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Pumpkin Seed Fixed Oil Granulate (Cucurbita sp.): A Multi-Target Phytotherapeutic Approach to Benign Prostatic Hyperplasia and Inflammatory Modulation in Wistar Rats

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Research Article

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Pumpkin Seed Fixed Oil Granulate (*Cucurbita sp.*): A Multi-Target Phytotherapeutic Approach to Benign Prostatic Hyperplasia and Inflammatory Modulation in Wistar Rats

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Abstract

Benign prostatic hyperplasia (BPH) is characterized by the progressive growth of prostate cells. Pumpkin seeds (*Cucurbita sp.*) are rich in unsaturated fatty acids, phytosterols, and phenolic compounds, exhibiting significant anti-inflammatory and antitumor potential. The present study developed a granulate containing *Cucurbita sp.* seed fixed oil (GOFSC) and evaluated its anti-inflammatory activity and effects on BPH in Wistar rats. Phytochemical analysis identified linoleic acid (44.95%) and oleic acid (32.17%) as the major components, accounting for approximately 77% of the composition. The optimized granulate formulation demonstrated satisfactory flow properties (compressibility index of 13.73% and Hausner ratio of 1.15) and adequate physicochemical stability. In anti-inflammatory activity assays, GOFSC significantly reduced carrageenan-induced paw edema ($p < 0.001$), with results comparable to indomethacin, and decreased the weight of granulomatous tissue ($p < 0.001$) in the cotton pellet granuloma test. In the testosterone propionate-induced BPH model, GOFSC reduced prostate volume, decreased PSA levels ($p < 0.05$), and promoted histopathological changes consistent with regression of hyperplasia, including reduced epithelial thickness, decreased inflammatory infiltrate, and preservation of glandular architecture. Testosterone levels remained normal without endocrine impairment. Hepatic and renal markers stayed within normal limits, confirming the product's safety. The reduction in seminal vesicle weight in the treated groups was the only expected finding, consistent with dihydrotestosterone (DHT) inhibition. This

study reinforces the therapeutic potential of *Cucurbita sp.* seed oil in innovative solid pharmaceutical formulations for the multi-target management of BPH.

Keywords: *Cucurbita sp.*, inflammation, benign prostatic hyperplasia, pharmaceutical granulate, phytotherapy, linoleic acid.

Introduction

Benign prostatic hyperplasia (BPH) is a non-malignant proliferative condition of the prostate, characterized by the excessive growth of the stroma and glandular epithelium, resulting from an imbalance between cell proliferation and apoptosis. It is estimated to affect between 50% and 75% of men over 50 years old, exceeding 80% after age 70 (Liedtke et al., 2024; Wang et al., 2022).

Testosterone is converted into dihydrotestosterone (DHT) by the enzyme 5α-reductase, which has a 5-fold higher affinity for the androgen receptor (AR), activating growth factors such as FGF and TGF-β. With advancing age, pro-inflammatory cytokines like IL-6, IL-8, and TNF-α, associated with NF-κB activation and the accumulation of reactive oxygen species (ROS), establish a vicious cycle of inflammation and cell proliferation, consolidating BPH as a chronic inflammatory-hormonal pathology (Bahuguna et al., 2023; Singh et al., 2025).

Conventional therapies, such as alpha-1 adrenergic blockers (tamsulosin) and 5-alpha-reductase inhibitors (finasteride), although effective, are frequently associated with adverse effects like postural hypotension, erectile dysfunction, and decreased libido (Halawani et al., 2024; Lauck et al., 2024). This justifies the search for advanced phytotherapeutic formulations that offer efficacy along with a better safety profile (Alfred & Ama, 2024; Mbyemeire et al., 2026).

Cucurbita sp. seeds contain 30% to 50% fixed oil rich in linoleic and oleic acids, as well as phytosterols (especially Δ7-phytosterols), tocopherols, and phenolic compounds. These compounds act on multiple BPH pathways: 5α-reductase inhibition, NF-κB blockade, induction of cell apoptosis, and modulation of PPAR-α and PPAR-γ (Kang et al., 2021; Ramak & Mahboubi, 2019). However, crude oil exhibits oxidative instability and low oral bioavailability, necessitating its transformation into a solid pharmaceutical form. Dry granulation converts the oil into a stable matrix, improving intestinal absorption and therapeutic adherence (Adepu & Ramakrishna, 2021; García et al., 2022).

Given the above, this study aimed to develop and characterize a granulate containing

Cucurbita sp. seed fixed oil (GOFSC) and to evaluate its anti-inflammatory and anti-hyperplastic efficacy in an experimental model of BPH in Wistar rats.

Material and Methods

Fixed oil procurement and chemical analysis

The fixed oil of *Cucurbita sp.* seeds (OFSC) was provided by the company Mi Agiti Indústria e Comércio de Alimentos e Cosméticos Ltda. (Brazil). Fatty acid characterization was performed by gas chromatography coupled with mass spectrometry (GC-MS) following prior esterification with a solution of hydrochloric acid in anhydrous methanol. A Shimadzu PQ2010SE instrument equipped with an RTX-5MS fused silica capillary column was used. The saponification value (SV) and acid value (AV) were determined in accordance with the Brazilian Pharmacopeia (7th edition, 2025).

Granulate preparation and characterization

The granulate (GOFSC) was obtained by dry granulation. Four formulations (F1–F4) were tested with variations in excipients (colloidal silicon dioxide, corn starch, lactose, carboxymethylcellulose, and lecithin). Flow properties were evaluated by determining bulk density, tapped density, Hausner ratio (HR), compressibility index (CI), and angle of repose, in accordance with the United States Pharmacopeia (USP 43). Morphology was evaluated by scanning electron microscopy (SEM) using a T3030 Plus Tabletop microscope.

In vivo assays

The study was submitted to and approved by the Ethics Committee on Animal Use of the Federal University of Amapá (CEUA-UNIFAP) under Protocol No. 02/2022. Male Wistar rats (*Rattus norvegicus albinus*) were used.

Carrageenan-induced paw edema

Acute anti-inflammatory evaluation was performed using the carrageenan-induced paw edema model, as described by Posadas et al. (2004) and adapted by Carvalho et al. (2021). The animals were randomly divided into 6 experimental groups (n = 5 rats/group): Negative control (CON, treated with 0.9% NaCl orally), Positive control (INDO, treated with indomethacin orally), OFSC 200 mg/kg, OFSC 400 mg/kg, GOFSC 200 mg/kg, and GOFSC 400 mg/kg. All

treatments were administered orally, 30 minutes before edema induction. Edema was induced by a subplantar injection of 0.1 mL of 1% carrageenan (Sigma-Aldrich, USA) into the left hind paw. In the right hind paw, 0.1 mL of 0.9% NaCl solution was administered and used as a contralateral control. Edema formation was quantified by measuring paw volume with a 6" digital caliper (0–150 mm, Zaas Precision Co., Piracicaba, SP, Brazil), with measurements taken hourly for 5 consecutive hours after carrageenan application (Posadas et al., 2004).

Cotton pellet-induced granulomatous tissue formation

Subchronic inflammation was evaluated using the foreign body-induced granuloma model (cotton pellets), adapted from Posadas et al. (2004) and Carvalho et al. (2021). The animals were randomly divided into 6 groups (n = 5 rats/group): Negative control (CON, 0.9% NaCl orally), positive control (DEX, dexamethasone 0.5 mg/kg orally), OFSC 200 mg/kg, OFSC 400 mg/kg, GOFSC 200 mg/kg, and GOFSC 400 mg/kg. All treatments were administered by gavage for 7 consecutive days. Cotton pellets weighing 20 ± 2 mg were previously sterilized in an autoclave at 120 °C for 30 minutes. The animals were anesthetized with sodium thiopental (45 mg/kg, intraperitoneally), and the ventral region was trichotomized. A longitudinal ventral incision was made, and through blunt dissection of the subcutaneous tissue, four pellets were implanted at equidistant points along the incision, in the axillary and inguinal regions. Treatment began 2 hours after pellet implantation and was maintained until the 7th day. On the 8th day, the animals were euthanized by anesthetic overdose, the cotton pellets with the granulomatous tissue were removed, and dried in an oven at 60 °C for 24 hours until reaching a constant weight. Granulomatous tissue weight was calculated by the difference between the final dry weight and the initial pellet weight (Kumar et al., 2016; Posadas et al., 2004).

Induction of Benign Prostatic Hyperplasia (BPH)

BPH was induced according to the methodology described by Afryie et al. (2014) and adapted by Sorokina et al. (2022) via subcutaneous administration of testosterone propionate at 30 mg/kg for 15 consecutive days. After induction, the animals were divided into 4 groups (n = 5 rats/group) and treated for 28 days: Control Group (CON, treated with 0.9% NaCl orally), Finasteride Group (FIN, 5 mg/kg orally), OFSC Group (400 mg/kg orally), and GOFSC Group (400 mg/kg orally). Before induction and after the end of the 28-day treatment, blood was collected via the orbital plexus for laboratory measurement of prostate-specific antigen (PSA), total testosterone, creatinine, aspartate aminotransferase (AST), and alanine

aminotransferase (ALT). At the end of the experiment, the prostates were collected, individually weighed on an analytical balance, and sent for histopathological processing.

Histopathology

The collected prostates were histologically processed as described by Carvalho et al. (2018). The samples were successively dehydrated in graded alcohols (70%, 80%, 90%, and 100%), cleared in xylene, and embedded in paraffin at 60 °C. Sections with a thickness of 5 μm were obtained using a semi-automatic rotary microtome (model CUT 5062, SLEE, Germany), stained by the Hematoxylin-Eosin (H&E) method, and analyzed under an Olympus optical microscope (model CX31 trinocular) with a 40× objective. Foci of inflammatory infiltrate, tissue organization, epithelial thickness, and cell death were evaluated according to criteria described by Souza et al. (2019).

Statistical Analysis

For statistical analysis, all quantitative results from the experimental models were expressed as mean ± standard deviation (or standard error of the mean, depending on the assay). Normality was previously assessed using the Shapiro-Wilk test. Comparison among multiple groups was performed using one-way Analysis of Variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons between treated groups and the control group (Zar, 2010). Results with $p < 0.05$ were considered statistically significant. The statistical programs used were GraphPad Instat and GraphPad Prism (versions 5.03 and 7.0, GraphPad Software Inc., San Diego, CA, USA).

Results

Phytochemical and pharmacotechnical characterization

GC-MS analysis of the *Cucurbita sp.* fixed oil identified linoleic acid (44.95%, retention time 19.15 min) and oleic acid (32.17%, retention time 19.22 min) as the major fatty acids, totaling approximately 77% of the fatty acid composition (Figure 1). The obtained Acid Value was 0.56 mg KOH/g, which complies with the maximum limit of the Brazilian Pharmacopeia (< 4.0 mg KOH/g). The Saponification Value was 208.13 mg KOH/g, slightly above the standard range of 180-200 mg KOH/g, but considered an acceptable variation for this product of natural origin (Table 1).

In the development of the granulate, formulation F4 (composed of 40% fixed oil, 25% corn starch, 30% silicon dioxide, and 5% lecithin) presented the best results. It achieved a flow classified as excellent by USP 43, characterized by a compressibility index of 13.73 ± 0.01% (Table 2), Hausner ratio of 1.15 ± 0.03, and angle of repose of 27°. Scanning electron microscopy (SEM) analysis confirmed the formation of aggregates with a predominantly rounded morphology, suitable for industrial processing (Figure 2 and Table 3).

Anti-inflammatory activity

In the carrageenan-induced paw edema model, the negative control group showed a peak in edema after 3 hours. The groups treated with GOFSC at doses of 200 and 400 mg/kg exhibited a significant reduction in edema ($p < 0.001$) compared to the control group. Analysis of the Area Under the Curve (AUC) revealed that the inhibition induced by GOFSC was dose-dependent and comparable to that of the positive control (indomethacin). The inhibition observed with GOFSC surpassed that of the free oil (OFSC) at the same dosages (Figure 3).

In the cotton pellet granuloma assay, treatment with GOFSC at 400 mg/kg reduced granulomatous tissue weight to approximately 120 mg ($p < 0.001$), in sharp contrast to the negative control group, which reached approximately 300 mg. The effect of GOFSC was statistically comparable to that of the dexamethasone-treated group (which showed granulomas with an average weight of ~105 mg) (Figure 4).

Effects on Benign Prostatic Hyperplasia (BPH)

Induction with testosterone propionate significantly increased prostate weight in the control group, reaching 3.2 g. Treatment with GOFSC (400 mg/kg) reduced the prostate weight to ~1.8 g ($p < 0.001$), demonstrating an effect statistically similar to that of finasteride (1.5 g) (Figure 5). Macroscopic evaluation revealed that the prostate in the GOFSC group exhibited reduced volume and vascular congestion compared with the control group (Figure 6).

Histopathological analysis of the control group showed dilated acinar glands, papillary projections into the lumen, intense stromal inflammatory infiltrate, and cellular swelling. In the group treated with GOFSC, well-preserved glandular architecture, organized stromal tissue, normal epithelial morphology (reduction of cellular layers), and minimal inflammation with abundant apoptosis were observed (Figure 7).

Serum PSA levels were significantly reduced in the GOFSC group ($p < 0.05$) compared to the control (Figure 8). In contrast, serum total testosterone levels showed no significant differences among the groups, remaining at around 0.66 ng/dL in animals treated with the phytotherapeutic agent (Figure 9).

Safety evaluation

Biochemical assays evaluating hepatic and renal functions revealed that the activity of the enzymes AST and ALT, as well as serum creatinine concentration, remained within normal limits in all groups, with no statistically significant differences between the control and the groups treated with OFSC or GOFSC (Figures 10 and 11). Additionally, the relative weight of organs (heart, liver, kidneys, and testes) showed no significant changes. Only the weight of the seminal vesicles was reduced in the FIN, OFSC, and GOFSC groups compared to the control (Table 4).

Discussion

The phytochemical characterization confirmed that *Cucurbita sp.* seed oil is a rich source of polyunsaturated and monounsaturated fatty acids, particularly linoleic and oleic acids. The abundance of these compounds supports their potential to modulate oxidative processes and inhibit inflammatory pathways, including NF-κB signaling, thereby justifying their applicability in the treatment of BPH (Kang et al., 2021; Zerafatjou et al., 2021). The exceptionally low acid values attest to the quality and oxidative stability of the raw material used.

The dry granulation technology, utilizing lecithin as a replacement for carboxymethylcellulose (CMC), was decisive in obtaining a solid product with excellent flow performance. Lecithin, due to its amphipathic nature, not only reduced friction between particles and improved powder fluidity but also facilitated the incorporation of a high lipid load (40% oil) into the excipient matrix without compromising granulate stability (Adepu & Ramakrishna, 2021).

The results of the in vivo inflammation assays confirmed that the granulation process did not degrade the oil's active principles. On the contrary, the anti-inflammatory efficacy of GOFSC was superior to that of the free oil in the paw edema model (Figure 3). This potentiation

can be attributed to micelle formation, facilitated by lecithin in the gastrointestinal tract, which increases the intestinal solubilization and absorption of fatty acids and phytosterols (García et al., 2022). Furthermore, the marked inhibition of granulomatous tissue formation indicates that the formulation acts effectively on the proliferative and tissue-remodeling phases of chronic inflammation (Figure 4), which are fundamental underlying pathophysiological mechanisms in BPH (Wang et al., 2022).

In the experimental model of BPH, GOFSC demonstrated potent anti-hyperplastic action. The reduction in prostate weight and PSA levels, combined with the regression of histopathological lesions, confirms that the phytotherapeutic agent acts directly on androgen-induced epithelial and stromal proliferation (Amante et al., 2026). A crucial clinical differentiator of this formulation, compared with synthetic 5α-reductase inhibitors (such as finasteride), was its ability to attenuate hyperplasia without altering serum testosterone levels (Figures 8 and 9). This suggests local modulation of androgen receptors or specific tissue inhibition of prostatic 5α-reductase, avoiding the systemic endocrine adverse effects that frequently lead to the abandonment of conventional treatment (Lauck et al., 2024; Singh et al., 2025).

The safety profile was attested by the normality of hepatic and renal biomarkers and the absence of changes in vital organ weights. The isolated decrease in seminal vesicle weight was an expected anatomical effect, since these structures strongly depend on androgenic stimulation mediated by DHT.

Conclusion

The *Cucurbita sp.* seed fixed oil granulate (GOFSC) proved to be a viable and effective pharmacotechnical innovation. The solid formulation optimized the oil's flow properties and stability, resulting in potent in vivo anti-inflammatory and anti-hyperplastic activity. By attenuating prostate growth, reducing PSA levels, and reversing histopathological damage without causing systemic endocrine alterations or organ toxicity, GOFSC presents itself as a promising, effective, and safe multi-target phytotherapeutic agent for the clinical management of benign prostatic hyperplasia.

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Author contributions

Conceptualization, data curation, and formal analysis: Crislany da Costa Furtado, Bianca Vitória Monteiro Ferreira, Juliane Almeida de Sousa, Gabriel da Costa Furtado, and Danna Emanuelle Santos Gonçalves. Analysis validation and visualization: Abrahão Victor Tavares de Lima Teixeira. Funding and Project administration: José Carlos Tavares Carvalho. Original draft: Helison de Oliveira Carvalho; Revised manuscript: Thiago Afonso Carvalho Celestino Teixeira.

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Data availability statement

All sources analyzed during this study are included in the reference list and are publicly available.

Declarations

Conflict of interest

The authors declare no conflict of interest, financial or otherwise.

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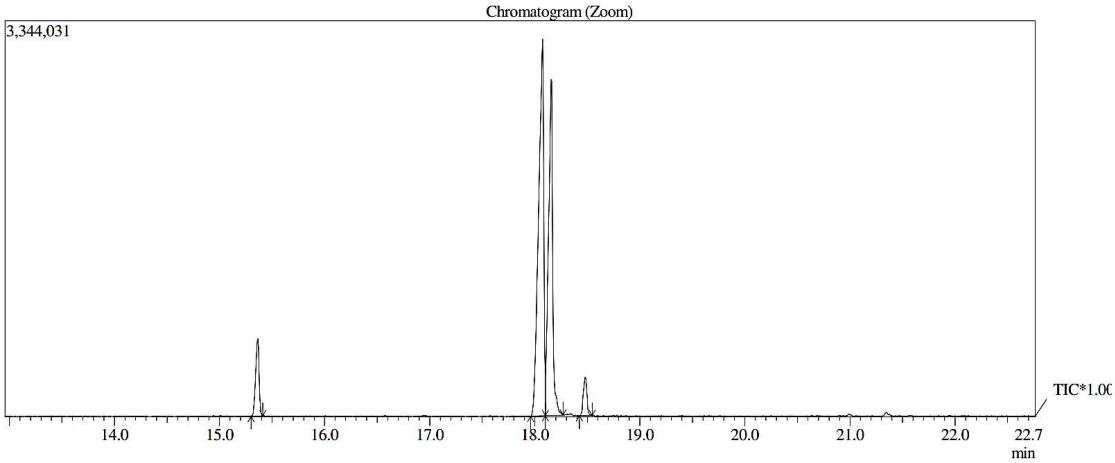


Figure 1. Chromatographic profile of *Cucurbita sp.* seed fixed oil (OFSC) obtained by gas chromatography coupled with mass spectrometry (GC-MS), following esterification with HCl in anhydrous methanol. The chromatogram shows two major peaks at retention times of 19.15 min (linoleic acid, C18:2 ω-6) and 19.22 min (oleic acid, C18:1 ω-9), representing 44.95% and 32.17% of the total integrated area, respectively, totaling approximately 77% of the fatty acid composition. Compound identification was performed by comparison with the NIST 8.0 spectral library and integration using LabSolutions GC-MS software (Shimadzu PQ2010SE).

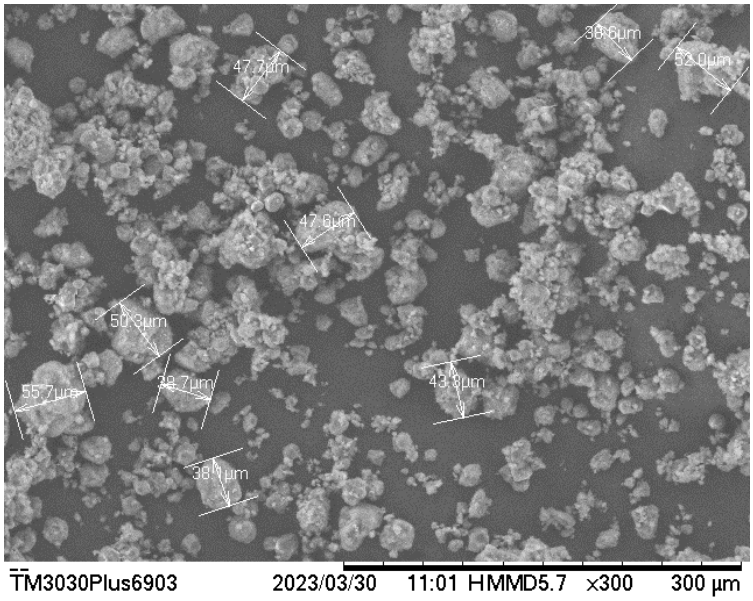


Figure 2. Scanning electron microscopy (SEM) of *Cucurbita sp.* seed fixed oil granulate (GOFSC, optimized formulation F4). Magnification ×300; scale bar = 300 µm. Equipment: TM3030Plus Tabletop (Hitachi). Aggregates with predominantly rounded morphology and moderately heterogeneous size distribution are observed. The spherical particle shape reduces intermolecular cohesion and interparticle contact area, favoring superior flow properties and confirming concordance with experimentally determined pharmacotechnical parameters (HR = 1.15; CI = 13.73%; α = 27°). The observed morphology is suitable for encapsulation and compression operations at industrial scale.

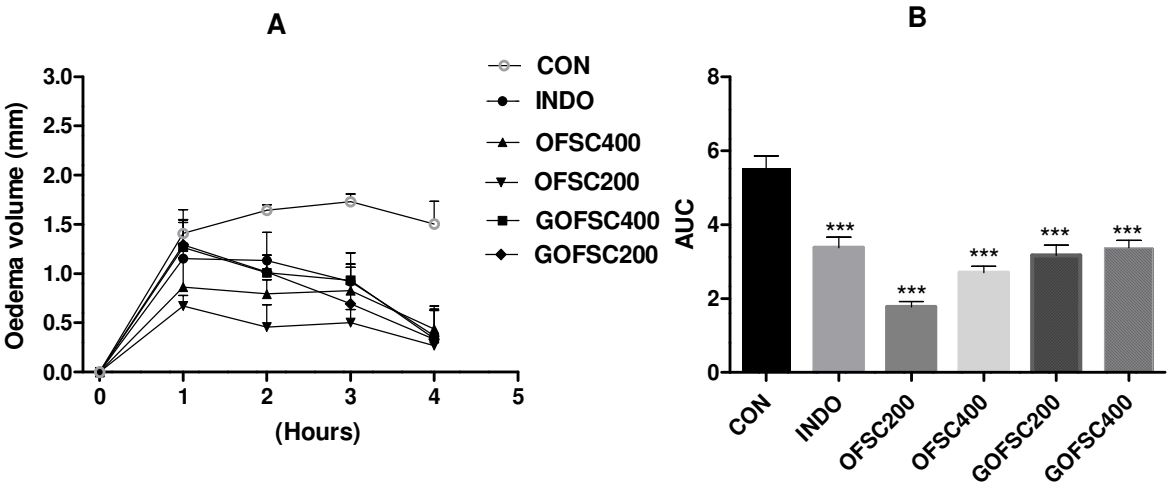


Figure 3. Effect of single-dose oral treatment by gavage with indomethacin (INDO, positive control), *Cucurbita sp.* seed fixed oil (OFSC, 200 and 400 mg/kg), and *Cucurbita sp.* seed fixed oil granulate (GOFSC, 200 and 400 mg/kg), administered 30 minutes before induction, on paw edema induced by subplantar injection of 1% carrageenan (0.1 mL) in the left hind paw of male Wistar rats. **(A)** Temporal evolution of edema volume (mm) measured by digital caliper hourly for 5 consecutive hours post-induction. **(B)** Area under the curve (AUC) corresponding to cumulative edema progression across the different experimental groups, used as an integrated parameter of anti-inflammatory efficacy. Data are expressed as mean ± standard deviation (n = 5 per group). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test. ***$p < 0.001$ compared to the negative control group (CON, 0.9% NaCl). GOFSC demonstrated dose-dependent inhibition of edema, comparable to indomethacin and surpassing free oil (OFSC) at the same doses.

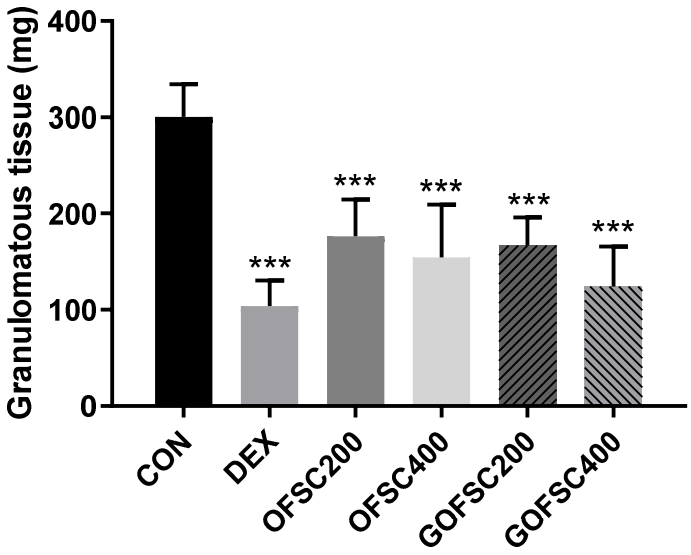


Figure 4. Effect of 7-day consecutive oral treatment by gavage with dexamethasone (DEX, 0.5 mg/kg, positive control), *Cucurbita sp.* seed fixed oil (OFSC, 200 and 400 mg/kg), and *Cucurbita sp.* seed fixed oil granulate (GOFSC, 200 and 400 mg/kg) on granulomatous tissue formation induced by subcutaneous implantation of sterilized cotton pellets (20 ± 2 mg) in the axillary and inguinal regions of male Wistar rats. Columns represent the mean dry weight of granulomatous tissue (mg), obtained after pellet removal on day 8 and oven drying at 60 °C for 24 hours. Data are expressed as mean ± standard deviation (n = 5). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test. ***$p < 0.001$ compared to the negative control group (CON, 0.9% NaCl). The significant reduction in granulomatous tissue indicates that GOFSC effectively inhibits the proliferative and tissue remodeling phases of chronic inflammation.

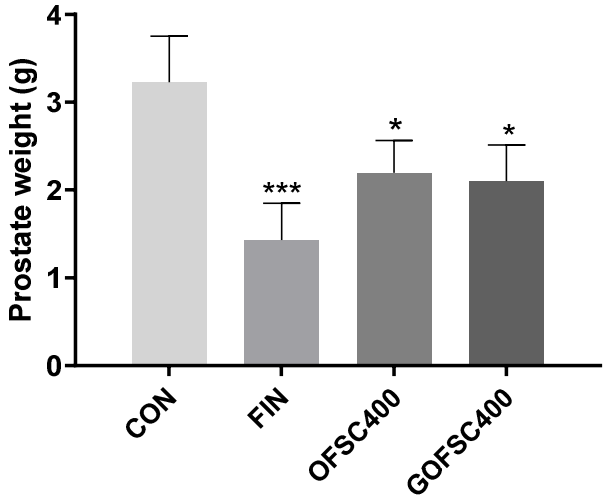


Figure 5. Effect of 28-day oral treatment by gavage with finasteride (FIN, 5 mg/kg), *Cucurbita sp.* seed fixed oil (OFSC, 400 mg/kg), and *Cucurbita sp.* seed fixed oil granulate (GOFSC, 400 mg/kg) on prostate weight (g) of male Wistar rats subjected to BPH induction by testosterone propionate (30 mg/kg, s.c., 15 days). Data are expressed as mean ± standard deviation (n = 5). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test. ***$p < 0.001$ (FIN vs. CON) and *$p < 0.05$ (OFSC and GOFSC vs. CON). GOFSC promoted a reduction in prostate weight statistically comparable to finasteride, quantitatively confirming the histopathological and macroscopic findings.

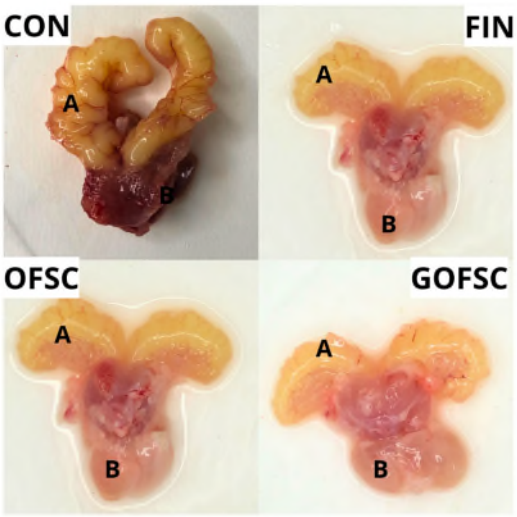


Figure 6. Effect of 28-day oral treatment by gavage with finasteride (FIN, 5 mg/kg), *Cucurbita sp.* seed fixed oil (OFSC, 400 mg/kg), and *Cucurbita sp.* seed fixed oil granulate (GOFSC, 400 mg/kg) on the gross morphology of the prostate and seminal vesicles of male Wistar rats subjected to BPH induction by testosterone propionate (30 mg/kg, s.c., 15 days). Groups: (1) CON — BPH control; (2) FIN — finasteride; (3) OFSC — fixed oil; (4) GOFSC — granulate. (A) Seminal vesicles; (B) Prostate. Progressive reduction in prostate volume and vascular congestion is observed in the treated groups compared to the control, with the GOFSC group showing macroscopic volume and coloration similar to the FIN group (gold standard). The reduction in seminal vesicles in the treated groups is an expected physiological effect, consistent with local DHT inhibition.

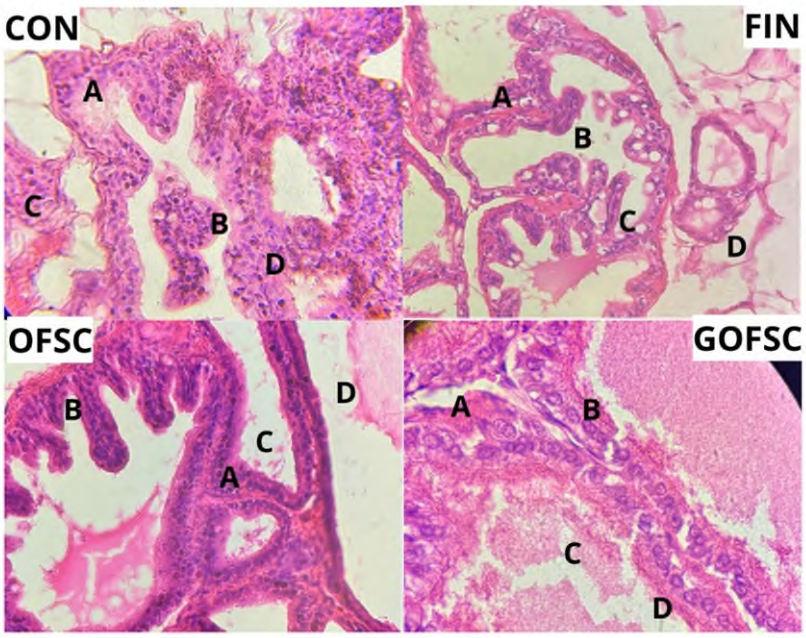


Figure 7. Effect of 28-day oral treatment by gavage with finasteride (FIN, 5 mg/kg, positive control), *Cucurbita sp.* seed fixed oil (OFSC, 400 mg/kg), and *Cucurbita sp.* seed fixed oil granulate (GOFSC, 400 mg/kg) on prostatic histopathology of male Wistar rats subjected to benign prostatic hyperplasia (BPH) induction by testosterone propionate (30 mg/kg, subcutaneously, for 15 consecutive days). Photomicrographs stained with Hematoxylin-Eosin (H&E), 40× objective, Olympus CX31 microscope. **1 — CON (BPH control):** (A) marked epithelial proliferation with multiple cell layers; (B) intraluminal papillary projections; (C) intense stromal inflammatory infiltrate composed of macrophages, neutrophils, and lymphocytes; (D) acute cellular swelling with hyperchromatic nuclei and frequent mitotic figures. **2 — FIN (finasteride 5 mg/kg):** (A) flat atrophied epithelium with marked reduction in cell layers; (B) reduced cell density; (C) dilated acinar lumens with scant secretion; (D) well-preserved stroma with minimal inflammatory infiltrate. **3 — OFSC (fixed oil 400 mg/kg):** (A) mild epithelial reduction; (B) partially preserved glandular architecture; (C) moderate cuboidal epithelium; (D) reduced but still present inflammatory infiltrate. **4 — GOFSC (granulate 400 mg/kg):** (A) well-preserved glandular architecture with morphological pattern similar to the FIN group; (B) organized stromal tissue; (C) nearly normal epithelial morphology with marked reduction in cell layers; (D) minimal inflammation with evidence of abundant apoptosis. The histopathological superiority of GOFSC over OFSC confirms that the granulate formulation optimizes the bioavailability of bioactive compounds in prostatic tissue.

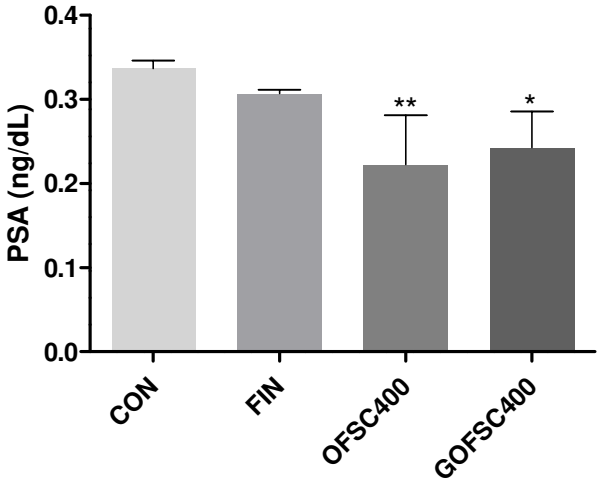


Figure 8. Effect of 28-day oral treatment by gavage with finasteride (FIN, 5 mg/kg), *Cucurbita sp.* seed fixed oil (OFSC, 400 mg/kg), and *Cucurbita sp.* seed fixed oil granulate (GOFSC, 400 mg/kg) on serum prostate-specific antigen (PSA, ng/dL) levels of male Wistar rats subjected to BPH induction by testosterone propionate (30 mg/kg, s.c., 15 days). Blood samples were collected via the orbital plexus before induction and after treatment completion. Data are expressed as mean ± standard deviation (n = 5). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test, with a significance level of $p < 0.05$. **$p < 0.01$ (OFSC vs. CON) and *$p < 0.05$ (GOFSC vs. CON). The reduction in PSA levels is directly consistent with the regression of prostatic epithelial proliferation observed in histopathological analysis, confirming the anti-hyperplastic efficacy of GOFSC.

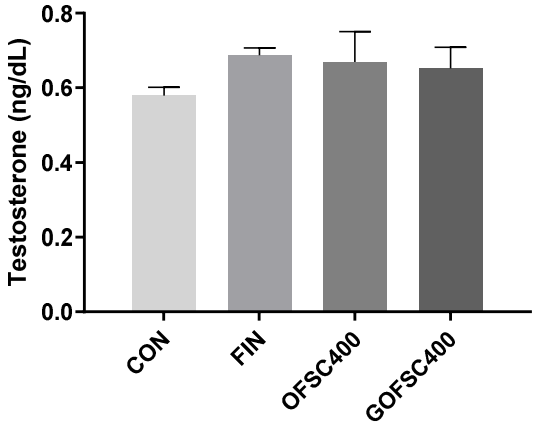


Figure 9. Effect of 28-day oral treatment by gavage with finasteride (FIN, 5 mg/kg), *Cucurbita sp.* seed fixed oil (OFSC, 400 mg/kg), and *Cucurbita sp.* seed fixed oil granulate (GOFSC, 400 mg/kg) on serum total testosterone levels (ng/dL) of male Wistar rats subjected to BPH induction by testosterone propionate (30 mg/kg, s.c., 15 days). Blood samples were collected via the orbital plexus. Data are expressed as mean ± standard deviation (n = 5). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test. The absence of statistically significant differences among groups demonstrates that OFSC and GOFSC treatments do not compromise the systemic androgenic endocrine axis, representing a relevant clinical advantage over synthetic 5α-reductase inhibitors, which are frequently associated with sexual dysfunction due to systemic DHT reduction.

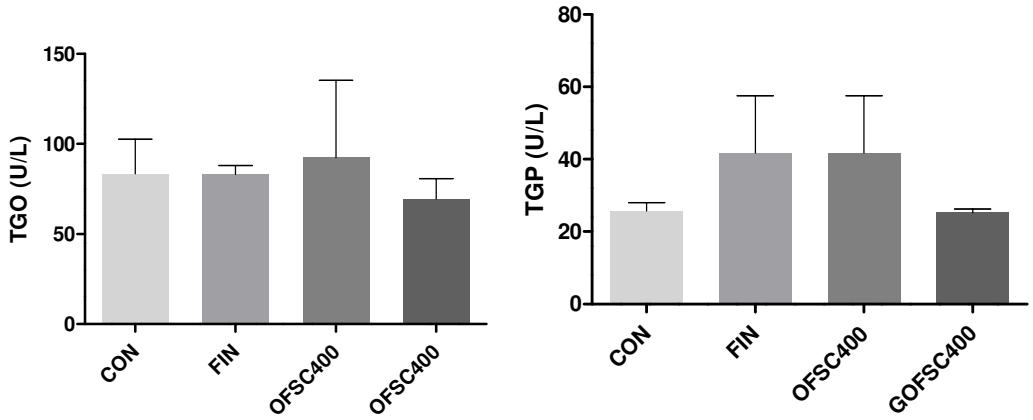


Figure 10. Effect of 28-day oral treatment by gavage with finasteride (FIN, 5 mg/kg), *Cucurbita sp.* seed fixed oil (OFSC, 400 mg/kg), and *Cucurbita sp.* seed fixed oil granulate (GOFSC, 400 mg/kg) on serum enzymatic activity of aspartate aminotransferase (AST, U/L) and alanine aminotransferase (ALT, U/L) of male Wistar rats subjected to BPH induction by testosterone propionate (30 mg/kg, s.c., 15 days). Data are expressed as mean ± standard deviation (n = 5). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test. The maintenance of AST and ALT values within normal physiological limits in all groups, with no statistically significant differences, confirms the absence of hepatotoxicity of the treatments over 28 days of continuous oral administration.

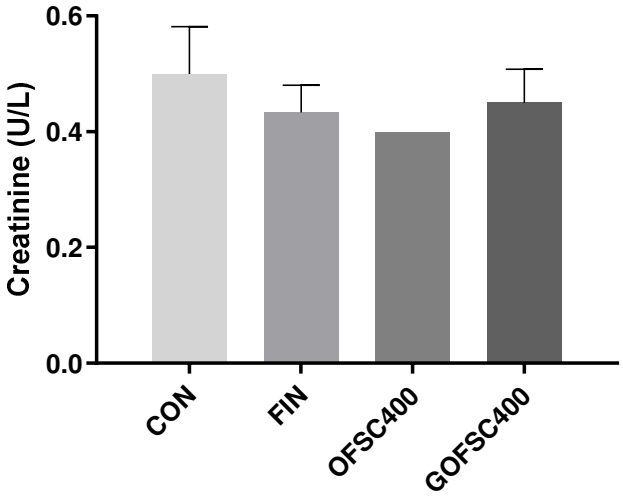


Figure 11. Effect of 28-day oral treatment by gavage with finasteride (FIN, 5 mg/kg), *Cucurbita sp.* seed fixed oil (OFSC, 400 mg/kg), and *Cucurbita sp.* seed fixed oil granulate (GOFSC, 400 mg/kg) on serum creatinine concentration (U/L) of male Wistar rats subjected to BPH induction by testosterone propionate (30 mg/kg, s.c., 15 days). Data are expressed as mean ± standard deviation (n = 5). Statistical analysis was performed by one-way ANOVA followed by Tukey's post hoc test. The maintenance of creatinine values within normal physiological limits in all groups, with no statistically significant differences, confirms the absence of nephrotoxicity of the treatments over 28 days of continuous oral administration, attesting to the renal safety profile of GOFSC.

Supplementary Files

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- GraphicalAbstractInflammopharmacology.png