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ORIGINAL ARTICLE

Influence of β-Sitosterol on Neurobehavior after Intermittent Fasting and Binge Eating in Experimental Animal

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ABSTRACT

Intermittent Fasting (IF) and Binge Eating (BE) have gained popularity, but their impact on neurobehavioral aspects raises concerns. This study explores the potential mitigating effects of β -Sitosterol, known for its diverse health benefits, on anxiety-related behaviors associated with IF and BE in Swiss Albino mice. The study aims to assess the impact of β-Sitosterol on neurobehavioral aspects, body weight, and blood glucose levels in mice subjected to IF and BE. Experimental subjects included Control, Standard, and Test groups. IF and BE were induced, and neurobehavioral assessments were conducted using the Elevated Plus Maze, Open Field, and Social Interaction tests. β-Sitosterol (10 mg/kg) and Diazepam (5 mg/kg) were administered orally. Statistical analysis employed ANOVA followed by the Tukey-Kramer test. IF and BE mice exhibited anxiety-like behaviors, reduced locomotor activity, and impaired social interaction. β-Sitosterol significantly ameliorated these effects in both groups, increasing open arm entries, central zone exploration, and social interaction parameters. This study suggests that Beta-Sitosterol has potential anxiolytic effects, alleviating anxiety-related behaviors induced by IF and BE. It improves exploration, social interaction, and reverses the adverse impact on body weight and blood glucose levels.

Keywords*: Intermittent Fasting, Binge Eating, Beta Sitosterol, Anxiety, Behavior, Elevated Plus Maze Test, Open Field Test, Social Interaction Test.*

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INTRODUCTION

Intermittent Fasting has gained popularity for its potential health benefits, including weight loss, increased energy, and enhanced mental clarity (1). The connection between the brain and gut is crucial for mental well-being, with 95% of serotonin synthesized in the gut (2). Various types of Intermittent Fasting, such as Time-Restricted, Modified-Calorie, and Every Other Day Eating, offer flexible approaches (3). Additionally, Binge Eating Disorder (BED) is a prevalent concern, and a new cognitive and behavioral model aims to enhance understanding and treatment (4). This research emphasizes the impact of calorie restrictions and binge eating on mental health, proposing that β-sitosterol, a phytosterol with diverse properties, may play a role in managing neurobehavioral issues for improved overall well-being (5).

MATERIAL AND METHODS

Drugs and Chemicals

β-Sitosterol was procured as a gift sample from Pharmed Ltd. Solan, Himachal Pradesh (India). Marketed Preparation of Diazepam was used and Sodium CMC and other chemicals from IPS Academy College of Pharmacy.

Preparation of Dosage Form

In the Control group, the Sodium Carboxymethyl Cellulose (CMC) was administered orally as vehicle. The Standard group, Diazepam (5 mg/kg) was administered orally (p.o.) in suspension of 0.5% w/v Sodium CMC. The Test group, β-sitosterol (10 mg/kg) was administered orally (p.o.) in suspension of 0.5% w/v

Sodium CMC.

Animals

The *Swiss Albino* mice of either sex weighing 20-30g were utilized for the study. The animals were kept in a cage made of polypropylene with a paddy husk bed coated in stainless steel wire mesh. The animals were kept in groups of five mice per cage at a temperature of 24°C, a relative humidity level of 45–55%, and a day/night cycle of 12°C. The animals had unlimited access to water and regular chew pellets for meals. Up until the start of the experiment, normal ambient, food, and water conditions were kept.

Approval of Protocol

The research protocol for this study received ethical approval from the Institutional Animal Ethics Committee (IAEC) at IPS Academy College of Pharmacy, Indore, in strict adherence to the guidelines set forth by the Committee for Control and Supervision of Experiments on Animals (CCSEA), under the Ministry of Environment and Forest, Government of India. The IAEC, constituted to ensure the ethical treatment of animals in research, thoroughly reviewed and granted approval for all experimental procedures outlined in the protocol. The Protocol Approval number was CPCSEA/219/2023.

Grouping

The animals were divided into three groups. The Control group, Standard group, and the Test group, each further divided into Intermittent Fasting (IF) and Binge Eating (BE) subgroups. In the Elevated plus Maze (EPM) test, the Control group receive no specific drug, the Standard group was administered Diazepam (5 mg/kg) orally, and the Test group was given β-sitosterol (10 mg/kg) orally. Corresponding IF and BE subgroups were established within each main category. The Open Field and Social Interaction tests follows a similar grouping pattern, with distinct drug administrations and doses. The grouping ensures a comprehensive examination of neurobehavioral responses to intermittent fasting and binge eating, with 6 animals in each subgroup, amounting to a total of 84 animals showing in Table 1 (a) & (b).

Intermittent Fasting and Binge Eating in Experimental Mice

For Intermittent Fasting mice were subjected to the Every Other Day Fasting (EOD) regime where mice had free access to food for 24 h alternating with deprivation to food for the next 24 h but had a free access to water for 6 weeks. Food was provided to mice at 9 a.m. and were withdrawn at 9 a.m. on the next morning (6). In Binge Eating Mice were fed with standard diet in their cages, and they were expose to BE every alternate day for 6 weeks (7). The BE Diet consisted of High Fat Diet (HFD) with composition of 20 g of fat/100 g of diet (19 g of coconut oil + 1 g of soybean oil) and 10% of sucrose in tap water (8).

Body Weight and Blood Sugar level

The body weight was monitored weekly throughout the experiment (9), and Blood sugar Concentration was measured using glucometer. Glucose profiles were analyzed on 2 occasions, at baseline, and after 5 weeks on the respective feeding regimes (10).

Evaluation of Neurobehavioral Parameter

Elevated Plus Maze Test

The testing setup comprised two arms exposed to the environment and two arms enclosed with cardboard to block out light. All four arms extended outward from a central platform, which was raised 38.5 cm above the floor. The test initiation involved placing the mouse on the central platform, oriented toward one of the open arms, and observing its behaviour for a duration of 5 minutes. The mouse was considered on the central platform when it had two paws positioned on it and inside one of the arms when all four paws were located within that specific arm. Recorded behavioural variables encompassed the count and duration of entries into an open arm, entries into a closed arm, and head-dipping without protection, where the animal extended its head into the open space beneath the open arm (11).

Open Field Test

The open field test was conducted in a white acrylic plastic chamber measuring 50 cm in length, 50 cm in width, and 38 cm in height. The chamber floor was divided into a 4x4 grid pattern, creating 10x10 squares, with a central 20x20 cm square zone marked. Individual mice were placed in the center of the arena, and their activity was monitored for a period of 10 minutes. Various parameters were recorded for each mouse during this 10-minute interval, including the time spent in the central zone, time spent in the four corners of the square grid, the path length travelled (measured by tracking the distance between the mouse's nose and the 10x10 cm grid lines on the chamber floor), and the number of times the mouse reared. All data collection was carried out manually in a double-blind fashion, with each recording being observed three times to minimize any potential errors (12).

Social Interaction Test

Two mice, previously housed separately, were introduced into a box measuring 40 × 40 × 30 cm and were allowed to freely explore their surroundings for a duration of 10 minutes. During this time, the behaviour of the mice was closely observed and analysed. Various behavioural parameters were recorded, including

the total duration of contacts (in seconds), the number of contacts, the total duration of active contacts (in seconds), the mean duration per contact, and the total distance travelled (in centimetres). To define "active contact," images were captured at a rate of three frames per second, and the distance covered by each mouse between two consecutive frames was calculated. If the two mice came into contact with each other, and the distance travelled by either mouse was 5 cm or more during that interaction, it was categorized as an "active contact" (13).

Statistical Analysis

The data was presented as the Mean ± Standard Error of Mean (S.E.M.), and statistical analysis was conducted using Ordinary One-Way Analysis of Variance (ANOVA), followed by the "Tukey-Kramer" multiple comparison test, with a significance level set at $p<0.05$. The statistical analysis was performed utilizing "GraphPad Prism" version 10.0.3 for Windows, a software developed by GraphPad Software located in San Diego, California, USA (www.graphpad.com).

RESULT

Elevated Plus Maze Test

In the elevated plus maze test, the introduction of Beta-Sitosterol to mice undergoing intermittent fasting (IF) brought about notable changes in their behavior. Specifically, Beta-Sitosterol resulted in a decrease in the number of entries into the closed arm and an increase in entries into the open arm, indicating reduced anxiety-like behavior. Moreover, mice in the Beta-Sitosterol group spent more time in the open arm, further suggesting a decrease in anxiety when compared to the control and standard groups. In contrast, the control IF group displayed heightened anxiety-like behaviors, characterized by more time spent in the closed arm and fewer entries into the open arm. The standard group, which received Diazepam, exhibited the least anxiety-like behaviors, displaying a preference for the open arm and reduced time spent in the closed arm (shown in figure 1). Similar trends were observed in the binge-eating group. Beta-Sitosterol alleviated anxiety-like behaviors by reducing the number of entries into the closed arm and increasing entries into the open arm, with these mice also spending more time in the open arm. Conversely, the control binge-eating group exhibited increased anxiety-like behaviors, spending more time in the closed arm and making fewer entries into the open arm. The standard group, treated with Diazepam, displayed the least anxiety-like behaviors (shown in figure 2).

Open Field Test

In the intermittent fasting group, mice supplemented with Beta-Sitosterol also spent more time in the central zone of the open field. This finding was significant because the central zone is typically considered riskier and more anxiety-inducing for rodents due to its exposure. The increased time spent in the central zone suggests that Beta-Sitosterol may boost the mice's confidence in exploring and navigating unfamiliar and potentially anxiety-inducing environments. These observations collectively indicate an enhancement in exploratory behaviour and a reduction in anxiety-like responses in the Beta-Sitosterol group during intermittent fasting (shown in figure 3). Similarly, in the binge-eating group, Beta-Sitosterol exhibited a positive impact on exploratory behaviour. The mice in this group also displayed an increased path length travelled, reduced time in corner squares, and an increased duration in the central zone. These results were consistent with those observed in the intermittent fasting group, reinforcing the notion that Beta-Sitosterol may possess anxiolytic properties, promoting exploration, and alleviating anxiety-like behaviours, regardless of the eating pattern (shown in figure 4).

Social Interaction Test

In the intermittent fasting group, mice receiving Beta-Sitosterol exhibited increased social contacts, as indicated by a higher total duration of contact and a greater number of contacts. These findings suggest that Beta-Sitosterol improved social interaction, potentially by reducing social anxiety in these mice. Increased social contacts and duration of contact point toward enhanced sociability and reduced aversion to social interaction (shown in figure 5). Similarly, in the binge-eating group, Beta-Sitosterol led to improved social behaviour. The mice in this group displayed increased social contacts, similar to the intermittent fasting group. The increased duration and number of social contacts in the Beta-Sitosterol group indicate improved social interaction and reduced social anxiety (shown in figure 6).

Body Weight and Blood Sugar level

The research investigated the effects of intermittent fasting and binge eating on body weight and blood glucose levels. Intermittent fasting led to a decrease in body weight, potentially linked to anxiety-related behaviours. Changes in weight, whether its loss or fluctuations, can induce stress in both animals and humans. Furthermore, intermittent fasting resulted in a reduction in blood glucose levels. Low blood sugar levels, known as hypoglycaemia, can trigger sensations of anxiety, restlessness, and heightened stress responses. Hence, the observed anxiety-like behaviours in the intermittent fasting group might be

influenced by the decrease in body weight and blood glucose levels (Table 2). Conversely, in the binge-eating group, there was an increase in body weight, a common consequence of binge eating. Excessive calorie intake during binge episodes can lead to obesity, which is linked to anxiety and depression in both animals and humans. Furthermore, binge eating led to elevated blood glucose levels, which may also contribute to anxiety-like responses (Table 3).

Table 1 (a) Grouping of Swiss Albino Mice for Elevated Plus Maze

S.No.	Group	Drug	Dose	Route of Administration	No. of Animals*
1.	Control				6
2.	Control IF	Sodium CMC	0 .5 ml of 0.5%w/v	Oral (p.o.)	6
3.	Control BE				6
4.	Standard IF	Diazepam	5 mg/kg	Oral (p.o.)	6
5.	Standard BE				6
6.	Test drug IF	β-sitosterol	10 mg/kg	Oral (p.o.)	6
7.	Test drug BE				6
Total No. of Animals Required					**42**

(IF = Intermittent Fasting mice, BE= Binge Eating mice)
6 animals/group for Elevated Plus Maze Test

Table 1 (b) Grouping of Swiss Albino Mice for Open field and Social Interaction Test

S.No.	Group	Drug	Dose	Route of Administration	No. of Animals*
1.	Control				6
2.	Control IF	Sodium CMC	0 .5 ml of 0.5%w/v	Oral (p.o.)	6
3.	Control BE				6
4.	Standard IF	Diazepam	5 mg/kg	Oral (p.o.)	6
5.	Standard BE				6
6.	Test drug IF	β-sitosterol	10 mg/kg	Oral (p.o.)	6
7.	Test drug BE				6
Total No. of Animals Required					**42**

(IF = Intermittent Fasting mice, BE= Binge Eating mice)
**For each group 6 animals/group for Open field Test and Social Interaction Test*

Table 2. Body Weight and Blood Glucose Level of Intermittent Fasting Group

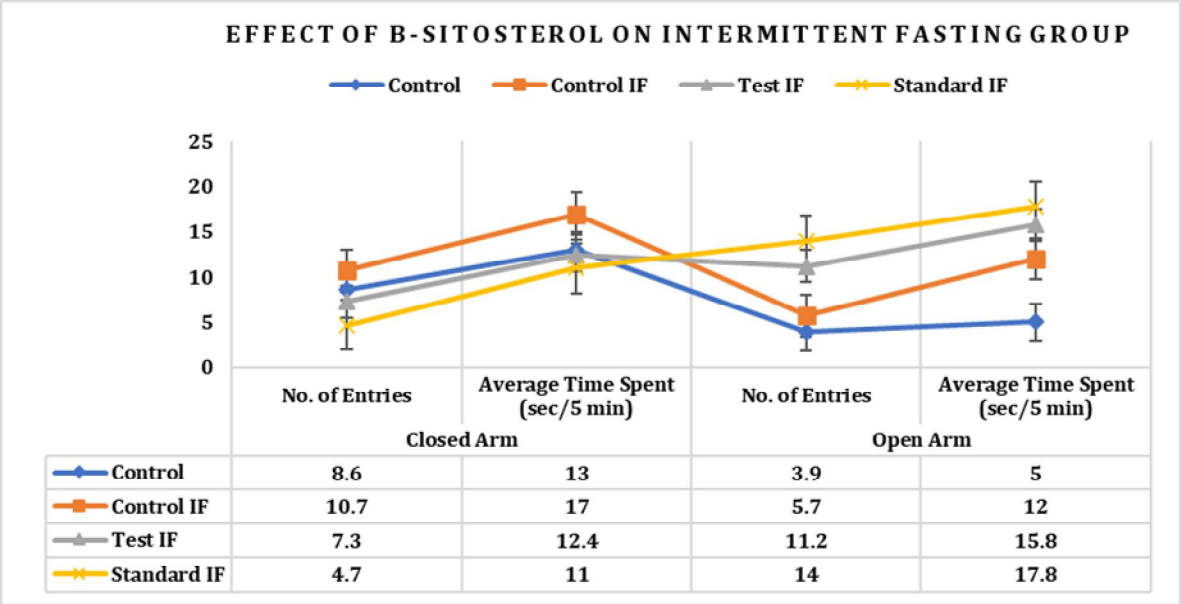
S.No.	Grouping	Body Weight		Blood Glucose Level	
		Baseline	After 5 Weeks	Baseline	After 5 Weeks
1	**Control**	21 ± 0.4	22 ± 0.4	96 ± 1	98 ± 1
2	**Control IF**	21 ± 0.2	20 ± 0.3	95 ± 1	75 ± 1
3	**Test IF**	22.5 ± 0.2	21.5 ± 0.1	92 ± 1	75 ± 1.2
4	**Standard IF**	22 ± 0.4	20.8 ± 0.2	93 ± 0.5	75 ± 1.3

Intermittent Fasting (IF), Negative Control (Control)
Values are expressed in Mean ± SEM, (n=6); Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparison test.

Table 3. Body Weight and Blood Glucose Level of Binge Eating Group

S.No.	Grouping	Body Weight		Blood Glucose Level	
		Baseline	After 5 Weeks	Baseline	After 5 Weeks
1	**Control**	21 ± 0.3	22 ± 0.4	96 ± 1.3	97 ± 1
2	**Control BE**	22 ± 0.2	24 ± 0.2	94 ± 1	116 ± 1
3	**Test BE**	22 ± 0.3	24 ± 0.1	92.33 ± 1	119 ± 1.5
4	**Standard BE**	22 ± 0.3	23 ± 0.3	95 ± 0.7	120 ± 1.5

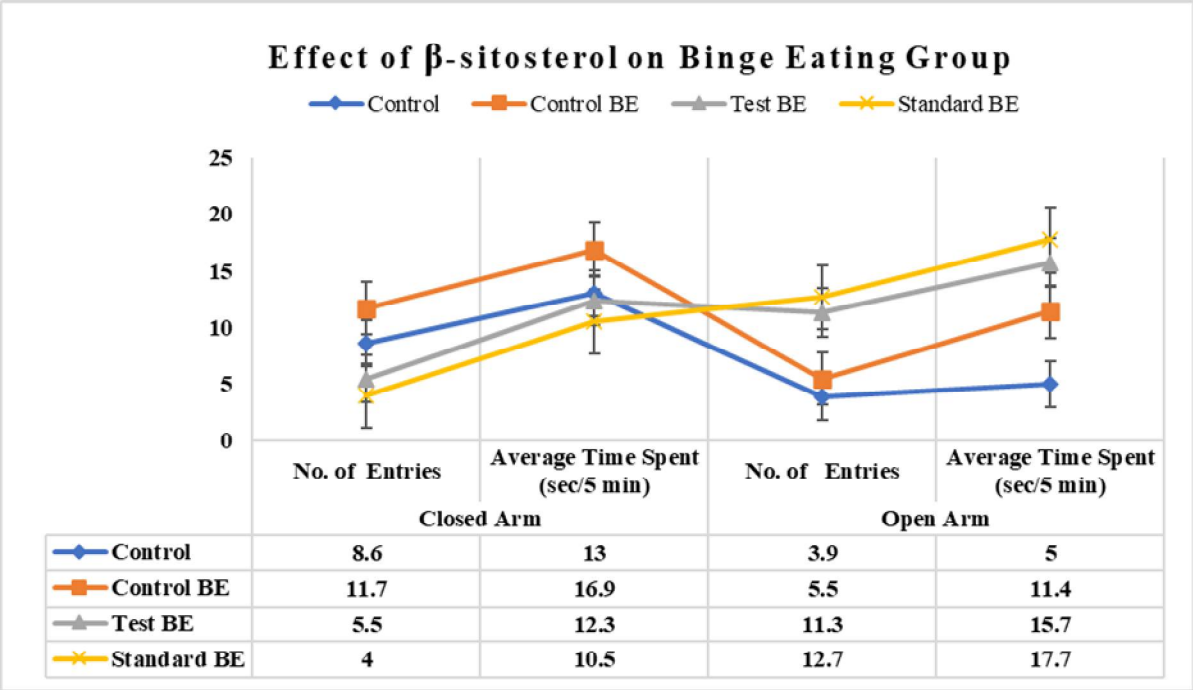
Binge Eating (BE), Negative Control (Control)
Values are expressed in Mean ± SEM, (n=6); Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple comparison test.



	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	13	3.9	5
Control IF	10.7	17	5.7	12
Test IF	7.3	12.4	11.2	15.8
Standard IF	4.7	11	14	17.8

Intermittent Fasting (IF), Negative Control (Control)
Values are expressed in Mean ± SEM, (n=6); Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple Comparison Test

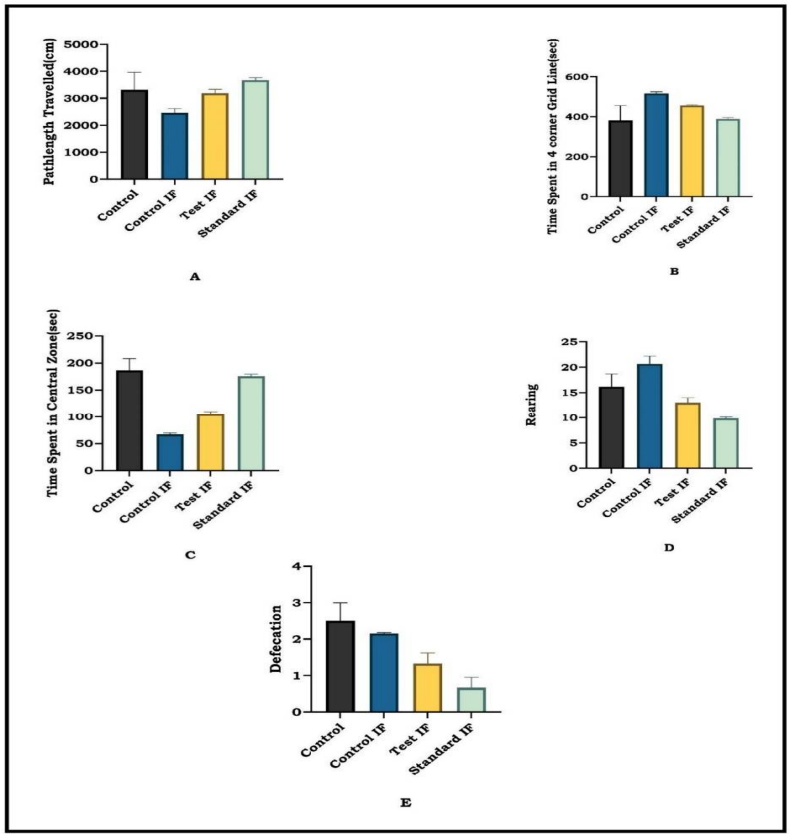
Fig. 1 Graphical Presentation of the Effect of β-Sitosterol on Intermittent Fasting group in Elevated Plus Maze



	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	13	3.9	5
Control BE	11.7	16.9	5.5	11.4
Test BE	5.5	12.3	11.3	15.7
Standard BE	4	10.5	12.7	17.7

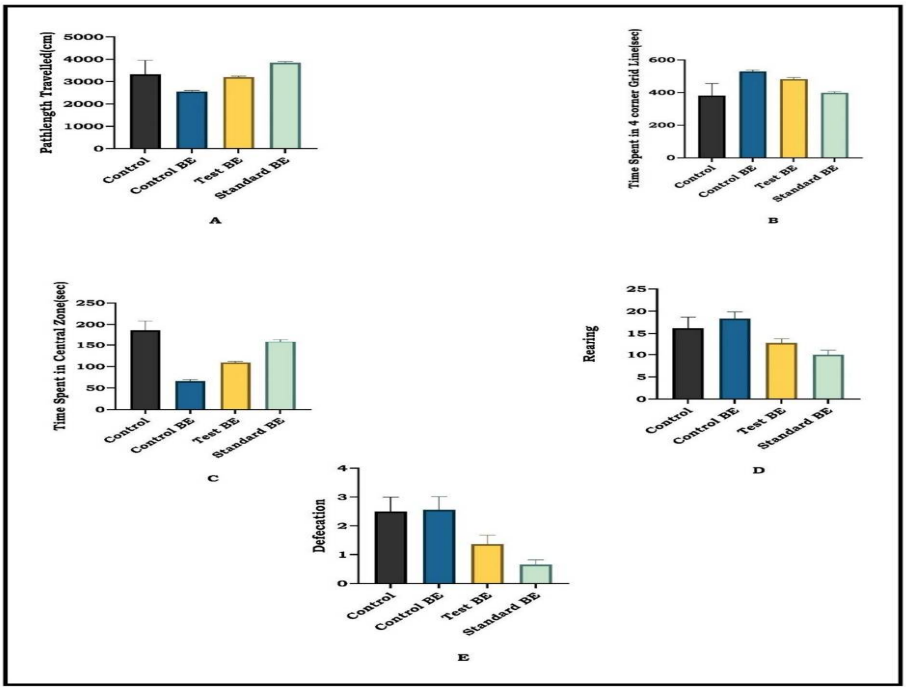
Binge Eating (BE), Negative Control (Control) Values are expressed in Mean ± SEM, (n=6); Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple Comparison Test.

Fig. 2 Graphical Presentation of the Effect of β-Sitosterol on Binge Eating Group in Elevated Plus Maze



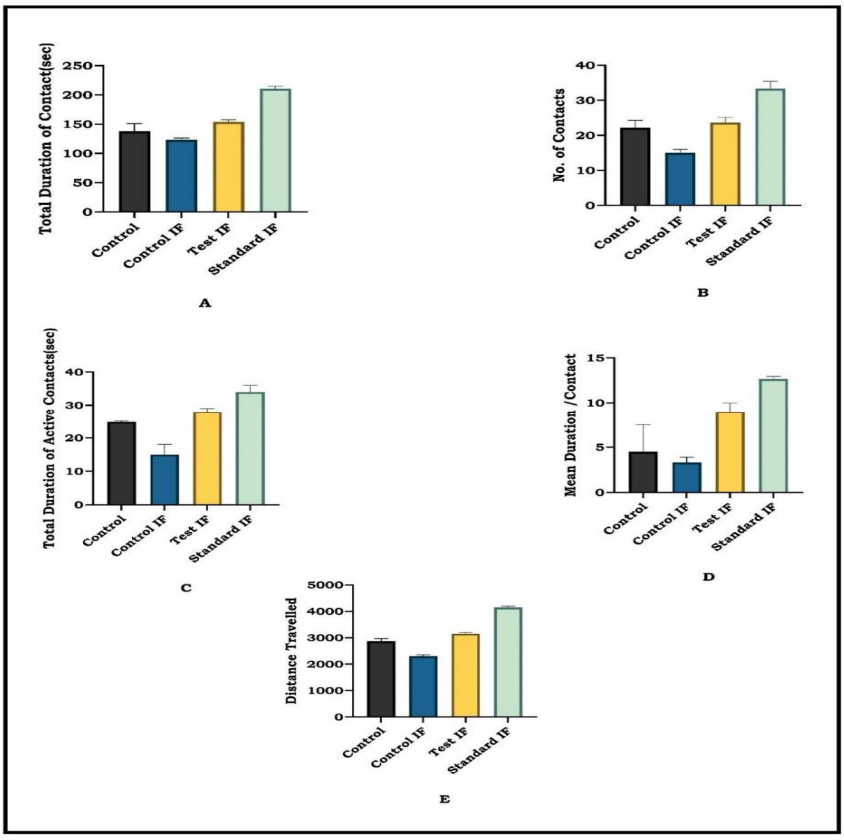
Intermittent Fasting (IF), Negative Control (Control) Values are expressed in Mean ± SEM, (n=6); Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple Comparison Test

Fig. 3 Graphical Presentation of the Effect of β-Sitosterol on Intermittent Fasting group in Open Field Test



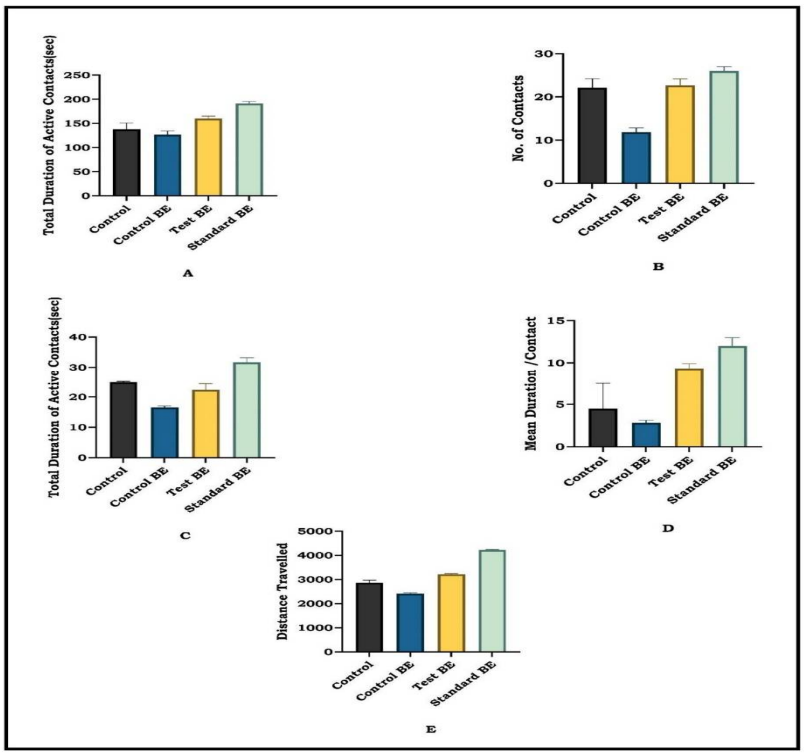
Binge Eating (BE), Negative Control (Control) Values are expressed in Mean ± SEM, (n=6); Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple Comparison Test

Fig. 4 Graphical Presentation of the Effect of β-Sitosterol on Binge Eating group in Open Field Test



Intermittent Fasting (IF), Negative Control (Control) Values are expressed in Mean ± SEM, (n=6); Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple Comparison Test

Fig. 5 Graphical Presentation of the Effect of β-Sitosterol on Intermittent Fasting Group Social Interaction Test



Binge Eating (BE), Negative Control (Control) Values are expressed in Mean ± SEM, (n=6); Data were analyzed using one-way ANOVA followed by Tukey-Kramer multiple Comparison Test

Fig. 6 Graphical Presentation of the Effect of β-Sitosterol on Binge Eating Group in Social Interaction Test

DISCUSSION

The study investigate the neurobehavioral impacts of intermittent fasting (IF) and binge eating (BE) in Swiss Albino mice, focusing on anxiety and depression-like behaviors. Beta-Sitosterol, a natural compound, was compared to the standard drug diazepam for its effects. Behavioral tests, including the Elevated Plus Maze, Open Field Test, and Social Interaction Test, revealed that Beta-Sitosterol mitigated anxiety-like behaviors and improved exploratory and social interactions in both IF and BE groups. Additionally, the research explored the physiological effects, indicating that IF reduced body weight and blood glucose levels, potentially associated with anxiety, while BE increased body weight and blood glucose levels.

Limitation: This study was faces challenges in generalizing mouse findings to humans, requiring further investigation for clinical relevance. Additionally, it lacks exploration of the molecular mechanisms underlying Beta-Sitosterol anxiolytic effects, limiting targeted therapeutic insights.

CONCLUSION

Beta-Sitosterol demonstrated anxiolytic effects in mice subjected to intermittent fasting and binge eating, enhancing social interaction and reducing anxiety-like behaviors. Diazepam showed similar effectiveness. Body weight and blood glucose fluctuations correlated with anxiety responses, highlighting potential links between metabolic changes and behavioral outcomes in dietary stress paradigms.

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Statement of Contribution: Nil

Conflict of Interest: None declared

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