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Ameliorative Effects of Supplements of Papaya Seed, Watermelon Seed, and Clove buds on Testosterone-DMBA Induced Prostate Cancer in Wistar Rats: Oxidative Stress, Inflammation, and Histological Analysis

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Abstract

Background: Prostate cancer is the second most common cancer among men worldwide with escalating incidence and mortality rates. This study investigated the protective effects of papaya seed, watermelon seed, and clove bud on testosterone-DMBA-induced prostate cancer in male Wistar rats.

Methods: Seventy male Wistar rats were randomly divided into seven groups: normal control, cancer control, combination supplementation (papaya seed, watermelon seed and clove bud, individual supplementations of papaya seed, watermelon seed, clove bud, and flutamide treatment. Prostate cancer was induced by single dose intraperitoneal injection of DMBA 65mg/kg) and subcutaneous testosterone (3mg/kg) continued for 12 weeks. The intervention groups received their respective supplementations 2 weeks before induction and continued after the induction. At the end of the intervention period, oxidative stress markers, inflammatory markers, and histopathological changes were assessed.

Results: Watermelon seed supplementation provided optimal balanced protection, significantly preserving catalase activity (6.87±0.63 vs. 4.67±1.17 µ/ml) and reducing CRP levels (2.70±0.25 vs. 12.47±5.17 mg/L). Clove bud supplementation effectively reduced nitric oxide levels (193.92±43.85 vs. 588.35±127.24 µm/mL) and IL-6 (31.47±4.24 vs. 34.88±4.03 pg/mL). Combination treatment demonstrated complete prostate and liver tissue protection. All dietary interventions provided complete hepatoprotection, contrasting with flutamide's hepatotoxicity. COX-2 levels were significantly lower in dietary groups (199.04±89.42 to 324.78±17.96 pg/mL) versus flutamide (613.07±201.71 pg/mL). Watermelon supplementation achieved complete renoprotection with preserved glomerular architecture.

Conclusion: Natural dietary supplements demonstrate organ-specific protective effects against prostate cancer with superior safety profiles compared to conventional treatment. These findings support their potential as supplementary therapeutic strategies for minimizing treatment-associated toxicities while enhancing outcomes.

Keywords: Prostate cancer, testosterone-DMBA, oxidative stress, inflammation, papaya seed, watermelon seed, clove bud, dietary supplements.

How to cite this article

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INTRODUCTION

Prostate cancer poses a major global health challenge, with 1460000 new cases and 396,000 deaths reported in 2022.[1] Despite advances in conventional treatments including radiotherapy, surgery, and hormone therapy, mortality rates remain high, particularly in advanced stages where only 31% of patients survive five years with the metastatic disease.[2] This concerning reality has driven research into alternative approaches, particularly dietary interventions and natural products that may target key pathways involved in prostate cancer development.

Prostate cancer development involves multiple interconnected mechanisms including hormonal imbalances, oxidative stress, chronic inflammation, and dysregulated cellular signaling.[3,4,5,6,7] Testosterone, while essential for normal prostate function, contributes to cancer development through its conversion to dihydrotestosterone (DHT), which promotes cell growth by activating androgen receptor pathways.[8,9,10,11,12,13] Environmental carcinogens like 7,12-dimethylbenz[a]anthracene (DMBA) cause DNA damage, trigger inflammatory cascades, and accelerate malignant transformation.[14,15.16]

Oxidative stress, characterized by an imbalance between reactive oxygen species production and antioxidant defences, plays a crucial role in cancer initiation and progression.[17] In prostate cancer, elevated oxidative stress markers correlate with disease advancement, with studies showing up to 3-fold increases in lipid peroxidation products and significant decreases in antioxidant enzymes compared to normal tissue.[18,19,20,21,22,23] Chronic inflammation also contributes significantly, with pro-inflammatory cytokines like interleukin-6 and tumour necrosis factor-alpha creating an environment conducive to tumour development.[24,25,26]

Traditional medicinal plants and their bioactive compounds have gained attention for their potential cancer-fighting properties.[27,28,29,30,31] Three promising natural products show particular relevance. Papaya seeds contain benzyl isothiocyanate, polyphenols, and flavonoids with potent antioxidant, anti-inflammatory, and potential anti-androgenic properties. Recent studies demonstrate their ability to reduce prostate-specific antigen levels in experimental models.[32,33,34] Watermelon seeds are rich in citrulline, arginine, and phenolic compounds with documented antioxidant and anti-inflammatory effects, capable of reducing inflammatory markers in various models.[35,36,37] Cloves contain high levels of eugenol and possess exceptional free radical scavenging abilities and significant anti-inflammatory properties. Studies show they can inhibit NF-κB signalling pathways and reduce pro-inflammatory cytokine production.[38,39,40,41]

Despite individual documented benefits, the combined effects of these natural products on prostate cancer remain unexplored. This study aims to investigate the protective effects of the supplements of papaya seed, watermelon seed, and clove bud on oxidative stress parameters, inflammatory markers, and tissue changes in testosterone-DMBA induced prostate cancer in male rats, potentially identifying novel therapeutic approaches for cancer prevention and management.

MATERIALS AND METHODS

Chemicals/Reagents

All chemicals and reagents used in this study were of analytical or higher grade. All standards and calibrators were prepared according to manufacturers' instructions. All reagents were stored as recommended by the manufacturers, and their stability was verified prior to use.

Testosterone propionate (≥99% purity, CAS 57-85-2), 7,12-Dimethylbenz[a]anthracene (DMBA) (≥95% purity, CAS 57-97-6), Testosterone (≥98% purity, CAS 58-22-0), Flutamide (≥98% purity, CAS 13311-84-7), and Carboxymethylcellulose (pharmaceutical grade, CAS 9004-32-4) were all obtained from Beijing Solarbio Science & Technology Co., Ltd. (Tongzhou Dist, Beijing, China). Corn oil (pharmaceutical grade, C8267) used as a vehicle for testosterone and DMBA administration was obtained from Sigma-Aldrich (St. Louis, MO, USA).

Collection of plant material and identification

Fresh samples of papaya fruits, watermelon fruits, and clove buds were obtained from a local market in Dutsin-Ma, Katsina State, Nigeria (12.4672° N, 7.4947° E) in July 2024. The specimens were identified and authenticated by a botanist at the Federal University Dutsin-Ma's Plant Biology Department Herbarium, and voucher specimens were deposited with accession numbers FUDMA/PSB/00004 (papaya),

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FUDMA/PSB/00118 (watermelon), and FUDMA/PSB/00087 (clove). The papaya and watermelon fruits were processed by washing, dissecting, and extracting the seeds. The seeds, along with the clove buds, underwent separate cleaning and drying procedures. They were then dried for 72 hours in a well-ventilated area, with frequent stirring to ensure uniform drying. After drying, the seeds were ground into fine powders using a laboratory-grade blender and sieved to achieve uniform particle size. The resulting powders were stored at room temperature for further analysis.

Feed formulation

A standardized rodent diet was formulated by mixing the following ingredients in specific proportions: corn starch (554.5 g/kg), soya bean meal (SBM) (320 g/kg), methionine (2.5 g/kg), vitamin and mineral premix (2.5 g/kg), salt (2.5 g/kg), cellulose (45 g/kg), palm oil (60 g/kg), and bone meal (12.5 g/kg). The ingredients were thoroughly blended to create a homogeneous and nutritionally balanced diet that meets established rodent nutritional requirements.[42]

Diet supplementation

The combined supplement diet was made by blending 98g of standard rodent diet with 2g of a custom mixture comprising papaya seed powder, watermelon seed powder, and clove powder in a 4:4:2 ratios, aiming to optimize their combined benefits and mitigate potential risks. The diet formulation was refined to balance nutritional and pharmacological properties, prioritizing animal welfare. The choice of 4g for papaya and watermelon seeds versus 2g for clove bud in the combined custom supplement was deliberate, as clove bud is known for its potent bioactive properties, necessitating a lower concentration to avoid adverse effects. The concentrations and ratios used in this study were selected based on preliminary assessment and represent an exploratory investigation to establish baseline effects before conducting comprehensive dose-response studies.

Also, dedicated supplement diets from each plant were made by blending 96g of standard rodent diet with 4g (4%) of seed powder in cases of papaya and watermelon; and 98g of standard rodent diet with 2g (2%) of bud powder in case of cloves.

Experimental animals

Seventy male Wistar rats, aged 8-10 weeks, were obtained from the Ebonyi State University's Department of Biological Sciences Animal House. Upon arrival, the rats were housed in standard plastic cages and acclimated to laboratory conditions (25°C, 50% humidity) for two weeks. During this period, they had unrestricted access to standard rodent chow and water, ensuring their optimal health and well-being before the experiment.

Ethical Clearance

This study was conducted in accordance with international ethical standards for animal experimentation and received approval from the Animal Care and Use Research Ethics Committee (ACUREC) at Bayero University, Kano, with assigned animal use protocol number BUK/ACUREC/CAP/PG46

Experimental design

After a two-week acclimatization period, 70 male Wistar rats were randomly assigned to seven groups (n=10) with similar average weights. The groups were as follows:

Grouping of experimental animals

Group 1: Normal control (no cancer, fed normal rodent diet)
Group 2: Cancer-Induced animals fed standard rodent diet (negative control)
Group 3: Cancer-Induced animals fed a combination supplement containing 98g standard rodent diet and a 2g blend of papaya seed powder, watermelon seed powder and clove bud powder in a 4:4:2 ratio
Group 4: Cancer-Induced animals fed a supplement containing 96g standard rodent diet and 4g papaya seed powder
Group 5: Cancer-Induced animals fed a supplement containing 96g standard rodent diet and 4g watermelon seed powder
Group 6: Cancer-Induced animals fed a supplement containing 98g standard rodent diet and 2g clove powder
Group 7: Cancer-Induced animals treated with 10mg of Flutamide

Induction protocol

Following a two-week acclimatization period, animals received respective supplements for 2 weeks before

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induction, which continued after induction until the end of the study period. The induction protocol was adapted from previous studies.[43,44] The protocol consisted of a series of administrations:

1. Flutamide (25 mg/kg) was administered daily via gavage for 2 weeks.
2. Twenty-four hours after initiating flutamide treatment, testosterone propionate (100 mg/kg) was injected subcutaneously.
3. Fifty-six hours later, an intraperitoneal injection of 7,12-Dimethylbenz(a)anthracene (DMBA) (65 mg/kg) was administered.
4. One week after DMBA induction, testosterone (3 mg/kg) was administered subcutaneously every 48 hours for 10 weeks.

Collection and preparation of sera samples

At the end of the 16-week study, the rats were weighed and humanely euthanized by chloroform inhalation. Blood samples were then collected from each rat via cardiac puncture and placed in red-top tubes to obtain serum. Following clot formation, the samples were centrifuged (5430R, Eppendorf AG, Hamburg, Germany, 250 rpm, 10 minutes) to separate the serum.

Estimation of oxidative stress markers

Estimation of Superoxide Dismutase (SOD)

This was determined in accordance with the protocols outlined in the Superoxide dismutase (SOD) assay kit (19160, Sigma-Aldrich, St. Louis, MO, USA). Serum samples collected via cardiac puncture were analysed for SOD activity. The reaction mixture contained 2.5 ml of 0.05 M carbonate buffer (pH 10.2), 0.3 mM EDTA, and 0.2 ml of 0.3 mM epinephrine, with 0.1 ml serum added. Monitoring at 480 nm for 5 minutes (30-second intervals) using a Shimadzu UV-1800 spectrophotometer showed SOD inhibited epinephrine auto-oxidation. Activity was expressed in U/ml serum (1 unit = 50% inhibition). Samples were analysed in triplicate; results were mean values. The SOD activity was calculated based on the percentage inhibition of epinephrine auto-oxidation, using the formula:

$$\%\ Inhibition\ = \frac{\Delta A_o\ -\ \Delta A_1}{\Delta A_o} X\ 100$$

Where:

ΔA_o is the change in absorbance of the reference mixture per unit time.

ΔA_1 is the change in absorbance of the sample mixture per unit time.

Estimation of Reduced Glutathione (GSH)

This was determined in accordance with the protocols outlined in the reduced glutathione (GSH) assay kit (CS0260, Sigma-Aldrich, St. Louis, MO, USA). A protein-free filtrate was prepared by mixing 0.5 ml serum with 2.0 ml of 10% trichloroacetic acid (TCA), vortexed, stood for 5 minutes, and centrifuged at 1,200 g for 10 minutes. For GSH estimation, 0.5 ml supernatant was mixed with 2.0 ml of 0.1 M phosphate buffer (pH 7.4) and 0.25 ml DTNB solution (40 mg/100 ml). Absorbance was measured at 412 nm against a blank (using 10% TCA) using a Shimadzu UV-1800 spectrophotometer. GSH concentration was determined via a standard curve (1-10 µg/ml GSH) and expressed as µg/ml serum. Analyses were triplicate; results were mean values.

$$GSH\ concentration\ (\mu g/mL) = \Delta A412/min\ X\ (1/\varepsilon)\ X\ \frac{1}{sample\ volume}$$

Where:

ΔA412/min = Rate of increase in absorbance at 412 nm.

ε = Extinction coefficient of TNB. The extinction coefficient (ε) of TNB is typically 13.6 $mM^{-1}\ cm^{-1}$.

Sample volume = Volume of the sample used in the assay.

Estimation of Malondialdehyde (MDA)

This was determined in accordance with the protocols outlined in the malondialdehyde (MDA) assay kit (MAK085, Sigma-Aldrich, St. Louis, MO, USA). Serum samples were centrifuged at 250 rpm for 10 minutes using an Eppendorf 5430R centrifuge. Proteins were precipitated by mixing 0.5 ml serum with 2.5 ml of 10% TCA, incubated for 15 minutes, and centrifuged at 3,500 rpm for 10 minutes. 2.0 ml supernatant was mixed with 1.0 ml of 0.67% TBA in 0.05 M NaOH and incubated at 95-100°C for 30 minutes for MDA-TBA complex formation. Absorbance was measured at 532 nm (A532) and 600 nm (A600) using a Shimadzu UV-1800 spectrophotometer; ΔA = A532 - A600. MDA concentration was determined via a standard curve (0.5-5.0 nmol/ml TEP) and expressed as nmol/ml serum. Analyses were triplicate; results were mean values.

$$MDA\ content\ (nmol/mL)\ =\ \frac{(\Delta A\ x\ DF)}{(\varepsilon\ x\ L)}$$

Where:

ΔA = A532 - A600

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Dilution factor = (total volume of reaction mixture) / (volume of sample)
ε = extinction coefficient of MDA-TBA complex (typically 1.56 x 10^5 M^{-1} cm^{-1})
L = path length of cuvette (typically 1 cm)

Estimation of Catalase (CAT)

This was determined in accordance with the protocols outlined in the Catalase (CAT) assay kit (CAT100, Sigma-Aldrich, St. Louis, MO, USA). Serum collected via cardiac puncture was centrifuged at 250 rpm for 10 minutes using an Eppendorf 5430R centrifuge. Catalase activity was assayed by mixing 0.1 ml serum with 0.5 ml of 0.2 M H_2O_2 in 0.05 M phosphate buffer (pH 7.0) for 60 seconds; reaction stopped with 2.0 ml dichromate/acetic acid reagent (5% potassium dichromate: glacial acetic acid, 1:3). Tubes were heated for 10 minutes at boiling water bath; absorbance measured at 570 nm using Shimadzu UV-1800 spectrophotometer. Activity calculated via H_2O_2 standard curve, expressed in U/ml serum (1 unit decomposes 1 µmol H_2O_2/minute). Analyses triplicate; results mean values.

The rate of decrease in absorbance (ΔA240/min) was then calculated from these readings.

$$Catalase\ Activity\ (U/mL) = (\Delta A240/min)\ X\ (1/0.0436)\ X\ \frac{1}{sample\ volume\ in\ mL}$$

Where: 0.0436 is the extinction coefficient of H_2O_2 at 240 nm.

Estimation of Nitric Oxide (NO)

This was determined using the Nitric oxide (NO) assay kit (23479, Sigma-Aldrich, St. Louis, MO, USA) based on Griess reagent. Serum collected via cardiac puncture was deproteinized with 10% zinc sulphate, vortexed, and centrifuged at 10,000 g for 15 minutes at 4°C. Nitrate was converted to nitrite using nitrate reductase (0.2 U/mL), 5 µM FAD, 0.1 mM NADPH in phosphate buffer (pH 7.4) for 1 hour at 37°C. 100 µL processed sample was mixed with Griess reagent (1:1 ratio of 1% sulphanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl) ethylenediamine dihydrochloride) and incubated 10 minutes; absorbance measured at 540 nm using BioTek microplate reader. NO concentration (as nitrite equivalents) calculated via 0-100 µM sodium nitrite standard curve, expressed in µmol/ml serum. Analyses triplicate; results mean values.

$$NO\ concentration\ (\mu mol/ml) = \frac{A540\ of\ sample\ -\ A540\ of\ blank}{A540\ of\ standard\ -\ A540\ of\ blank}\ X\ standard\ concentratio/ml)$$

Where:
A540 = absorbance at 540 nm
Blank = reagent blank without sample or standard
Standard = known concentration of sodium nitrite ($NaNO_2$) standard
Sample = test sample containing NO

Estimation of Inflammatory Markers

Estimation of C-Reactive Protein (CRP)

The C-reactive protein (CRP) assay was performed using a high-sensitivity CRP ELISA kit (RAB1121, Sigma-Aldrich, St. Louis, MO, USA).

Estimation of Interleukin-6 (IL-6)

The Interleukin-6 (IL-6) ELISA kit (R6000B, R&D Systems, Minneapolis, MN, USA) was used for this study.

Estimation of Tumour Necrosis Factor-Alpha (TNF-α)

The Tumour Necrosis Factor-alpha (TNF-α) ELISA kit (RTA00, R&D Systems, Minneapolis, MN, USA) was used.

Estimation of Cyclooxygenase 1 (COX-1)

The COX-1 was determined using the Cyclooxygenase-1 (COX-1) ELISA kit (MyBioSource, San Diego, CA, USA).

Estimation of Cyclooxygenase 2 (COX-2)

The Cyclooxygenase-2 (COX-2) ELISA kit (Sigma-Aldrich, St. Louis, MO, USA) was used.

Estimation of Interleukin-10 (IL-10)

IL-10 was determined using the Interleukin-10 (IL-10) ELISA kit (R1000, R&D Systems, Minneapolis, MN, USA).

Histopathological Studies

Organs of interest, kidney, liver and prostate were dissected, washed in ice-cold saline, and processed for further analysis. A portion of the tissues was fixed in 10% neutral buffered formalin fixative solution to facilitate histological studies. Following fixation, the tissues were embedded in paraffin, and 5 mm thick sections were cut and stained with haematoxylin and eosin.[45] The stained sections were examined under a light microscope at a magnification of 400x, and photomicrographs were taken to assess microscopic changes of pathological importance.

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Data Analysis

Data analysis was conducted using Statistical Package for Social Sciences (SPSS) software, version 21 (SPSS Inc., Chicago, IL, USA). Results are expressed as mean ± standard error of the mean (SEM). Duncan's multiple comparison test was used to ascertain significant variations among group means. The threshold for statistical significance was established at $p < 0.05$.

RESULTS

Table 1: Oxidative Stress Markers of Testosterone-DMBA-Induced Prostate Cancer in Wistar Rats Fed Papaya seed, Watermelon seed, and Clove Supplemented Diet.

Group	SOD(U/mL)	GSH(µg/mL)	MDA (nmol/mL)	CAT(µ/mL)	NO (µm/mL)
1	11.00±1.77^{a}	7.37±0.35^{a}	316.67±22.05^{a}	10.60±0.45^{a}	444.71±102.09^{ab}
2	11.87±2.68^{a}	6.90±1.22^{a}	370.10±41.75^{a}	4.67±1.17^{bc}	588.35±127.24^{a}
3	16.50±4.58^{a}	7.97±1.73^{a}	384.17±30.35^{a}	4.63±0.64^{bc}	429.29±103.63^{ab}
4	22.40±5.45^{a}	8.17±2.44^{a}	358.60±22.02^{a}	4.93±1.52^{bc}	454.69±107.63^{ab}
5	18.50±5.58^{a}	7.47±0.55^{a}	299.27±76.78^{a}	6.87±0.63^{b}	363.63±175.61^{ab}
6	10.20±1.99^{a}	4.30±0.66^{a}	367.80±17.28^{a}	3.23±0.23^{cd}	193.92±43.85^{b}
7	12.40±3.66^{a}	5.17±1.96^{a}	259.33±44.36^{a}	1.43±0.27^{d}	538.98±64.48^{ab}

The result represents the average of three determinants with their Mean ± SEM and statistical significance indicators. Groups marked with the same letter (e.g., 'a' or 'b') are not significantly different from each other, while groups marked with different letters (e.g., 'a' vs. 'b') are significantly different from each other in terms of oxidative stress. Key: SOD: Superoxide dismutase, GSH: Glutathione, MDA: Malondialdehyde, CAT: Catalase, NO: Nitric oxide. The seven groups consisted of the following: normal rats fed a standard diet (Group 1); testosterone-DMBA-induced prostate cancer rats fed a standard diet (Group 2); testosterone-DMBA-induced prostate cancer rats fed a diet supplemented with papaya seed, watermelon seed, and cloves (Group 3); testosterone-DMBA-induced prostate cancer rats fed a papaya seed-supplemented diet (Group 4); testosterone-DMBA-induced prostate cancer rats fed a watermelon seed-supplemented diet (Group 5); testosterone-DMBA-induced prostate cancer rats fed a clove-supplemented diet (Group 6); and testosterone-DMBA-induced prostate cancer rats administered 10 mg/kg flutamide (Group 7).

Table 2. Inflammation and Anti-Inflammation Markers of Testosterone-DMBA-Induced Prostate Cancer in Wistar Rats Fed Papaya, Watermelon, and Clove Supplemented Diet

Group	CRP (mg/L)	IL-6 (pg/mL)	TNF (pg/mL)	COX-1 (pg/mL)	COX-2 (pg/mL)	IL-10(pg/mL)
1	10.23±1.78^{ab}	39.44±2.79^{ab}	154.52±4.79^{b}	109.11±6.21^{ab}	346.88±23.57^{ab}	793.03±109.89^{a}
2	12.47±5.17^{ab}	34.88±4.03^{ab}	157.63±29.69^{b}	100.59±4.58^{abc}	417.43±16.14^{ab}	607.88±174.08^{a}
3	20.50±7.57^{a}	54.89±12.56^{a}	479.29±160.78^{a}	84.15±4.18^{bcd}	199.04±89.42^{b}	610.54±203.73^{a}
4	10.80±3.84^{ab}	39.47±4.22^{ab}	222.43±39.44^{b}	104.09±10.04^{abc}	324.78±17.96^{b}	801.31±101.62^{a}
5	2.70±0.25^{b}	40.14±5.48^{ab}	161.55±9.34^{b}	65.27±6.08^{d}	248.10±15.68^{b}	738.36±192.35^{a}
6	7.70±1.90^{ab}	31.47±4.24^{b}	138.04±19.23^{b}	75.46±7.58^{cd}	210.64±12.25^{b}	527.49±49.06^{a}
7	19.60±8.60^{ab}	41.36±5.08^{ab}	194.46±30.88^{b}	118.77±18.52^{a}	613.07±201.71^{a}	822.24±80.69^{a}

The result represents the average of three determinants with their Mean ± SEM and statistical significance indicators. Groups marked with the same letter (e.g., 'a' or 'b') are not significantly different from each other, while groups marked with different letters (e.g., 'a' vs. 'b') are significantly different from each other in terms of inflammation markers. Key: CRP: C-reactive protein, IL-6: Interleukin-6, TNF: Tumour necrosis factor; COX-1: Cyclooxegenase 1; COX-2: Cyclooxegenase 2; IL-10: Interleukin-10. Tsnihe seven groups consisted of the following: normal rats fed a standard diet (Group 1); testosterone-DMBA-induced prostate cancer rats fed a standard diet (Group 2); testosterone-DMBA-induced prostate cancer rats fed a diet supplemented with papaya seed, watermelon seed, and cloves (Group 3); testosterone-DMBA-induced prostate cancer rats fed a papaya seed-supplemented diet (Group 4); testosterone-DMBA-induced

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prostate cancer rats fed a watermelon seed-supplemented diet (Group 5); testosterone-DMBA-induced prostate cancer rats fed a clove-supplemented diet (Group 6); and testosterone-DMBA-induced prostate cancer rats administered 10 mg/kg flutamide (Group 7).

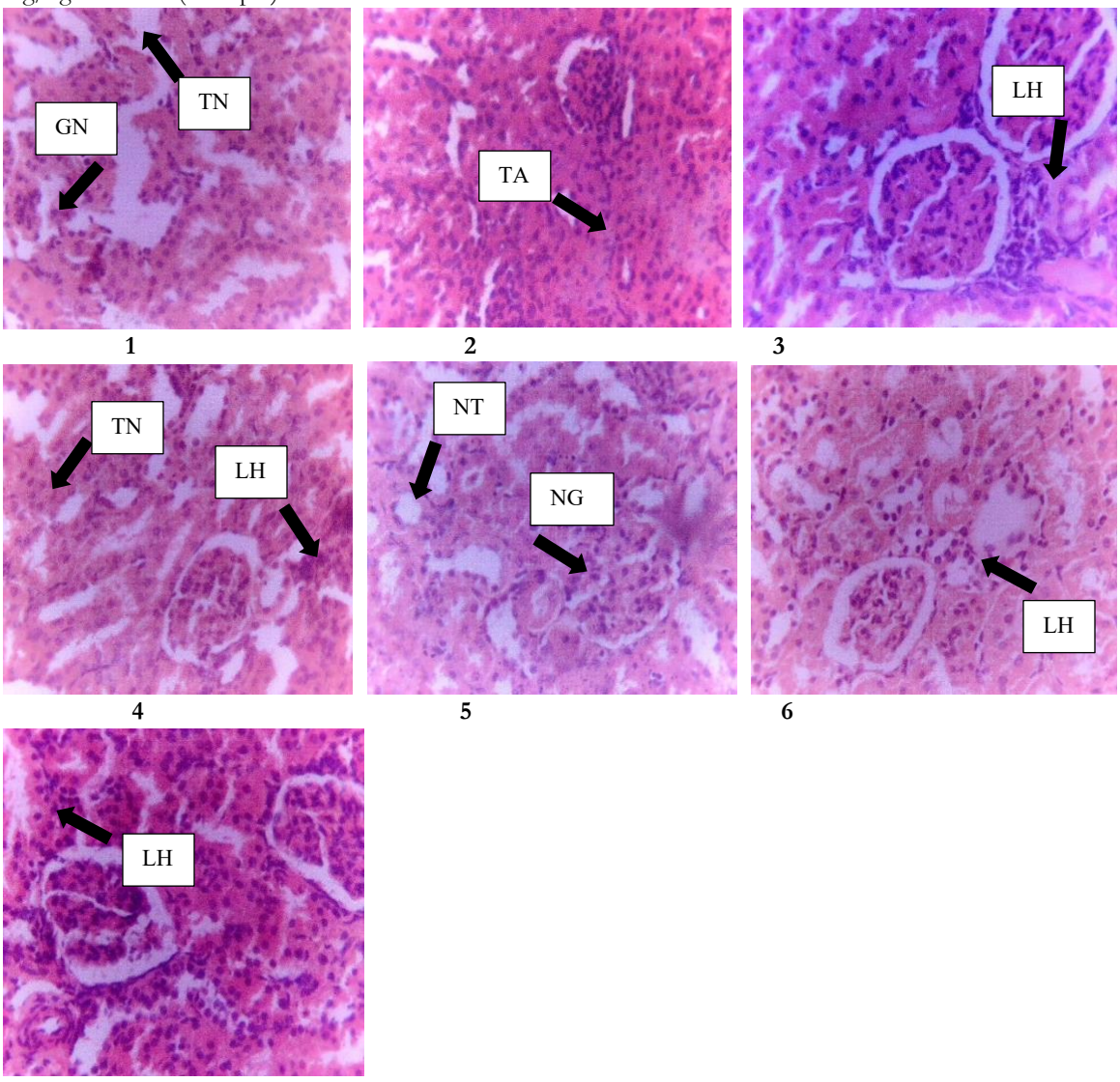

Figure 1: Photomicrograph of Kidney of Testosterone-DMBA-Induced Prostate Cancer in Wistar Rats Fed Papaya, Watermelon, and Clove Supplemented Diet. H & E Stain (X 250)
Group 1: normal rats fed a standard diet (Group 1); Group 2: Testosterone-DMBA-induced prostate cancer rats fed a standard diet; Group 3: Testosterone-DMBA-induced prostate cancer rats fed a diet supplemented with papaya, watermelon, and cloves; Group 4: Testosterone-DMBA-induced prostate cancer rats fed a papaya-supplemented diet; Group 5: Testosterone-DMBA-induced prostate cancer rats fed a watermelon-supplemented diet; Group 6: Testosterone-DMBA-induced prostate cancer rats fed a clove-supplemented diet; Group 7: Testosterone-DMBA-induced prostate cancer rats administered 10 mg/kg flutamide (Group 7). Key: GN: Glomerular necrosis; LH: Hyperplasia of inflammatory cells; TA: Tubular adhesion; TN: Tubular necrosis; NG: Normal glomerulus; NT: Normal tubules

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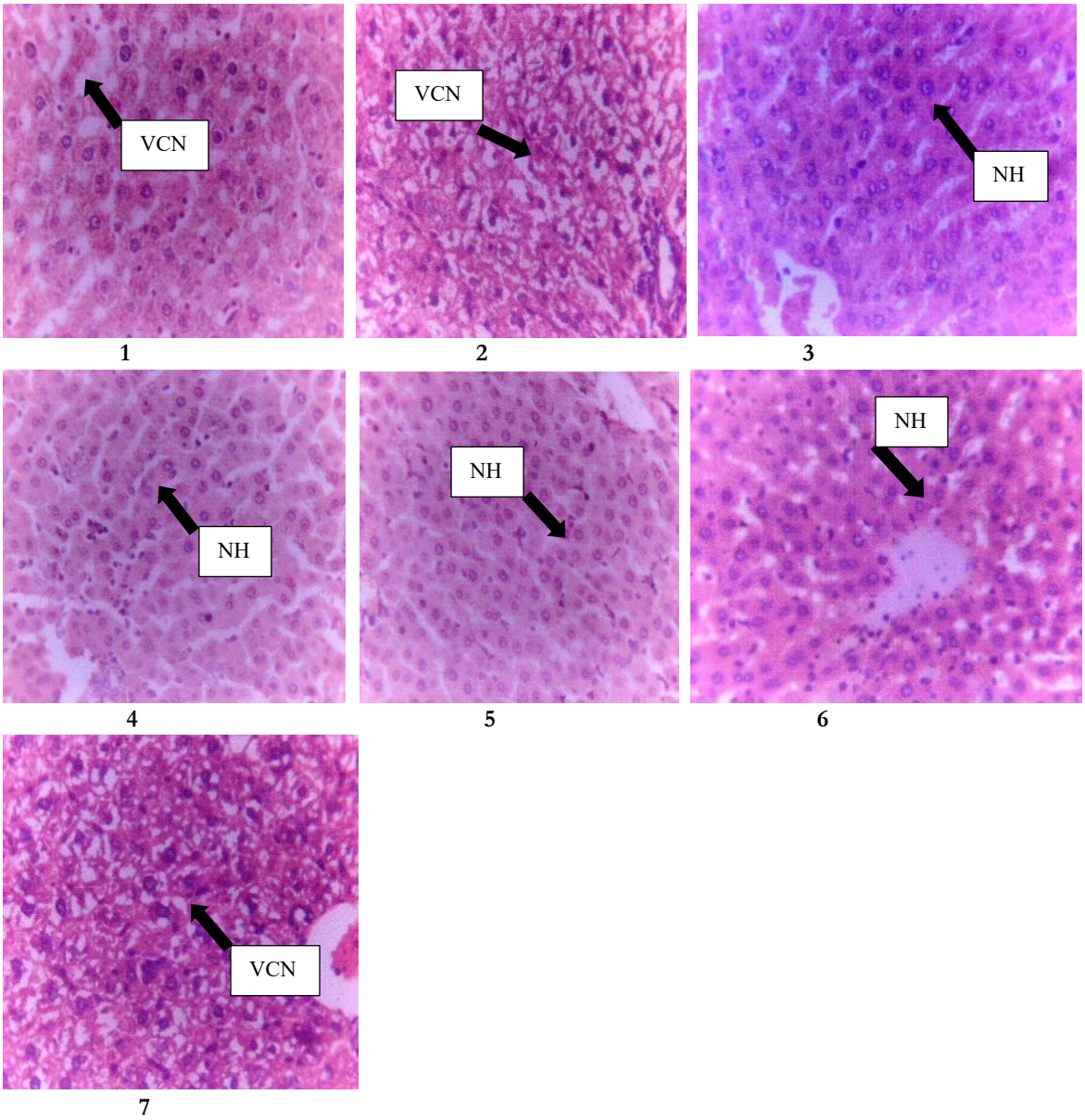

Plate 4.2: Photomicrograph of Liver of Testosterone-DMBA-Induced Prostate Cancer in Wistar Rats Fed Papaya, Watermelon, and Clove Supplemented Diet. H & E Stain (X 250)
Group 1: normal rats fed a standard diet (Group 1); Group 2: Testosterone-DMBA-induced prostate cancer rats fed a standard diet; Group 3: Testosterone-DMBA-induced prostate cancer rats fed a diet supplemented with papaya, watermelon, and cloves; Group 4: Testosterone-DMBA-induced prostate cancer rats fed a papaya-supplemented diet; Group 5: Testosterone-DMBA-induced prostate cancer rats fed a watermelon-supplemented diet; Group 6: Testosterone-DMBA-induced prostate cancer rats fed a clove-supplemented diet; Group 7: Testosterone-DMBA-induced prostate cancer rats administered 10 mg/kg flutamide (Group 7). Key: NH: Normal hepatocytes; VCN: Vacoulation and necrosis.

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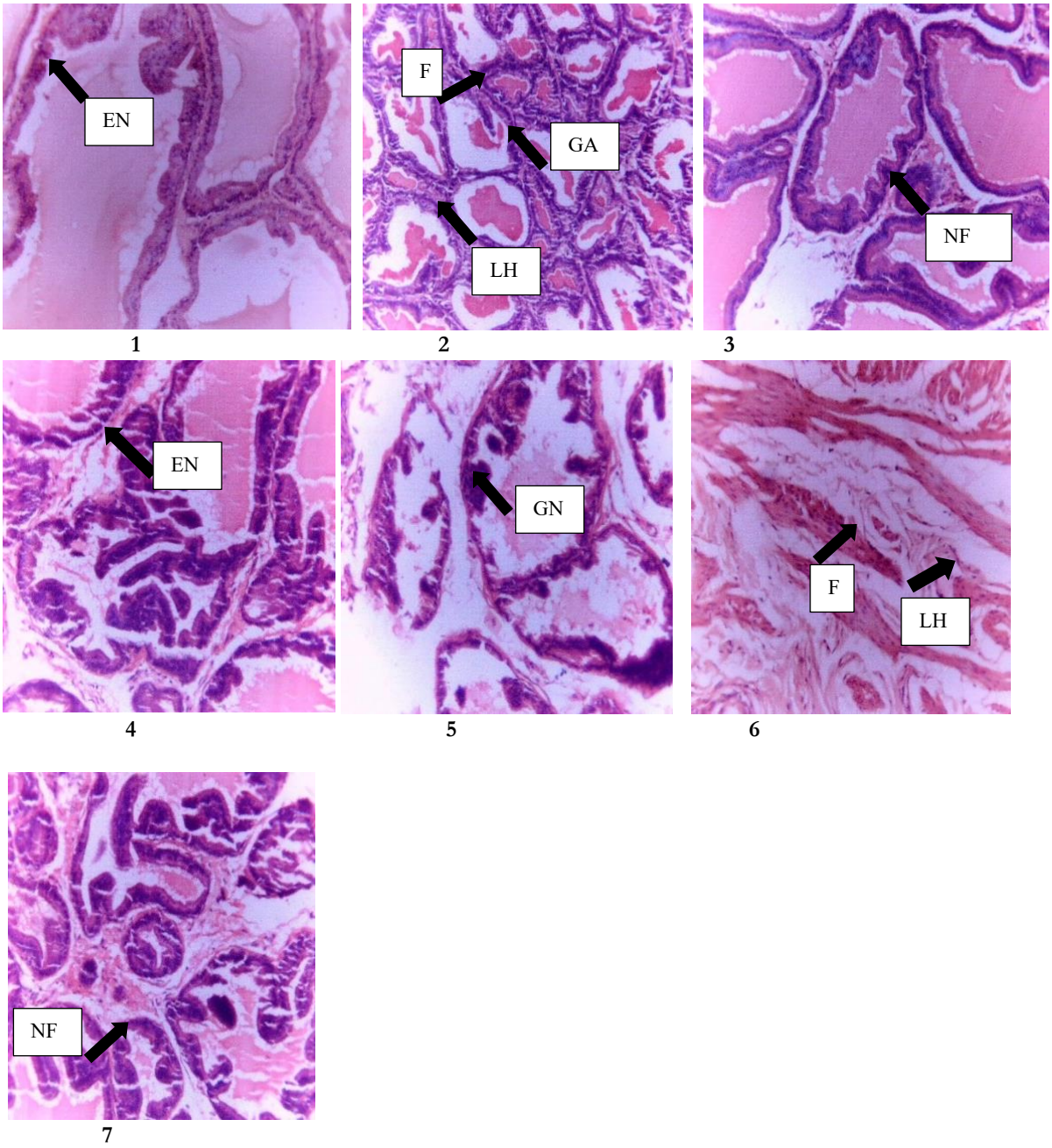

Figure 3: Photomicrograph of Prostate of Testosterone-DMBA-Induced Prostate Cancer in Wistar Rats Fed Papaya, Watermelon, and Clove Supplemented Diet. H & E Stain (X 250)

Group 1: normal rats fed a standard diet (Group 1); Group 2: Testosterone-DMBA-induced prostate cancer rats fed a standard diet; Group 3: Testosterone-DMBA-induced prostate cancer rats fed a diet supplemented with papaya, watermelon, and cloves; Group 4: Testosterone-DMBA-induced prostate cancer rats fed a papaya-supplemented diet; Group 5: Testosterone-DMBA-induced prostate cancer rats fed a watermelon-supplemented diet; Group 6: Testosterone-DMBA-induced prostate cancer rats fed a clove-supplemented diet; Group 7: Testosterone-DMBA-induced prostate cancer rats administered 10 mg/kg flutamide (Group 7). Key: EN: Epithelium necrosis; F: Fibrosis; GA: Glandular atrophy; GN: Glandular necrosis; LH: Hyperplasia of inflammatory cells; NF: Normal features.

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DISCUSSION

The study revealed that superoxide dismutase (SOD) activity showed no statistically significant differences across all experimental groups. However, the numerical trends, particularly the elevated SOD levels in Groups 3 (combination diet group), 4 (papaya seed diet group), and 5 (watermelon seed diet group) suggests a potential antioxidant-enhancing effect of papaya, watermelon, and their combination. These findings align with a study showing significant increases in SOD activity in rats treated with watermelon seed oil, showing that the antioxidant-rich nature of watermelon seed oil, abundant in unsaturated fatty acids such as oleic acid, presumably facilitated the upregulation of SOD, validating its antioxidant efficacy.[46] Similarly, another study demonstrated that papaya extract significantly restored SOD activity in CCl_4-induced oxidative damage models, highlighting that the antioxidant rich phytochemicals in papaya seeds (phenolics, flavonoids, terpenoids) mitigated CCl_4-induced oxidative stress by replenishing SOD and other antioxidants.[47] The lack of statistical significance in our study, despite numerical increases, might be due to the relatively short intervention period or the complexity of the testosterone-DMBA model.

Similar to SOD, reduced glutathione (GSH) levels showed no statistically significant differences across all groups. However, the numerical data suggests that papaya supplementation (Group 4) and the combination treatment (Group 3) maintained slightly higher GSH levels compared to cancer control (Group 2). Interestingly, the clove-supplemented group (Group 6) showed the lowest GSH levels, even lower than the flutamide treatment group (Group 7). These findings contrast with those of another study that reported significant GSH elevation with clove extract in radiation-induced oxidative stress, stating that bioactive chemicals found in cloves, including as ferulic acid, rutin, and isoquercitrin, enhance glutathione (GSH) synthesis via modulating antioxidant pathways (e.g., Nrf2/ARE).[48] Ethanolic extraction concentrates phenolic compounds compared to whole plant material and enhances bioavailability by pre-dissolving active ingredients. In contrast, dried clove powder requires gastrointestinal digestion to release bioactives from the plant cell matrix, potentially limiting absorption and reducing effective concentrations of GSH-modulating compounds. This preparation difference likely explains why the previous study[48] observed significant GSH elevation while our study showed more modest effects. Future studies should consider standardized clove extract preparations to achieve therapeutic concentrations of bioactive compounds for optimal antioxidant effects.
Research demonstrated that papaya peel extract significantly increased GSH levels in HepG2 liver cancer cells by enhancing antioxidant enzyme activity (SOD, CAT, GPx, GR).[49] The discrepancy might be related to the complex interaction between clove compounds and the testosterone-DMBA model.

The malondialdehyde (MDA) levels, indicators of lipid peroxidation, showed no statistically significant differences across groups. However, the flutamide treatment (Group 7) and watermelon supplementation (Group 5) showed numerically lower MDA levels compared to the cancer control (Group 2). This trend aligns with findings from other study, reported that lycopene (abundant in watermelon) enhances the antioxidant response in prostate cells, countering oxidative stress mechanisms like lipid peroxidation.[50] The lack of statistical significance in our study might be attributed to the high variability in MDA measurements, particularly in Group 5.

Catalase activity demonstrated the most pronounced statistically significant differences among all measured parameters. The normal control group (Group 1) exhibited significantly higher CAT activity compared to all treatment groups. Among the treatment groups, watermelon supplementation (Group 5) maintained significantly higher CAT activity compared to other interventions, particularly flutamide (Group 7) and clove supplementation (Group 6). These findings corroborate a research demonstrating that citrulline and other antioxidants (e.g., lycopene and polyphenols) from watermelon preserved catalase activity in oxidative stress conditions by scavenging reactive oxygen species (ROS) in diabetic rats.[51] The significant reduction in CAT activity in flutamide-treated rats aligns with a study that reported similar findings and attributed this to the potential pro-oxidant effects of long-term flutamide administration.[52] The intermediate values for papaya seed supplement (Group 4) and combination treatment (Group 3) suggest a moderate protective effect, consistent with studies demonstrating dose-dependent effects of papaya extracts on antioxidant enzymes.[49,53]

Nitric oxide (NO) levels showed significant differences, particularly between clove supplementation (Group 6)

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and cancer control (Group 2). All other treatments demonstrated intermediate effects without statistical significance compared to the control groups. This significant reduction in NO with clove supplementation aligns with findings that reported that eugenol, the primary bioactive compound in cloves, blocks the activation of NF-κB, a pathway significant for inflammation and cancer growth, and hence suppresses COX-2, TNF-α, and iNOS.[41] Similarly, other findings showed that eugenol exhibits anti-inflammatory effects by inhibiting iNOS and nitric oxide (NO) production, which is mediated through the inhibition of the NF-κB and MAPK pathways.[54] Also, a study demonstrated that eugenol suppresses iNOS expression by inhibiting NF-κB and AP-1 signalling pathways, which are key regulators of iNOS transcription.[55] This mechanism was observed in macrophages and other inflammatory models. Elevated NO levels in cancer control rats corroborate with research reporting that Chronic inflammation, prevalent in prostate cancer, induces iNOS expression, resulting in elevated NO levels that suppress androgen receptor (AR) activity.[56] This facilitates the progression of cancer independent of androgens. Similarly, another finding reported that chronic inflammation in the prostate generates reactive nitrogen species (e.g., NO), contributing to oxidative DNA damage and neoplastic transformation.[57] The relatively high NO levels in the flutamide group contrast with some previous findings and potentially related to the contradicting effects of androgen deprivation on inflammatory processes as described by a previous study, men undergoing androgen deprivation (through orchidectomy or androgen deprivation therapy (ADT)) exhibited improved endothelium-dependent vasodilation, associated with elevated nitric oxide bioavailability.[58] This systemic elevation of NO contrasts with localised pro-tumour effects observed in the prostate. Similarly, findings from another study showed that administration of testosterone in rats resulted in elevated levels of nitric oxide and associated enzymes, establishing a pro-inflammatory "feed-forward" mechanism that enhances the progression of prostate carcinogenesis.[59] ADT reduces androgen-driven proliferation; however, it may not effectively suppress, and could potentially enhance, inflammation-driven NO production, thereby promoting castration-resistant prostate cancer (CRPC) progression.

The data revealed statistically significant differences in C-reactive protein (CRP) levels among the experimental groups. Notably, the combined supplementation group (Group 3) showed significantly elevated CRP levels compared to the watermelon-supplemented group (Group 5). Other groups demonstrated intermediate CRP values without statistical significance compared to these extremes. The remarkably low CRP level in the watermelon-supplemented group aligns with findings that reported significant reductions in CRP with lycopene supplementation in clinical inflammation models.[60] This anti-inflammatory effect may be attributed to lycopene's ability to suppress NF-κB signalling pathways, as demonstrated by a mechanistic study, showing that lycopene inhibits the enzyme IκB kinase beta (IKKβ), suppressing NF-κB signalling, thereby reducing cell proliferation.[61] The unexpectedly high CRP in the combination treatment contradicts the anticipated additive anti-inflammatory effects and suggests potential antagonistic interactions between bioactive compounds when administered together.

Interleukin-6 (IL-6) levels showed significant differences, particularly between the combination treatment (Group 3) and clove supplementation (Group 6). The clove-supplemented group exhibited the lowest IL-6 levels among all groups, suggesting potent anti-inflammatory effects. This finding corroborates recent work demonstrating that eugenol from cloves significantly suppressed IL-6 production in prostate cancer cell lines through inhibition of STAT3 phosphorylation.[54] The relatively elevated IL-6 in the combination treatment, potentially due to adverse interactions in specific contexts (e.g., high doses, specific populations).

Tumour necrosis factor (TNF) levels exhibited the most dramatic differences among all measured inflammatory markers. The combination treatment group (Group 3) showed significantly higher TNF levels compared to all other groups, which exhibited relatively similar values. This unexpected elevation in TNF with the combination treatment contradicts findings from individual supplementation studies. For instance, a study demonstrated significant TNF suppression with watermelon extract,[36] while another study reported similar effects with papaya leaf extract, by inhibition of NF-κB and MAPK pathways (ERK1/2, JNK, p38), which regulate TNF-α production.[62] Similarly, another

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research highlighted that clove water extract inhibited TNF-α production in lipopolysaccharide-stimulated cells and mouse peritoneal macrophages by blocking NF-κB nuclear translocation and MAPK signalling.[63] The synergistic increase in TNF with combined supplementation suggests that certain phytochemical combinations may trigger complex immunomodulatory responses that differ substantially from their individual effects potentially due to dose, bioavailability, or unique molecular interactions.

Cyclooxygenase-1 (COX-1) levels demonstrated significant variations across groups. The flutamide treatment group (Group 7) maintained the highest COX-1 levels, significantly different from watermelon (Group 5) and clove supplementation (Group 6). The combination treatment (Group 3) showed intermediate effects. The significantly reduced COX-1 levels with watermelon supplementation align with findings showing that citrulline from watermelon modulates COX-1 expression through nitric oxide-dependent pathways.[64] The preserved COX-1 levels in the flutamide group corroborate with a study reporting that androgen receptor antagonists preferentially target COX-2 rather than COX-1,[65] potentially explaining the differential effects observed between these two cyclooxygenases in our study.
The significantly lower COX-2 levels in all dietary intervention groups compared to flutamide treatment suggest potent COX-2 inhibitory effects of the phytochemicals present in papaya, watermelon, and cloves. This aligns with findings from multiple studies, that identified specific flavonoids in papaya with selective COX-2 inhibitory properties,[49] and a review demonstrated that eugenol in clove inhibits COX-2 gene expression by blocking NF-κB signalling.[66] The elevated COX-2 in the flutamide group, despite its therapeutic efficacy, suggests potential compensatory inflammatory responses, a phenomenon also reported in a comprehensive analysis of inflammatory side effects associated with androgen deprivation therapies[67] and in a clinical study, demonstrating that COX-2 overexpression may reflect unresolved inflammation due to androgen deprivation, which long-term ADT (LTAD) could mitigate.[68]

Interestingly, IL-10, an anti-inflammatory cytokine, showed no statistically significant differences across all experimental groups. However, numerical trends suggest slightly elevated IL-10 levels in the flutamide (Group 7), papaya (Group 4), and normal control (Group 1) groups compared to others. The lack of significant modulation in IL-10 levels across intervention groups contrasts with several in vitro studies, such as those which reported significant IL-10 induction with various phytochemicals present in the supplements tested.[69,70] This discrepancy might be related to the complex in vivo environment and the specific testosterone-DMBA model used, which could potentially override certain immunomodulatory pathways. A study similarly reported minimal IL-10 modulation in their In-vivo cancer model despite significant effects in corresponding in vitro systems, suggesting context-dependent immunomodulatory responses.[71]

The watermelon group, which demonstrated the most balanced antioxidant profile with preserved catalase activity and moderate SOD induction, along with significantly reduced CRP and COX-1 levels, exhibited complete renoprotection histologically. This strong correlation supports the mechanistic link between oxidative stress, inflammation, and structural renal damage proposed in some comprehensive reviews.[72,73] Similarly, the clove-supplemented group showed significant reductions in both nitric oxide and IL-6 in previous analyses, correlating with the absence of structural damage despite mild inflammatory infiltrates observed histologically. This relationship was previously highlighted by a study, that demonstrated that selective inflammatory pathway modulation can preserve tissue architecture even in the presence of mild residual inflammation.[74] The incomplete protection observed in the papaya and combination groups despite some improvements in oxidative stress markers suggests that threshold effects may exist, requiring more substantial modulation of specific pathways to achieve complete renoprotection. This concept of threshold-dependent tissue protection was similarly proposed in a dose-response analysis of phytochemical interventions in renal injury models.[75] While watermelon supplementation demonstrated complete protection in both hepatic and renal tissues, papaya and clove supplementation exhibited organ-specific efficacy, providing complete hepatoprotection but only partial renoprotection. This differential tissue response might reflect organ-specific vulnerability to testosterone-DMBA-induced damage, differential bioaccumulation

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of protective compounds, or tissue-specific activation of protective pathways.

The complete hepatoprotection observed with the combination treatment, despite showing only partial renoprotection, further highlights these organ-specific responses. Some studies reported similar organ-specific protective profiles with phytochemical interventions and attributed these to differences in tissue perfusion, metabolic activity, and expression of specific receptors and transporters across organ systems.[76,77,78] Notably, the flutamide treatment exhibited significant toxicity in both hepatic and renal tissues, revealing the substantial adverse effect profile of conventional pharmacological approaches despite their established therapeutic efficacy. This observation supports the growing interest in complementary phytochemical interventions to mitigate treatment-associated toxicities while potentially enhancing therapeutic outcomes, as reviewed comprehensively in a study.[79] The complete hepatoprotection observed with dietary interventions correlates with their previously reported effects on oxidative stress and inflammatory markers. The watermelon group, which demonstrated the most balanced antioxidant profile and significant anti-inflammatory effects, exhibited complete protection in both hepatic and renal tissues.

The clove-supplemented group, which showed significant reductions in nitric oxide and IL-6, demonstrated complete hepatoprotection despite partial renoprotection. However, the combination treatment, which showed paradoxical increases in certain inflammatory markers like TNF, still provided complete hepatoprotection. This observation suggests that the liver might possess superior resilience against inflammatory damage compared to the kidneys, potentially related to its robust regenerative capacity and extensive detoxification systems. This theory is supported by studies, demonstrating that the liver maintains structural integrity despite elevated inflammatory markers in various experimental models through balanced pro-/anti-inflammatory signalling, immune modulation and regenerative responses and compensatory extracellular matrix (ECM) dynamics in early injury phases.[80,81]

An analysis of prostatic outcomes among therapeutic groups indicates a hierarchy of effectiveness. The combination supplementation and flutamide groups demonstrated complete protection, the papaya and watermelon groups showed substantial but incomplete protection, and the clove group exhibited limited protection. This pattern differs from the previously observed hepatic outcomes, where all dietary interventions provided complete protection, and renal outcomes, where only watermelon demonstrated complete protection. This differential efficacy across organ systems suggests complex interactions between the interventions and tissue-specific pathophysiological processes as with the liver histology. The combination supplementation group demonstrated equivalent prostatic protection to flutamide but without the significant hepatotoxicity and nephrotoxicity associated with the pharmaceutical intervention. This observation suggests a potentially superior therapeutic profile for the phytochemical combination, providing target organ efficacy while sparing non-target organs from toxicity. This finding supports the increasing interest in phytochemical interventions as adjunctive or alternative methods to traditional cancer treatments, as thoroughly examined by a previous study.[82]

These prostatic histopathological observations with previously discussed oxidative stress and inflammatory markers reveals interesting correlations. The combination treatment group, which showed paradoxical increases in certain inflammatory markers like TNF but balanced effects on oxidative stress parameters, demonstrated complete prostatic protection, suggesting that local tissue effects may not directly correlate with systemic biomarkers. The watermelon group, which demonstrated the most balanced antioxidant profile and significant anti-inflammatory effects systemically, exhibited substantial but incomplete prostatic protection. This partial discordance between systemic biomarkers and target organ outcomes suggests complex relationships between circulating mediators and tissue pathophysiology. The clove-supplemented group, which showed significant reductions in nitric oxide and IL-6 systemically, demonstrated limited prostatic protection also reveals the complex relationship between systemic biomarkers and organ-specific outcomes, potentially reflecting differences in tissue penetration, target expression, or the relevance of specific inflammatory pathways to prostatic pathophysiology.

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Strengths and limitations of the study

This study presents a comprehensive investigation of natural supplement combinations for prostate cancer treatment, demonstrating strong methodological rigor through multi-organ assessment, robust experimental design, and extensive biomarker evaluation. As the first research to examine these three natural products in combination, it offers valuable insights into potential adjunctive therapies with reduced toxicity profiles and organ-specific protective effects.

However, significant limitations constrain the findings' immediate clinical applicability. The animal model design, short intervention period, single-dose testing, and small sample sizes limit generalizability to human patients.

Despite these constraints, the results suggest promising clinical implications for adjunctive cancer therapy, prevention strategies for high-risk populations, and personalized medicine approaches. The research provides a foundation for future human studies, though larger-scale trials with extended follow-up periods are essential before clinical translation can be considered safe and effective.

Research implications

This study's findings necessitate comprehensive follow-up research including mechanistic pathway investigations, dose optimization studies, and human clinical trials to establish safety and efficacy before clinical translation. The research also requires bioavailability studies and detailed analysis of combination therapy interactions to understand the underlying mechanisms. From a public health standpoint, the results suggest potential integration of these natural supplements into dietary guidelines as cost-effective cancer prevention interventions. This approach is particularly valuable for resource-limited settings where traditional pharmaceutical treatments may be inaccessible, providing evidence-based validation for complementary medicine approaches.

Policy Implications

The study suggests policy reforms in various domains, including health regulatory agencies, national healthcare policies, insurance frameworks, food safety, and agricultural policies. It also calls for updated standards for medicinal plant cultivation, processing, and contamination limits.

Practice Implications

Clinical practice transformation necessitates training for healthcare providers on evidence-based natural supplement use, including patient selection protocols and interaction monitoring. Pharmacy practice should incorporate specialized natural supplement counselling, quality assessment capabilities, and interaction screening systems. Primary care providers should integrate supplement assessment into routine preventive visits. Community health programs should develop accessible cancer prevention initiatives. Quality assurance protocols are essential for safe implementation.

CONCLUSION

The study demonstrates that watermelon supplementation provides the most comprehensive multi-organ protection against testosterone-DMBA induced prostate cancer through balanced modulation of oxidative stress and inflammatory parameters. Importantly, dietary interventions achieved organ protection comparable to conventional pharmacological treatment (flutamide) without associated toxicity, suggesting significant potential as adjunctive therapeutic approaches. The findings support the development of evidence-based dietary recommendations and natural product formulations that could complement conventional treatments or serve as preventive strategies for high-risk populations, while showing the need for further mechanistic studies and clinical translation.

Declarations:

Ethical considerations: All animal handling and experimental procedures were conducted in accordance with and adhered to the ARRIVE (Animal Research: Reporting of In Vivo Experiments) guidelines. Ethical approval was sought from the Animal Care and Use Research Ethics Committee (ACUREC) at Bayero University, Kano, where animal use protocol number (AUP): BUK/ACUREC/CAP/PG46 was assigned.

Authors' contributions: Karimah Mohammed Rabiu and Lawal Nura conceptualized the study and study design. Karimah Mohammed Rabiu conducted experiments, and drafted the manuscript. Lawal Nura conceived the original idea, provided guidance and reviewed the manuscript. Aderounmu I. Ganiyu provided additional guidance, analysed data, and offered insights. All authors approved the final manuscript.

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