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Protective effect of *Curcubita pepo* seed extract against some prostatic and biochemical markers of Testosterone propionate-Induced benign prostatic hyperplasia in male albino rats

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Abstract

Aim: This research was designed to determine the protective effect of *Curcubita pepo* seed-extract (CPSE) on some prostatic and biochemical markers of testosterone propionate-induced Benign Prostatic Hyperplasia (BPH) in male albino rats. **Methods**: Standard methods were used to determine the chemical constituents of *Curcubita pepo* seed-extract. After one week of adaptation, a total of 60 male albino rats were randomly divided into six experimental groups (A-F) of ten rats per group. Group A received only the vehicle, olive oil and served as normal control. Testosterone propionate (TP), at 14 $mgKg^{-1}$ body weight was administered intraperitoneally daily to groups B to F for 4 weeks to induce BPH. While group C was administered 10 $mgKg^{-1}$ of finasteride (F) through oral intubation, groups D, E, and F were orally administered extract of CPSE at the doses of 200, 400, and 800 mg/Kg body weight daily for four weeks. The albino rats were euthanized and sacrificed, while the tissues were harvested for analysis. **Results**: Testosterone propionate-induced benign prostatic hyperplasia in our albino rats (group B) discloses a significant ($P<0.05$) increase in the levels of PSA, ACP, MDA, dihydrotestosterone, estrogen and androgen with a significant ($P<0.05$) decrease in the activities of CAT, SOD and levels of GSH compared to the Group A that received only the vehicle, olive oil. However, treatments with both standard drug (finasteride) and CPSE at the stipulated doses reversed the trends of these markers to a level comparable to the normal control. The **GC**-MS analysis results of *Cucurbita pepo* seed revealed the existence of fifteen compounds. Docking results showed that 9,12-Octadecadienoic acid and 2-Hydroxycyclopentadecanone exhibited good binding affinities against PSA similar to the standard drug (-5.4, and -7.8, compared to -7.9), and ACP, -5.5, and -7.0 compared to the standard drug, -8.2. These findings indicate that CPSE could be effective in the management of BPH and its associated disorders.

Keywords: *Curcubita pepo* seed-extract, prostatic biomarkers, testosterone propionate-induced Benign Prostatic Hyperplasia (BPH), dihydrotestosterone, estrogen, androgen, GC-MS, Docking
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Introduction

Benign prostate enlargement is a multifactorial, genitourinary disorder and the most common noncancerous form of unusual prostate cell growth affecting aging men (Gossel and Wuest, 2004). It involves concurrent inflammation around the prostate gland, fibrosis, increased adrenergic tone in prostate smooth muscle, renal insult, and suppression of apoptosis of prostatic cells (Novara *et al.,* 2006; Lui *et al.,* 2007).

The actual causes of BPH are not fully understood but the excessive growth of smooth muscle tissues and glandular epithelial tissue is attributed to several various causes such as old age, late activation of cell growth, genetic factors, and hormonal changes (Culig *et al.,* 1998). Heart and circulatory diseases, obesity, and type-2 diabetes are medical conditions linked to BPH (Farley, 2011). Androgen and estrogen are also risk factors widely reported to constitute the primary factors responsible for BPH disorder (Shin *et al.,* 2012; De Nunzo and Tuban, 2011).

Due to the adverse effects of the available synthetic drugs including higher incidence of impotency, dizziness, hypotension there is need for complementary alternative herbal medicine which are easily accessible and affordable to the common man of which one of such plants is *Cucurbita pepo* (Pumpkin), hence the present study.

Cucurbita pepo (Pumpkin) one of the natural products with antioxidant potential is a leafy green vegetable belonging to the Cucurbitaceae family. Pumpkin have a central seed cavity that holds the seeds, and a thick, edible flesh. Furthermore, the fruits have different weights, color, shapes, and sizes. (Robinson and Decker-Walters, 1997). Oil from Pumpkin seed is one of the edible oils used in making salads, giving them a very agreeable taste (Murkovic *et al.,* 2004). Pumpkin common names are apala (Yoruba), "akwato or bàkánùwaà" (Hausa), squash (English) while it is known as "ụ́gbọ̀gùlù", "ányụ̄" and "ụ́gbọghùrù or ụ́gbọ̀ghọ̀rọ̀" in Igbo-Onitsha, Owerri and Umuahia language respectively (Adebayo *et al*., 2013, Smith, 1997). Part of Ebonyi State in Nigeria, Izzi refers to pumpkin seed as "Ugboma".

Pumpkin seed extract supplementation has been reported to avert changes in plasma lipids and blood pressure linked to low estrogen availability (Gossell-Williams *et al.,* 2008). It has also been shown too be useful in the management of hypertension and atherosclerosis (Al-Zuhair *et al.,* 2000).



Plate 1: *Cucurbita pepo* Leaves and Fruit (Gong *et al.,* 2012)

Ethno-pharmacological studies have shown that *Cucurbita pepo* is used in many countries for the treatment of numerous diseases, such as, an anti-inflammatory, antiviral, analgesic urinary disorders, anti-ulcer, Anti-diabetic and antioxidant (Carvalho *et al.,* 2014).. Report has equally shown that *Cucurbita pepo* exhibits important physiological properties as wound healing, tumour growth inhibition, hypoglycaemic effects and immunomodulating potential (Chaturvedi, 2012).

Materials and Methods

Materials

Chemicals and Reagents

The chemicals and reagents used were of good analytical quality. The chemicals were sourced from May and Baker, England; BDH, England and Merck, Darmstadt, Germany. The reagents used were commercial kits and products of Randox, QCA, USA, and Biosystem Reagents and Instruments, Spain.

Collection and Authentication of Biological Materials

Fresh seeds of *Cucurbita pepo* were collected from Ishieke Igbudu village in Igbudu Community in Ikwo Local Government Area of Ebonyi State, Nigeria, and were identified by Dr. O. E. Nwankwo, a Taxonomist in the Department of Applied Biology, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria. Parts of the identified plants with voucher number EBSU-H-1034 were kept in the Applied Biology Department, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria, for reference purposes.

The albino rats used were purchased from the Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu, Nigeria. The rats were acclimatized for one week before the commencement of the experiment in the Animal House of the Department of Biochemistry, Ebonyi State University, Abakaliki, Ebonyi State, Nigeria.

Methods

Extraction of *Cucurbita pepo* Seed Extract

Whole seeds from pumkin fruit were separated and dried at room temperature. Thereafter, the dried seeds were ground with a good grinder (Hanil Science Industrial Co. Ltd., Korea) to reduce the particle size. Distilled water (2500 ml) was measured into a beaker and 250g of the pulverised seed was soaked into it. This was allowed to stay overnight, and subsequently sieved (Chung Gye Sang Gong Sa, Korea). It was kept in a plastic container and stored in the laboratory pending the commencement of the experiment.

Experimental Design for the Study

Following acclimatization of the animals, a total of 60 albino rats were randomly divided into six experimental groups (A-F) of ten rats per group. Group A received olive oil only, the vehicle and served as normal control. Testosterone propionate (TP), at 14 $mgKg^{-1}$ body weight was administered intraperitoneally daily to groups B to F for 4 weeks to induce BPH. While group C was administered 10 $mgKg^{-1}$ of finasteride (F) through oral intubation, groups D, E, and F were orally administered extract of CPSE at the doses of 200, 400, and 800 mg/Kg body weight daily for four weeks.

Biochemical Analysis

Serum Prostate Specific Antigen (PSA) Estimation

The method of Nilson *et al*. (1997) was used in prostate specific antigen (PSA) estimation

Principle and procedure

The PSA–Elisa test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The test system makes use of a goat anti-PSA antibody positioned against prostate specific antigen for solid phase immobilization for the microliter well. A monoclonal anti-PSA antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution.

At room temperature our specimen reacted with the immobilized goat antibody for 60 minutes. The wells were cleaned to eliminate any unbound antigen. The immobilized antigen reacted with monoclonal anti-PSA-HRP

conjugate upon addition at room temperature and was allowed for 60 minutes leading to the PSA molecules being loaded between the solid phase and enzyme-linked antibodies. Unbound-labelled antibodies was eliminated with water from the wells. The specimen was incubated for 20 minutes upon addition of tetramethyl benzidine (TMB) at room temperature leading to blue colour development. Stop solution was used to change the blue color development to yellow upon addition. However, the colour intensity of the specimen is directly proportional to the concentration of the PSA while absorbance was read at 450nm.

Determination of Total Acid Phosphatase

The method of Allen (1995) was used to estimate total acid phosphatase (ACP).

Principle

Acid phosphatase at pH 4.9 metabolizes the release of inorganic phosphate from organophosphates. ACP activity is directly proportional to the concentration of nitrophenol released. For determination of prostatic acid phosphatase, the determination is carried out in the presence of tartarate. The prostatic enzyme is inhibited by tartarate. The difference in activity between total acid phosphatase and non-prostatic form gives activity of prostate form.

Procedure

The serum acid phosphatase (ACP) activity were measured with the ACP Elisa Kit according to the manufacturer's instructions.

Determination of Dihydrotestosterone, Estrogen, and Androgen Levels

Dihydrotestosterone, estrogen, and androgen levels were determined using the methods of Ismail (1981) and Yen and Jaffe (1986).

Principle

This method uses an enzyme-linked hormone and measures a colorimetric change (usually after adding a substrate) to determine hormone levels.

Procedure

The blood sample was centrifuged to separate the serum, which contains the hormones of interest. The serum samples were stored at -20°C until analysis. Wells of a microtiter plate were coated with a specific antibody against the hormone of interest.

Enzyme-linked hormone (ELISA) were added to the standards and sample wells/test tubes. The hormone in the sample competes with the labeled hormone for binding to the antibody. The mixture was incubated for a specific period (e.g., 1-2 hours) at room temperature or 37°C to allow binding equilibrium.

After the incubation, the wells were washed to remove unbound components. Substrate that reacts with the enzyme linked to the bound hormone were added, producing a color change. The absorbance using a spectrophotometer were measured. The intensity of the color is inversely proportional to the hormone concentration.

Calculation

Hormone concentration in serum was gotten using Standard curve by plotting the radioactivity or absorbance against the concentration of the standards

Determination of Oxidative Stress Parameters

Catalase Activity

Activity of catalase (CAT) was determined according to method of Aebi (1983).

Principle

The principle was based on ultraviolet absorption of hydrogen peroxide which was measured at 240 nm. On the decomposition of hydrogen peroxide (H_2O_2) with catalase, the absorption decreased with time and from this reduction, catalase activity was measured.

Procedure

Approximately 0.5 ml of the sample was intoduced into a test tube containing 2 ml of hydrogen peroxide (H_2O_2) and 2.5 ml of phosphate buffer. After that, 2 ml of dichromate acetic acid reagent was put into 1 ml portion of the reaction mixture while absorbance was measured per minute at 240 nm against the bank.

Calculation

$$\text{Catalase concentration (U/L)} = \frac{0.23 \times \log A}{}$$

Activity of Superoxide Dismutase

Superoxide dismutase (SOD) was assayed by the method of Marklund and Marklund (1974).

Principle

The superoxide dismutase ability to block the autoxidation of adrenaline was the basis of the SOD assay. Superoxide generated by xanthine oxidase is known to cause the oxidation of adrenaline to adenochrome. The production of the adenochrome formed per superoxide added increased with elevation of pH and also with increased adrenaline.

Procedure

Approximatly 0.2 ml of the sample was put into 2.5 ml of 0.05 phosphate buffer. Thereafter, 0.3 ml of freshly prepared adrenaline solution at pH of 7.8 was introduced into the reaction mixture and mixed by cuvette inversion. However, 0.3 ml of adrenaline and 2.5 ml buffer was the make up of the blank. For 3 minutes absorbance was read per 30 seconds at 480 nm.

Calculation

Super Oxide Dismutase (SOD) activity was evaluated by measuring the inhibition of auto oxidant of adrenalins as shown below.

$$\% \text{ Inhibition} = \frac{(\text{O. D ref} - \text{O. D test}) \text{ x } 100}{\text{O. D ref}}$$

Level of Reduced Glutathione

Reduced glutathione level was determined by the method of Jollow *et al.* (1974).

Principle

This was based on the formation of relative stable yellow colour when ellman's reagent is added to a sulforhydryl compound, 2-nitro-5 thiobenzoic acid. Ellman's reagent produce chromophoric product as a result of the reaction with reduced gluthathione absorbing at 412 nm. The absorbance at 412 nm is proportional to the gluthathione content. Reduced glutathione (GSH) form the bulk of non protein sulfhydryl groups.

Procedure

Approximatly One mililitre of sample was put into 4.0 % sulfo-salicyclic acid and centrifuged at 2^0C for 15 minutes at 3000 rpm. After centrifugation 4.5 ml of Ellman reagent was added to the mixture and absorbance read at 412 nm.

Calculation

$$\text{GSH concentration} = \frac{\text{Asample - Ablank}}{\text{Astandard - Ablank}} \text{X Cstandard}$$

$$\frac{\text{Asample - Ablank}}{\text{Astandard - Ablank}} \text{X Cstandard}$$

Levels of Malondialdehyde (MDA)

Malondialdehyde level was estimated by measuring thiobarbituric acid reactive substances (TBARS) by method of Ohkawa *et al.* (1979).

Principle

The principle of this test was based on the fact that malondiadehyde (MDA) reacted with thiobarbituric acid to form a red or pink colored complex which in acid solution absorbed maximally at the wavelength of 532 nm.

MDA + 2TBA ⟶ MDA: TBA adult + H_2O

Procedure

Approximately 0.1 ml of sample, 0.9 ml of distilled H_2O, and 0.5 ml of 1% TBA reagent in 0.3% NaOH were put into a test tube. At 95^0C the test tube was incubated for 40 minutes and was placed in water to cool. After cooling 0.1 ml of 20% SDS (sodium dodecyl sulphate) was introduced and absorbance read at 532 and 600nm against the blank reagent.

Calculation

$$\%\text{TBARS} = \frac{\text{A532} - \text{A600}}{0.5208 \times 0.1} \times 100$$

Chemical Constituents of *Cucurbita pepo* Seed Extract

GC-MS Analysis of the *Cucurbita pepo* Seed Extract

The GC-MS analysis was done using GC-MS (Model: QP2010 PLUS Shimadzu, Japan) comprising an AOC-20i auto-sampler and chromatograph interfaced to a mass spectrometer (GC-MS).

In silico studies of chemical profile of *Curcubita pepo* Seed

The gas chromatography-mass spectrometry (GCMS) examination of the plant produced the chemical profile. The recognized chemical entities were obtained from the PubChem repository (https://pubchem.ncbi.nlm.nih.gov/) while targeted proteins Prostate specific antigens (PDB-ID: 2ZCL) and prostatic acid phosphatase (PDB-ID: 1CVI) protein structures were extracted from the Protein Data Banks database (https://www.rcsb.org/). The proteins were prepared using the software Chimera1.14 (https://www.cgl.ucsf.edu/chimera/). Here nonstandard residues were removed, other chains of the receptors deleted, hydrogen atoms and Gasteiger charges added, and allowed to run 200 descent steps, and 10 conjugated gradient steps for refining of the proteins. The prepared proteins were saved in protein data bank file format (pdb). The chemical components of the plant downloaded were uploaded into another software PyRx as chemical table fie (SDF) where it was also prepared by minimizing all imported ligands and converting to PDBQT format alongside the proteins in preparation for the docking using Autodock Vina. The grid boxes were set at X-84.16, Y-102.70, Z-26.56 Prostate specific antigens (PDB-ID: 2ZCL), X52.95, Y-62.72, and X-25.1 prostatic acid phosphatase (PDB-ID: 1CVI) protein respectively.

Virtual high throughput screening of 15 compounds plus standard drug finasteride (PubChem CID: 57363) based on binding energy scores with targeted proteins prostate-specific antigens (PDB-ID: **2ZCL**) and prostatic acid phosphatase (PDB-ID: 1CVI) was carried out with the PyRx software in the PDBQT format. The 2D and 3D structural interactions and configurations were viewed and prepared with the Discovery Studio visualizer 2024. (https://discover.3ds.com/discovery-studio-visualizer-download).

Statistical Analysis

Our generated data was displayed as mean ± standard deviation (SD). However, the data was analyzed with Statistical Package for Social Scientists (SPSS version 21.0) using one-way ANOVA as well as GraphPad Prism 8.0.2 (263). The Post Hoc Tukey test was used to compare between the groups. Differences were considered significant at $p<0.05$.

RESULTS

Effect of whole pumpkin seed extract on PSA and ACP levels of Tp-induced Benign Prostatic hyperplasia in albino rats.

The results on the effect of whole pumpkin seed extract on PSA and ACP levels on Tp-induced benign prostatic hyperplasia in albino rats revealed a significant ($p<0.05$) increase in PSA and ACP concentrations of the BPH-induced group compared to control group as shown in Figures 1 and 2, respectively. However, on treatment with standard drug and seed extract the effect was reversed to levels comparable to the control when compared with the untreated group.

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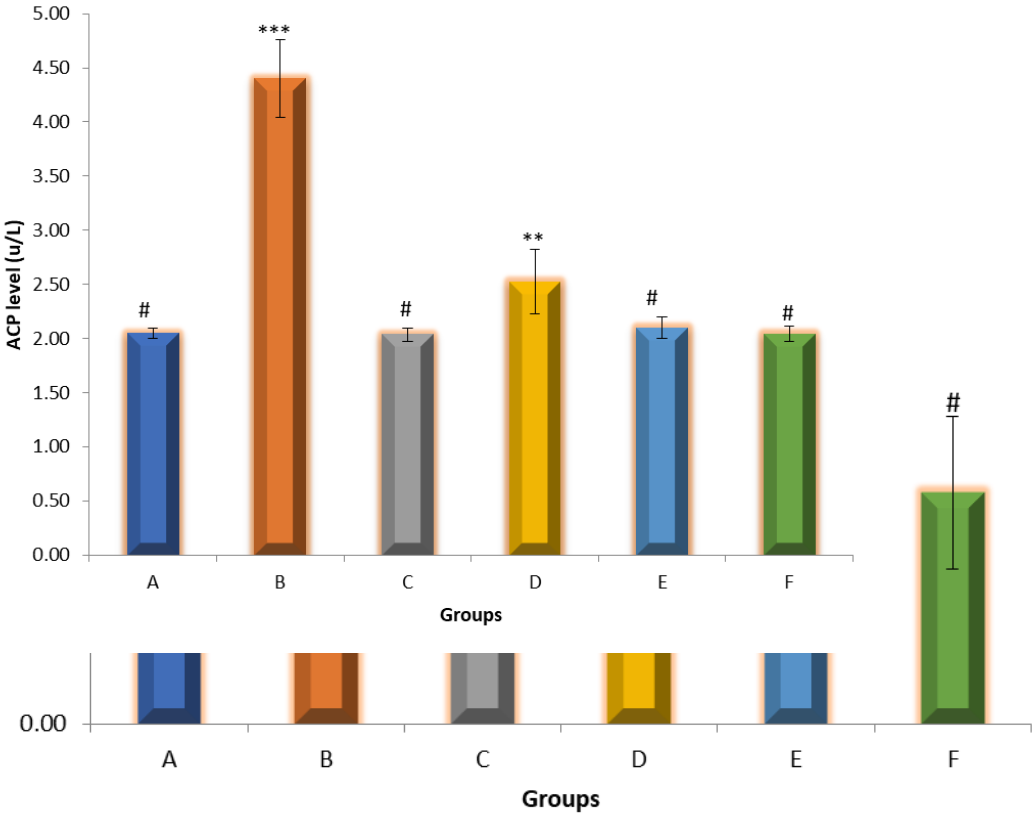


Fig 1: Effect of CPSE on PSA level in Tp-induced benign prostatic hyperplasia in albino rats. Data are unveiled as mean ± Standard Deviation (n=10). Mean values with different symbol(s) are significantly different at p<0.05.

Fig 2: Effect of CPSE on ACP level in Tp-induced benign prostatic hyperplasia in albino rats. Data are unveiled as mean ± Standard Deviation (n=10). Symbols with different mean values are significantly different at p<0.05.

Effect of CPSE on Hormonal Markers of Tp-induced Benign Prostatic hyperplasia in albino rats.

The protective effect of CPSE on some hormones of Tp-induced Benign Prostatic hyperplasia in albino rats showed a significant (P<0.05) increase in the levels of dihydrotestosterone, estrogen, and androgen in BPH induced group compared to the normal control group as shown in Figure 3, 4 and 5 respectively. However, treatment with both standard drug and CPSE at the stated doses revealed a significant reversal in the trends of these hormones to a level comparable to the normal control when compared to the untreated group.

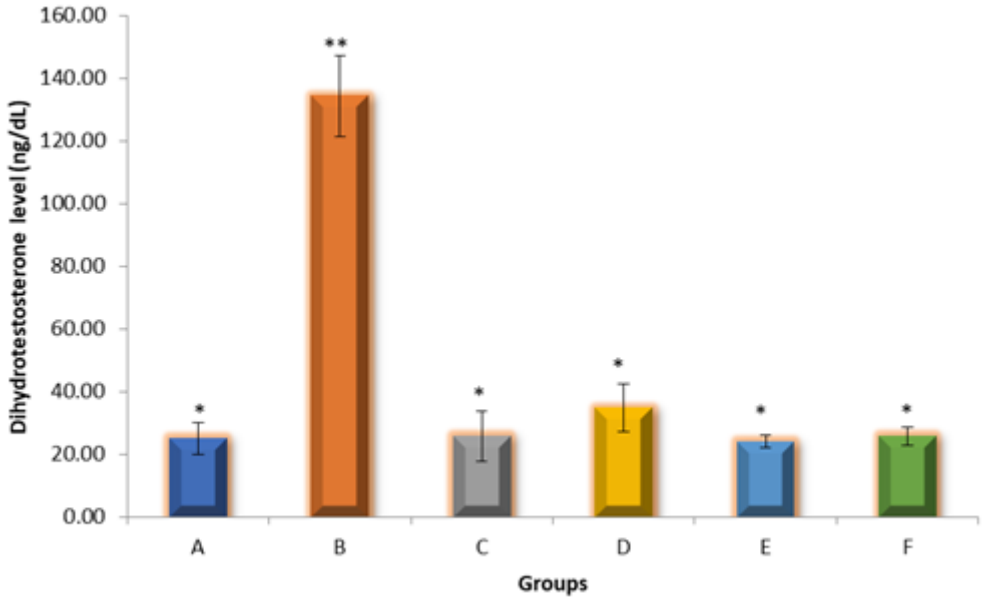


Fig 3: Effect of CPSE on dihydrotestosterone level of Tp-induced benign prostatic hyperplasia in albino rats. Data are shown as mean ± Standard Deviation (n=10). Mean values with different symbols are significantly different at p<0.05.

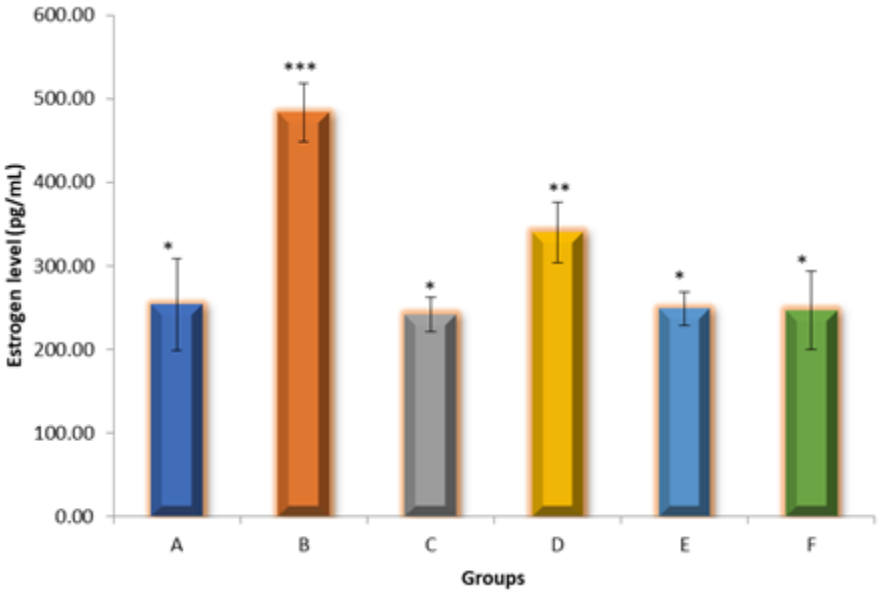


Fig 4: Effect of CPSE on estrogen level of Tp-induced benign prostatic hyperplasia in albino rats. Data are shown as mean ± Standard Deviation (n=10). Mean values with different symbols are significantly different at p<0.05.

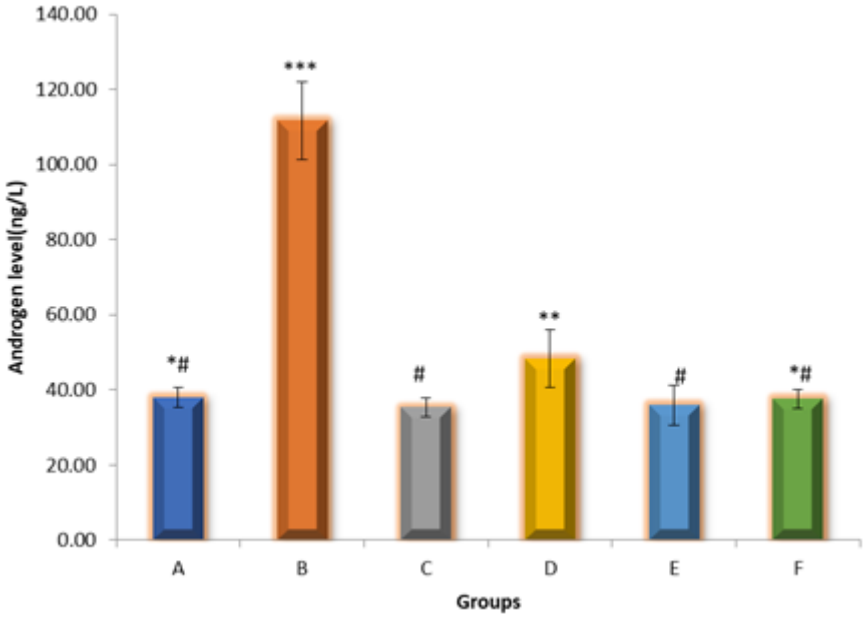


Fig 5: Effect of CPSE on androgen level of Tp-induced benign prostatic hyperplasia in albino rats. Data are shown as mean ± Standard Deviation (n=10). Mean values with different symbols are significantly different at p<0.05.

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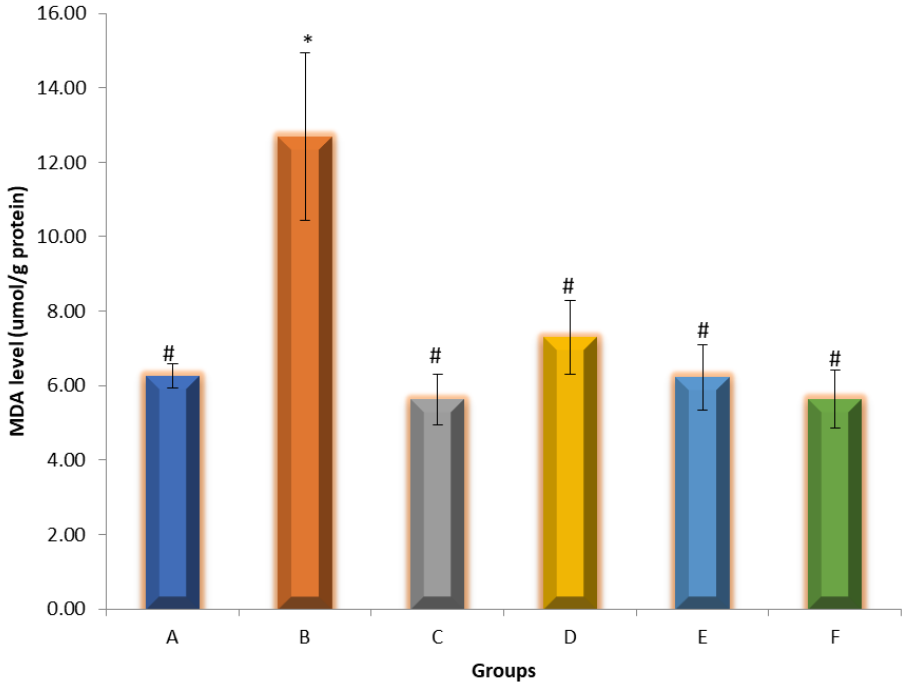
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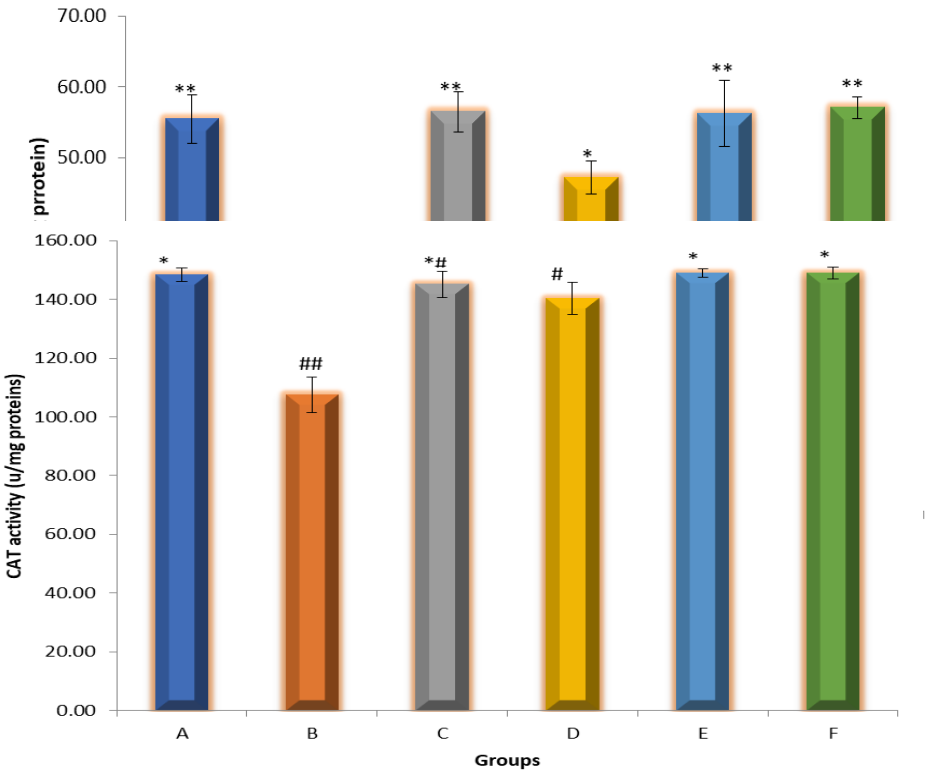
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Fig 7: Effect of CPSE on SOD activity of Tp-induced benign prostatic hyperplasia in albino rats. Data are shown as mean ± Standard Deviation (n=10). Mean values with different symbol(s) are significantly different at p<0.05.

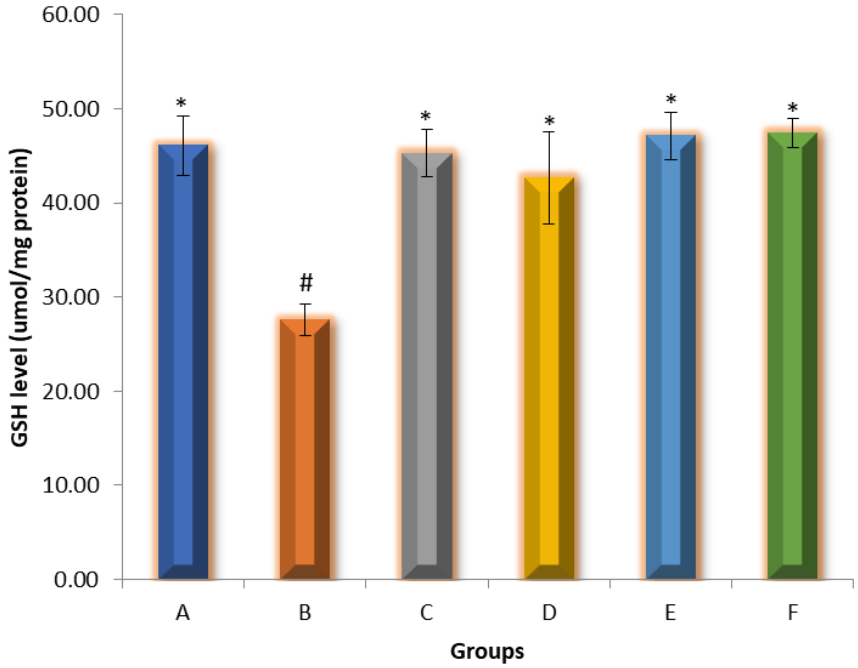


Fig 8: Effect of CPSE on GSH level of Tp-induced benign prostatic hyperplasia in albino rats. Data are shown as mean ± Standard Deviation (n=10). Mean values with different symbols are significantly different at p<0.05.

Fig 9: Effect of CPSE on MDA level of Tp-induced benign prostatic hyperplasia in albino rats. Data are unveiled as mean ± Standard Deviation (n=10). Symbols with different mean values are significantly different at p<0.05.

GC-MS profile of *Cucurbita pepo* Seed

The results of GC-MS analysis of the *Cucurbita pepo* seed showed seventeen peaks from the chromatogram of the seed. These peaks indicate the presence of fifteen compounds (1-15) in the oil (Table 1). The retention time, corrected area, % total area, and bioactive function of the compounds are shown in Table 1. These compounds comprise mainly fatty acids, alcohols, and esters. The compositions of the seed extract comprise; n-Propyl Nonyl ether, Dimethoxymethyl-silane, Hydrazinecarbothioamide, Benzyloxymethylimine, Heptadecanoic acid, methyl ester, Hexadecanoic acid, methyl ester, Methyl 10-methyl-hexadecanoate, Hexadecanoic acid, Pentadecanoic acid, 14-methyl-ethyl ester, 9,12-Octadecadienoic acid (Z, Z)-Oleic acid, 9,12-Octadecadienoic acid, Methyl ester-cis-13-Octadecenoic acid, 2-hydroxy-Cyclopentadecanone, Methyl ester-11,14-Octadecadienoic acid, Methyl ester-9-Octadecenoic acid, Methyl ester-9,12-Octadecadienoic acid and 6-Octadecenoic acid as the major chemical constituents found in the oil.

Table 1: GC-MS profile of chemical constituent of *Cucurbita pepo* Seed Extract collected from Ishieke Igbudu village in Igbudu Community in Ikwo Local Government Area of Ebonyi State, Nigeria.

Peak	Compound	Retention Time	Corrected Area	% Total Area	Bioactive Function
1	n-Propyl Nonyl ether	7.488	24632	1.96	Antibacterial
2	Dimethoxymethyl-silane	7.938	171281	43.90	**Antibacterial, inhibition of Apoptosis**
3	Hydrazinecarbothioamide	7.957	169760	4.95	Antioxidant, Antimicrobial
4	Benzyloxymethylimine	7.984	167502	26.25	Inhibit the Production of Uric Acid.
5	Heptadecanoic acid, methyl ester	30.967	10652	0.60	Skin cancer protein
6	Hexadecanoic acid, methyl ester	31.041	23580	1.09	Anti-inflammatory, Antioxidant, hypocholesterolemic nematicide, Inhibition of apoptosis, modulation of enzymes and receptors.
7	Methyl 10-methyl-hexadecanoate	31.062	26246	0.51	Antioxidant, Cancer-preventive, Hypercholesterolemic,
8	Hexadecanoic acid	31.080	22755	0.90	Antioxidant, Inhibition of Apoptosis, Modulation of Enzymes and Receptors.
9	Pentadecanoic acid, 14-methyl-thyl ester	31.134	18751	1.02	Antimicrobial, Membrane Stabilizing Effect
10	9,12-Octadecadienoic acid (Z,Z)-Oleic acid	36.329	8210	0.25	Anti-inflammatory, hypocholesterolemic, antioxidant, neuroprotective
11	9,12-Octadecadienoic acid	36.430	24730	1.52	Antibacterial, Anti-inflammatory, Hypocholesterolemic, Cancer preventive, Hepatoprotective, Antioxidant, Neuroprotective
12	Methyl ester-cis-13-Octadecenoic acid	36.490	38316	1.90	Anti-inflammatory, Antioxidant, Neuroprotective
13	2-hydroxy-Cyclopentadecanone	36.573	45541	3.20	Radical Scavengers, Anti-inflammatory and Anti-cancer
14	Methyl ester-11,14-Octadecadienoic acid	36.616	47097	2.83	Anti-inflammatory, Antiandrogenic, Cancer Preventive, Hypocholesterolem-ic, Neuroprotective
15	Methyl ester-9-Octadecenoic acid	36.674	48054	2.54	Antibacterial

Molecular Docking Result

From the docking results, the following compounds; 9,12-Octadecadienoic acid, and 2-Hydroxycyclopentadecanone exhibited good binding affinities against PSA closer to the standard drug finasteride (-7.9, and -8.2, compared to -7.9 Kcal/mol), and ACP, -5.5 Kcal/mol, and -7.0 Kcal/mol compared to the standard drug, finasteride -8.2. However, the study of the 2D and 3D diagrams exposes the bonding interactions of the top posed bioactive compounds in Figure, 1, 2, 3 (A&B). Molecular docking analysis exposed high interactions by clear covalent interactions, hydrogen bonding, with mutiple *van der Waal* forces between the selected compounds; 9,12-Octadecadienoic acid, and 2-Hydroxycyclopentadecanone akin

to the noticed stable bonds among finasteride (standard drug) and prostatic acid phosphatase (target protein).

Table 2: Docking Scores

S/N	Name of compound	Molecular Formula	PubChem ID	Protein target	
				PSA(PDB-ID: 2ZCL)	ACP (PDB-ID: 1CVI)
1	9,12-Octadecadienoic acid	C18H32O2	3931	-5.4	-5.5
2	2-Hydroxycyclopentadecanone	C15H28O2	543400	-7.8	-7.0
6	Finasteride	C23H36N2O2	57363	-7.9	-8.2

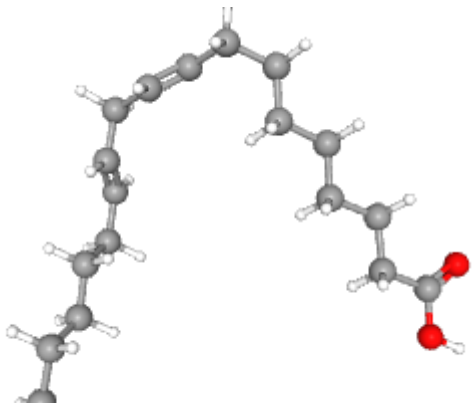


Plate 2: 39319,12-Octadecadienoic acid

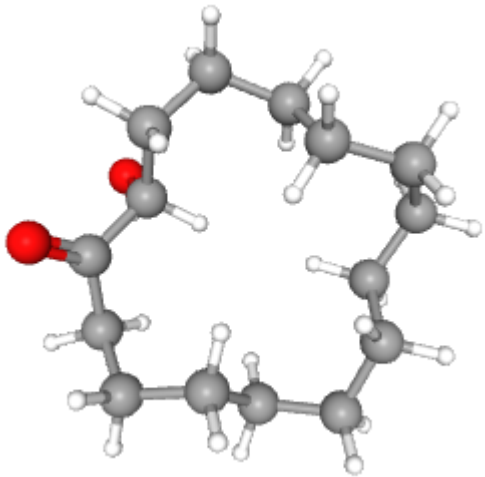


Plate 3: 5434002-Hydroxycyclopentadecanone

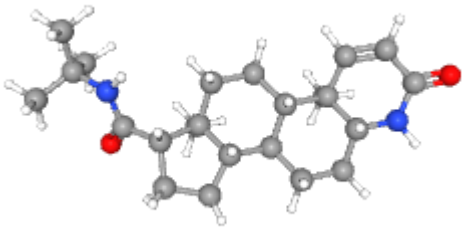


Plate 4: 57363 Finasteride

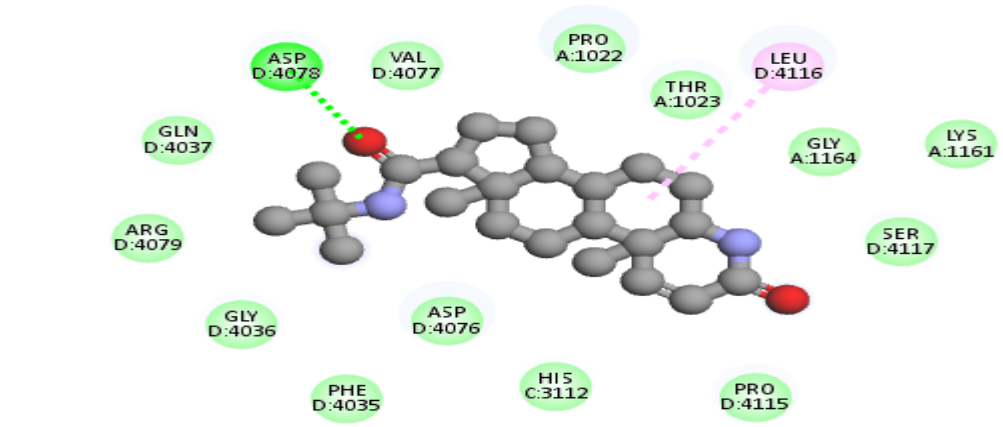


Plate 5: Standard 57363 2D structure interaction with ACP

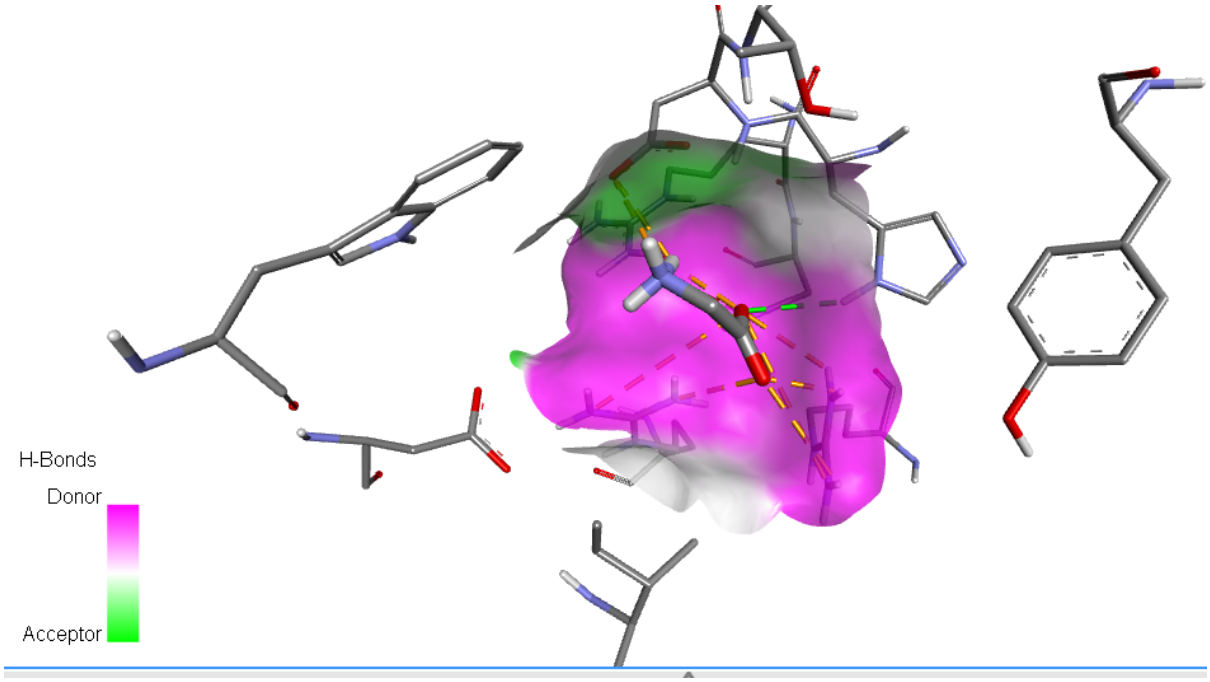


Plate 6: Standard 57363 3D structure interaction with ACP

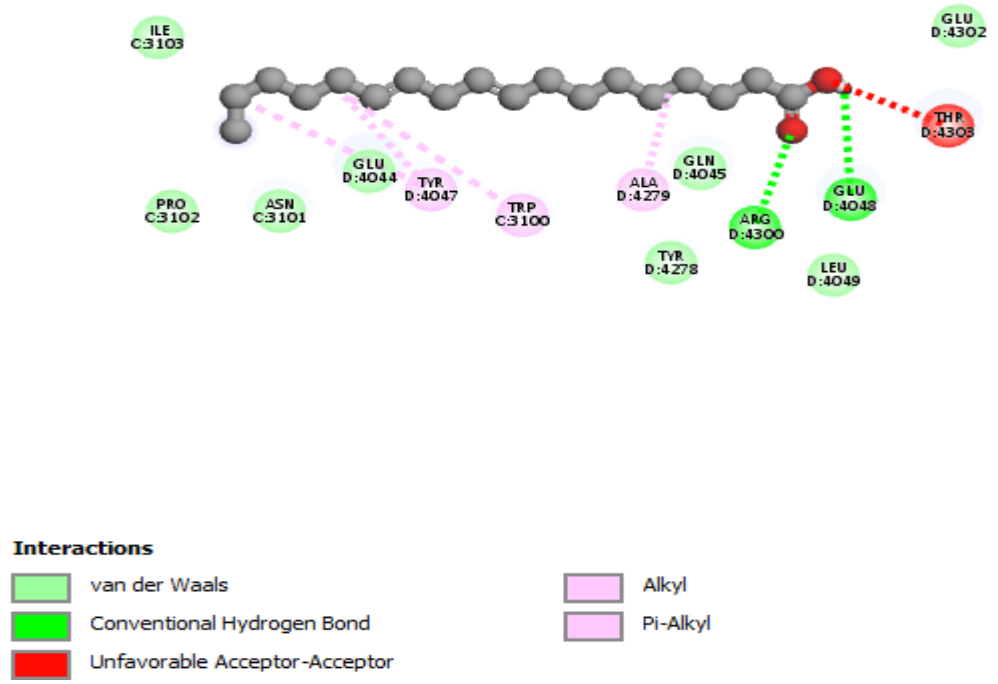


Plate 7: 3931 2D structure interaction with ACP

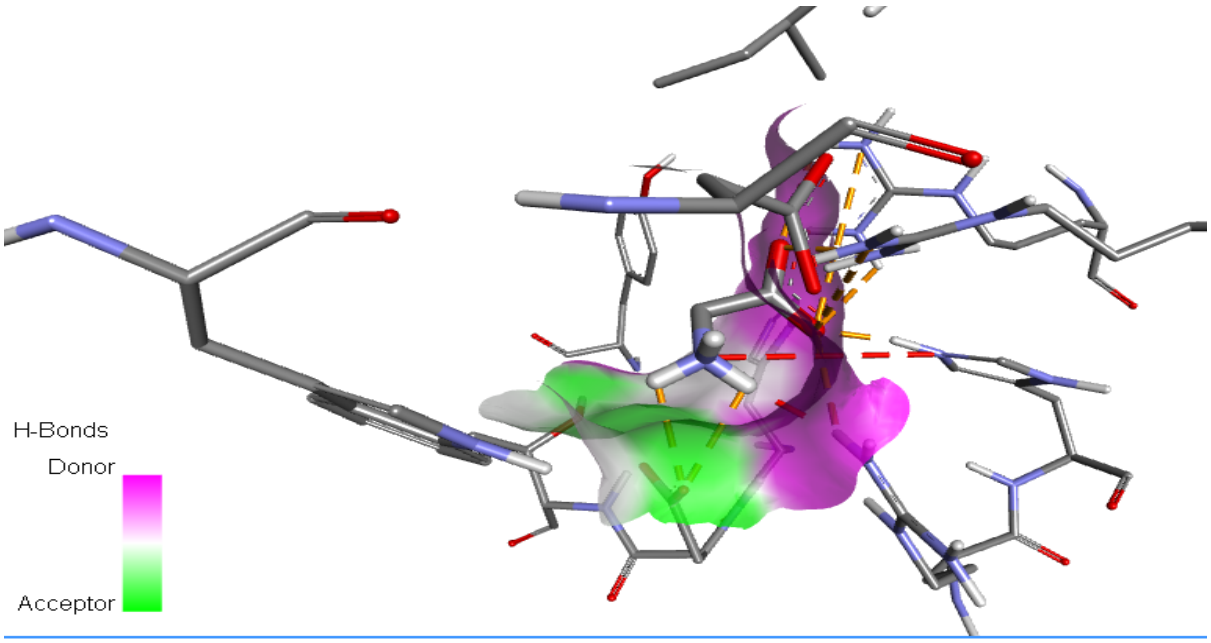


Plate 8: 3931 3D structure interaction with ACP

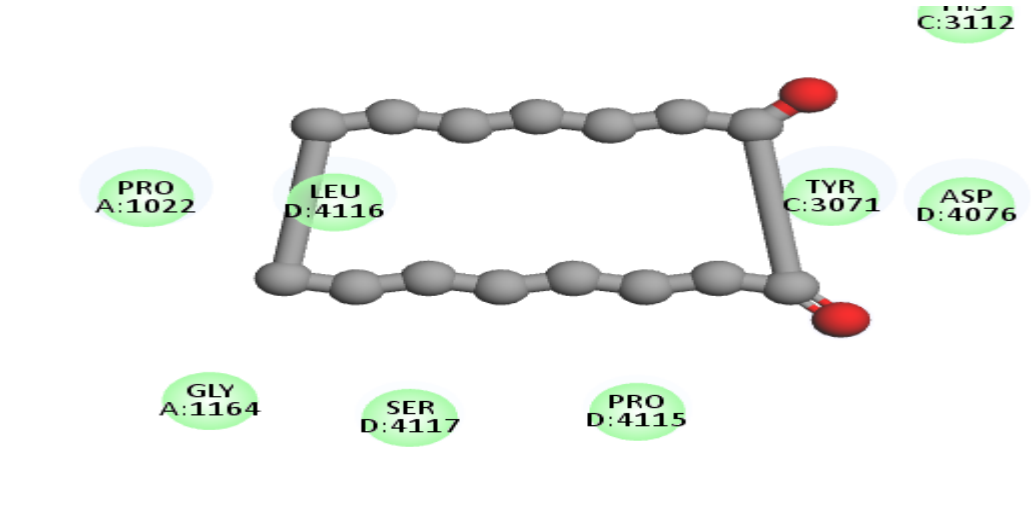


Plate 8: 543400 2D interaction versus ACP

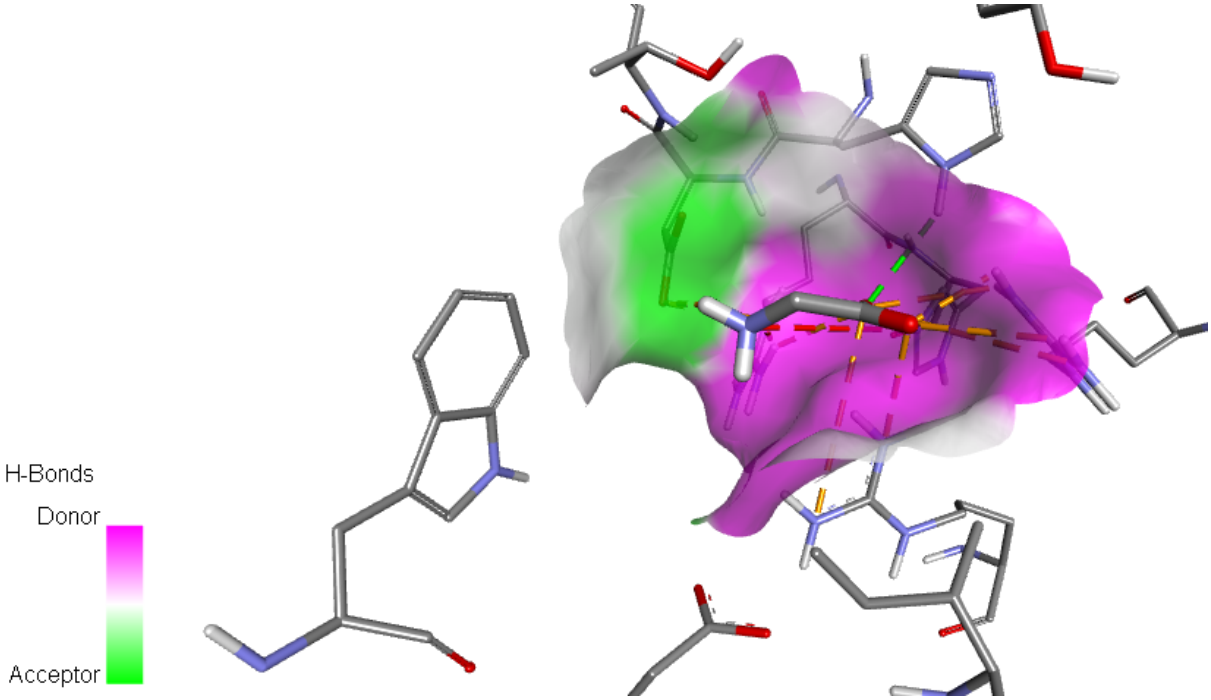


Plate 9: 543400 3D interaction versus ACP

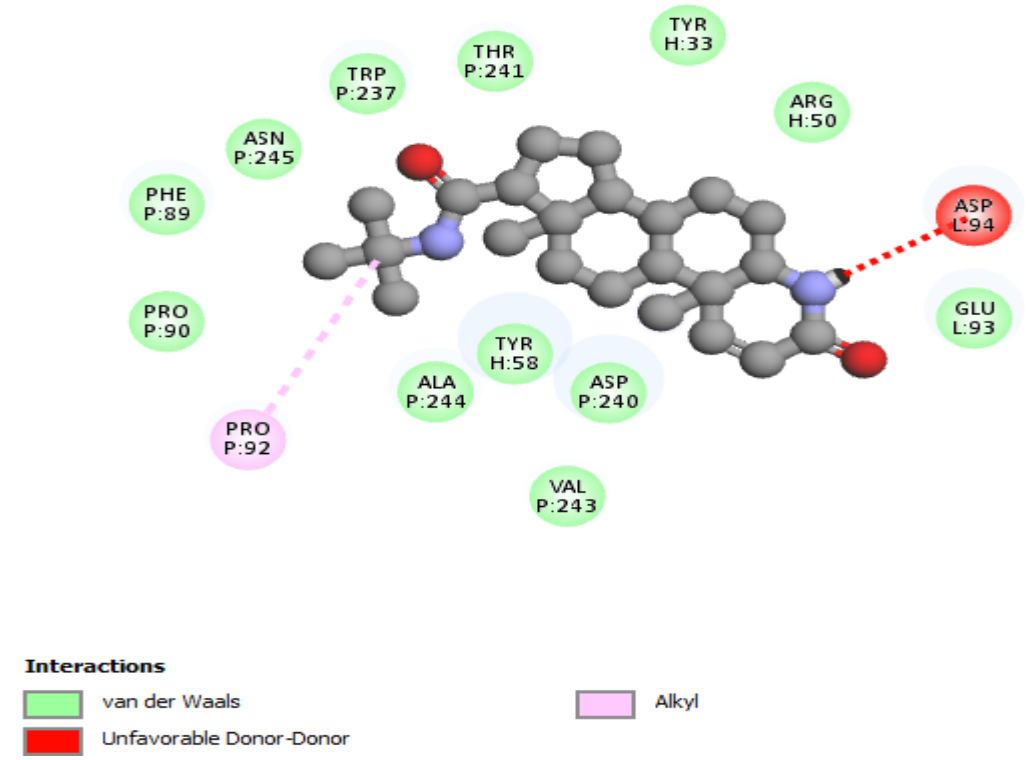


Plate 10: Standard 57363 2D versus PSA

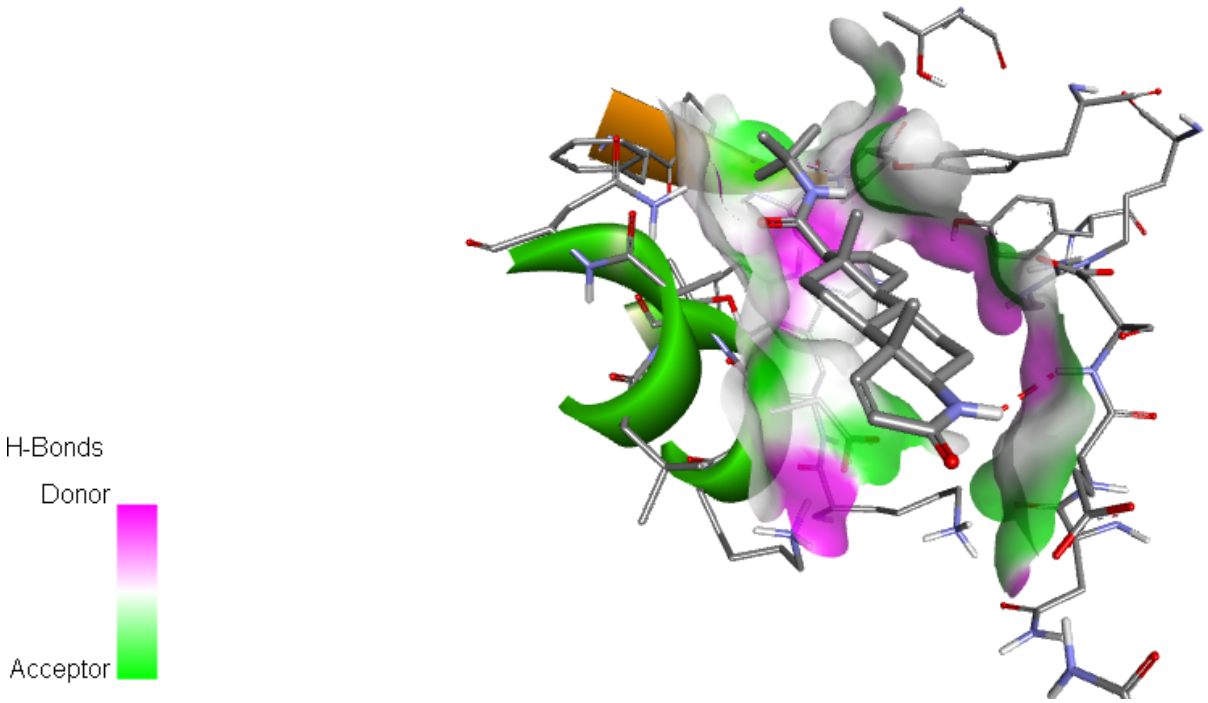


Plate 11: Standard 57363 3D versus PSA

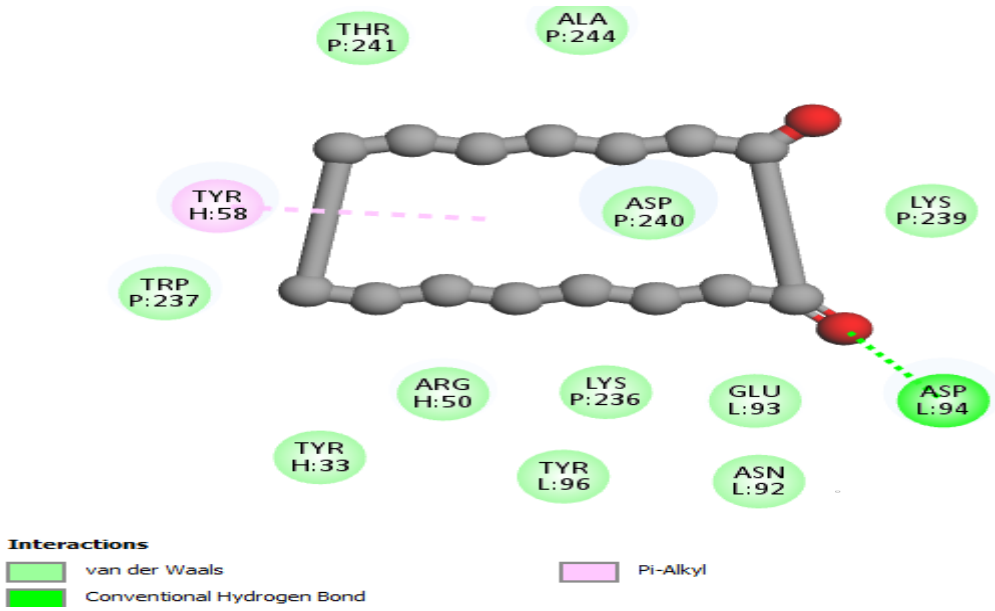


Plate 12: 543400 2D versus PSA

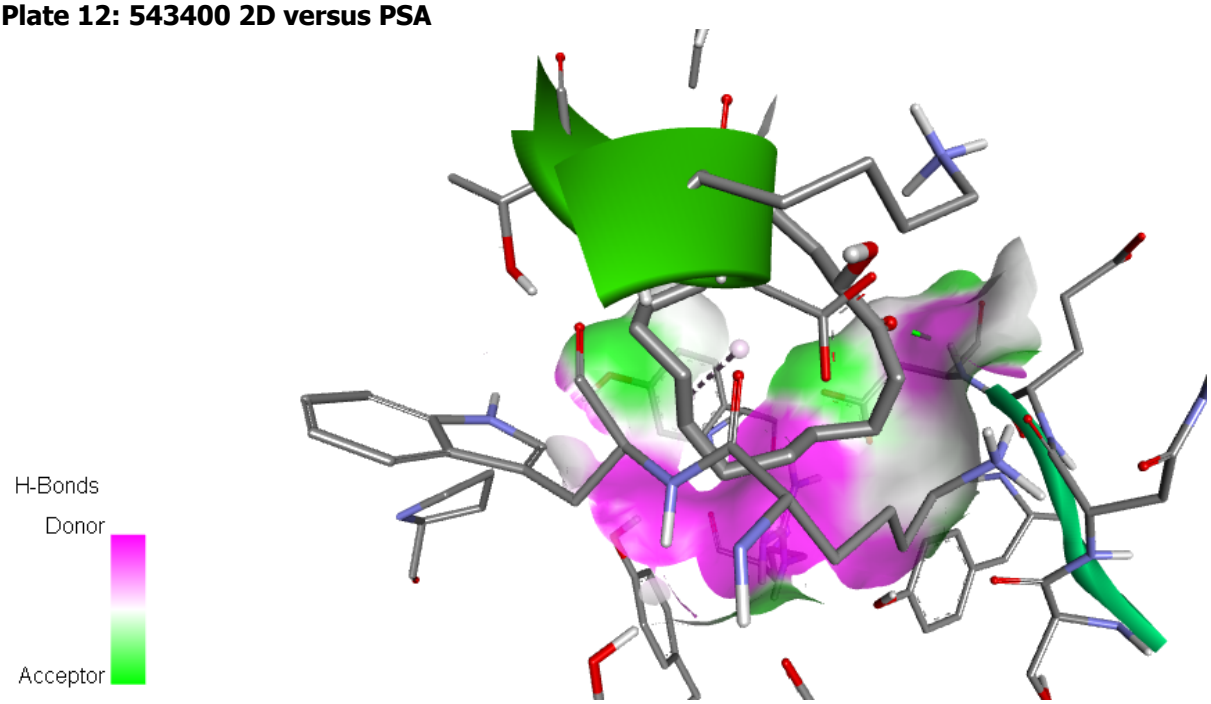


Plate 13: 543400 3D versus PSA

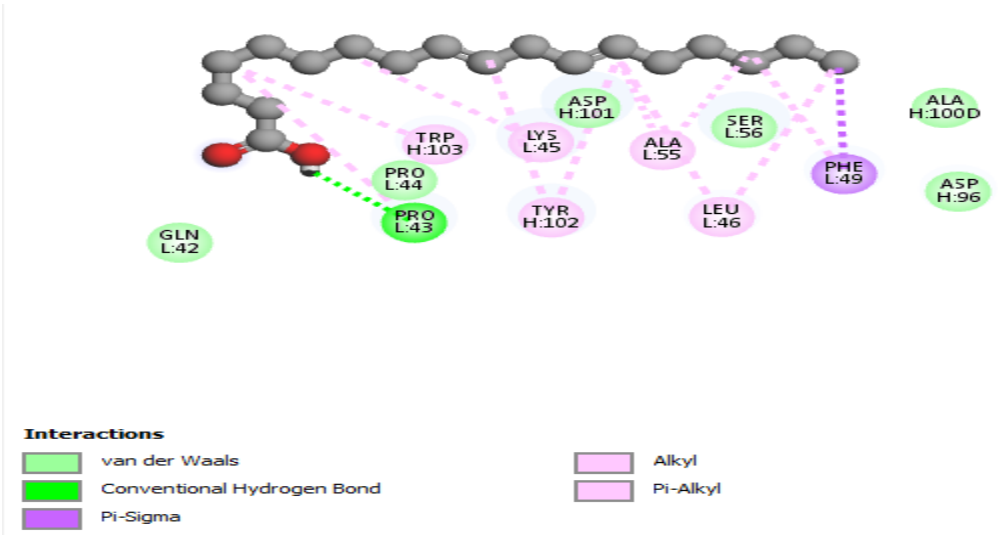


Plate 14: 3931 vers PSA 2D interaction

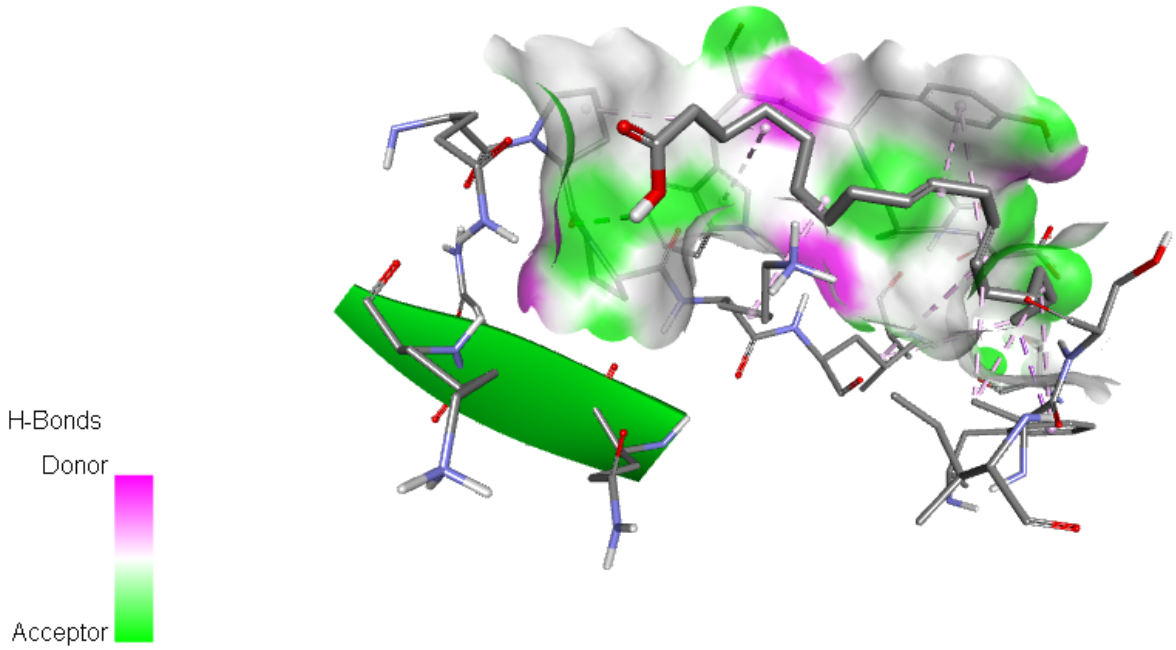


Plate 15: 3931 vers PSA 3D interaction

Table 3: Absorption, distribution, and metabolism profile of top two compound and standard drug finasteride

	CPD 1	CPD 2	Standard
Drug likeness			

LogS	-5.23	-3.124	-4.693
LogD	3.58	3.359	1.734
LogP	6.652	4.304	3.824
Pgp-inh	0	0.109	0.862
		Absorption/metabolism profile	
Pgp-sub	0.002	0.009	0.996
HIA	0.01	0.001	0.004
F(20%)	0.009	0.999	0.002
F(30%)	0.549	0.993	0.005
Caco-2	-4.733	-4.761	-6.497
MDCK	1.93E-05	2.17E-05	4.71E-05
BBB	0.196	0.955	0.005
PPB	98.39%	94.88%	97.76%

CPD 1=9,12-Octadecadienoic acid; **CPD 2=**2-Hydroxycyclopentadecanone; Standard=Finasteride

Table 3 presents a comparison of the absorption, distribution, and metabolism, characteristics of two substances (9,12-Octadecadienoic acid and 2-Hydroxycyclopentadecanone, with the reference medication Finasteride. Important factors considered were drug-likeness, and absorption/metabolism characteristics. Both compounds have good solubility, distribution coefficient, and partition coefficient compared to finasteride. Additionally, they demonstrated little inhibition of P-glycoprotein, which impacts the efflux and absorption of drugs compared to finasteride. Like finasteride, they have low level of absorption in the human intestines, but has a higher degree of bioavailability and permeability when taken orally. Distribution is quantified by the assessment of BBB (Blood-Brain Barrier Penetration), plasma protein binding, and volume of distribution. CPD 2 has the highest distribution across the BBB compared to CPD 1 and Finasteride.

Discussion

Prostate-specific antigen (PSA) and acid phosphatase (ACP) are critical biomarkers for prostate function and inflammation, with elevated levels often indicating prostate enlargement, inflammation, or malignancy. In this study, the results of the effect of *Cucurbita pepo* seed extract on prostate-specific antigen (PSA) and acid phosphatase (ACP) levels in testosterone propionate (Tp)-induced benign prostatic hyperplasia (BPH) in albino rats demonstrate a significant protective effect. The significant elevation in PSA and ACP levels ($P<0.05$) in BPH-induced group only compared to the control group indicates the successful induction of BPH and the presence of prostate inflammation or abnormal growth. However, treatment with *Cucurbita pepo* seed extract, as well as the standard drug, reversed these elevated PSA and ACP levels, bringing them back to values comparable to the control group. This normalization suggests that the seed extract effectively counteracted the pathological changes induced by testosterone propionate, likely due to its anti-inflammatory, antioxidant, and hormone-regulating properties. This agrees with previous reports on the therapeutic effects of *Cucurbita pepo* seed oil reported by Almohaimeed *et al.* (2021) and Neamah *et al.* (2023). In this study, the ability of *Cucurbita pepo* to restore PSA and ACP levels implies that it may inhibit abnormal prostate growth and reduce inflammation, thereby mitigating the progression of BPH. Findings from this study highlight the seed extract's potential as a natural therapeutic agent in managing BPH and reducing related biomarkers of prostate dysfunction.

Hormonal dysregulations are key to BPH manifestations. In this study, the hormonal assay on the protective effects of *Cucurbita pepo* seed extract on testosterone propionate (Tp)-induced benign prostatic hyperplasia (BPH) in albino rats demonstrate significant disruptions in hormone levels. In the BPH-induced group, there was a notable ($P<0.05$) increase in the levels of dihydrotestosterone (DHT), estrogen, and androgen compared to the normal control group. Elevated DHT is a well-known driver of prostate enlargement, as it stimulates excessive growth of prostate

cells, which contributes to the development of BPH (Carson and Rittmaster, 2003). Similarly, an increase in estrogen levels in males, especially relative to androgen levels, can further promote abnormal prostate growth and contribute to the progression of BPH. The elevated androgen levels reflect hormonal imbalances that exacerbate the condition. However, treatment with both the standard drug and *Cucurbita pepo* seed extract resulted in a significant reversal of these hormonal imbalances. These observations corroborate the previous reports on the potentials of *C. esculata* and *Xylopia aethiopica* against BPH (Tusubira *et al.,* 2023[a]; Ibiam *et al.,* 2023). DHT, estrogen, and androgen levels were reduced to levels comparable to the normal control group, suggesting a restoration of hormonal homeostasis. The normalization of DHT levels is particularly important for reducing prostate enlargement, as DHT is the primary hormone responsible for prostatic hyperplasia. The reduction in estrogen and androgen levels further indicates that *Cucurbita pepo* seed extract can help regulate hormonal fluctuations, thereby mitigating the factors driving BPH progression. These findings suggest that *Cucurbita pepo* seed extract has a regulatory effect on hormone levels, likely by inhibiting the conversion of testosterone to DHT or modulating the body's response to these hormones. Therefore, this study proposes that the hormonal regulation is a key mechanism by which the *Cucurbita pepo* seed extract exerts its protective effects against BPH, helping to reduce prostate size and alleviate related symptoms.

Oxidative stress is a pathophysiological condition exacerbating benign prostatic hyperplasia (BPH). Antioxidants have capabilities in mitigating oxidative damage. In the BPH-induced group, there was a significant ($P<0.05$) decrease in the activities of key antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD), as well as a reduction in the levels of glutathione (GSH), an important intracellular antioxidant. These decreases indicate impaired antioxidant defenses, leading to heightened oxidative stress, which is a known contributor to BPH progression. Concurrently, there was a significant ($P<0.05$) increase in malondialdehyde (MDA) levels, a marker of lipid peroxidation, suggesting increased oxidative damage to cellular lipids in the prostate (Cordiano *et al.,* 2023). However, treatment with both the standard drug and pumpkin seed extract reversed these effects, significantly restoring CAT, SOD, and GSH levels to values comparable to those in the normal control group. Additionally, MDA levels were reduced, indicating decreased lipid peroxidation and oxidative stress. Similar to existing pieces of literature, the reversal of these oxidative stress markers implies that pumpkin seed extract, rich in antioxidants, helped restore the balance between pro-oxidant and antioxidant systems in the prostate (Oh *et al.,* 2016). Our findings are similar to Ibiam *et al.,* (2023) on *Xylopia aethiopica* against BPH and *C. esculata* against BPH (Tusubira *et al.,* 2023). In this study, the observed protective effect could be attributed to the bioactive compounds in the seed extract, such as tocopherols, flavonoids, and fatty acids, which enhance antioxidant enzyme activities and reduce oxidative damage. Therefore, the findings suggest that pumpkin seed extract has potent antioxidant properties that may play a crucial role in preventing or alleviating oxidative stress-related prostate damage in BPH.

In the present study, the GC-MS analysis of *Cucurbita pepo* seed extract identified fifteen bioactive compounds, predominantly consisting of fatty acids, alcohols, and esters, which suggest significant therapeutic potential. This obsrvation agrees with recent report from Mwangi *et al.* (2024) on Kenyan cultivated *Cucurbita pepo*. Notable among these are Heptadecanoic acid, Hexadecanoic acid, and various derivatives of 9,12-Octadecadienoic acid, which are known for their anti-inflammatory and antioxidant properties (Rizvi *et al.,* 2023). These compounds can help reduce oxidative stress and inflammation, key contributors to benign prostatic hyperplasia (BPH). Additionally, the presence of methyl esters, such as Methyl 10-methyl-hexadecanoate and Methyl ester-9-Octadecenoic acid, enhances lipid solubility, aiding in the absorption and effectiveness of these bioactive compounds (Mazumder *et al.,* 2020). Alcohols and ethers like n-Propyl Nonyl ether may contribute antimicrobial properties, potentially preventing secondary infections and promoting healing in the background of prostate enlargement (Vaou *et al.,* 2021). In this study, these findings suggest that the overall composition of *Cucurbita pepo* seed

extract, rich in beneficial fatty acids and esters, positions it as a promising candidate for mitigating testosterone propionate-induced BPH through mechanisms involving oxidative stress reduction, lipid metabolism regulation, and anti-inflammatory action.

The molecular docking results of *Cucurbita pepo* seed extract compounds, specifically 9,12-Octadecadienoic acid and 2-Hydroxycyclopentadecanone, reveal promising interactions with key biomarkers of benign prostatic hyperplasia (BPH), namely prostate-specific antigen (PSA) and acid phosphatase (ACP). These compounds exhibited strong binding affinities against PSA, with 9,12-Octadecadienoic acid and 2-Hydroxycyclopentadecanone showing binding scores of -7.9 and -8.2 Kcal/mol, respectively. Findings from this investigation align with reports from Tusubira *et al.* (2023[b]) on In-silico elucidation of the molecular mechanism of *C. esculenta* compounds against BPH. Similar to the authors, these values are comparable to the standard drug, finasteride, which also exhibited a binding score of -7.9 Kcal/mol. In this study, the binding affinities suggest that these compounds could potentially inhibit PSA activity in a manner similar to finasteride, which is known to reduce prostate growth by blocking the conversion of testosterone to dihydrotestosterone (DHT), a key factor in BPH progression. In addition, the compounds also showed notable binding affinities against ACP, a marker associated with prostate health. 2-Hydroxycyclopentadecanone exhibited a binding score of -7.0 Kcal/mol, while 9,12-Octadecadienoic acid showed a slightly lower binding affinity of -5.5 Kcal/mol, compared to finasteride's score of -8.2 Kcal/mol. Although these compounds displayed lower affinities than finasteride, their interaction with ACP suggests potential for reducing ACP activity, which may contribute to lowering inflammation and prostatic growth. Therefore, these docking results indicate that 9,12-Octadecadienoic acid and 2-Hydroxycyclopentadecanone possess significant potential as natural inhibitors of PSA and ACP, comparable to the standard drug finasteride. Their strong binding affinities suggest strong therapeutic benefits in managing BPH by targeting the enzymes that regulate prostate function and growth. These findings support further exploration of *Cucurbita pepo* seed extract for BPH management.

Furthermore, the analysis of the 2D and 3D diagrams depicting the bonding interactions of the top bioactive compounds, 9,12-Octadecadienoic acid and 2-Hydroxycyclopentadecanone, reveals key molecular interactions that contribute to their strong binding affinity to prostatic ACP, a critical marker in BPH. Molecular docking analysis showed that these compounds establish multiple stable interactions with the target protein, similar to those observed between finasteride, the standard drug for BPH treatment, and ACP. In this study, both 9,12-Octadecadienoic acid and 2-Hydroxycyclopentadecanone formed strong hydrogen bonds, which are essential for stabilizing the compound within the binding pocket of the target protein. Hydrogen bonding enhances the specificity and stability of drug-target interactions, making these compounds effective at binding ACP. Additionally, the presence of covalent interactions, which involve the sharing of electrons between the compound and the protein, further strengthens the attachment, contributing to long-lasting effects in inhibiting ACP activity. Moreover, van der Waals forces, which are weaker interactions that occur due to the proximity of atoms, also played a significant role in the binding of these bioactive compounds to ACP. These non-covalent interactions help stabilize the binding conformation, ensuring that the compounds fit well into the protein's active site. The similarity between the interactions observed with the compounds and those of finasteride suggests that 9,12-Octadecadienoic acid and 2-Hydroxycyclopentadecanone could mimic the standard drug's mechanism of action in inhibiting ACP activity.

Importantly, the comparison of the pharmacokinetic characteristics of 9,12-Octadecadienoic acid (CPD 1) and 2-Hydroxycyclopentadecanone (CPD 2) with finasteride, as presented in Table 2, reveals important insights into their potential as therapeutic agents for benign prostatic hyperplasia (BPH). In this study, both bioactive compounds demonstrate favorable drug-likeness, with good solubility, distribution coefficient (log D), and partition coefficient (log P), indicating that they have the potential for efficient absorption and distribution in the body. These properties compare well with

finasteride, suggesting that *Cucurbita pepo* seed compounds could serve as effective alternatives or supplements to conventional BPH treatments. A key distinction is their interaction with P-glycoprotein, a transporter protein that impacts the absorption and efflux of drugs (Aja *et al.,* 2022; Agu *et al.,* 2023). Both CPD 1 and CPD 2 showed minimal inhibition of P-glycoprotein compared to finasteride, which may enhance their absorption and retention within cells, potentially improving their overall bioavailability. This reduced P-glycoprotein inhibition is advantageous as it reduces the likelihood of the compounds being expelled from cells too quickly, allowing for sustained therapeutic effects. Interestingly, despite having a low level of absorption in the human intestines, comparable to finasteride, CPD 1 and CPD 2 exhibited higher oral bioavailability and permeability. This suggests that although they may be absorbed at a slower rate in the intestines, once in the bloodstream, they maintain a higher concentration, which could translate into more potent and lasting effects for BPH treatment. In terms of distribution, CPD 2 demonstrated the highest level of penetration across the blood-brain barrier (BBB) compared to CPD 1 and finasteride (Tusubira *et al.,* 2023[b]; Agu *et al.,* 2024). While BBB penetration is not directly related to BPH, it indicates that CPD 2 could have additional central nervous system effects or potential off-target benefits or side effects, depending on the clinical context. Additionally, CPD 2's volume of distribution suggests that it can spread more widely throughout the body compared to CPD 1 and finasteride, potentially making it a more potent systemic treatment. Lastly, both compounds demonstrated effective plasma protein binding and volume of distribution, which are critical for maintaining therapeutic levels in the bloodstream. These characteristics make CPD 1 and CPD 2 promising candidates for further development as natural alternatives or supplements to conventional BPH treatments in regions where access to synthetic medications like finasteride may be limited or too costly.

Conclusion

The current study provides compelling evidence for the protective effects of *Cucurbita pepo* seed extract in the management of testosterone propionate-induced benign prostatic hyperplasia (BPH) in albino rats. The findings demonstrate that the seed extract significantly ameliorated the pathological alterations in biochemical, oxidative stress and hormonal, parameters associated with BPH. Notably, the seed extract showed strong regulatory effects on prostate-specific antigen (PSA) and acid phosphatase (ACP) levels, which are crucial markers of prostate health. Moreover, the extract restored antioxidant defense mechanisms by increasing the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), while reducing lipid peroxidation (MDA), thereby mitigating oxidative stress—a key factor in BPH pathogenesis. The hormonal modulation observed, particularly in dihydrotestosterone (DHT), estrogen, and androgen levels, further highlights the extract's ability to correct hormonal imbalances that drive prostate enlargement. Molecular docking analysis revealed that bioactive compounds like 9,12-Octadecadienoic acid and 2-Hydroxycyclopentadecanone exhibited strong binding affinities to PSA and ACP, comparable to the standard drug finasteride, which underscores their therapeutic potential. These compounds also showed favorable pharmacokinetic profiles, including good solubility, bioavailability, and blood-brain barrier (BBB) penetration, further supporting their viability as natural agents for BPH management. These findings suggest that CPSE has the potential to modulate BPH-related biochemical alterations in rats, warranting further investigation in preclinical and clinical models.

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