



ABSTRACT

Phytosterols, modulation of sex hormone receptors, endocrine effects – from animal model to clinical practice

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Benign prostatic hyperplasia (BPH) is a common condition in aging men, associated with lower urinary tract symptoms that significantly impact quality of life. Due to its multifactorial pathogenesis, BPH treatment is complex. Current therapeutic protocols include surgical interventions, conventional pharmacotherapy, lifestyle modifications, and phytotherapy.

As interest grows in natural therapies, β -sitosterol has emerged as a promising candidate for BPH due to its anti-androgenic, anti-inflammatory, and pro-apoptotic effects. This study explores β -sitosterol's therapeutic mechanisms and role in BPH symptom management through four focused research axes, each with defined objectives and methods.

The first study focused on investigating the molecular-level interactions between phytosterols (β -sitosterol, stigmasterol, and campesterol), and 5 α -reductase type 2 enzyme. Using complementary computational (*in silico*) and experimental (*in vitro*) approaches, we characterized the binding patterns between phytosterols and the enzyme's active site to determine their inhibitory potential. The combined *in vitro* and *in silico* analysis revealed a relatively weak inhibitory activity of phytosterols against 5 α -reductase type 2, significantly lower than dutasteride. β -sitosterol demonstrated the highest binding affinity, suggesting superior therapeutic potential compared to the other studied phytosterols. However, the clinical relevance of this mechanism appears limited, indicating that the therapeutic benefits of phytosterol-rich plant extracts likely stem more significantly from other mechanisms, such as anti-inflammatory and antioxidant effects.

The second study aimed to qualitatively and quantitatively analyze β -sitosterol-containing dietary supplements used for BPH. An HPLC analytical method was developed and validated for β -sitosterol quantification. Furthermore, the study assessed the compliance between declared and measured concentrations in commercial products, while evaluating the therapeutic relevance of the identified doses to determine if they fell within the active range required to induce the presumed beneficial effects. The results highlighted significant quality control and standardization issues. Labeling transparency deficiencies and substantial concentration variations between products were found to affect result reproducibility. These discrepancies may compromise both therapeutic efficacy and product safety, underscoring the need for stricter regulations and standardized analytical strategies in the manufacturing process.



The third research direction assessed healthcare students' knowledge and perceptions regarding phytosterol use in BPH treatment. Using a structured questionnaire, we evaluated familiarity with these complementary therapies to identify gaps in medical education and develop recommendations for better understanding phytosterols' therapeutic potential. The study revealed that future healthcare professionals generally hold favorable attitudes toward phytosterol-based supplements, despite limited in-depth knowledge of their mechanisms. This finding underscores both the need for evidence-based education on phytotherapy in medical curricula and the importance of ensuring market availability of clinically validated supplements with proven efficacy."

The fourth study aimed to evaluate β -sitosterol efficacy *in vivo* using a pharmacologically induced BPH animal model. The study objectives included: comparison with standard treatment (dutasteride), (2) investigation of anti-androgenic mechanisms through TST/DHT ratio analysis, assessment of pro-apoptotic effects via Bax/Bcl-2 protein expression, and evaluation of anti-inflammatory activity through quantification of proinflammatory cytokines IL-1 β and IL-6. Results demonstrated that β -sitosterol exhibits moderate anti-androgenic activity in the BPH model, evidenced by significant reduction in TST/DHT ratio, though with limited effects on prostate size and no significant impact on apoptosis. Combined administration of β -sitosterol with dutasteride reduced both prostate volume and IL-6 levels, suggesting potential synergistic effects - albeit without surpassing dutasteride monotherapy efficacy. These findings indicate that β -sitosterol may function more effectively as adjuvant therapy within combined treatment regimens targeting multiple BPH pathogenic mechanisms simultaneously.

This study implements an innovative strategy for investigating the therapeutic potential of phytosterols in BPH, integrating *in silico* simulations, *in vitro* testing, and *in vivo* animal model studies alongside quality analyses of dietary supplements and evaluations of healthcare professionals' perceptions. The research provides the first comparative assessment of binding affinities for three phytosterols (β -sitosterol, campesterol, and stigmasterol) to 5 α -reductase type 2 enzyme, revealing significantly lower inhibitory activity compared to synthetic inhibitors. Furthermore, it represents the pioneering investigation to simultaneously evaluate β -sitosterol's anti-androgenic, anti-inflammatory, and antiproliferative effects in monotherapy using a BPH animal model. The results demonstrate: (1) potential synergistic effects with standard therapy (e.g., IL-6 reduction), yet (2) limitations in prostate volume reduction - highlighting its potential as complementary rather than standalone therapy.

This work stands out through its integrative approach, bridging fundamental research with practical applications. On one hand, mechanistic studies provide detailed insights into molecular interactions, while on the other hand, quality analyses of dietary supplements and questionnaire-based research offer direct contributions to improving clinical practice. Moreover, the study provides a multidisciplinary perspective by combining diverse methodologies (drug analysis, biochemical investigations, pharmacological evaluations, and medical perception studies), thereby enabling a more comprehensive understanding of the research field.