REVIEW ARTICLE

Pharmacokinetic and Pharmacodynamics Formulation Aspects of β-Sitosterol

Durgawale TP[1*], Rahate AC[2], Nikam PP[2], Nemade LS[2]

[1]*Krishna Institute of Pharmacy KVV'S, Karad, Maharashtra, India.*
[2]*Govindrao Nikam College of Pharmacy, Sawarde, Maharashtra, India.*

Received: 20th May, 2024; Revised: 16th July, 2024; Accepted: 03rd August, 2024; Available Online: 31st August, 2024

ABSTRACT

Sterols have a crucial role in the structure of cells of plants and animals membranes. Animals and plants are rich in cholesterol and beta-sitosterol, respectively. Vegetarians consume a lot of β-sitosterol, but their absorption is quite low. Because of their structural similarities, beta-sitosterol competes with cholesterol for absorption and is consequently utilized as an antihyperlipidemic drug. ß-sitosterol's pharmacological screening showed a variety of non-toxic antibacterial, anti-inflammatory, antitumor, antifertility, angiogenic, antioxidant, immunomodulatory, and antinociceptive properties. It is also well-studied how β-sitosterol pharmacokinetics work. Numerous authors have produced several formulas. Nevertheless, dearth of research reported on the pharmacology, phytochemistry, and market outlook of β-sitosterol.

Keywords: Sterols, Pharmacokinetics, Pharmacodynamics, Cholesterol, Phytosterol.

International Journal of Pharmaceutical Quality Assurance (2024); DOI: 10.25258/ijpqa.15.3.91

How to cite this article: Durgawale TP, Rahate AC, Nikam PP, Nemade LS. Pharmacokinetic and Pharmacodynamics Formulation Aspects of β-Sitosterol. International Journal of Pharmaceutical Quality Assurance. 2024;15(3):1693-1702.

Source of support: Nil.

Conflict of interest: None

INTRODUCTION

Bioactive substances called phytosterols are present in plant cell membranes and chemically resembling that of cholesterol generated from mammalian cells. One of the numerous phytosterols that share a chemical structure with cholesterol isβ-sitosterol (BS). Plant foods high in lipids, like nuts, seeds, legumes, and olive oil, are rich in them. Out of all the phytosterols, the main one that is abundant in plants is called β-sitosterol (SIT).[1] β-sitosterol is a naturally arising phytosterol. It is a steroidal substance derived from plants that resembles cholesterol. It is found in a variety of plant and vegetable oils due to its steroidal lipophilic properties.[2]

Within the kingdom of plants, β-sitosterol is an old chemical. However, in vascular plants and single-celled creatures simple form of sterol gets converted into a more complex one. According to research published in the European Journal of Medicinal Plants, 4(5): 590–609, 2014 597, plants produce 24 α– ethyl sterols like sitosterols, whereas fungi, algae, and protozoa generate 24β– methyl sterols or ergosterols.[3] Plasma membrane fluidity and cellular permeability during stressful conditions or other aberrant metabolic disruptions. In mammals, sterols serve as a precursor to numerous steroidal-derived compounds, including testosterone, estrogen, glucocorticoids, and mineral corticoids. The primary sterol components found in human nutrition are represented by the plants we eat, where β-sitosterol makes up 65%, campesterol 30%, and stigmasterol 3% of the total nutritious content.[4] The research revealed that the absorption of β-sitosterol and other phytosterols is dependent on both concentration and time.[2] Figure 1 displayed information on the graphical introduction of β-sitosterol.

Pharmacokinetics of β-sitosterol

Pharmacokinetics (PK) is the reckonable, in-depth study of drug distribution, metabolism, excretion, and absorption, i.e., how the body metabolizes a medication as it is acting on it.[5] β-sitosterol experiences a range of pharmacokinetic factors such as absorption, distribution, metabolism and excretion. Figure 2 illustrates the pharmacokinetics of beta-sitosterol.

Mechanism of Absorption

Since humans cannot synthesize β-sitosterol, and acquire it from food. However, based on the unique sterol structure, the oral bioavailability of phytosterols is relatively low, ranging from 0.5 to 5%. Currently, it is unknown what the volume of distribution and specific clearance is for the two primary dietary phytosterols, β-sitosterol and campesterol. Nonetheless, plasma concentrations are only in the micromolar range and are far less as compared to typically seen for cholesterol (about 5 mM).

**Author for Correspondence: truptipdurgawale@gmail.com*

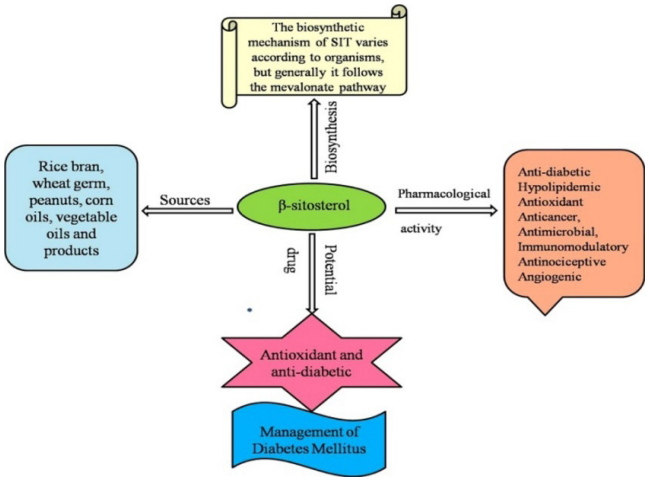

Figure 1: Graphical introduction of β-sitosterol

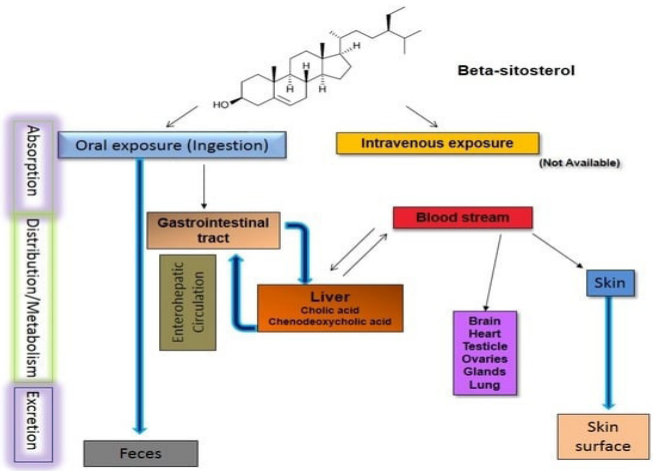

Figure 2: Pharmacokinetics of β-sitosterol

The human pharmacokinetic study was designed utilizing sitosterol as an example to obtain a better understanding of the precise bioavailability and disposition features of xenosterols. Due to their use as functional dietary ingredients and the advancement of our mechanistic understanding, it is crucial to comprehend how phytosterols behave in the gastrointestinal tract and throughout the body.[6]

In contrast to 45.50% of cholesterol, only about 5% of oral β-sitosterol is absorbed. It appears that during childhood and adolescence, there is a higher uptake of phytosterols than during adulthood (5–15 times higher plasma levels of phytosterols), and in the occasional autosomal receding condition known as sitosterolaemia (50–100 times higher plasma levels). β-sitosterol blocks a putative cholesterol transporter the enterocyte plasma membrane, which recirculates over the entero-hepatic circulation. Studies on absorption have revealed that hepatocytes' response to a negative cholesterol balance is enhanced production and decreased absorption of cholesterol.[7]

Factors Influencing Absorption

Certainly, a number of factors affect the absorption mentioned below,

Comparable Structure to Cholesterol

- An unsaturated double bond at positions C5 and C6, which is prone to oxidative stress by oxygen species. The structure of β-sitosterol is analogous to that of cholesterol that can give rise to several oxyphytosterols. The plasma and aortic valve tissue of individuals suffering cardiac disorders had higher concentrations of oxyphytosterols, according to this study.[8,9]
- The structural makeup of β-sitosterol and cholesterol is similar.
- Due to its structural similarity, during intestinal absorption, it competes with cholesterol.
- Consequently, because it lessens the absorption of dietary cholesterol, β-sitosterol is employed as an antihyperlipidemic drug, which lowers low-density lipoprotein (LDL) cholesterol levels.

Solubility in Micelles of Bile Salts

The fatty liver and metabolic anomalies triggered by HFWD, such as higher altitudes of hepatic total lipids, triacylglycerols, cholesterol, and liver histopathology, were considerably improved by β-sitosterol. Fecal lipid levels were significantly elevated in conjunction with a decrease in intestinal bile acid levels caused by β-sitosterol. Furthermore, they changed how genes related to lipid metabolism were expressed. The majority of these indicators were less effectively affected by β-sitosterol.[10] The intestinal absorption of β-sitosterol is greatly influenced *via* solubility in bile salt micelles. Plant sterols and cholesterol are both absorbed with the assistance of bile salt micelles. The absorption of β-sitosterol is influenced by its dissolution in these micelles. Figure 3 illustrates β-sitosterolreduces the absorption of cholesterol and interrupts bile circulation.

Pharmacokinetics and Formulations

When comparing β−sito−Alg/Ch/NPs through β−sito− suspension, the pharmacokinetic characteristics, such as C_{max}, T_{max}, half-life, and bioavailability, were examined. The stability of β−sito−Alg/Ch/NPs was evaluated at pH 7.4. According to reports, the percentage of drug released in phosphate buffer with a pH of 7.4 was 41 ± 6%, whereas for above both formulations, it was 11 ± 1%. The drug release observed for β-sito−Alg/Ch/NPs 75 ± 9% and for that of β-sito−suspension 12 ± 4% in an acidic pH 5.5. The γ−sito−Alg/Ch/NPs showed ~3.41-fold greater bioavailability after oral administration and increased cytotoxicity ($p < 0.05$) than β−sito−suspension. Remarkably, our work explored that, in β−sito−Alg/Ch/NPs exhibited increased cytotoxicity because of their enhanced bioavailability and antioxidant capacity.[11]

Aspects of Neutraceuticals and Toxicology:

A component of many plants, β-sitosterol has pharmacological properties that may be beneficial to humans. The compounds themselves have biological characteristics that are connected to the regulation of the immunological, neurological, and inflammatory systems. Because of this, a safety assessment of the compounds is required with respect to their potential

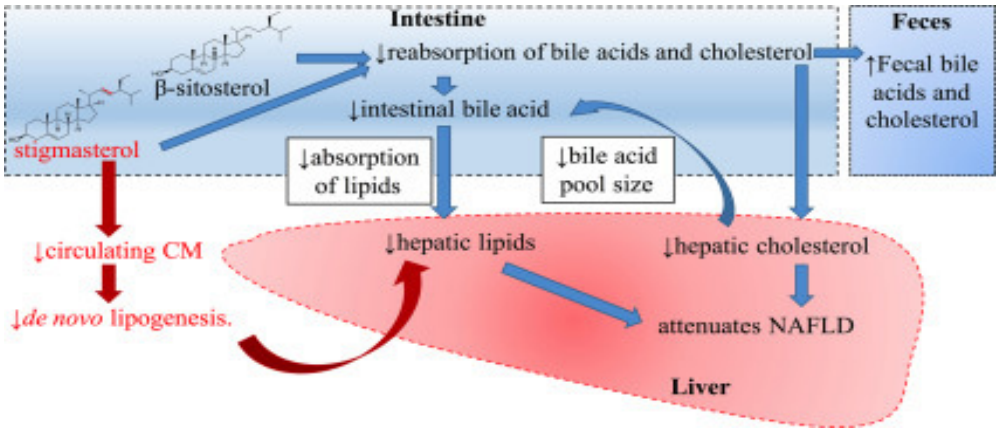

Figure 3: β-sitosterolreduces the absorption of cholesterol and interrupts in bile circulation

benefits for human health. The ability of the chemicals to cause sister chromatid exchanges (SCE), or to affect the mitotic index (MI) and cellular proliferation kinetics (CPK) in mouse bone marrow cells is how the current study assesses their genotoxic and cytotoxic potential. β-sitosterol's genotoxicity, which included an acute toxicity test that showed the drug's modest fatal potential (38%).[12]

Bioavailability Studies

Absolute oral bioavailability

In healthy individuals, the following β-sitosterol parameters were assessed: clearance, volume of distribution, oral bioavailability, and metabolic turnover. [C(14)] β-Sitosterol was given orally and intravenously, and it was utilized as an isotopic tracer to identify pulse dosages from dietary sources. The recommended dosages of [(14)C] β-sitosterol were between 3 and 4 µg, which is a low enough amount to avoid interfering with the kinetics of β-sitosterol obtained from food. Accelerator mass spectrometry's ultrasensitive isotope ratio analytical approach was employed since it was expected that quantities of [(14)C]β-sitosterol resulting from low-slung dosages would be extremely low. For [(14)C]β-sitosterol, the limit of quantification was about 0.1 pg/mL, the oral absolute bioavailability was about 0.41%, the volume of distribution was 46 L, the clearance was 85 ml/h, and the turnover was 5.8 mg/day. Based on the steady-state values of 2.83 µg/mL of β-sitosterol, the estimated dietary load was determined to be around 1400 mg per day.[13]

Formulations and stability

Researchers have also looked into β-sitosterol formulations, such as lipid carriers with nanostructures. Extensive research was conducted on physical, chemical and oxidative stability, storage, release pattern from compositions and bioavailability. [19] The important parameters that should be analyzed for the shelf life of formulation include physical, chemical, and oxidative stability. The physical and chemical stability of formulation was assessed by observing alterations in particle size analysis, zeta potential, polydispersity index (PDI), extent of degradation, and the concentration of β-sitosterol throughout the storage period. The ability of the nanostructured lipid carriers (NLCs) to neutralize free radicals was determined using the DPPH method. The resistance of the formulations to oxidative degradation was estimated by assessing the peroxide value (PV), the formation of substances reactive to thiobarbituric acid (TBARS), and through Fourier-transform infrared (FTIR) spectroscopy.[14,15]

DISTRIBUTION

After absorption, β-sitosterol is transported throughout the body via the lymphatic system, primarily incorporated into chylomicrons, and then into the bloodstream. It is distributed to various tissues but is not synthesized or metabolized by the body as efficiently as cholesterol. The tissue distribution of β-sitosterol within the human body is quite limited compared to endogenous sterols like cholesterol.

Plasma Protein Binding

The concept of "plasma protein binding" typically refers to how drugs interact with plasma proteins like albumin or alpha-1-acid glycoprotein in the bloodstream, affecting their distribution, elimination, and activity. The plasma protein binding of β-sitosterol, like other lipids, involves its association with lipoproteins rather than traditional plasma proteins such as albumin. Lipoproteins are the primary carriers of cholesterol, triglycerides, and similar sterols in the bloodstream. Given its structural similarity to cholesterol, β-sitosterol is transported in the plasma mainly by lipoproteins rather than plasma protein binding.

Metabolism

The metabolism of β-sitosterol in in the human body is a process that mainly focuses on limiting its absorption and promoting its excretion due to its limited physiological roles compared to cholesterol.

Enzymatic Pathway

The enzymatic pathway for β-sitosterol metabolism in the body involves several key steps. Let's explore this process:

Cholesterol biosynthesis

Cholesterol, a pioneer for steroid hormones, is synthesized de novo in vertebrates. The pathway begins with the two-carbon acetate group of acetyl-CoA.18 different enzymes participate in cholesterol synthesis.

Key enzymes in cholesterol biosynthesis

HMG-CoA reductase (Hmg1/2p) catalyzes the conversion of HMG-CoA to mevalonate. Mevalonate kinase (Erg12p) phosphorylates mevalonate. Phosphomevalonate kinase (Erg8p) further phosphorylates the product. Diphosphomevalonate decarboxylase (Erg19p) generates isopentenyl diphosphate (IPP). Polyprenyl synthetase (Erg20p) elongates IPP to form squalene. Squalene epoxidase (Erg1p) converts squalene to lanosterol. Lanosterol 14α-demethylase (Erg11p) removes a methyl group from lanosterol. C-4 methyl sterol oxidase (Erg25p) and C-5 sterol desaturase (Erg3p) are also involved in post-sqalene steps. [22,23,24]

Metabolites identification

Identifying the metabolites of β-sitosterol in the human body involves a multi-step process that combines *in-vitro* experimentation and animal studies. Here's a general outline of how metabolites of β-sitosterol are identified

In-vitro metabolism studies

- Microsomal Incubation: β-sitosterol can be incubated with liver microsomes or hepatocytes obtained from animals or humans. This simulates the metabolic transformation that occurs in the liver.
- Enzymatic Reactions: Specific enzymes accountable for phase I (oxidation, reduction, hydrolysis) and phase II (conjugation) metabolism can be utilized to determine the potential metabolites.
- Identification Techniques: Analytical techniques such as mass spectrometry coupled with chromatography (GC-MS, LC-MS) are used to recognize the metabolites generated in-vitro studies.

Excretion

β-sitosterol, due to its limited absorption in the human digestive system, has a unique pattern of excretion. The primary route of β-sitosterol excretion is through feces. Because only a small fraction (estimated to be less than 5%) of dietary β-sitosterol is absorbed in the intestines, the majority of it passes directly through the gastrointestinal tract and is excreted in the feces. This unabsorbed portion includes beta-sitosterol which is inherently present in foods as well as any phytosterols that might have been added to foods to enhance their cholesterol-reducing properties.

Biliary Excretion

The absorbed β-sitosterol is about 80% excreted more rapidly as free sterol in bile as compared to cholesterol. Of the small amount of beta-sitosterol that is absorbed, a significant portion is transported to the liver, where it can be incorporated into bile. It is then secreted into the digestive tract through the biliary system and eventually excreted in the feces. This excretion mechanism is part of the body's way of regulating sterol levels, preventing the accumulation of excess sterols, including both cholesterol and plant sterols like β-sitosterol.[16]

Fecal Excretion

The primary route of β-sitosterol excretion is through feces. Because only a small fraction (estimated to be less than 5%) of dietary β-sitosterol is absorbed in the intestines, the majority of it passes directly through the gastrointestinal tract and is excreted in the feces. This unabsorbed portion includes beta-sitosterol, which is inherently present in foods, as well as any phytosterols that might have been added to foods to enhance their cholesterol-lowering properties.

Minor Routes

While fecal and biliary excretions are the primary routes, research on the minor routes of β-sitosterol excretion (such as urinary excretion) suggests that they play a negligible role in its elimination from the body. The exact mechanisms and extent of these minor routes are less well-understood and are considered to be of limited physiological significance in the context of β-sitosterol metabolism.

Elimination Kinetics

The elimination kinetics of β-sitosterol have a structure analogous to cholesterol and are influenced by its limited absorption, minimal metabolism, and excretion patterns.

Absorption

Bioavailability: β-sitosterol has very low oral bioavailability, with less than 5% typically being absorbed. This impacts its pharmacokinetic parameters by limiting systemic exposure.

Distribution

Volume of Distribution (Vd): The Vd of β-sitosterol is difficult to quantify due to its minimal systemic absorption and distribution. β-sitosterol acts primarily in the gastrointestinal tract, and any absorbed fraction is likely distributed among lipoproteins in the plasma.

Metabolism

Metabolic Pathways: β-sitosterol undergoes little to no metabolism in humans. It is structurally resistant to the typical pathways that metabolize cholesterol, which limits the relevance of kinetic parameters related to metabolism.

Excretion

- *Clearance (CL)*

Specific clearance rates for beta-sitosterol are not well-documented, largely because it is excreted unchanged rather than being metabolized. The primary route of excretion is fecal, consisting of both unabsorbed β-sitosterol and any small amounts that were absorbed and then excreted into the bile.

- *Half-Life ($t_{1/2}$)*

The half-life of β-sitosterol is not explicitly defined due to its mode of excretion and the lack of systemic absorption and metabolism. The concept of half-life is more relevant to substances that are metabolized and cleared through the liver

and kidneys. [18] The half-life of β-sitosterol, which is the period required for half of the substance than the original to be metabolized or eliminated, isn't precisely documented in the literature, but it is generally considered to be short, with the compound being metabolized and excreted within a few hours after consumption.

Pharmacodynamics β-sitosterol

Mechanism of action

The mechanisms of action of β-sitosterol, a plant sterol, are varied and complex, influencing several physiological processes. Here are some of the key mechanisms:

- This process lowers low-density lipoprotein (LDL) cholesterol, which is typically stated to as "bad" cholesterol, without changing high-density lipoprotein (HDL), which is often stated to as "good" cholesterol.
- Cholesterol Absorption Inhibition: Beta-sitosterol races with cholesterol for absorption in the intestine. Due to its structural resemblance to cholesterol, it can block the absorption of dietary cholesterol, reducing its entry into the bloodstream. This mechanism helps to lower LDL without affecting HDL.[17] Phytosterol with a daily intake of 2.0 to 2.5 g recommended that lowers cholesterol up to 10% and risk of cardiac disorders.
- Anti-Inflammatory property: Beta-sitosterol demonstrates anti-inflammatory qualities by reduction in the synthesis of inflammatory modulators prostaglandins and cytokines. This exercise helps lessen inflammation brought on by ailments, including inflammatory bowel disease and arthritis.[1]
- Immunomodulation: Studies have shown that beta-sitosterol affects T cells' and macrophages' activity, among other immune cells, hence modifying immune function. Since it can occasionally increase immune responses while suppressing excessive immune responses in others, it may aid in the treatment of autoimmune diseases or immunological-related disorders.[3]
- Effects of Antioxidants: The body uses beta-sitosterol's antioxidant properties to help reduce oxidative stress and neutralize free radicals. By scavenging free radicals, beta-sitosterol protects cells, prolonging life and improving general health.[5]
- Anticancer Activity: Research has shown that beta-sitosterol may have anticancer properties, particularly with regard to prostate cancer. It can stop cancer cells from growing, trigger programmed cell death in cancer cells, and block many signaling pathways that are implicated in the start and spread of cancer.[1,2]

Molecular targets

- Beta-sitosterol, a phytosterol found in plants, can interact with various molecular targets in the body, influencing different physiological processes. Here are some of the molecular targets of beta-sitosterol:
- NPC1L1 (Niemann-Pick C1-Like 1): Beta-sitosterol inhibits the Niemann-Pick C1-Like 1 protein, which is primarily expressed in the small intestine. NPC1L1 plays a crucial role in the absorption of cholesterol from the diet into enterocytes. By inhibiting NPC1L1, beta-sitosterol reduces the absorption of dietary cholesterol, leading to decreased levels of LDL cholesterol in the bloodstream.[17,18]
- 5-alpha-Reductase: The enzyme that catalyzes the transformation of testosterone into dihydrotestosterone (DHT) is inhibited by beta-sitosterol. DHT influences prostate gland growth and development. Beta-sitosterol can help reduce the symptoms of benign prostatic hyperplasia (BPH), a disorder marked by enlargement of the prostate, by blocking 5-alpha-reductase.[18]
- Inflammatory Mediators: The synthesis of inflammatory mediators, such as prostaglandins and cytokines (such as interleukins and tumor necrosis factor-alpha), is modulated by β-sitosterol. It can increase the release of anti-inflammatory cytokines while inhibiting the function of pro-inflammatory mediators. The potential therapeutic effects of β-sitosterol in situations marked by inflammation are facilitated by its anti-inflammatory activity.[19]
- Immune cells: T cells, macrophages, and dendritic cells are just a few of the immune cells that β-sitosterol contacts. Conditions involving immunological dysregulation, like autoimmune illnesses, may benefit from beta-sitosterol's immunomodulatory properties.[20]
- Antioxidant Pathways: β-sitosterol reduces oxidative stress and scavenges free radicals to exhibit antioxidant capabilities. It has the ability to both boost the activity of antioxidant enzymes like glutathione peroxidase (GPx) and superoxide dismutase (SOD) and reduce the generation of reactive oxygen species (ROS). Overall health and longevity are enhanced by these antioxidant properties, which shield cells from oxidative harm.[21]
- Beta-sitosterol impacting cholesterol metabolism: Maintaining Homeostasis in Cholesterol The induction of apoptosis, or programmed cell death, in cancer cells can be triggered by beta-sitosterol through a variety of pathways. The activation of caspases and the modification of Bcl-2 family proteins are two apoptotic signaling pathways that are impacted. One reason for the possible anticancer qualities of beta-sitosterol, especially in prostate cancer, is its pro-apoptotic activities.[21]

Cellular Effect

The phytosterol β-sitosterol, which is present in a variety of plants, influences multiple physiological processes within cells. In the intestinal tract, it prevents cholesterol from being absorbed and competes with cholesterol for inclusion in membranes of cells. The fluidity of the cell membrane can be changed and processes associated with the membrane might be affected when beta-sitosterol molecules replace cholesterol ones within the cell.

Protecting cells against oxidative stress: By eliminating free radicals and lowering oxidative stress, β-sitosterol functions as an antioxidant within cells. While it prevents cells from producing reactive oxygen species (ROS), it increases the countenance of antioxidant enzymes together

with glutathione peroxidase (GPx) and superoxide dismutase (SOD). Biomolecules like proteins, lipids, and DNA are shielded from oxidative damage by these antioxidant properties. The enzyme 5-alpha-reductase, included in the conversion pathway of testosterone to dihydrotestosterone (DHT), is inhibited *via* β-sitosterol. In particular, in prostate cells, beta-sitosterol's inhibition of this enzyme lowers intracellular DHT levels, which may have an impact on cellular processes linked to androgen signaling. Anti-Inflammatory actions: By modifying different cellular pathways implicated in inflammation, β-sitosterol has anti-inflammatory actions at the cellular level. It may impede.[19]

Formulating β-sitosterol in a solid dosage form involves overcoming its solubility challenges to enhance bioavailability while ensuring the stability and efficacy of the final product. A thorough understanding of the physicochemical properties of beta-sitosterol, along with meticulous formulation and processing techniques, are essential for successful product development.

Pharmaceutical formulation

Pharmaceutical formulations of beta-sitosterol include the following,

Tablets/Capsules

The most common form, used mainly for managing cholesterol and BPH. These are usually standardized to contain a specific amount of beta-sitosterol.

Topical Creams/Ointments

For skin conditions, beta-sitosterol is formulated into creams and ointments. It is believed to help with wound healing and reducing inflammation.

Suppositories

In some cases, especially for treating hemorrhoids, beta-sitosterol can be formulated into suppositories for rectal administration.

Fortified Foods

Not strictly a pharmaceutical formulation, but worth mentioning. Some foods are fortified with beta-sitosterol to help lower cholesterol levels. These include margarines, yogurts, and granola bars.

Powders

Beta-sitosterol is also available in powder form, which can be mixed into drinks or smoothies. This form is often used by people looking to add beta-sitosterol to their diet without taking pills.[22]

Solid Dosage Forms

Formulating beta-sitosterol in a solid dosage form, such as tablets or capsules, requires careful consideration of several factors to ensure stability, bioavailability, and patient compliance. Here are key considerations:

Solubility and bioavailability

Beta-sitosterol has poor water solubility, which can limit its bioavailability. Strategies to enhance solubility and bioavailability include the use of solid dispersions, nanoparticles, or complexation with cyclodextrins.

Excipients selection

The choice of excipients is crucial. Since beta-sitosterol is lipophilic, using lipid-based excipients or surfactants can help improve its solubility. Additionally, choosing the right binders, fillers, disintegrants, and lubricants that are compatible with beta-sitosterol is essential to ensure the stability and integrity of the dosage form.

Compatibility

It's important to conduct compatibility studies between beta-sitosterol and the chosen excipients to prevent any potential interactions that could affect the stability or efficacy of the final product.

Formulation technique

Techniques such as direct compression, wet granulation, or dry granulation can be employed depending on the properties of the drug and excipients. Direct compression might be challenging due to the poor flowability of beta-sitosterol, so granulation techniques could be more appropriate to improve flow properties.

Dosage form design

Designing the dosage form to ensure adequate dose and ease of administration. For beta-sitosterol, which is often taken for cholesterol management, ensuring a consistent dose in each tablet or capsule is critical for efficacy.

Stability

Assessing and ensuring the stability of beta-sitosterol in the solid dosage form under various conditions (temperature, humidity, light) is necessary. Antioxidants might be needed if the compound is prone to oxidation.

Dissolution profile

Testing the dissolution profile of the final product is essential to ensure that beta-sitosterol is released at the desired rate, ensuring its availability for absorption.

Regulatory compliance

Ensuring that the formulation and manufacturing process complies with regulatory guidelines for dietary supplements or pharmaceuticals, depending on the market and intended use.

Formulating beta-sitosterol in a solid dosage form involves overcoming its solubility challenges to enhance bioavailability while ensuring the stability and efficacy of the final product. A thorough understanding of the physicochemical properties of beta-sitosterol, along with meticulous formulation and processing techniques, are essential for successful product development.[23]

Liquid Dosage Form

Formulating beta-sitosterol in a liquid dosage form presents a unique set of challenges, primarily due to its poor water solubility. However, liquid formulations can offer advantages in terms of bioavailability, ease of ingestion, and flexibility

in dosing. Here are key considerations for formulating beta-sitosterol in a liquid dosage form:

Solubility enhancement

The primary challenge is to enhance the solubility of beta-sitosterol. This can be achieved through various strategies, such as the use of co-solvents (ethanol, propylene glycol), surfactants to form micelles (Tween, Span), or the creation of self-emulsifying drug delivery systems (SEDDS).

Selection of vehicle

The choice of vehicle is critical. It must be compatible with beta-sitosterol and the other formulation components. Options include aqueous solutions, oils (medium-chain triglycerides), or hydro-alcoholic solutions, depending on the solubility enhancement strategy employed.

Stability

Beta-sitosterol must remain stable throughout the shelf life of the product. This includes physical stability (preventing precipitation of the drug) and chemical stability (protecting against degradation). Antioxidants may be necessary if the compound or any excipient is prone to oxidation. The formulation pH may need to be adjusted to optimize stability.

Emulsification

For oil-based systems or systems requiring surfactants for solubility enhancement, creating a stable emulsion is critical. The choice of emulsifying agents and the method of emulsification will impact the stability and bioavailability of the formulation.

Taste masking

Liquid formulations often require taste masking to improve patient compliance. Techniques can include the use of flavors, sweeteners, or encapsulation strategies to mask the taste of beta-sitosterol.

Preservatives

Liquid formulations are susceptible to microbial contamination. Including preservatives is essential to ensure the safety and longevity of the formulation. The choice of preservatives must be compatible with all formulation components and acceptable for the intended route of administration.

Packaging

The packaging material must protect the formulation from light, air (oxygen), and moisture, which could degrade beta-sitosterol or other sensitive components. The use of amber glass or opaque plastic containers is common. The packaging should also support ease of use and accurate dosing.

Dosing and administration

The formulation should allow for easy and accurate dosing. This might involve the use of droppers, measuring cups, or other dosing aids. The viscosity of the liquid formulation should be considered to ensure it can be easily measured and administered.

Regulatory compliance

Ensuring that the formulation, manufacturing process, and packaging comply with the regulatory requirements for dietary supplements or pharmaceuticals, depending on the intended market.[24]

Formulating beta-sitosterol in a liquid dosage form requires a multifaceted approach to overcome its solubility challenges, ensure stability, enhance bioavailability, and achieve patient compliance. Each aspect, from solubility enhancement to packaging, must be carefully considered to develop a successful product.

Excipient Selection

Excipient selection is crucial in the formulation of beta-sitosterol-based products to ensure stability, bioavailability, and efficacy. Beta-sitosterol, a phytosterol found in plants, is commonly used in dietary supplements and pharmaceuticals for its potential health benefits, particularly in managing cholesterol levels and supporting prostate health

Excipient selection for the solid dosage form of beta-sitsterol:

Solublizers

In solid dosage forms of beta-sitosterol, solubilizers are typically used to enhance the dissolution and bioavailability of the active ingredient. While solubilizers are more commonly associated with liquid formulations, they can also be utilized in solid dosage forms to improve the solubility of poorly water-soluble compounds like beta-sitosterol. However, it's important to remember that the selection and use of solubilizers in solid dosage forms may differ from those in liquid formulation.[26]

Some solubilizers that may be used in solid dosage forms of beta-sitosterol include:

Surfactants

Surfactants can help solubilize hydrophobic compounds like beta-sitosterol by reducing the interfacial tension between the solid particles and the dissolution media. Common surfactants used in solid dosage forms include:

Polysorbates (e.g., Tween series)

Polysorbates are nonionic surfactants commonly used to enhance the solubility and dispersion of lipophilic compounds in aqueous solutions. They can be incorporated into solid dosage forms as wetting agents or dispersants.[26]

Sodium lauryl sulfate

This anionic surfactant is sometimes used in solid dosage forms to improve wetting and dissolution.

Co-solvents

Co-solvents can be added to solid dosage forms to improve the solubility of beta-sitosterol by forming a co-solvent system with the dissolution medium. Common co-solvents include:

Ethanol

Ethanol is often used as a co-solvent in solid dosage forms to enhance the solubility of poorly water-soluble drugs. It can help improve the dissolution rate of beta-sitosterol.

Propylene glycol

Propylene glycol is another commonly used co-solvent that can enhance the solubility and bioavailability of lipophilic compounds in solid dosage forms.

Cyclodextrins

Cyclodextrins form inclusion complexes with hydrophobic molecules like betasitosterol, improving their aqueous solubility. Cyclodextrins such as hydroxypropyl-beta-cyclodextrin (HPβCD) or casually methylated-beta-cyclodextrin (RMβCD) may be used in solid dosage forms to enhance the solubility and dissolution of beta-sitosterol.

Lipid-based excipients

They include fatty acids, oils, or lipid nanoparticles can also be used to improve the solubility and dissolution of lipophilic drugs like betasitosterol in solid dosage forms.

Stabilizers

Stabilizers are essential in solid pharmaceutical preparations containing active pharmaceutical ingredients (APIs), such as beta-sitosterol. Beta-sitosterol, a plant sterol present in the variability of foods and used for its possible health advantages, can be formulated into solid dosage forms such as tablets or capsules. Stabilizers are used to ensure the dosage forms' integrity, stability, and shelf-life. Some popular stabilizers used in solid dose formulations include:a. Microcrystalline Cellulose (MCC): MCC is a common stabilizer and filler in tablet formulations. It adds weight to the tablet and aids in compression.

Lactose

Lactose serves as both a filler and a stabilizer. It helps maintain the integrity of the tablet by providing cohesion and improving the flow properties during manufacturing.

Magnesium stearate

Magnesium stearate is a lubricant and a stabilizer. It aids in the tableting process by reducing friction between the tablet formulation and the equipment, thus preventing sticking and ensuring uniform tablet weight.

Silica

Silica is often used as a desiccant and a stabilizer. It helps in controlling moisture content, which can be crucial for the stability of the active ingredient.

Croscarmellose Sodium

Croscarmellose sodium is a super-disintegrant commonly used in tablets to enhance disintegration and dissolution. It ensures rapid release of the active ingredient, which can contribute to its stability.

Povidone (Polyvinylpyrrolidone, PVP)

Povidone is a binder and a stabilizer. It enhances the tablet's cohesive properties and improves dissolution characteristics.

Sodium Lauryl Sulfate (SLS)

SLS can be used as a surfactant and stabilizer. It aids in wet granulation processes and can enhance the solubility and dissolution of poorly water-soluble drugs. These stabilizers are carefully chosen according to their compatibility with beta-sitosterol as well as their capacity to keep the solid dosage form stable and of high quality throughout its shelf life. The particular formulation and amounts of stabilizers can differ depending on the desired release profile, manufacturing technique, and compatibility with other excipients in the formulation.

Excipient selection for the liquid dosage form of beta-sitosterol

Solublizer

Solubilizers are often utilized in liquid dosage forms containing beta-sitosterol in order to improve the active ingredient's solubility and rate of dissolution. Because beta-sitosterol dissolves readily in oils and organic solvents but not in water, solubilizers are essential for creating stable and potent liquid formulations.

The following are some typical solubilizers found in liquid beta-sitosterol dose forms:

Ethanol: Capable of dissolving both hydrophilic and lipophilic substances, ethanol is a versatile solubilizer. In liquid formulations, it is frequently utilized as a co-solvent to improve the solubility of hygroscopic solvent propylene glycol. It is frequently employed as a co-solvent and solubilizer in liquid formulations to increase the stability and solubility of beta-sitosterol.

Water-soluble polymers known as polyethylene glycol (PEG) can be used as surfactants and solubilizers in liquid formulations. They are frequently used to improve the bioavailability and solubility of medications that are not very soluble in water, such as beta-sitosterol.

Propylene glycol

A variety of hydrophobic and hydrophilic substances can be dissolved by medications that are not very soluble in water, such as beta-sitosterol.

Polysorbates

Polysorbates are nonionic surfactants that can dissolve lipophilic substances in aqueous solutions. One example of a polysorbate is polysorbate 80 (Tween 80). To increase the stability and dispersibility of beta-sitosterol in liquid formulations, they are frequently employed as emulsifiers and solubilizers.[26]

Lecithin

Lipophilic substances can be dissolved in aqueous solutions by lecithin, a phospholipid that occurs naturally. In liquid formulations containing beta-sitosterol, it is frequently employed as an emulsifier and solubilizer to enhance the dispersibility.[27]

Stabilizers

In order to guarantee the chemical and physical stability of liquid dosage forms containing beta-sitosterol, stabilizers are essential. They aid in preserving the active ingredient's quality and effectiveness over time by preventing its deterioration,

aggregation, or precipitation. The following stabilizers are frequently used in liquid beta-sitosterol dose forms:
Antioxidants are substances that prevent oxidation, which can cause beta-sitosterol to degrade. Antioxidants that are frequently utilized in liquid formulations include Vitamin E, or tocopherols. By acting as scavengers of free radicals, tocopherols prevent beta-sitosterol from oxidizing. Synthetic antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) are regularly employed in formulations that contain sensitive substances like beta-sitosterol to stop oxidation.

Chelating agents

Chelating agents help in complexing metal ions, which can catalyze oxidation reactions or promote the degradation of beta-sitosterol. Common chelating agents include:

- *Ethylenediaminetetraacetic acid (EDTA)*

EDTA binds to metal ions, preventing them from catalyzing oxidation or degradation reactions.

- *Citric acid*

Citric acid can act both as a chelating agent and a buffer, helping to maintain the pH of the formulation.

Absorption enhancer

Absorption enhancers may be added to solid dosage forms containing beta-sitosterol in order to improve the active ingredient's bioavailability. Beta-sitosterol, like many other plant sterols, can have low solubility and bioavailability, which may reduce its efficiency. Absorption enhancers try to overcome these barriers by enhancing the permeability of the intestinal membrane or improving solubility.[27] Some favored absorption enhancers used in solid dosage forms are:

- *Phospholipids*

Phospholipids like phosphatidylcholine have been shown to progress the absorption of lipophilic substances like beta-sitosterol by generating mixed micelles in the intestinal lumen, enhancing solubility and transport across the membrane. b) Tocopherols: Vit E derivatives, such as tocopherol, can improve lipophilic chemical absorption by increasing their solubility in the digestive tract and perhaps regulating membrane permeability

- *Black pepper extract (Piperine)*

Piperine has been demonstrated to increase the bioavailability of different substances by blocking drug-metabolizing enzymes and enhancing intestinal absorption. It can be utilized to improve absorption in formulations that contain beta-sitosterol.

- *Polysorbates*

Surfactants such as Polysorbate 80 are routinely employed in pharmaceutical formulations to advance the solubility and bioavailability of poorly water-soluble medicines.

- *Sodium caprate*

Sodium caprate, a medium-chain fatty acid, can improve paracellular transport across the intestinal epithelium, improving the absorption of lipophilic substances like beta-sitosterol.

These are often used in the form of absorption enhancers for solid dosage forms to increase the bioavailability and therapeutic activity of beta-sitosterol. The type of absorption enhancer to be used depends on the physicochemical characteristics of beta-sitosterol, its intended release profile, and its compatibility with other compounds in the formulation.[28]

CONCLUSION

β-Sitosterol, a plant-derived phytosterol similar to cholesterol, offers notable health benefits. Despite a low oral absorption rate (0.5–5%) and limited systemic impact, β-sitosterol effectively competes with dietary cholesterol for incorporation into bile salts. Its pharmacokinetic profile emphasizes its role in cholesterol management rather than as a major metabolic substrate. Its primary advantage is lowering LDL cholesterol, which helps manage hypercholesterolemia and reduce cardiovascular disease risk. Additionally, it has potential anti-diabetic effects, can alleviate benign prostatic hyperplasia (BPH), and exhibits anti-inflammatory and antioxidant properties.

REFERENCES

1. Babu S, Jayaraman S. An update on β-sitosterol: A potential herbal nutraceutical for diabetic management. Biomedicine & Pharmacotherapy 2020; 131:110702. DOI: https://doi.org/10.1016/j.biopha.2020.110702
2. Novotny L, Abdel-Hamid ME, Hunakova L, Saeidnia S, Manayi A, Gohari AR, et al. Anticancer potential of β-sitosterol. International Journal of Clinical Pharmacology & Pharmacotherapy. 2017; 2(10.15344):2456–3501. DOI: https://doi.org/10.15344/2017/2456-3501/129
3. Saeidnia S, Manayi A, Gohari AR, Abdollahi M. The story of beta-sitosterol- A review. European Journal of Medicinal Plants 2014; 4(5):590–609.
4. Abdulqader DH, AL-Zubaydi SR, Kadhim EJ. Isolation of Beta-Sitosterol, Campesterol and Stigmasterol from Alcea Kurdica roots petroleum extract using preparative HPLC. International Journal of Pharmacy and Pharmaceutical Studies 2023; 7(1): 6-12. DOI: http://www.bharatpublication.com/current-issue.php?jID=33/IJPPS
5. Li Y, Meng Q, Yang M, Liu D, Hou X, Tang L, et al. Current trends in drug metabolism and pharmacokinetics. Acta Pharmaceutica Sinica 2019; 9(6):1113–1144. DOI: https://doi.org/10.1016/j.apsb.2019.10.001
6. Sayeed B, Shahdaat M, Karim SMR, Sharmin T, Morshed MM. Critical analysis on characterization, systemic effect, and therapeutic potential of beta-sitosterol: A plant-derived orphan phytosterol. Medicines Basel 2016;3(4):29. DOI: https://doi.org/10.3390/medicines3040029
7. Salen G, Shore V, Tint GS, Forte T, Shefer S, Horak I, et al.. Increased sitosterol absorption, decreased removal, and expanded body pools compensate for reduced cholesterol synthesis in sitosterolemia with xanthomatosis. Journal of lipid research. 1989;30(9):1319–1330. DOI: https://doi.org/10.1016/S0022-2275(20)38259-6
8. Potter RM, Harikumar KG, Wu SV, Miller LJ. Differential

sensitivity of types 1 and 2 cholecystokinin receptors to membrane cholesterol. Journal of lipid research. 2012; 53(1):137–148. DOI: https://doi.org/10.1194/jlr.M020065

9. Desai AJ, Harikumar KG, Miller LJ. A type 1 cholecystokinin receptor mutant that mimics the dysfunction observed for wild type receptor in a high cholesterol environment. Journal of Biological Chemistry 2014; 289(26):18314–18326. DOI: https://doi.org/10.1074/jbc.M114.570200
10. Feng S, Dai Z, Liu AB, Huang J, Narsipur N, Guo G, et al. Intake of stigmasterol and β-sitosterol alters lipid metabolism and alleviates NAFLD in mice fed a high-fat western-style diet. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids 2018; 1863(10):1274–1284. DOI: https://doi.org/10.1016/j.bbalip.2018.08.004
11. Afzal O, Akhter MH, Ahmad I, Muzammil K, Dawria A, Zeyaullah M, et al.. A β–Sitosterol encapsulated biocompatible alginate/chitosan polymer nanocomposite for the treatment of breast Cancer. Pharmaceutics 2022; 14(8):1711. DOI: https://doi.org/10.3390/pharmaceutics14081711
12. Pareek A, Pant M, Gupta MM, Kashania P, Ratan Y, Jain V, Pareek A, Chuturgoon AA. Moringa oleifera: An updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. International journal of molecular sciences. 2023;24(3):2098. DOI: https://doi.org/10.3390/ijms24032098
13. Duchateau G, Cochrane B, Windebank S, Herudzinska J, Sanghera D, Burian A, et al. Absolute oral bioavailability and metabolic turnover of sitosterol in healthy subjects. Drug metabolism and disposition: The biological fate of chemicals 2012; 40(10):2026-2030. DOI: https://doi.org/10.1124/dmd.112.046623
14. Soleimanian Y, Goli S, Varshosaz J, Di Cesare Mannelli L, Ghelardini C, Cirri M, et al.. β-Sitosterol loaded nanostructured lipid carrier: Physical and oxidative stability, *in vitro* simulated digestion and hypocholesterolemic activity. Pharmaceutics 2020;12(4):386. DOI: https://doi.org/10.3390/pharmaceutics12040386
15. Soleimanian Y, Goli SAH, Varshosaz J, Maestrelli F. β-Sitosterol lipid nano carrier based on propolis wax and pomegranate seed oil: Effect of thermal processing, pH, and ionic strength on stability and structure. European journal of lipid science and technology 2019; 121(1):1800347. DOI: https://doi.org/10.1002/ejlt.201800347
16. Ambavade SD, Misar AV, Ambavade PD. Pharmacological, nutritional, and analytical aspects of β-sitosterol: A review. Oriental Pharmacy and Experimental Medicine 2014; 14:193–211. DOI: https://doi.org/10.1007/s13596-014-0151-9
17. Lomenick B, Shi H, Huang J, Chen C. Identification and characterization of β-sitosterol target proteins. Bioorganic & medicinal chemistry letters 2015; 25(21):4976–4979. DOI: https://doi.org/10.1016/j.bmcl.2015.03.007
18. Cabeza M, Bratoeff E, Heuze I, Ramírez E, Sánchez M, Flores E. Effect of beta-sitosterol as inhibitor of 5 alpha-reductase in hamster prostate. Proceedings of the Western Pharmacology Society 2003 46:153-155.
19. Fraile L, Crisci E, Córdoba L, Navarro MA, Osada J, Montoya M. Immunomodulatory properties of beta-sitosterol in pig immune responses. International Immunopharmacology 2012; 13(3):316–321. DOI: https://doi.org/10.1016/j.intimp.2012.04.017
20. Chanioti S, Katsouli M, Tzia C. β-Sitosterol as a functional bioactive. A centum of valuable plant bioactives. Academic Press. 2021;193–212. DOI: https://doi.org/10.1016/B978-0-12-822923-1.00014-5
21. Bin Sayeed MS, Ameen SS. Beta-sitosterol: A promising but orphan nutraceutical to fight against cancer. Nutrition and cancer. 2015;67(8):1216–1222. DOI: https://www.tandfonline.com/doi/abs/10.1080/01635581.2015.1087042
22. Zahid, Sara, Arif Malik, Suleyman Waqar, Fatima Zahid, Nusrat Tariq, Ali Imran Khawaja, Waqas Safir, Faisal Gulzar, Javeid Iqbal, and Qurban Ali. Countenance and implication of B-sitosterol, B-amyrin and epiafzelechin in nickel exposed Rat: in-silico and in-vivo approach. Scientific Reports. 2023;13(1):21351. DOI: https://doi.org/10.1038/s41598-023-48772-4
23. Bartke, Nana, and Yusuf A. Hannun. Bioactive sphingolipids: metabolism and function. Journal of lipid research. 2009;50:S91-S96.DOI: https://doi.org/10.1194/jlr.R800080-JLR200
24. Chavez, Jose A., M. Mobin Siddique, Siew Tein Wang, Jianhong Ching, James A. Shayman, and Scott A. Summers. Ceramides and glucosylceramides are independent antagonists of insulin signaling. Journal of Biological Chemistry. 2014;289(2):723-734. DOI: https://doi.org/10.1074/jbc.M113.522847
25. Kumar N, Pahuja S, Sharma R. Pharmaceutical Polymers - A Review. International Journal of Drug Delivery Technology. 2019; 9(1); 27-33 DOI: 10.25258/ijddt.9.1.5
26. Prasad D, Parashar T, Khaudiyal S, Semwal A, Jakhmola V. Gastro Retentive Drug Delivery System: Latest Approach towards Novel Drug Delivery. International Journal of Pharmaceutical Quality Assurance. 2023;14(4):1256-1263. DOI: http://dx.doi.org/10.52711/0974-360X.2023.00077
27. Patil MR, Kshirsagar SK. Formulation, Optimization and Evaluation of Solid SMEDDS of Pioglitazone. International Journal of Drug Delivery Technology. 2024;14(1):145-159. DOI: 10.25258/ijddt.14.1.21
28. Bhairam M, Shukla SS, Gidwani B, Pandey Rk. Solid Dispersion of Dolutegravir: Formulation Development, Characterization, and Pharmacokinetic Assessment. International Journal of Pharmaceutical Quality Assurance. 2022;13(4):496-503. DOI: 10.25258/ijpqa.13.4.24