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Prasad**

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(54) **COMPOSITION AND METHOD FOR  
TREATING PROSTATE DISORDERS**

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30, 2018.

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**A61K 9/00** (2006.01)

**A61P 13/08** (2006.01)

**A61K 36/889** (2006.01)

(52) **U.S. Cl.**

CPC ..... **A61K 31/575** (2013.01); **A61K 9/0056**  
(2013.01); **A61K 9/0095** (2013.01); **A61K**  
**36/889** (2013.01); **A61P 13/08** (2018.01)

(58) **Field of Classification Search**

CPC .. **A61K 31/575**; **A61K 9/0095**; **A61K 36/889**;  
**A61K 9/0056**; **A61P 13/08**

See application file for complete search history.

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Brown

(57) **ABSTRACT**

The invention provides a composition and method for treat-  
ing prostate disorders. The composition can be formulated to  
provide a saw palmetto oil having about 3% w/w  $\beta$ -sitos-  
terol.

**13 Claims, 11 Drawing Sheets**

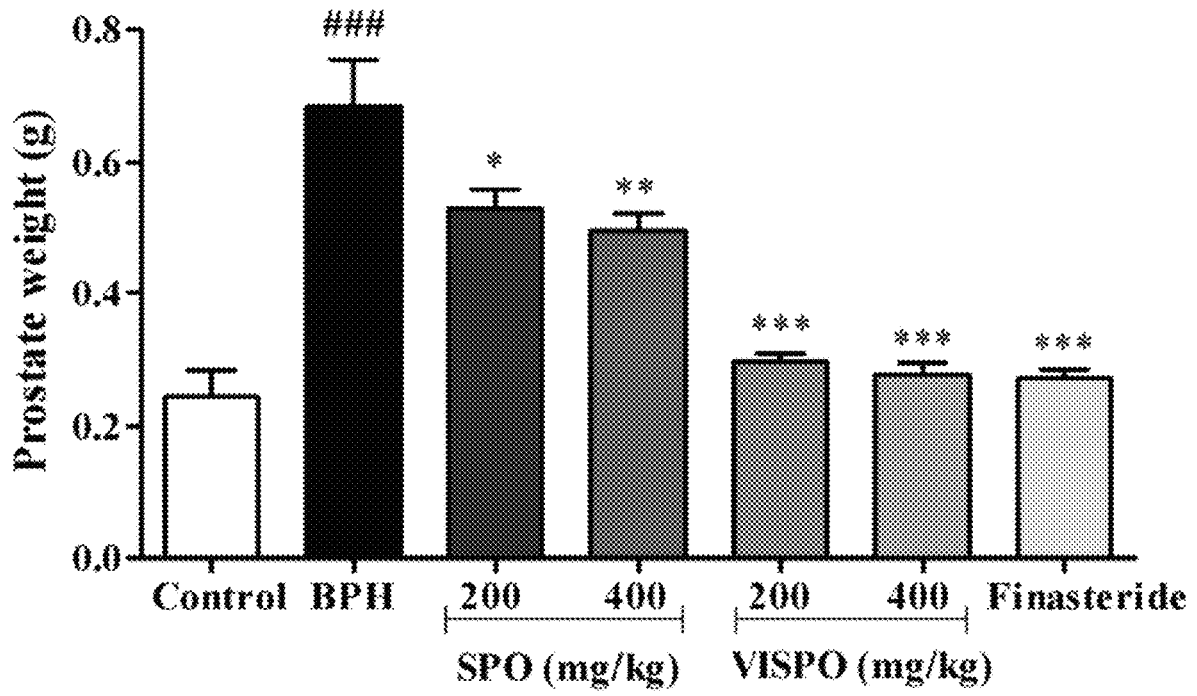


FIG. 1

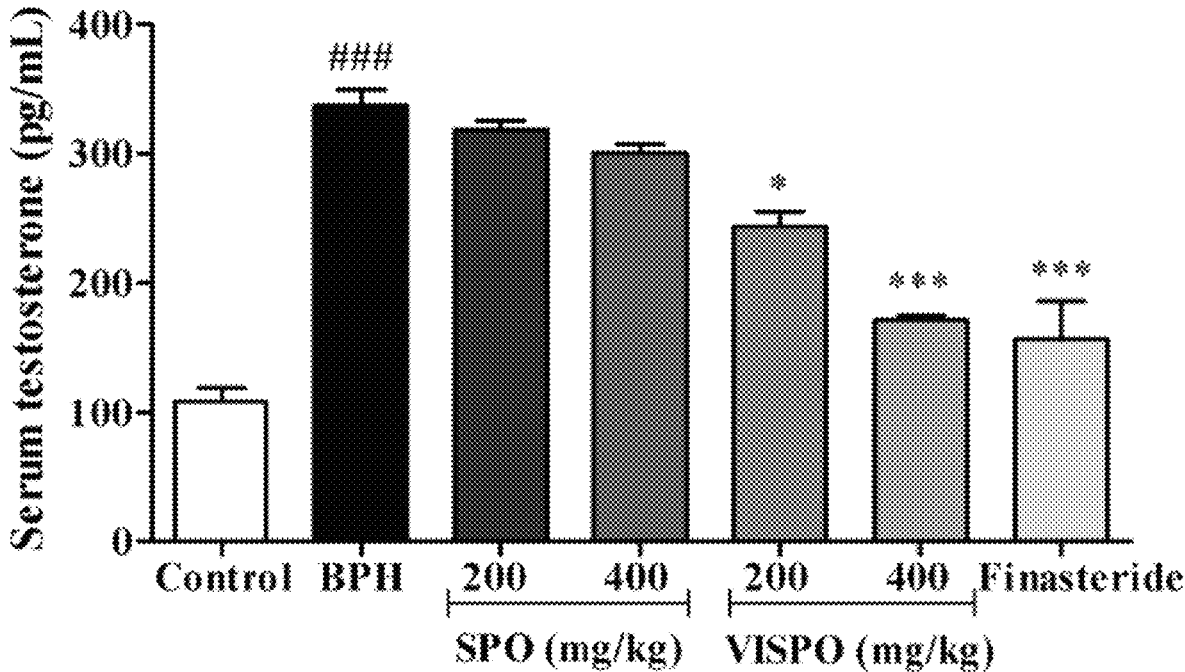


FIG. 2

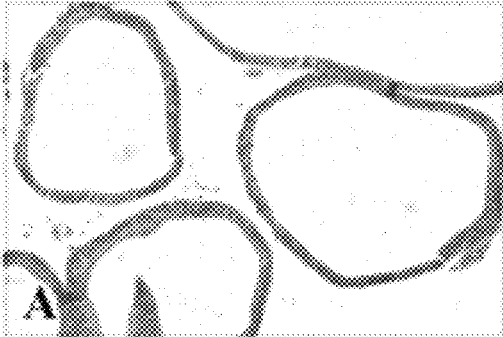


FIG. 3A

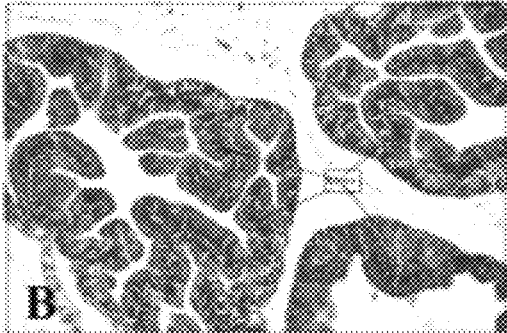


FIG. 3B

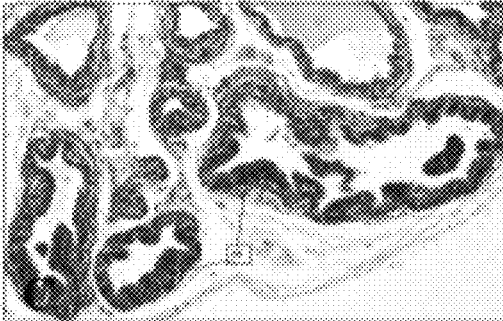


FIG. 3C

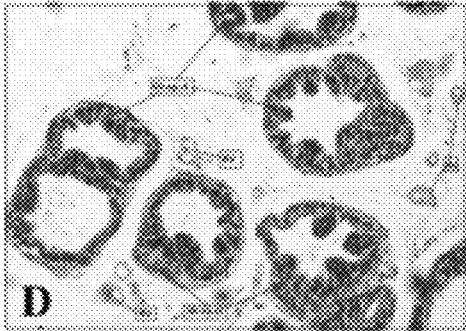


FIG. 3D

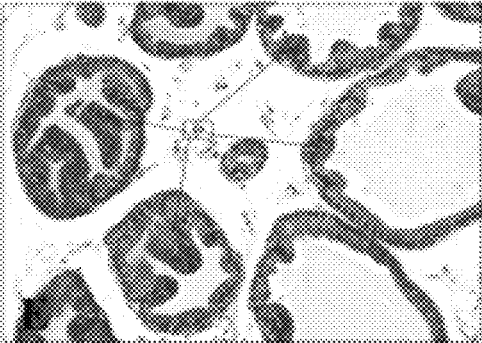


FIG. 3E

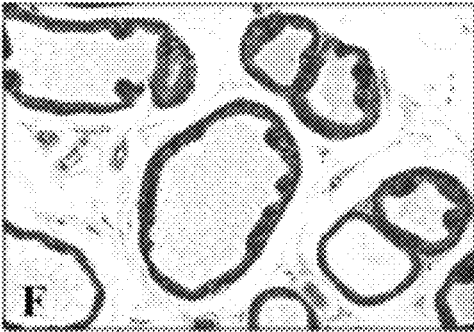


FIG. 3F

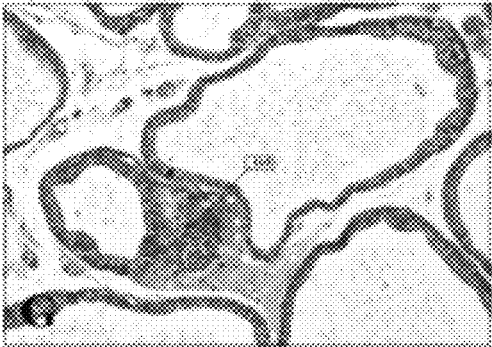


FIG. 3G

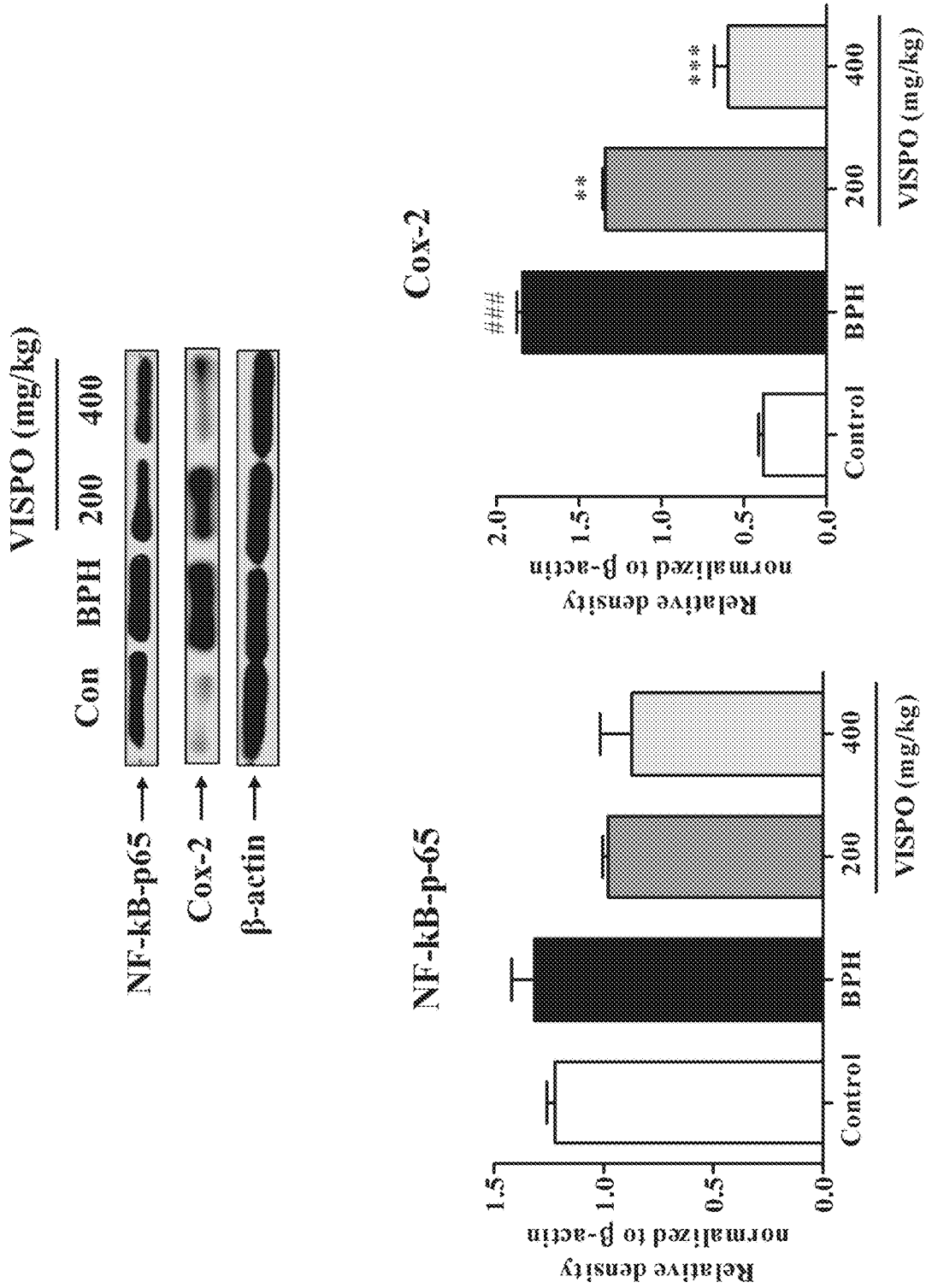


FIG. 4

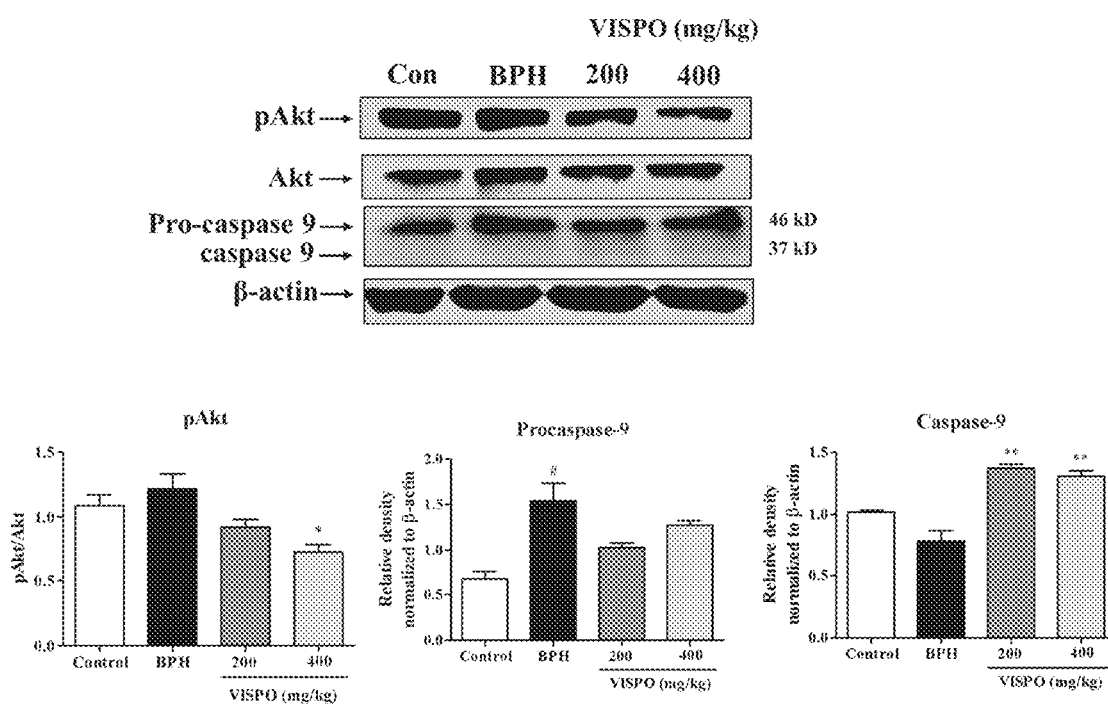


FIG. 5

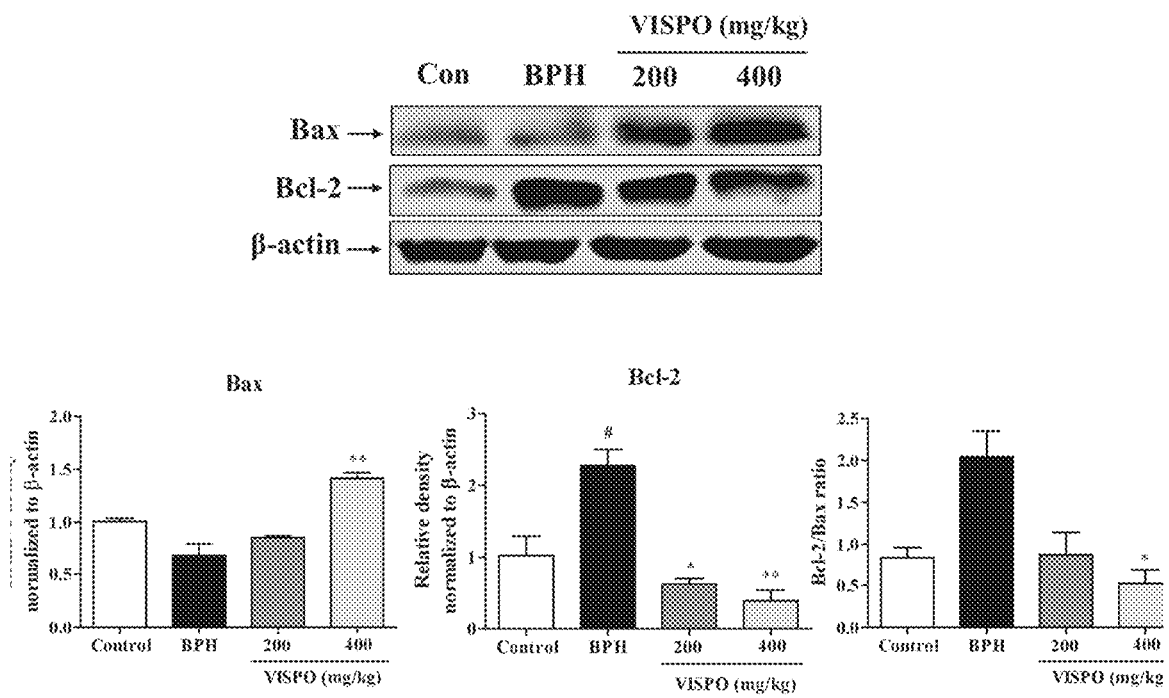


FIG. 6



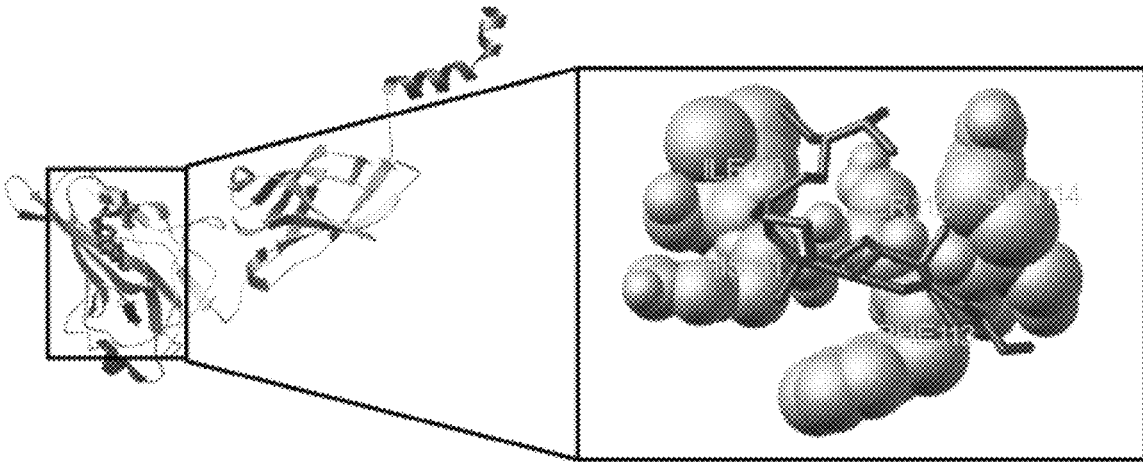


FIG. 7A

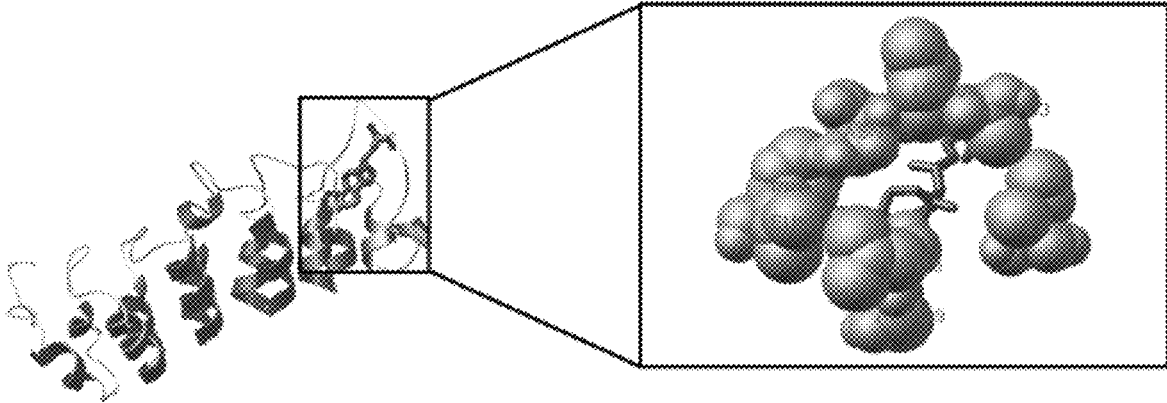


FIG. 7B

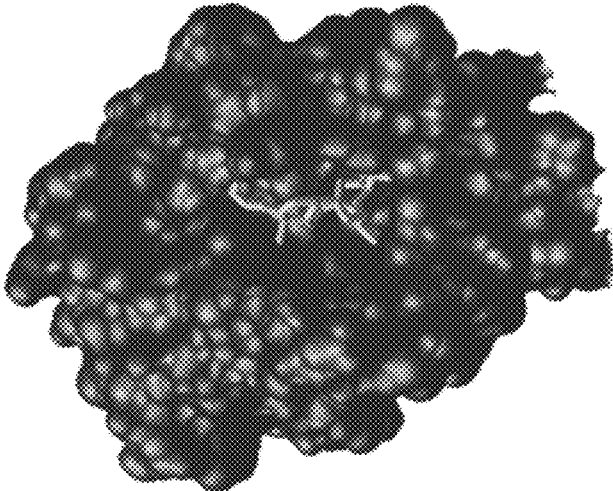


FIG. 8C

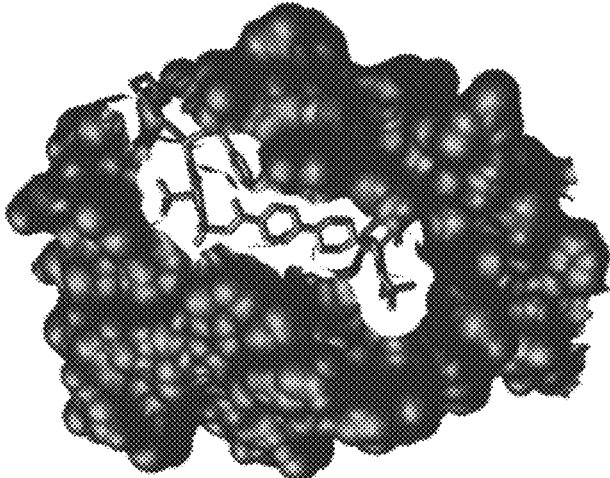


FIG. 8B

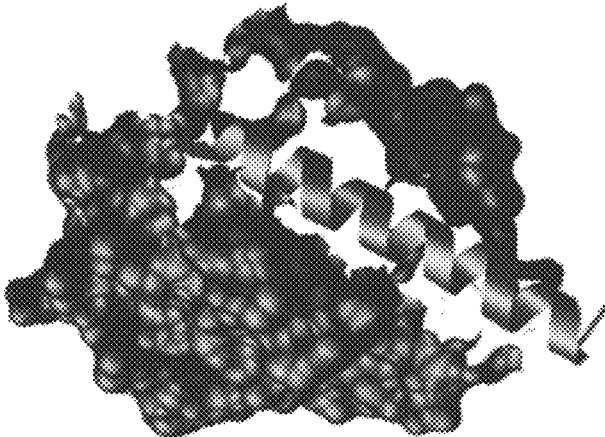


FIG. 8A

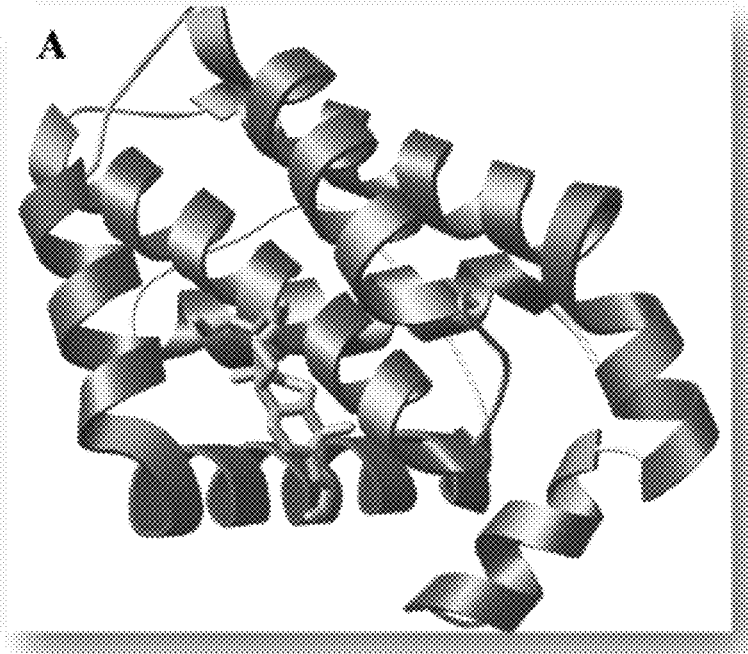


FIG. 9A

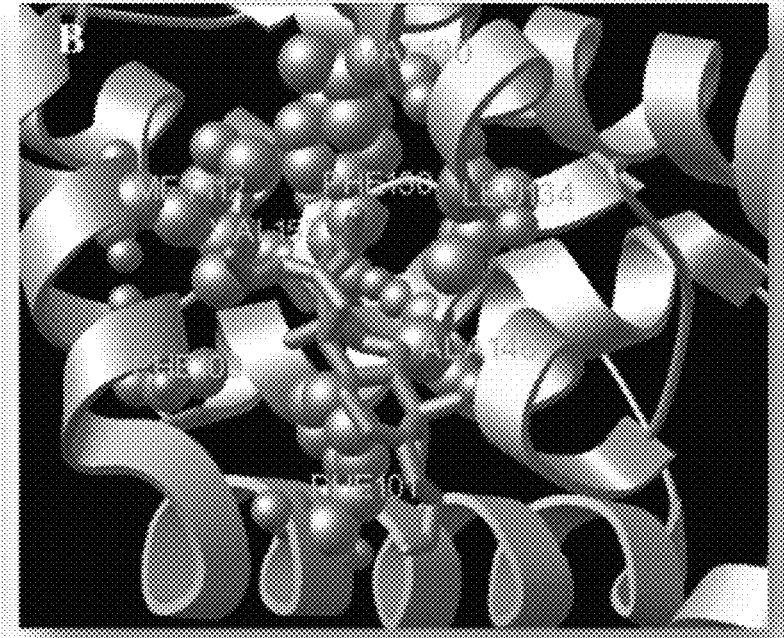


FIG. 9B

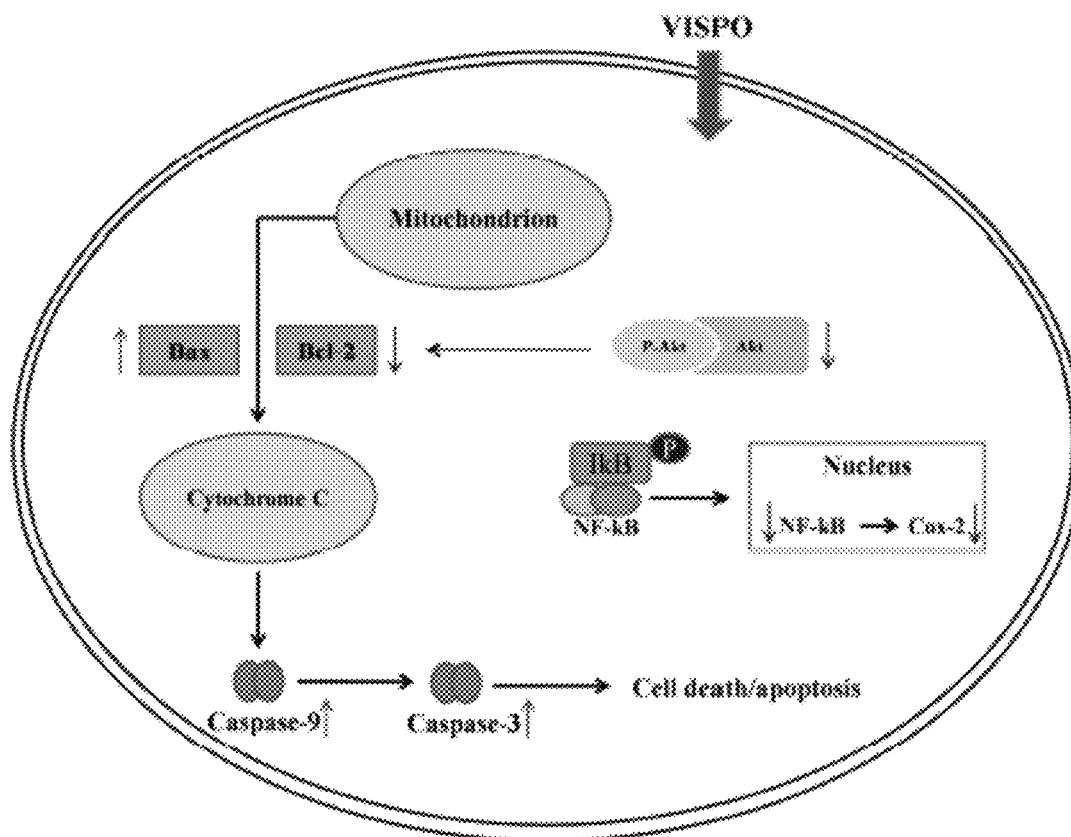


FIG. 10

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**COMPOSITION AND METHOD FOR  
TREATING PROSTATE DISORDERS****CROSS-REFERENCE TO RELATED  
APPLICATIONS**

This application claims priority to U.S. Provisional Application No. 62/711,834 filed Jul. 30, 2018, the entire contents of which are incorporated herein by reference for all purposes.

**FIELD OF INVENTION**

The invention generally relates to compositions and methods for the treatment of prostate disorders. More particularly, the invention relates to a composition of  $\beta$ -sitosterol and method of its use in treating benign prostate hyperplasia and prostatitis.

**BACKGROUND OF THE INVENTION**

Benign prostate hyperplasia (BPH) is a common prostate disease affecting older men. It is a major cause of lower urinary tract symptoms which deteriorate quality of life (Gupta et al. 2015). BPH pathogenesis has been associated with factors such as genetic predisposition, hormonal imbalance, imbalance in cell proliferation and death, and inflammation (Pawlicki et al. 2004). The current therapy for BPH involves administration of 5- $\alpha$  reductase inhibitors, phosphodiesterase-5 inhibitors, and laser therapy. 5- $\alpha$  reductase inhibitors effectively reduce the conversion of testosterone to dihydrotestosterone (DHT), and hence decrease the BPH progression. While these treatments can improve the quality of life, some patients feel worse due to the side effects of the treatment. For example, Finasteride™ is a 5- $\alpha$  reductase inhibitor that can effectively alleviate the complications of BPH (Nickel et al. 2011). However, Finasteride™ is associated with undesirable side effects such as erectile dysfunction, increased risk of impotency, and ejaculation disorder. Moreover, natural treatments for BPH, such as saw palmetto oil, lack the degree of efficacy needed to effectively treat BPH.

What is needed in the art therefore is a treatment for BPH that lacks the side effects of small molecule BPH therapy and provides greater efficacy than available natural treatments for BPH.

**SUMMARY OF THE INVENTION**

It is an object of the invention to provide a composition and method for treating a prostate disorder that is free of the side effects of small molecule prostate disorder therapies and has greater efficacy than natural prostate disorder treatments that are currently available.

In some aspects, the composition comprises 3% w/w  $\beta$ -sitosterol.

In a further aspect of the invention, the composition comprises saw palmetto oil having 3% w/w  $\beta$ -sitosterol.

In some aspects of the invention, the prostate disorder is BPH, prostatitis, or a combination thereof.

In some aspects, the invention treats one or more prostate disorder symptoms selected from enlarged prostate, increased prostate weight, inflammation, inhibited apoptosis of prostate tissue, and prostate growth.

The present disclosure demonstrates that the inventive composition surprisingly possesses superior in vivo efficacy compared to saw palmetto oil. Study subjects showed a

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significant increase in prostate growth following sub-cutaneous administration of testosterone, while a 21-day treatment with the inventive composition appreciably improved hyperplastic conditions as evident from parameters such as prostate weight, prostate weight to body weight ratio, growth inhibition of prostate, and serum testosterone levels. The inventive composition effectively suppressed prostatic inflammation and strongly induced apoptosis in the prostate tissue of the study subjects.

**BRIEF DESCRIPTION OF THE DRAWINGS**

FIG. 1 shows a comparison of the effect of saw palmetto oil (SPO) and an embodiment of the inventive composition on prostate weight and prostate to body weight ratio.

FIG. 2 shows a comparison of the effect of saw palmetto oil (SPO) and an embodiment of the inventive composition on serum testosterone levels.

FIGS. 3A-3G show a comparison of the effect of saw palmetto oil (SPO) and an embodiment of the inventive composition on the histology of prostate tissue.

FIG. 4 shows the effect of an embodiment of the inventive composition on the expression of inflammatory markers.

FIG. 5 shows the effect of an embodiment of the inventive composition on the expression of phosphorylated Akt and caspase-9 proteins in prostate tissue.

FIG. 6 shows the effect of an embodiment of the inventive composition on the expression of Bcl-2 family in prostate tissue.

FIGS. 7A and 7B show the lowest energy binding poses of  $\beta$ -sitosterol with I $\kappa$ B and NF- $\kappa$ B proteins.

FIGS. 8A-8C show a representation of molecular interactions between  $\beta$ -sitosterol and the BH-3 binding groove of Bcl-2.

FIGS. 9A and 9B show hydrophobic interactions between  $\beta$ -sitosterol and the BH-3 binding groove of Bcl-2.

FIG. 10 is a schematic representation of the potential mechanism for the efficacy of an embodiment of the inventive composition in treating BPH.

**DETAILED DESCRIPTION OF THE  
INVENTION**

The invention provides a composition and method of treating disorders of the prostate without producing the side effects associated with contemporary prostate disorder treatments and with greater efficacy than present-day natural therapies.

The invention provides a composition for treating prostate disorders. In one non-limiting embodiment of the invention, the composition comprises about 3% w/w  $\beta$ -sitosterol. As used herein, the term "about" means the quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length that is referenced, or that varies (plus or minus) by as much as 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2% or 1% from the referenced quantity, level, value, number, frequency, percentage, dimension, size, amount, weight or length. In some embodiments of the invention, the composition comprises a saw palmetto oil having about 3% w/w  $\beta$ -sitosterol. The saw palmetto oil for formulating the composition can be obtained from, for example, *Serenoa repens*. The composition can be formulated by combining saw palmetto oil with  $\beta$ -sitosterol to provide a saw palmetto oil composition having about 3% w/w  $\beta$ -sitosterol.

As demonstrated by the accompanying examples, the inventor surprisingly discovered that formulating saw pal-

metto oil with about 3% w/w  $\beta$ -sitosterol provides greater efficacy in treating the symptoms and inflammatory mechanisms associated with prostate disorders compared to a saw palmetto oil that lacks the formulation of the inventive composition.

In some embodiments of the invention, the composition further comprises one or more substances selected from a vitamin, mineral, extract, amino acid, protein, carbohydrate, lipid, fatty acid, excipient, pharmaceutically acceptable carrier, bulking agent, binding agent, caffeine, flavouring, sweetener, and preservative. Excipients for use with the composition can be selected on the basis of their compatibility with the composition's active ingredients and the properties of the desired dosage form. Suitable excipients include, but are not limited to, binders, fillers, flow aids/glidants, disintegrants, lubricants, stabilizers, surfactants, and the like. Suitable excipients include, but are not limited to, those disclosed in the following publications, the disclosures of which are incorporated herein by reference in their entirety for all purposes: Remington: The Science and Practice of Pharmacy, 19<sup>th</sup> Ed (Easton, Pa.: Mack Publishing Company, 1995); Hoover, John E., Remington's Pharmaceutical Sciences, (Easton, Pa.: Mack Publishing Co 1975); Liberman, H. A. and Lachman, L., Eds., Pharmaceutical Dosage Forms (New York, N.Y.: Marcel Dekker 1980); and Pharmaceutical Dosage Forms and Drug Delivery Systems, Seventh Ed (Lippincott Williams & Wilkins 1999). The composition can be formulated, and administered as, a food, food supplement, beverage, or nutritional supplement.

In at least one aspect of the invention, the composition is provided and/or manufactured in bulk. The composition can be provided in bulk for the manufacture of foods, nutritional supplements, nutraceuticals, dietary supplements and/or food supplements for the treatment of pain. Bulk quantities of the composition can be packaged, stored and/or distributed in drums, bags, boxes, containers and the like. Such containers can be configured to prevent or inhibit the oxidation of the active ingredients of the composition.

In at least one embodiment, the invention provides a method of treating a prostate disorder in a patient in need thereof. As used herein, the terms "treat," "treating," "treatment," and the like, can refer to the clinical intervention of a disease or condition in an attempt to alter, alleviate, ameliorate, prevent, inhibit or reverse the progression or symptoms of the disease or condition.

The method can be practiced by administering, to a patient in need of treatment for a prostate disorder, an effective amount of the composition disclosed herein. The prostate disorder can be BPH, prostatic inflammation, or a combination thereof. Administering the composition can treat one or more symptoms of the prostate disorder, including, without limitation, frequent or urgent need to urinate, increased frequency of urination, difficulty starting urination, weak urine stream, urine stream that stops and starts, dribbling at the end of urination, involuntary urination, painful urination, inability to completely empty the bladder, urinary tract infection, inability to urinate, and blood in the urine. In at least one aspect of the invention, administering the composition treats the underlying causes of the symptoms of the prostate disorder. For example, administering the composition can inhibit or reverse prostatic inflammation, increase apoptosis of prostate tissue, decrease serum testosterone, reduce prostate size, inhibit increases in prostate size, or combinations thereof.

The composition can be administered systemically. Suitable administration routes for the composition include, but are not limited to, auricular, buccal, conjunctival, cutaneous,

dental, endocervical, endosinusal, endotracheal, enteral, epidural, extra-amniotic, interstitial, intra-abdominal, intra-amniotic, intra-arterial, intra-articular, intrabiliary, intrabronchial, intrabursal, intracardiac, intracartilaginous, intracaudal, intracavernous, intracavitary, intracerebral, intracisternal, intracorneal, intracoronal dental, intracoronary, intracorporus cavernosum, intradermal, intradiscal, intraductal, intraduodenal, intradural, intraepidermal, intrasophageal, intragastric, intravaginal, intraileal, intralesional, intraluminal, intralymphatic, intramedullary, intrameningeal, intramuscular, intraocular, intraovarian, intrapericardial, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrasinal, intraspinal, intrasynovial, intratendinous, intratesticular, intrathecal, intrathoracic, intratubular, intratumor, intratympanic, intrauterine, intravascular, intravenous, intravenous bolus, intravenous drip, intraventricular, intravitreal, laryngeal, nasal, nasogastric, ophthalmic, oral, oropharyngeal, parentera, percutaneous, periarticular, peridural, perineural, periodontal, rectal, inhalation, retrobulbar, soft tissue, subarachnoid, subconjunctival, subcutaneous, sublingual, submucosal, topical, transdermal, transmucosal, transplacental, transtracheal, transtympanic, ureteral, urethral, vaginal, or combinations thereof. The composition can be administered by irrigation, drip, infusion, or topically by a dressing, patch, or bandage that is in contact with the composition.

The present invention is explained in detail by the following examples. Example 1 describes BPH induction and dosage of the composition and Finasteride™ to experimental rats. Example 2 illustrates the effect of the composition and Finasteride™ on prostate growth in rats. Example 3 describes the influence of treatments on serum testosterone levels in BPH rats. Example 4 illustrates the effect of the composition and Finasteride™ on the histology of prostate tissue. Example 5 relates to the effect of the composition on the expression of inflammatory markers in the prostate tissue of testosterone induced BPH rats. Example 6 describes the influence of the composition on the regulation of apoptosis in the prostate tissue of rats. Example 7 describes the binding mode of  $\beta$ -sitosterol with I $\kappa$ B and NF- $\kappa$ B proteins through in silico docking. Example 8 illustrates molecular interaction of  $\beta$ -sitosterol with the BH-3 binding groove of antiapoptotic Bcl-2 protein.

#### Example 1: Induction of Benign Prostatic Hyperplasia in Rats Animals

Forty-two male Wistar rats (10 week old) were purchased from Biogen, Bangalore, India (CPCSEA Reg. No. 971/bc/06). The animals were housed in accordance with the CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines. After a one-week acclimatization period the animals were housed in rooms maintained at 22±2° C. and 30-70% humidity. Water and standard pellet diet were given ad libitum. The animals were randomly divided into seven groups, each group consisting of six animals with less than 20% mean body weight range.

#### BPH Induction and Dosage

BPH was induced in rats using testosterone 5 mg/kg subcutaneously and a simultaneous treatment with different doses of saw palmetto preparations viz. Saw palmetto oil (SPO) and the inventive composition (200 and 400 mg/kg b.w.) or Finasteride (1 mg/kg b.w.) p.o., was followed for 21 days (Table 1). The inventive composition (VISPO) was a composition of saw palmetto oil having 3%  $\beta$ -sitosterol. At the end of the treatment period, blood was collected prior to

the necropsy. Animals were sacrificed by overdose of anesthetics. The vital parameters such as prostate weight, prostate weight to body weight ratio, growth inhibition of prostate, serum testosterone level, expression of inflammatory and apoptotic proteins in prostate tissue were evaluated.

TABLE 1

Experimental design (n = 6 in each group)	
Groups	Treatment mg/kg b.w.
Control	0.1% CMC in saline
BPH	Testosterone 5 mg/kg b.w. + 0.1% CMC in saline
SPO	Testosterone 5 mg/kg b.w. + 200 mg/kg p.o. Testosterone 5 mg/kg b.w. + 400 mg/kg p.o.
VISPO	Testosterone 5 mg/kg b.w. + 200 mg/kg p.o. Testosterone 5 mg/kg b.w. + 400 mg/kg p.o.
Finasteride	Testosterone 5 mg/kg b.w. + Finasteride 1 mg/kg b.w.

Example 2: Effect on Prostate Growth

Following a 21-day treatment with the saw palmetto oil preparations and/or Finasteride, prostate tissues were excised, rinsed and weighed immediately after removal. The PW/BW ratio was calculated using the following equation: PW/BW ratio=(Prostate weight of each animal from experimental group/Body weight of each animal from experimental group)×1000. The percentage of growth inhibition was calculated as follows: 100-[(treated group-control group)/(BPH group-control group)]×100.

The mean prostate weight of animals in the BPH group was significantly increased as compared to the normal rats (p<0.001) indicating testosterone induced BPH in rats. The prostate weights were reduced considerably in the Finasteride and VISPO treated groups (p<0.001). There was a dose dependent decrease of prostate weight in treated animals (FIG. 1). The prostate weight in BPH group was 2.83 fold higher than control group of rats (Table 2). The treated groups exhibited appreciable reduction in prostate weight. There was a reduction of 1.28 and 1.38 fold in prostate weight of 200 and 400 mg/kg SPO treated rats respectively. Interestingly, groups with VISPO showed a highly significant decrease in the prostate weight compared to the BPH group (p<0.001). At the 400 mg/kg dose, VISPO effectively reduced the prostate weight by 2.43 fold which was comparable to the Finasteride treatment (2.51 fold). Further, the PW/BW ratio in BPH group was significantly increased as compared to the control group (p<0.001). Administration of SPO preparations reduced the PW/BW ratio significantly in a dose dependent manner (FIG. 1B). The data were highly significant in VISPO treatment as compared to BPH group (p<0.001). The percentage growth inhibition of prostate was 90.9% at 400 mg/kg in the VISPO treated group.

TABLE 2

Prostate growth in BPH model rats		
Group	Prostate weight (g)	Inhibition of growth (%)
Control	0.24 ± 0.04	—
BPH	0.68 ± 0.07 <sup>###</sup>	—
SPO (200 mg/kg)	0.53 ± 0.03*	34.09
SPO (400 mg/kg)	0.49 ± 0.03**	43.18

TABLE 2-continued

Prostate growth in BPH model rats		
Group	Prostate weight (g)	Inhibition of growth (%)
VISPO (200 mg/kg)	0.29 ± 0.01 <sup>***</sup>	88.63
VISPO (400 mg/kg)	0.28 ± 0.02 <sup>***</sup>	90.9
Finasteride (1 mg/kg)	0.27 ± 0.01 <sup>***</sup>	93.18

The data were represented as mean + SEM (n = 6).  
<sup>###</sup>p < 0.001 vs. control group;  
<sup>\*</sup>p < 0.05,  
<sup>\*\*</sup>p < 0.01 and  
<sup>\*\*\*</sup>p < 0.001 vs. BPH group.  
 Growth inhibition = 100 - ((treated group-control group)/(BPH group-control group) × 100)  
 SPO: Saw palmetto oil;  
 VISPO: saw palmetto oil with 3% β-sitosterol

Example 3: Effect on Serum Testosterone Levels

Blood samples were collected at the end of experiment and coagulated at room temperature for 20 min Serum was separated by centrifuging at 3000 g, 4° C. Serum samples were analyzed for testosterone levels using commercial ELISA kit (582701; Cayman Chemical Co., Ann Arbor, Mich., USA) following manufacturer's protocol.

The serum testosterone level was significantly higher in the BPH group (337.5±12.15 pg/mL) as compared to control rats (108.6±14.92 pg/mL). As shown in FIG. 2, the level of testosterone was decreased in the treatment groups. The values were highly significant in the 400 mg/kg VISPO treated group and comparable to Finasteride (p<0.001).

Example 4: Effect on Hyperplastic Patterns in Prostate Tissue of BPH Rats

The prostate tissue samples were fixed in 4% formalin, dehydrated with a graded alcohol series, embedded in paraffin, and then cut into 4 μm thickness. The sections were stained with hematoxylin and eosin (H&E, Sigma-Aldrich, St. Louis, Mo., USA). The images were captured using a microscope (Leica, Germany).

Histological examination of prostate tissue revealed that the testosterone treatment induced glandular hyperplasia with decreased glandular luminal area compared to the control animals (FIG. 3B). SPO (200 and 400 mg/kg) and 200 mg/kg VISPO treatment groups exhibited moderate suppression of hyperplastic patterns (FIGS. 3C-E). 400 mg/kg VISPO treatment markedly restored the cellular architecture in BPH rats (FIG. 3F). The epithelial cell thickness was reduced and the luminal area increased considerably. The finasteride group also showed near normal prostatic glands with mild focal inflammation (FIG. 3G).

Example 5: Effect on Expression of Inflammatory Markers in Prostate Tissue

The prostatic tissue from each animal was homogenized in lysis buffer and incubated for 20 minutes to induce cell lysis. Tissue extracts were centrifuged at 12000 rpm for 20 minutes and the supernatants were transferred to clean tubes. Aliquots of protein samples (30 μg) were resolved on 8-15% sodium dodecyl sulphate-polyacrylamide gel electrophoresis gels and transferred on to a nitrocellulose membrane. The membranes were incubated for 1 hr with blocking solution and subsequently incubated with 1:500 dilution of primary antibodies, anti-NF-kB and anti-COX-2 overnight at 4° C.

The membranes were washed three times with 0.1% Tween 20 in TB S followed by incubation with Horseradish peroxidase conjugated goat IgG antibody (1:4000 dilution) for 1 h at room temperature. Detection was performed on ImageQuant™ LAS 500 (GE Healthcare Life Sciences). Densitometry analyses of the results were made using Image J software (version 1.46, National Institutes of Health, Bethesda, Md.).  $\beta$ -actin was used as a loading control.

Testosterone treatment markedly increased the expression of NF-kB ( $p < 0.05$ ) and COX-2 ( $p < 0.001$ ) in prostate tissue of BPH rats as compared to the control group. However, treatment with VISPO significantly down-regulated the expression of these proteins in a dose dependent manner (FIG. 4).

#### Example 6: Effect on the Induction of Apoptosis in Prostate Tissue of BPH Rats

The prostate tissue lysates were subjected to western blotting as described in Example 5 for the analysis of phosphorylated Akt (Ser473), caspase-9, Bcl-2 and Bax protein expression in the VISPO treated BPH rats.

In the prostate tissue of BPH rats, there was increased phosphorylation of Akt at Ser-473. VISPO treatment effectively down-regulated the pAkt levels. There was a considerable decrease in the procaspase-9 level in the prostate tissue of BPH rats after treatment with respective doses of VISPO- $\beta$  (FIG. 5). Further, VISPO treated groups showed a significant decrease in the antiapoptotic protein Bcl-2, but increased expression of proapoptotic Bax as compared to BPH group ( $p < 0.01$ ) (FIG. 6).

#### Example 7: Binding of $\beta$ -Sitosterol with IkB and NF-kB Proteins

Molecular docking is the study of the interaction of ligands with target macromolecules. The three-dimensional crystal structures of IkB (PDB ID: 1IKN) and NF-kB (1NFI) were retrieved from the Protein Data Bank (<http://www.rcsb.org/>). AutoDock tool was utilized to generate grids, calculate dock score and evaluate the conformers of  $\beta$ -sitosterol bound in the active sites of target proteins. AutoDock 4.2 was employed to get docking and binding scores; which is implemented by Lamarckian genetic algorithm method. The ligand molecule was designed and the structure was analyzed using ACD/Chemsketch. The PRODRG server was used to minimize energy of drug compounds and 3D coordinates were prepared. A grid box was generated using AutoGrid to define docking spaces for the respective proteins. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters (Rodriguez and Infante, 2011). The lowest binding energy and the inhibitory constant for each protein were obtained from the docking log files (Dlg).

NF-kB is a transcriptional factor responsible for the activation of inflammatory genes such as COX-2 and iNOS. However, under normal conditions in the cell, NF-kB remains in inactive form, bound to specific inhibitor &B. From the western blot analysis of prostate tissue lysates, it became apparent that VISPO treatment down-regulated the NF-kB. The higher content of  $\beta$ -sitosterol in VISPO can prevent the dissociation of IkB from the IkB-NF-kB complex leading to the maintenance of NF-kB in its idle form. Hence, a docking analyses of  $\beta$ -sitosterol with IkB and NF-Kb was performed. As shown in Table 3,  $\beta$ -sitosterol preferably bound to IkB with a binding energy of  $-9.17$  kcal/mol, while the phytosterol had a lower affinity towards

NF-kB ( $-8.7$  kcal/mol). Further,  $\beta$ -sitosterol had more hydrophobic interactions with the binding site of IkB. The docking of  $\beta$ -sitosterol into the target proteins are shown in FIG. 7. The higher affinity of  $\beta$ -sitosterol with IkB can show that the molecule inhibits the phosphorylation and degradation of IkB keeping NF-kB in its inactive form.

TABLE 3

Molecular docking score of $\beta$ -sitosterol to IkB and NF-kB proteins				
Protein	Binding energy kcal/mol	Inhibitory constant (nM)	H-bond	Hydrophobic interactions
IkB	-9.17	190.27	Ala127	Phe103, Leu104, Ile94, Pro114, Leu115, Leu130, Ala129
NF-kB	-8.7	421.04	Asn115	Ile110, Phe113

#### Example 8: Molecular Docking of $\beta$ -Sitosterol with the BH-3 Binding Groove of Antiapoptotic Bcl-2 Protein

The three-dimensional crystal structure of Bcl-2 in complex with a Bax BH-3 peptide (PDB ID: 2XA0) and co-crystal structure of Bcl-2 and Navitoclax (PDB ID: 4LVT) were retrieved from Protein Data Bank. Further docking analysis of  $\beta$ -sitosterol was performed as mentioned in Example 7.

In silico docking study was performed on the BH-3 binding groove of Bcl-2 protein in order to show the mechanism by which VISPO can induce apoptosis. The active principle  $\beta$ -sitosterol was made to dock to the co-crystal structure of Bcl-2 with its selective inhibitor, Navitoclax. The detailed molecular interaction of  $\beta$ -sitosterol and hydrophobic residues of BH-3 binding groove of Bcl-2 is shown in FIG. 9A.  $\beta$ -sitosterol interacted with hydrophobic enclosure of Phe101, Phe109, Met112, Val130, Leu134, Ala146, Phe150 and Val153 (FIG. 9B). The lowest binding energy was  $-8.79$  kcal/mol.

#### INDUSTRIAL APPLICABILITY

As evident from the experimental data described herein, a composition of saw palmetto (*S. repens*) oil having 3%  $\beta$ -sitosterol has high efficacy, compared to conventional saw palmetto oil, in the treatment of benign prostate hyperplasia and associated inflammatory response.

The foregoing description, examples and accompanying drawings are intended to describe some, but not necessarily all, examples or embodiments of the invention. The described and exemplified embodiments are to be considered in all respects only as illustrative and not restrictive. The contents of the detailed description, examples and accompanying drawings do not limit the scope of the invention in any way.

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The invention claimed is:

1. A method of treating a prostate disorder, comprising administering to a patient in need thereof saw palmetto oil having about 3% w/w  $\beta$ -sitosterol, wherein said saw palmetto oil is administered at a dosage of about 400 mg/kg body weight, and wherein administering said saw palmetto oil treats said prostate disorder and reduces serum testosterone in said patient by about 33%.

2. The method of claim 1, wherein said disorder is benign prostatic hyperplasia, prostatitis, or a combination thereof.

3. The method of claim 1, wherein administering said saw palmetto oil treats one or more symptoms of said prostate disorder.

4. The method of claim 3, wherein said symptoms are selected from frequent and/or urgent need to urinate, increased frequency of urination, difficulty starting urination, weak urine stream, urine stream that stops and starts, dribbling at the end of urination, involuntary urination, painful urination, inability to completely empty the bladder, urinary tract infection, inability to urinate, blood in the urine, and combinations thereof.

5. The method of claim 1, wherein administering said saw palmetto oil treats one or more of an enlarged prostate, increased prostate weight, prostate inflammation, inhibited apoptosis of prostate tissue, and prostate growth.

6. The method of claim 1, wherein administering said saw palmetto oil reduces or inhibits prostate inflammation in said patient.

7. The method of claim 1, wherein administering said saw palmetto oil decreases prostate weight or prevents an increase in prostate weight in said patient.

8. The method of claim 1, wherein administering said saw palmetto oil inhibits hyperplasia in the prostate of said patient.

9. The method of claim 1, wherein administering said saw palmetto oil increases apoptosis of prostate tissue in said patient.

10. The method of claim 1, wherein said patient is human.

11. The method of claim 1, wherein said saw palmetto oil is administered orally.

12. The method of claim 1, wherein said saw palmetto oil is combined with a carrier, excipient, or combination thereof.

13. The method of claim 1, wherein said saw palmetto oil is combined with a nutritional supplement, food supplement, or beverage.

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