0.1^{abc}
Standard SI	5721 ± 216^{ab}	184 ± 4.1^{ab}	337 ± 6.2^{ab}	23.3 ± 0.6^{ab}	1.7 ± 0.21^{ab}

Social Isolation (SI), Negative Control (Control)

Values are expressed in Mean ± SEM, (n=6 animal in each group); the Data were analyzed using one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where $p^a<0.05$ when compared with Control, $p^b<0.05$ when compared with Control SI, $p^c<0.05$ when compared with Standard SI.

Table 5: Effect of β-sitosterol on Group Housed Animals in Social Interaction Test

Groups	Total Duration of Contact(sec)	No. of Contacts	Total Duration of Active Contact(sec)	Mean Duration /Contact	Distance Travelled(cm)
Control	138 ± 5.2	22 ± 0.8	25 ± 0.1	4.5 ± 1.2	2870 ± 41.9
Control GH	136 ± 3.4	21 ± 1.08	24 ± 0.6	4 ± 0.2	2800 ± 15.8
Test GH	171 ± 4.6^{abc}	30 ± 1.6^{ac}	30 ± 1.3^{bc}	10.7 ± 0.1^{abc}	3750 ± 11.4^{abc}
Standard GH	205 ± 1.3^{ab}	33 ± 0.8^{ab}	35 ± 0.7^{ab}	11.8 ± 0.4^{ab}	4634 ± 31.3^{ab}

Group Housed (GH), Negative Control (Control)

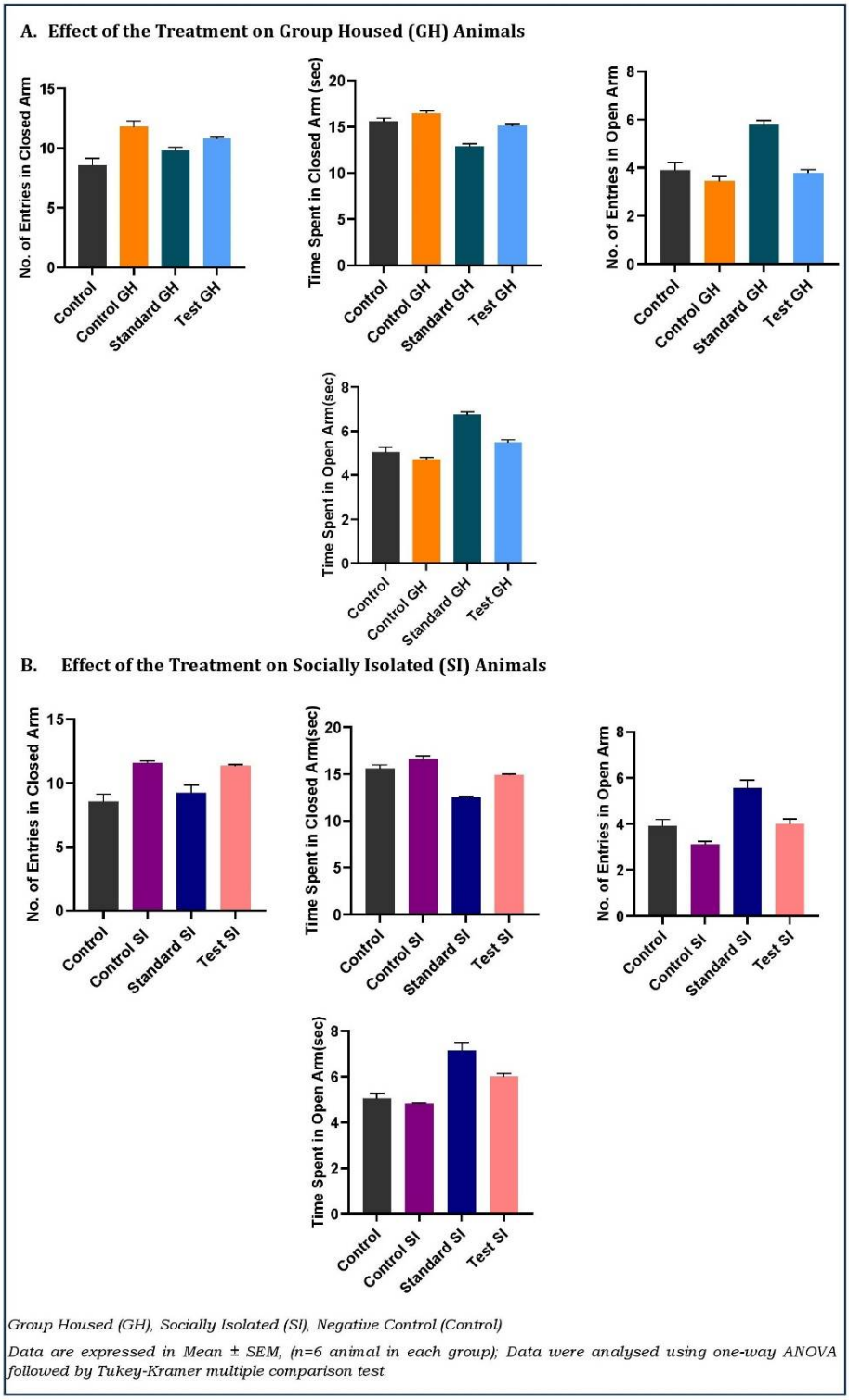
Values are expressed in Mean ± SEM, (n=6 animal in each group); the Data were analyzed using one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where $p^a<0.05$ when compared with Control, $p^b<0.05$ when compared with Control GH, $p^c<0.05$ when compared with Standard GH.

Table 6: Effect of β-sitosterol on Socially Isolated Animals in Social Interaction Test

Groups	Total Duration of Contact(sec)	No. of Contacts	Total Duration of Active Contact(sec)	Mean Duration /Contact	Distance Travelled(cm)
Control	138.2 ± 5.2	22.1 ± 0.8	24.8 ± 0.1	4.5 ± 1.2	2870 ± 42
Control SI	124 ± 3.5	12.1 ± 0.4	14.3 ± 1.8	2.1 ±0.3	2634 ± 18
Test SI	160.2 ± 1.8^{abc}	23.8 ± 0.1^{abc}	26.8 ± 2.9^{bc}	9.7 ± 0.1^{ab}	3416 ± 23^{abc}
Standard SI	193 ± 3.2^{ab}	25.5 ± 0.2^{ab}	30 ± 3.3^{b}	11.5 ± 0.7^{ab}	4400 ± 29^{ab}

Social Isolation (SI), Negative Control (Control)

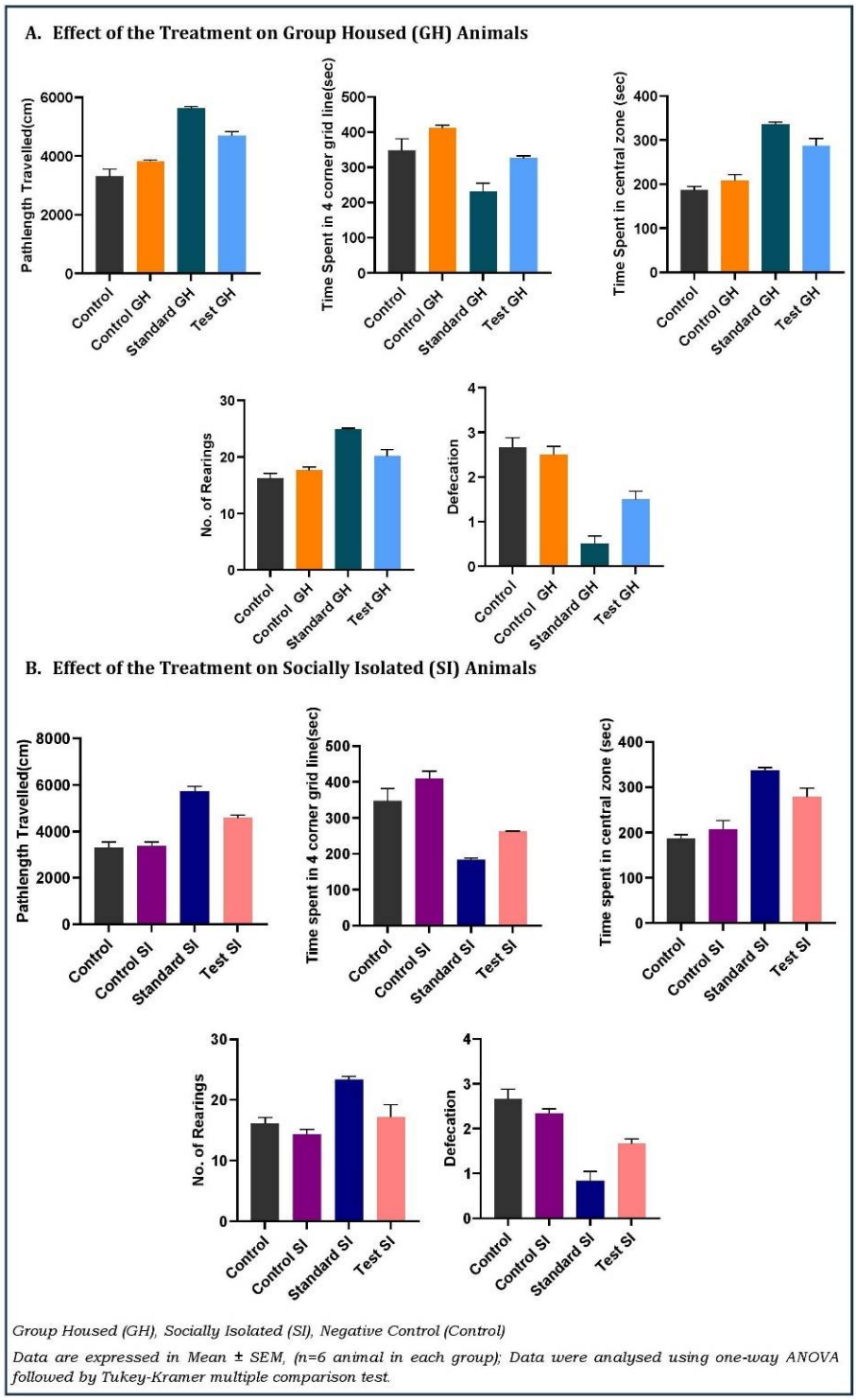
Values are expressed in Mean ± SEM, (n=6 animal in each group); the Data were analyzed using one-way ANOVA followed by Tukey-Kramer Multiple Comparison Test, where $p^a<0.05$ when compared with Control, $p^b<0.05$ when compared with Control SI, $p^c<0.05$ when compared with Standard SI.



Group Housed (GH), Socially Isolated (SI), Negative Control (Control)

Data are expressed in Mean ± SEM, (n=6 animal in each group); Data were analysed using one-way ANOVA followed by Tukey-Kramer multiple comparison test.

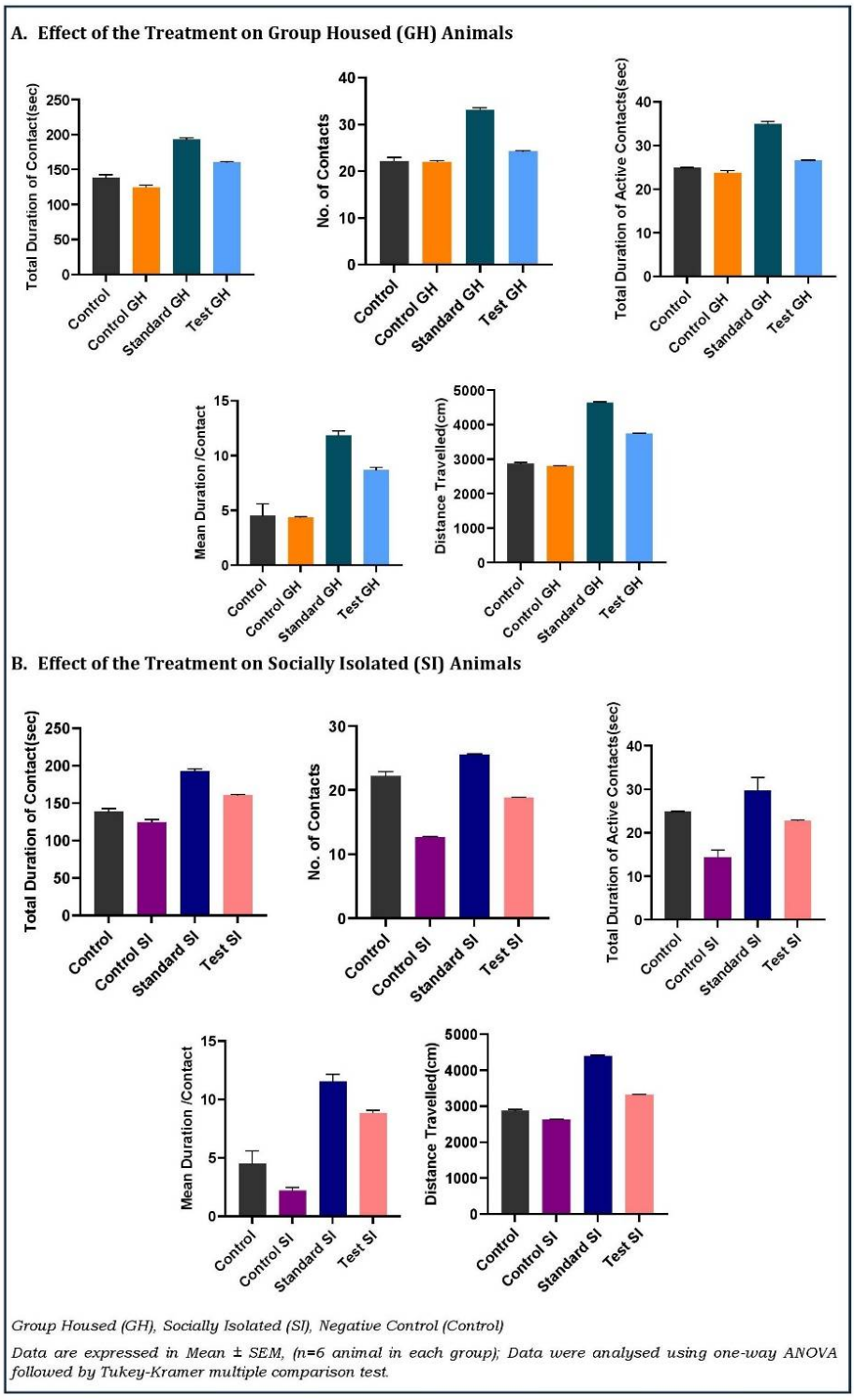
Figure 1: Graphical Presentation of effect of β-Sitosterol on animals in Elevated Plus Maze



Group Housed (GH), Socially Isolated (SI), Negative Control (Control)

Data are expressed in Mean ± SEM, (n=6 animal in each group); Data were analysed using one-way ANOVA followed by Tukey-Kramer multiple comparison test.

Figure 2: Graphical Presentation of effect of β-Sitosterol on animals in Open Field Test



Group Housed (GH), Socially Isolated (SI), Negative Control (Control)

Data are expressed in Mean ± SEM, (n=6 animal in each group); Data were analysed using one-way ANOVA followed by Tukey-Kramer multiple comparison test.

Figure 3: Graphical Presentation of effect of β-Sitosterol on animals in Social Interaction Test in Novel Environment

CONCLUSION

In conclusion, this study explored the potential adaptogenic, anxiolytic and neuroprotective effects of β-Sitosterol in animals through stress induced by environmental deprivation and Social Isolation. β-Sitosterol demonstrated promising adaptogenic, anxiolytic properties, mitigating anxiety-like behaviours and enhancing social interaction and locomotor activity. Further research is warranted to elucidate its mechanisms and therapeutic potential for stress-related neurobehavioral alterations.

REFERENCES

1. Yaribeygi H, Panahi Y, Sahraei H, Johnston TP, Sahebkar A. (2017). The impact of stress on body function: A review. *EXCLI J.* 16:1057-1072. doi: 10.17179/excli2017-480.
2. Chu B, Marwaha K, Sanvictores T and Ayers D. (2021). Physiology, Stress Reaction. *In StatPearls [Internet]*; StatPearls Publishing.
3. Schaffer J, Fogelman N, Seo D, Sinha R. (2023). Chronic pain, chronic stress and substance use: overlapping mechanisms and implications. *Front Pain Res (Lausanne).* 4:1145934. doi: 10.3389/fpain.2023.1145934.
4. Ray A, Gulati K, Rai N. (2017). Stress, Anxiety, and Immunomodulation: A Pharmacological Analysis. *Vitam Horm.* ; 103:1-25. doi: 10.1016/bs.vh.2016.09.007.
5. Lucchini R, Albini E, Placidi D, Alessio L. (2000). Mechanism of neurobehavioral alteration. *Toxicol Lett.* 112-113:35-9. doi: 10.1016/s0378-4274(99)00251-9.
6. Hagan JF Jr, Balachova T, Bertrand J, Chasnoff I, Dang E, Fernandez-Baca D, *et al.* (2016). Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure Workgroup; American Academy of Pediatrics. Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure. *Pediatrics.*;138(4): e20151553. doi: 10.1542/peds.2015-1553.
7. Van der Merwe J, Van der Veeken L, Ferraris S, Gsell W, Himmelreich U, Toelen J, *et al.* (2019). Early neuropathological and neurobehavioral consequences of preterm birth in a rabbit model. *Sci Rep.*9(1):3506. doi: 10.1038/s41598-019-39922-8.
8. Si L, Xiao L, Xie Y, Xu H, Yuan G, Xu W, *et al.* (2023). Social isolation after chronic unpredictable mild stress perpetuates depressive-like behaviors, memory deficits and social withdrawal via inhibiting ERK/KEAP1/NRF2 signaling. *J Affect Disord.* 324:576-588. doi: 10.1016/j.jad.2022.12.092.
9. Singh D, Pandey S, Ghosh A and Aich P. (2023). Effects of Constant Darkness on Behaviour and Physiology of Male and Female Mice. *European Journal of Neuroscience*;57(9):1498-1515.
10. González MM. (2018). Dim Light at Night and Constant Darkness: Two Frequently Used Lighting Conditions That Jeopardize the Health and Well-Being of Laboratory Rodents. *Frontiers in Neurology* ; 9:609.
11. Lee DH, Lee, JY, Hong, DY, Lee EC, Park SW, Lee YK and Oh JS. (2022). Pharmacological Treatment for Neuroinflammation in Stress-Related Disorder. *Biomedicines* ;*10*(10):2518.
12. Rashed K. (2020). Beta-Sitosterol Medicinal Properties: A Review Article. *J. Sci. Innov. Technol*; *9:208*-212.
13. Babu S and Jayaraman S. (2020). An Update on β-sitosterol: A Potential Herbal Nutraceutical for Diabetic Management. *Biomedicine & Pharmacotherapy* ; *131:110702*.
14. Abbas MM, Al-Rawi N, Abbas MA and Al-Khateeb I. (2019). Naringenin Potentiated β-Sitosterol Healing Effect on the Scratch Wound Assay. *Research in Pharmaceutical Sciences*;14(6):566.
15. Khan SL and Siddiqui FA. (2020). Beta-Sitosterol: as Immunostimulant, Antioxidant and Inhibitor of SARS-CoV-2 Spike Glycoprotein. *Archives of Pharmacology and Therapeutics*;2(1):12-16.
16. Al Omran AJ, Shao AS, Watanabe S, Zhang Z, Zhang J, Xue C, *et al*. (2022). Social isolation induces neuroinflammation and microglia overactivation, while dihydromyricetin prevents and improves them. *J Neuroinflammation* 9(1):2. doi: 10.1186/s12974-021-02368-9.
17. Clément Y, Le Guisquet AM, Venault P, Chapouthier G, Belzung C. (2009). Pharmacological alterations of anxious behaviour in mice depending on both strain and the behavioural situation. *PLoS One* ;4(11): e7745. doi: 10.1371/journal.pone.0007745.
18. Shoji H, Takao K, Hattori S, Miyakawa T. (2016). Age-related changes in behavior in C57BL/6J mice from young adulthood to middle age. Mol Brain. 9:11. doi: 10.1186/s13041-016-0191-9.
19. Rinki S, Mishra RN. (2011). Adaptogenic Activity of Triphala megaext. *International Journal of Research in Pharmaceutical and Biomedical Sciences*;2(1):106-109.
20. Esimone CO, Adikwu MU, Nworu C, Okoye SC, and Odimegwu DC. (2007). Adaptogenic potentials of Camellia sinensis leaves, Garcinia kola and Kola nitida seeds. *Scientific Research and Essays* ;2(7):232–237.
21. Namgyal D, Chandan K, Ali S, Ahmad A, Hashim MJ, Sarwat M. (2021). Aberrant Lighting Causes Anxiety-like Behavior in Mice but Curcumin Ameliorates the Symptoms. *Animals (Basel).* 2021;11(9):2590. doi: 10.3390/ani11092590.
22. Mumtaz F, Khan MI, Zubair M, Dehpour AR. (2018). Neurobiology and consequences of social isolation stress in animal model-A comprehensive review. *Biomed Pharmacother.* 105:1205-1222. doi: 10.1016/j.biopha. 2018.05.086.
23. Tan T, Wang W, Liu T, Zhong P, Conrow-Graham M, Tian X and Yan Z. (2021). Neural Circuits and Activity Dynamics Underlying Sex-Specific Effects of Chronic Social Isolation Stress. *Cell Reports* ;34(12).

CITATION OF THIS ARTICLE

Yashashwi S, Neelam B, Gaurav P. Impact of β-Sitosterol on Stress-Induced Neurobehavioral Alteration in Experimental Animal. Bull. Env. Pharmacol. Life Sci., Vol 13[2] January 2024: 121-132.