EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 9
52-57
Year: 2022

# INFLUENCE OF B-SITOSTEROL ON NEUROBEHAVIORAL PARADIGM IN EXPERIMENTAL MICE

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**Article Received on 24/06/2022** **Article Revised on 15/07/2022** **Article Accepted on 07/08/2022**

## ABSTRACT
Beta-sitosterol belongs to the faction of phytosterols, which are dynamic trace components existing in natural plants, known as the "key of life", and have a steroid nucleus structure similar to cholesterol. Due to the insolubility issue of beta-sitosterol, most pharmacological studies and clinical applications are restricted. Therefore, the modification of beta-sitosterol into its derivatives to improve its pharmacologic activity is possible. In this study, β-sitosterol was administered orally to mice in two doses, 5mg/kg and 10mg/kg. In both of the experimental models of anxiety elevated plus maze and mirror chamber test used to evaluate the anti-anxiety effect, the results imply that the drug possesses an anti-anxiety effect.

**KEYWORDS:** Anxiety, Serotonin, Gama-amino butyric acid,β-sitosterol.

## INTRODUCTION
Anxiety is an unpleasant state of tension, apprehension, or uneasiness (a fear that seems to arise from an unknown source) induced in animals and humans by a threat to well-being or survival, either actual or potential. It is characterized by increased arousal, expectancy, autonomic and neuroendocrine activation, and specific behavior patterns, often with a behavioral transition from ongoing behaviors (like exploration, feeding) to an escape (flight) or other defensive behaviors. The function of these changes is to facilitate coping with an adverse or unexpected situation. However, suppose the adaptive part of anxiety is not successful. In that case, anxiety can become a pathological state, which may, later on, interfere with the ability to cope with various challenges or stressful events in daily life, and even alter body conditions. Pathological anxiety can also be a consequence of predisposing factors (or traits), which result from numerous gene-environment interactions during development (particularly during the perinatal period) and experience (life events). Conceptually, it is crucial to distinguish fear, a response to an immediate, real danger, from anxiety, which is a response to a threat, i.e., a potential danger (Steven *et al.*, 2007).

### β-sitosterol
Beta-sitosterol is part of a group of phytosterols known as the "key of life" because they contain a steroid nucleus structure similar to cholesterol. Due to the insolubility issue of beta-sitosterol, most pharmacological studies and clinical applications are limited. Therefore, modifying beta-sitosterol into its derivatives to enhance its pharmacologic activity is viable. Beta-sitosterol derivatives were obtained by an esterification reaction with beta-sitosterol, organic acids, EDCI, and DMAP in dichloromethane. The chemical structures were defined by IR and NMR. Beta-sitosterol and its derivatives were employed to conduct antidepressant research in the tail suspension test (TST) and forced swimming test (FST) in mice. Moreover, the roles of different parts of the central nervous system (CNS) in the antidepressant-like effect of sitosterol, which is one of the beta-sitosterol derivatives. The study showed that the derivative exhibited more vital antidepressant activity than beta-sitosterol. Among the products, administration of sit-S (4MG/KG) gave the lowest immobility time in the TST, demonstrating that Sit-t exhibited the most potent antidepressant-like activity. The role of different parts of the CNS in the antidepressant, like the effect of Sit-S, showed that agomelatine (40 mg/kg) haloperidol (0.2mg/kg). The author highlighted that the beta-sitosterol derivatives broaden the pharmacological effects of beta-sitosterol; sitosterol (4 mg/kg) exhibits antidepressant-like effects. This antidepressant-like effect on male adult mice is mediated by the 5-HT, DA, and GABA-ergic systems (Sayeed *et al.*,2015).

### Structure of Beta-Sitosterol
### Source
Various dietary and non-dietary plants have been shown to contain beta-sitosterol. It may be found in a variety of plant components, including leaves, rhizomes, and fruits.

Other plant tissue cultures have also been shown to contain it. Its membrane-stabilizing impact on the cell membrane has been explored, but its involvement in the cytoplasm and chloroplast has yet to be investigated. The most essential chemical substance for plant life is BS-derived phytoecdysteroid, however whether or not Beta-Sitosterol plays a substantial role in plant defence requires more investigation. (Sayeed *et al.,* 2016).

**Chemistry**

Animals and plants both manufacture sterols, which are key components of cell membranes. All sterols have the same sterol ring structure; the variances are in the side chains. They're alcohols with 28 or 29 carbons. The most prevalent plant sterol, ß-sit sterol, is comparable to cholesterol. Because of its structural similarities to cholesterol, ß-sit sterol can be used to substitute it in the human body. 4-desmethyl sterol is ß-sitosterol. The C-5 location in the ring features a double bond. It is often esterified with fatty acids for inclusion into anti-BPH properties such as Arjuna (Terminalia arjuna), Haridra (Curcuma longa), Kummari (Aloe vera), Shatavari (Asparagus racemosus), Pippili (Piper longum), and others to analyse and determine anti-BPH activity. (Lawford and colleagues, 2003).

**Biosynthesis**

Although the precise biosynthetic method of BS differs between species, it typically follows the mevalonate route. Biologically, BS is produced via the mevalonate and deoxyxylulose pathways, although it prioritises one or the other depending on the external environment. The mechanism of BS biosynthesis was investigated using a 13C-labeling method. Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are thought to mix to generate farnesyl diphosphate (FPP). Then, by methylation, hydride shift, reduction, and small alteration in the beta-ring, two molecules of FPP unite tail-to-tail to generate Squalene, a triterpene, and then cycloartenol, which finally becomes BS through methylation, hydride shift, reduction, and minor change in the beta-ring. (Rang *et al.*, 2012).

**Chemical Synthesis**

For more over 50 years, many techniques for the synthesis of BS have been documented. It was discovered that selective hydrogenation of the stigmasterol side-chain 2021 alkene results in BS contaminated with various percentages of recovered stigmasterol and completely saturated stigmastanol. The synthesis of sitosterol and related sterols, on the other hand, avoids the necessity for selective hydrogenation to safeguard the cyclopropyl carbonyl ether. Solvolysis of the cyclopropane reintroduces both the C3-alcohol and the 566 alkene after hydrogenation of the 2223 double bond. A novel method for synthesising the side chain, based on the protection of the 5.6 alkene as an epoxide, has recently been used to modify phytosterols. However, neither a comprehensive BS biosynthetic route nor a complete BS chemical synthesis have been documented. (Hernandez *et. al.,* 2007).

**Pharmacological activities β-Sit sterol**

**Immunomodulatory activity**

When stimulated by phytohaemagglutinin (PHA) at lower concentrations than optimal, minimal dosages of -sitosterol and daucosterol (3-O-D-glucoside) have been shown to increase T-lymphocyte proliferative activity in vitro. The essential sterolin formulation (ESF) resulted in a considerable increase in the expression of CD25 and HLA-Dr antigens on T-lymphocytes, as well as an increase in IL-2 and gamma interferon production. NK-cell activity was boosted by either -sitosterol or daucosterol, whereas ESF exhibited greater activity. (Timmerman *et al.*, 1999).

**Anti-inflammatory activity**

The impact of -sitosterol in vivo in a model of delayed-type hypersensitivity (DTH). They discovered that this substance might control cell-mediated edoema. Despite this, it had no effect on the arachidonate pathway in intact cells and had no effect on leukocyte infiltration as evaluated by myeloperoxidase activity in biopsies. They stressed that its reaction to oxazolone might be attributable to a route other than interleukin-4. Furthermore, -sitosterol was unable to block the cyclooxygenase (COX) pathway, which is responsible for the production of prostaglandin E2 (PGE2). (Loizouet et al., 2016) used ELISA to determine the activity of -sitosterol (dose ranged (0.1-200 M) on the expression of vascular adhesion and intracellular adhesion molecule 1 in TNF-alpha-stimulated human aortic endothelial cells (HAECs), as well as monocyte attachment (U937 cells) in TNF-alpha-stimulated human aortic end In TNF-alpha-stimulated HAEC, - (steven *et al.*, 2007).

**MATERIAL AND METHOD**

**Drugs and Chemicals**

All the chemicals and drugs required for the experiment were procured from IPS Academy College of Pharmacy, Indore.

**Preparation of dosage form**

In the control group, the vehicle was administered orally. In the standard group, diazepam (5mg/kg) was administered orally as a suspension of (0.5w/v) sodium CMC. In the treatment group, beta-sitosterol (5mg/kg) (10mg/kg) was administered orally as a suspension of (0.5w/v) sodium CMC Aqueous solution of beta-sitosterol was dissolved in the solution of sodium CMC (0.5 w/v) to produce respective stock solutions of different doses.

**Route of administration**

An aqueous solution of beta-sitosterol was given orally by oral gavage.

**Instruments and equipment used**
Digital balance (Shimadzu Corporation), Hot air oven, elevated plus maze apparatus mirror chamber apparatus.

**Animals**
Albino mice weighing between 20-25gm of either sex were used. Animals were placed in the respective apparatus for 5 min session period. Standard laboratory conditions of temperature and humidity 12h/12 light/dark cycles were maintained throughout the experiment. Animals had free access to water and a normal pellet animal diet. The animal was allowed to acclimatize for one week before the investigation.

**Approval of protocol**
All experimental procedures and protocols used in this study were revised and 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 Animals (CPCSEA). Ethical guidelines were strictly followed during the experiment.

**Elevated plus-maze**
The study employed Swiss albino mice of either sex weighing 20-25g. They were separated into three groups, each with six animals, and housed in separate cages. Group 1 was a control group, group 2 was a standard group (diazepam), and group 3 was T-1 and T-2. For the raised maze test, an apparatus consisting of two arms (16*5*12) crossed with two closed arms (16*5*12) and two arms (16*5cm) with an open ceiling and elevated at a height of 25cm was set up. The arms were attached to the centre square, forming the plus sign shape of the equipment. The maze was kept in a dimly lighted chamber, elevated 50cm from the ground. Individual mice were put in the maze's middle, facing an open arm (Kulkarni and Sharma 1991).

**Fig. 1: Entry of mice into open arm.**

**Mirror Chamber Test**
Swiss albino mice of either sex were used in the study. They were divided into three groups Control, Standard, and Treatment (T1 and T2) and weighed. In this experiment, the mirror chamber apparatus was set up having dimensions of 40 x 40 x 30.5 cm. Mice were brought to the testing room30 minutes before the experiment. At the start of the 5 min test, mice were placed gently in fixed corners in the mirror chamber, the stopwatch was started, and the following parameters were noted.

- Latency to enter into mirror chamber, i.e., the time in seconds for the first entry into the section of mirrors.
- Number of entries in mirror chamber during five minutes.
- Total time spent in the mirror chamber during the five minute test period (Kumar *et al.*, 2013

**Fig. 2: Entry of mice into the mirror chamber.**

**Anti-anxiety activity**
**Elevated plus-maze**
**Table 5: Effect of diazepam and beta-sitosterol on the behavior of mice in the elevated plus-maze test.**

| S.No | Treatment | Dose | Closed Arm | | Open Arm | |
|---|---|---|---|---|---|---|
| | - | - | No of entries | Time spent | No of entries | Time spent |
| 1 | Control | - | 10 ± 0.93 | 3.3 ± 0.23 | 2.1 ± 0.40 | 0.1 ± 0.02 |
| 2 | Standard (Diazepam) | 5mg/kg | 10 ±0.47$^{ns}$ | 2.0 ± 0.17$^{a}$ | 6.3 ± 0.42$^{a}$ | 1.0 ± 0.11$^{a}$ |
| 3 | β-sitosterol | 5mg/kg | 7.3 ±0.42$^{ns}$ | 2.5 ± 0.21$^{ns}$ | 4.5 ± 0.42$^{a}$ | 0.72 ± 0.16$^{a}$ |
| 4 | β-sitosterol | 10mg/kg | 7.3 ± 0.84$^{ns}$ | 2.5 ± 0.26$^{ns}$ | 5.3 ± 0.55$^{a}$ | 0.87 ± 0.12$^{a}$ |

Control vs. DZP, BS (1), BS (ii) ap<0.01.
All the data were subjected to ANOVA followed by the Turkeys' test, and the values are reported as Mean ± SEM ap<0.01 when compared to control.

In this test, the following parameters were observed
1. Number of entries in open and closed arms- an arm entry is defined as the entry of four paws into the arm
2. The formula calculates the average time spent by each animal in each component-

Average time = Total duration in arm
Number of entries
The following result was found:-

The Control group displayed mice spent most of the time in the close arm during the test period.
1. Diazepam increased the preference of animals in the open arm and increased the number of entries (ap<0.01) and time spent by the animal in the open arm as compared to control.

2. β-sitosterol (5mg/kg and 10 mg/kg) showed a dose-dependent increase in the number of entries and time spent (ap<0.01) by the animal in the open arm as compared to the control.

**Mirror Chamber Test**

**Table Effect of Diazepam and Beta-sitosterol on Behavior of mice in Mirror Chamber Test.**

| S.No | Treatment | Dose | N | Latency | No of entries | Time spent |
|---|---|---|---|---|---|---|
| 1 | Control | - | 6 | 205± 9.06 | 1.6± 0.33 | 7±1.41 |
| 2 | Standard (Diazepam) | 5mg/kg | 6 | 17±0.97[a] | 7.3± 0.80a | 69±2.13a |
| 3 | β –sitosterol | 5mg/kg | 6 | 24±0.82[a] | 5.5 ±0.42[a] | 71 ±1.81[a] |
| 4 | β –sitosterol | 10mg/kg | 6 | 21±1.06[a] | 6 ± 0.57[a] | 78±3.10[a] |

Control vs. DZP, BS (1), BS (ii) ap<0.01.
All the data were subjected to ANOVA followed by Tukeys' Test, and the values are reported as Mean± SEM ap<0.01, compared to control.

**The following result was found**

1. The Control group showed mice took time to enter the mirrored chamber and spent minimal time in the chamber during the test period.
2. Diazepam decreased the latency (ap<0.01) to enter the mirror chamber and increased the number of entries ( ap<0.01) and time spent in the chamber during the test period as compared to the control group.
3. β-sitosterol (5mg/kg and 10 mg/kg) showed a significant dose-dependent decrease in latency and an increase in the number of entries and time spent (ap<0.01) as compared to the control group.

## DISCUSSION

Anxiety is a psychological condition with physical, emotional, behavioural, and cognitive aspects. GABAergic, dopaminergic, and adrenergic systems all have a role in anxiety disorders. Because of the negative side effects of synthetic medications such as diazepam, experts are increasingly focusing on alternative remedies. Animal models are used to analyse anxiety-related behaviour based on the notion that animal anxiety is equivalent to human anxiety. Anxiety-related behavioural and psychological reactions equip a person to respond effectively to such situations, for as by demonstrating defence behaviour like fight or flight. As a result, anxiety allows a person to flee potentially hazardous circumstances. An optimal anxiety animal model should respond to anxiolytics like benzodiazepines with a positive response.The present study demonstrates the anti-anxiety effect of beta-sitosterol and standard anxiolytic drug diazepam, which showed significant impact in several experimental models such as elevated plus maze mirror chamber test.

The elevated plus-maze model is considered a valid model of anxiety as it uses natural stimuli that fear new, brightly light open space and fear of balancing on a relatively narrow raised platform. It is known that anxiolytic agents increase the frequency of entries and time spent in the open arms of elevated plus maze (Pellow *et al.*, 1985). In the present study, the number of entries and time spent in the open arm was found to be effective at a dose of (5mg/kg, 10mg/kg) of beta-sitosterol. It was found that there is a significant increase in the number of entries and time spent by the animal in the open arm when compared with the control. The standard anxiolytic drug diazepam also shows a significant increase in open arm entries and time spent, demonstrating that the beta-sitosterol has distinct potential for being developed as an anti-anxiety drug for this model.

The mirror chamber test was developed for the animal species which exhibit approach-avoidance conflict upon placement in the mirror. This apparatus is widely used for the evaluation of anxiolytics agents belonging to chemically different classes. When the mouse approaches to mirror chamber, it initially doesn't touch the surface but retracts to the corridor and circles the entire corridor. The response to apparent animals reflected in the mirror might also be a source of anxiety (Paterson *et al*., 2009). In the present study, the β-sitosterol showed a decrease in latency increase in the number ofEntries and time spent by the animal in the mirrored chamber when compared to control group. Thus from this study, it is concluded that β-sitosterol possesses anti-anxiety activity.

Diazepam is a long-acting medium potency BZD used as an anticonvulsant for anxiolysis, sedation, and myorelaxation. Diazepam is one of the most common BZDS used for anxiety. It is available in different dosage forms. Diazepam reacts with equal affinity on all BZD sensitive receptors in the central nervous system. Anxiolytic effects are seen at low doses because of diazepam's interaction with ∝ two-containing receptors in the limbic system; at higher doses, diazepam may provide myorelaxation. In addition to anxiolysis, the myorelaxation effect is primarily mediated through ∝2 containing receptors in the spinal cord and motor neurons and, to a lesser extent, through interaction with ∝ three receptors (Charles *et al.*, 2013).

β-sitosterol is one of the several plant sterols which are structurally similar to cholesterol. sterols are not synthesized in the human body; however, they are ubiquitous throughout the plant kingdom. Although poorly absorbed, they appear to have important beneficial activities in human physiology when

administered in the diet. A dose-response curve of β-sitosterol in the range 1 to 30mg/kg doses indicated that this compound produced an anxiolytic action from 1 to 10mg/kg and a sedative response when the dose is increased to 30mg/kg. These effects resemble those produced by diazepam (0.1mg/kg) β-sitosterol has been reported to have beneficial effects in different diseases, but it has not developed as an independent drug primarily because of its relatively lower efficacy and the development of other drugs with higher efficacy new study design should be made on drug delivery to compensate its lower effectiveness and poor absorptivity over-generalization of systemic pharmacological effects of all phytosterols by all regulatory agencies (Sayeed *et al.*, 2016).

## CONCLUSION

In this study, β-sitosterol was administered orally to mice in two doses, 5mg/kg and 10mg/kg. In both of the experimental models of anxiety elevated plus maze and mirror chamber test used to assess the anti-anxiety effect, the results suggest that the drug possesses an anti-anxiety effect**.** In the present work, it was concluded that beta-sitosterol has anti-anxiety activity. The elevated plus-maze test and mirror chamber test were used as an experimental model of anxiety. In the plus maze test, exposure of animals to a novel maze alley evokes an approach-avoidance conflict which is more vital in the open arm as compared to the closed arm. In this model, after the administration of the treated drug, the animal showed an increase in the number of entries and time spent in the open arm when compared to the control group, the preference for to open arm increased.

Significantly same in the case of other models used for accessing the anxiety (mirror chamber test), the drug decreased the latency to enter the mirror chamber and increased the number of entries and time spent by the animal in the mirrored section when compared to the control. By this, it is concluded that the drug possesses anti-anxiety activity.

## ACKNOWLEDGEMENTS

I wish to thank my supervisor Dr. Neelam Balekar, Professor, IPS Academy College of Pharmacy, Indore for her patience, attentive guidance and keen interest, valuable time and mentoring skills, thereby providing me a free and pleasant atmosphere to work against all odd situations. I fall short of words to express the greatness of my esteemed supervisor. I express with utmost cordiality, the loving sincere gratitude to Dr. D.K Jain, Principal, IPS Academy College of Pharmacy, Indore, for his worthy assistance, provision of all necessities and for truly morale support.

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