Original Article

The Possible Protective and Therapeutic Role of Aqueous Extract of Avocado Seeds on Experimentally Induced Prostate Cancer in Adult Male Albino Rats

Noha Mohamed, Ahmed Zolfokar, Neveen El-Sherif and Marwa Al-Gholam

Department of Anatomy and Embryology, Faculty of Medicine, Menoufia University, Egypt

ABSTRACT

Objectives: Prostate cancer is the second most common cancer in men. So, this work aims to study the effect of avocado seeds aqueous extract in the protection and treatment of the histopathological changes that happened in experimentally induced prostate cancer rat model.

Material and Methods: Eighty (80) adult male albino rats of Wistar strain were divided randomly into 5 groups: Group I: Control group (n= 10) and was subdivided into subgroups IA and IB. Group II: Aqueous extract of avocado seeds (n = 10): received aqueous extract of avocado seeds (500 mg/kg/day orally) for 10 weeks. Group III: Prostate cancer group (n = 20). Group IV: Protected group (n = 20): received aqueous extract of avocado seeds (500 mg/kg/day orally) for 10 weeks then prostate cancer was induced. Group V: Treated group (n = 20): Prostate cancer was induced first and rats with prostatic specific antigen (PSA) more than 20 ng/ml were selected and received aqueous extract of avocado seeds (500 mg/kg/day orally) for further 10 weeks. Then the ventral prostate lobes were subjected to biochemical, histopathological, immunohistochemical, morphometric, and statistical analysis.

Results: Avocado seeds aqueous extract significantly protected against the prostate cancer as the protected group showed marked histological improvements and significant (p<0.001) decrease in the PSA, Interleukin-8 (IL-8), Tumor Necrosis Factor α (TNF-α), and Dihydrotestosterone (DHT) levels when compared to prostate cancer group, but it had no effect in treating the prostate cancer.

Conclusion: Avocado seeds aqueous extract plays an impressive important role as a chemo preventive agent, but it is useless in treating the prostate cancer.

Received: 31 December 2021, **Accepted:** 30 March 2022

Key Words: Avocado seeds aqueous extract, prostate cancer, wistar albino rats.

Corresponding Author: Noha Mohamed Abd AL Aziz, MSc, Department of Anatomy and Embryology, Faculty of Medicine, Menoufia University, Egypt, **Tel.**: +20 04 8231 1847, **E-mail:** hashema877@yahoo.com

ISSN: 1110-0559, Vol. 46, No. 3

INTRODUCTION

Prostate Cancer (PCa) is the one of the most common cancers in men, about 1.1 million men worldwide were diagnosed with PCa[1].

About 1 man in 39 will die of prostate cancer, and about 1 man in 7 will be diagnosed with prostate cancer during his lifetime[2].

Many risk factors are responsible for developing PCa like race, family history, diet, intrauterine conditions, hormone exposure and inflammation[3].

Prostatic carcinoma is an ideal disease for chemo-preventive interventions. So, it is become important to develop chemoprevention agents that may decrease prostate cancer risk, delay, or prevent surgery or chemo-radiotherapy[4].

7, 12-Dimethylbenz (a) anthracene (DMBA) is a potent laboratory carcinogen. It can induce tumors in many organs of the experimental animals as skin, mammary gland, and prostate[5].

Avocado, known as "Persea americana", is a greenish pear-shaped fruit. Its components are rich in nutrients and phytochemicals[6]. It was considered a high source for proteins, unsaturated fats, iron, potassium, phosphorus, and many vitamins as C and E[7].

Many studies reported the potent anti-cancer, anti-diabetic and anti-inflammatory, activities of avocado seed preparations[8].

Avocado seeds extract induces cytotoxic and apoptotic activities[8]. Avocado seed contains 57% of the antioxidant capacities and 64% of the phenolic compounds among different parts of avocado fruit[9].

Consequently, the aim of this study was to demonstrate the protective and therapeutic effects of avocado seeds aqueous extract on experimentally induced prostatic adenocarcinoma in adult male wistar albino rats.

MATERIALS AND METHODS

Materials

Testosterone propionate (Tp)

a product of (Sigma Chem. Co., St. Louis, Mo, USA) is available in a vial form (250gm/ 1ml).

 DOI: 10.21608/ejh.2022.113931.1625

Cypretrone acetate

A product of (Sigma Chem. Co., St. Louis, Mo, USA) is available in a form of tablets (100 mg and 250 mg).

7, 12-Dimethylbenz (a) anthracene (DMBA)

Aproduct of (Sigma Chem. Co., St. Louis, Mo, USA) is available in 1g in serum bottle.

Aqueous extract of avocado seeds

Samples of ripe avocado pear was purchased from local market. The fleshy part of the fruit was removed. The seeds were dried in an oven at 50°C. It was ground to powder. Hundred grams (100g) of the sample was soaked in 1000ml water (100%) for 5 days. The final extracts were filtered by Whatman (No.1) filter paper. The filtrates were concentrated under vacuum using a rotary evaporator at 40 °C and stored at 4 °C[10]. The extract was prepared at Faculty of Science, Menoufia University, Egypt.

Animals and experimental design

Eighty (80) adult male wistar albino rats (10-12 weeks old, weighing 180-200 g) were used in this study. The animals were kept under controlled conditions of temperature and humidity and provided with water and a balanced diet. The procedure was according to the ethics committee on the animal experiment of the Faculty of Medicine, Menoufia University, Egypt.

The animals were divided into 5 groups as follows:

Group I control group (n= 10): consisted of 10 rats and were subdivided into 2 subgroups:

- Subgroup IA (n=5): animals were fed a regular diet.
- Subgroup IB (n = 5): The rats were injected with a single intra-peritoneal solution contained equal percentage of sesame oil and saline.

Group II avocado seeds extract (n=10): received aqueous extract of avocado seeds (500mg/kg/day orally) daily for 10 consecutive weeks[10].

Group III: PCa induced group (n = 20).

Group IV: Protected group (n = 20): received aqueous extract of avocado seeds (500 mg/kg/day orally) daily for 10 consecutive weeks (10) then stopped and prostate cancer was induced[11].

Group V: Treated group (n = 20): Prostate cancer was induced first and rats with PSA more than 20 ng/ml were selected[12]. Twelve rats were selected and received aqueous extract of avocado seeds (500 mg/kg/day orally) daily for further 10 consecutive weeks[10].

Prostate Cancer Induction Protocol

The rats received daily gavage administration of the cyproterone acetate (50mg/kg/d) for 21 days. One day after the last dose of cyproterone acetate, rats received testosterone propionate (100mg/kg/d) subcutaneously dissolved in 2 cm corn oil for another 21 days. One day after the testosterone propionate injection, all the rats received a single intraperitoneal administration of 7, 12-Dimethylbenz (a) anthracene (DMBA) (65 mg/kg) dissolved in 1 cm sesame oil added to 1 cm saline. After four weeks of induction of prostate cancer, blood samples were taken from the tail vein of rats and prostatic specific antigen (PSA) was assayed to determine the occurrence of prostate cancer[11,12,13].

At the end of the experiment, rats were weighed, and blood samples were collected, the rats were anesthetized with pentobarbital (30 mg/kg, intra-peritoneal injection, I.p.)[14]. Prostate tissues were immediately separated. The ventral prostate lobes were weighed, and part of each lobe was fixed with paraformaldehyde for histological and immunohistochemical analysis, the remaining prostate sections were stored at -80 ºC for tissue biochemical analysis, another part was fixed in gluterlaldehyde for electron microscopic analysis.

Biochemical study

1. PSA Plasma levels were estimated by enzyme immunoassay technique[15].
2. ELISA Assay of prostate Interleukin-8 (IL-8), Tumor Necrosis Factor α (TNF- α), and Dihydrotestosterone (DHT) levels were determined using an Enzyme-Linked Immunosorbent Assay (ELISA) kit according to the manufacturer's instructions (ALPCO Diagnostics, Salem, NH, USA)[16]
3. Catalase enzyme (CAT) activity in the prostate was determined by a commercially available kit (Beyotime, Nantong, China). MDA Concentration in prostate was detected by a commercial kit (AMEKO Inc., Shanghai, China) following the manufacturer's instructions[2].

Histological study

Light microscope

1. Haematoxyline and Eosin stain: routine stain for histological examination.
2. Mallory's trichrome (MT) stain: special stain for detection of collagen fiber.

Transmission Electron Microscope (TEM)

Samples of ventral prostate tissues were fixed in 2.5 % glutaraldehyde and then 1% osmium tetroxide was added. Semi-thin (1µm) sections were stained with 1% toluidine blue and examined using an Olympus BX61 light microscope. At thickness 1 mm, ultra-thin sections have been cut and then stained with 2.5% uranyl acetate. The examination of ultrathin sections has been done using JEM-2100 (JEOL, Japan) transmission electron microscope at the electron microscope unit at Faculty of Agriculture, Mansoura University.

Immunohistochemical studies

1. High-molecular weight cytokeratins (HMWCK): with anti CK5/6 antibody (mouse monoclonal antibody; ab86974, Abcam, Cambridge, UK). They have proved to be useful markers of basal epithelial cells which include antibodies to specific CK subsets such as CK5⁄6. Cytoplasm of prostate basal cells were stained dark brown.
2. Transforming growth factor-β1 (TGF-β1): with anti-TGF-B1antibody (rabbit monoclonal antibody; ab215715, Abcam, Cambridge, UK). It increased in neoplastic prostatic epithelium when compared to normal prostate tissue. Cytoplasm of stromal cells and/or cytoplasm of epithelial cells showed dark brown reactivity.
3. Activated Caspase-3: with anti-caspase-3 antibody (rabbit monoclonal antibody; ab32150, Abcam, Cambridge, UK). The cytoplasm and nuclei of these cells showed dark brown reactivity.

Morphometric and statistical studies

Six different sections from each rat of all subgroups were examined by image analysis system (Image j program, version 1.43o8, National Institutes of Health, USA and Digimizer version 4.3.5, MedCalc software). This was done at the Faculty of Medicine, Menoufia University. The different parameters including:

- Mean area % of collagen in Mallory's trichrome stained sections.
- Mean area % of positive Caspase-3, CK5⁄6 and TGF-β1 in immune-stained sections.

Mean ± SD was used to present the collected data. Data analysis was carried out by an IBM compatible personal computer with SPSS statistical package version 23 (SPSS Inc. Released 2015. IBM SPSS statistics for windows, version 23.0, Armnok, NY: IBM Corp.). The obtained data were analyzed using one way-ANOVA. The results were considered statistically significant when the *p-values* were <0.05.

RESULTS

There was no significant difference between the subgroups IA and IB in all results; therefore, they were considered as a control group and the group IA was considered the standard control group for other study groups.

General results

Control and avocado extract groups showed a non-significant difference ($p>0.05$) in their final weight when compared to their initial one. Both prostate cancer and treated groups showed a significant ($p<0.001$) decrease in their final body weight when compared to their initial ones. The protected group showed a non-significant decrease ($p>0.05$) in their final weight when compared to their initial one (Table 1).

PCa and treated groups showed a significant increase ($p <0.001$) in the ventral prostate weight when compared to the control group. protected group showed a significant decrease of its ventral prostate weight ($p <0.001$) when compared to the prostate cancer or treated groups (Table 2).

Biochemical results

PCa and treated groups showed a highly significant increase ($p <0.001$) in their PSA levels when compared to the control group. However, the protected group showed a great decrease in the PSA level significantly ($p <0.001$) when compared with the prostate cancer or treated groups (Table 2).

PCa and treated groups showed a highly significant increase ($p <0.001$) in Interleukin-8 (IL-8), Tumor Necrosis Factor α (TNF-α) and Dihydrotestosterone (DHT) levels in the prostate tissue. However, their levels decreased significantly ($p <0.001$) in the protected group when compared with the prostate cancer or treated groups (Table 3).

PCa and treated groups showed a significant decrease ($p <0.001$) in the CAT activity when compared to the control group. However, the protected group showed a significant increase ($p <0.001$) in the CAT activity when compared to prostate cancer or treated groups. But prostate cancer and treated groups showed a significant increase ($p <0.001$) in the MDA level when compared to the control group. However, the protected group showed a significant decrease in the MDA level ($p <0.001$) when compared with the prostate cancer or treated groups (Table 4).

Histological results

The control subgroups were summed and referred to as the control group, also, all sections of the control and avocado seeds extract animals showed the same histological pictures. So, the photomicrographs of the two groups were presented by one group of them: the control group.

Light microscopic study

H&E-stained sections: Control group showed a normal prostate architecture. The prostatic parenchyma was formed of simple regular acini with different sizes. The acini were enclosing a lumen containing eosinophilic and homogeneous secretions. They were separated by few fibromuscular stroma. They were lined by a single layer of simple columnar epithelial cells with abundant basophilic cytoplasm and basophilic rounded and regular basal nuclei. They were also lined with basal cells located in the lower parts of the epithelium directly above the basement membrane. The basement membrane was encircling the acinus appeared intact (Figure 1: A-D).

PCa group represented major pathological changes: The acini showed an abnormal architectural glandular pattern. They showed irregular growth and distribution. Hyperproliferative epithelial sites were seen in the glandular epithelium, also long papillary projections were noticed inside the lumen. Nuclear enlargement and prominent

nucleoli were present. The acini contained abundant intraluminal secretions. The basement membranes were thin, irregular, and not intact in some acini. The interstitial stroma showed severe congested blood vessels infiltration (Figure 2: A-D).

Protected group, the irregularity of the acini decreased dramatically. The acini showed few papillary projections and contained intraluminal secretions. The lining epithelium of the acini showed atrophic changes. The basement membranes were regular and intact in most of the acini. The interstitial stroma showed mild inflammation. The lining epithelium having basophilic nucleus with focal hyperplastic changes (Figure 3: A-D).

Treated group, the acini still showed irregular growth and distribution with extended intra acinar papillary projections and contained abundant intraluminal secretions. The interstitial stroma showed marked inflammation. Basement membranes were irregular. Hyperproliferative epithelium were also noticed. Nuclear enlargement and prominent nucleoli were present (Figure 4: A-D).

Mallory's Trichrome-stained sections: PCa and treated groups showed a significant increase (p <0.001) in the amount of collagen fibers deposition in the fibro-muscular stroma However, the protected group showed a significant decrease (p <0.001) in this amount (Figure 5, Histogram 1).

Transmission Electron Microscopic study

Ventral prostate of control showed that the acini were lined by single layer of simple columnar cells containing basally located nucleus with an intact envelope and condensed chromatin at the periphery. Basal cells were triangular and integrated within the epithelial cells with basal rounded nucleus. The cellular cytoplasm showed a clearly visible supranuclear region. The Golgi complex was well developed. The mitochondria appeared to be normal in shape and distribution. Secretory vesicles were also noticed. Homogeneous flocculent secretion was observed in the lumen. Junctional complex was present between the cells. The basement membrane was intact and well defined. Small microvilli were homogeneously distributed on the cell surface (Figure 6: A,B).

PCa induced group revealed that the epithelial cells lining the acini ware stratified with hyperplastic changes and extreme ultra-structural polymorphism in which some cells had variable sized, and irregular nuclei, other cells were destroyed and devoid of nuclei. The cancer cells illustrated an irregular heterochromatic large nucleus and often with a prominent nucleolus. Indentations and lobulations of their nuclei were also noticed. Focal intercellular separation was seen. Mitochondria were swollen with pale matrix. Secretory vacuoles contained abundant secretion materials. The cisterns of the rough endoplasmic reticulum (RER) were dilated (Figure 6: C,D).

Protected group showed that the lining epithelium restored its structural organization, where most sections showed tall columnar cells with basal rounded nuclei, however other cells showed enlarged nucleus. Basal cells were present. Mitochondria showed normal shape and distribution. Well-developed Golgi apparatus was noticed. Microvilli covered the surface of the cell facing the lumen. parallel reticulum cisterns of the rough endoplasmic reticulum were verified (Figure 6: E,F).

In the treated group, the prostatic cells showed extreme variations in the nucleus shape and chromatin as the nucleus was large with a prominent nucleolus. Inter-cellular lacunae were noticed. Pale large mitochondria were also detected. Basal cell with degenerated nucleus was observed (Figure 6: G,H).

Immunohistochemical results

In Caspase-3-stain, prostate cancer and treated groups showed a significant decrease (p <0.001) in the percentage area of caspase-3 when compared to control group. However, the protected group showed a significant increase (p <0.001) in this percentage when compared to PCa and treated groups (Figure 7: A-D, Histogram 2).

In CK5 /6-stain, prostate cancer and treated groups showed a significant decrease (p <0.001) in the percentage area of CK5/6 immunoreaction when compared to control group. However, the protected group showed a significant increase (p <0.001) in this percentage when compared to PCa and treated groups (Figure 7: E-H, Histogram 3).

In TGF- β1-stain, prostate cancer and treated groups showed a significant decrease (p <0.001) in the percentage area of TGF- β1 immunoreaction when compared to control group. But the protected group showed a significant decrease (p <0.001) in this percentage when compared to PCa and treated groups (Figure 7: I-L, Histogram 4).

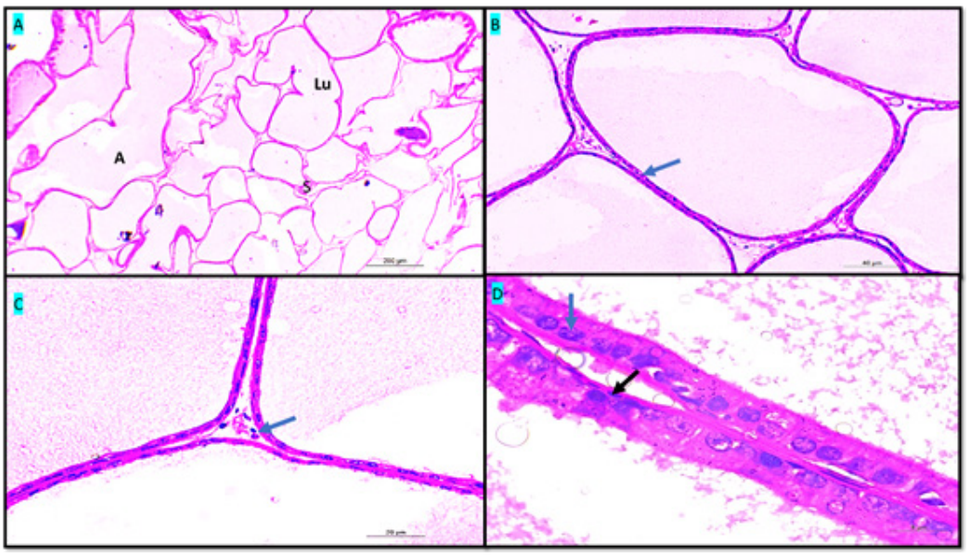


Fig. 1 (A-D): Photomicrographs of H&E- stained ventral prostate sections taken from the control group: The ventral lobe of the prostate of control group is composed of acini with variable sizes and shapes (A). The acini encircling lumen (LU) contains homogenous acidophilic secretion. There is a fibromuscular stroma (S) in between the acini (A) (x40). Prostatic acini lined with single layer of simple columnar to cuboidal epithelial cells (arrow) (B) (x200). The epithelium rests on intact and regular basement membrane (arrow) (C) (x400). The epithelium lined the acini has single basophilic basal rounded nuclei and abundant basophilic cytoplasm (black arrow). The acini also lined with basal cells located in the lower parts of the epithelium (blue arrow). Basement membrane appears regular and intact (black arrow) (D) (x1000).

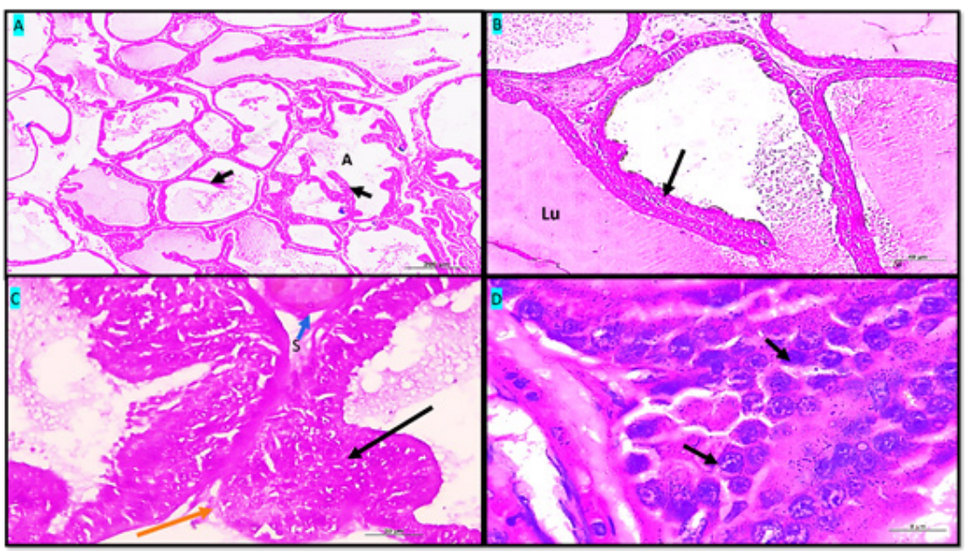


Fig. 2 (A-D): Photomicrographs of H&E- stained ventral prostate sections of PCa group: Prostatic acini (A) are crowded with extended intra acinar villous projections (arrow) (A) (x40). The acinus lined with very long papillary projection (arrow) and abundant luminal secretion (LU) (B) (x200). The prostate cancer group showing epithelial proliferation outside the acinus toward the stroma in the shape of nodule (arrow). Stroma contains congested blood vessels (S). Basement membrane was thin, irregular, and not intact (orange arrow) (C) (x400). The prostate cancer group showing hyperproliferative epithelium with pleomorphic and hyperchromatic nuclei with prominent nucleoli (arrow) (D) (x1000).

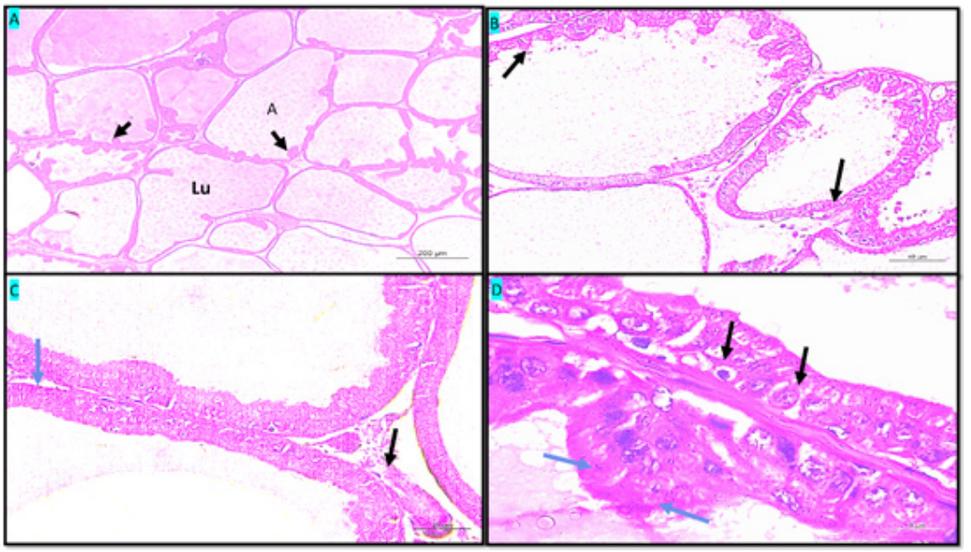


Fig. 3 (A-D): Photomicrographs of H&E- stained ventral prostate sections of the protected group: Prostatic acini (A) lined with few papillary projections (arrow) and homogenous luminal secretion (LU) (A) (x40). The acini lined by atrophic epithelium (B) (x200). The epithelium rests on regular intact basement membrane (blue arrow) and there is mild stromal inflammation (black arrow) (C) (x400). Some acinus lined by tall columnar epithelium with basal basophilic nuclei (black arrows), another showed epithelial proliferation with hyperpigmented and enlarged nuclei (blue arrows) (D) (x1000).

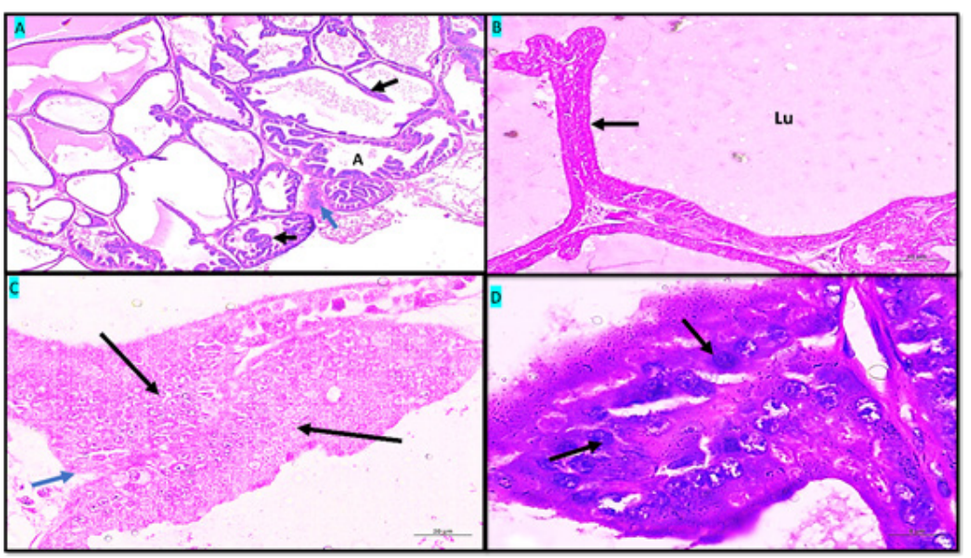


Fig. 4 (A-D): Photomicrographs of H&E- stained ventral prostate sections of the treated group: Prostatic acini (A) crowded with multiple papillary projections (black arrows). The interstitial stroma showed marked inflammation (blue arrow) (A) (x40). The acinus lined by long papillary projection (arow) and abundant luminal secretion (LU) (B) (x200). Hyperproliferative epithelium (black arrows) and irregular basement membrane (blue arrow) were noticed (C) (x400). The acinus showing hyperproliferative epithelium with large nuclei and prominent nucleoli (arrows) (D) (x1000).

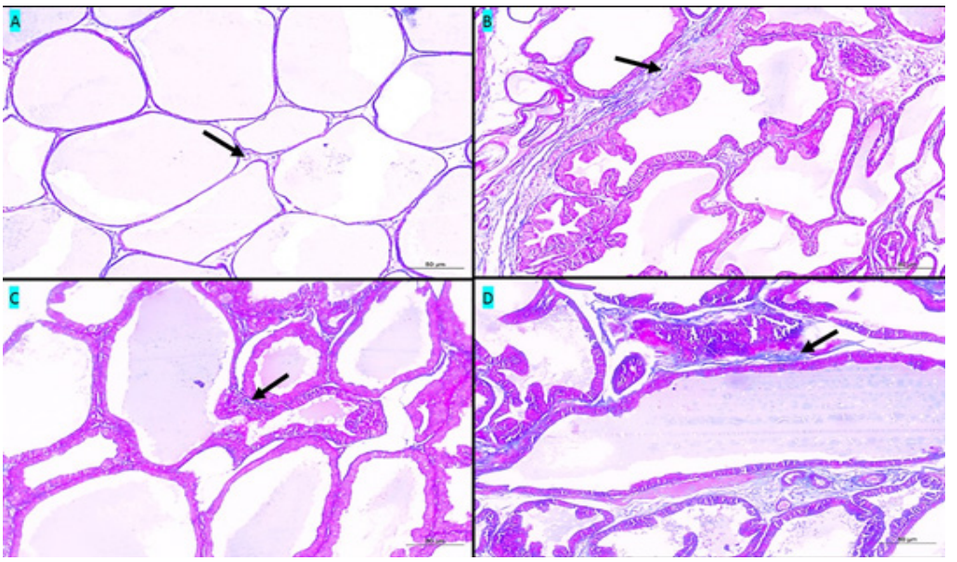


Fig. 5 (A-D): Photomicrographs of the Mallory Trichome-stained ventral prostate sections in all experimental groups (x100): Control group contains minimal amount of collagen fiber in between the acini (arrow) (A). PCa group contains large amount of collagen fiber in between the acini (arrow) (B). Protected group contains few amounts of collagen fiber in between the acini (arrow) (C). Treated group contains great amount of collagen fiber in between the acini (arrow) (D).

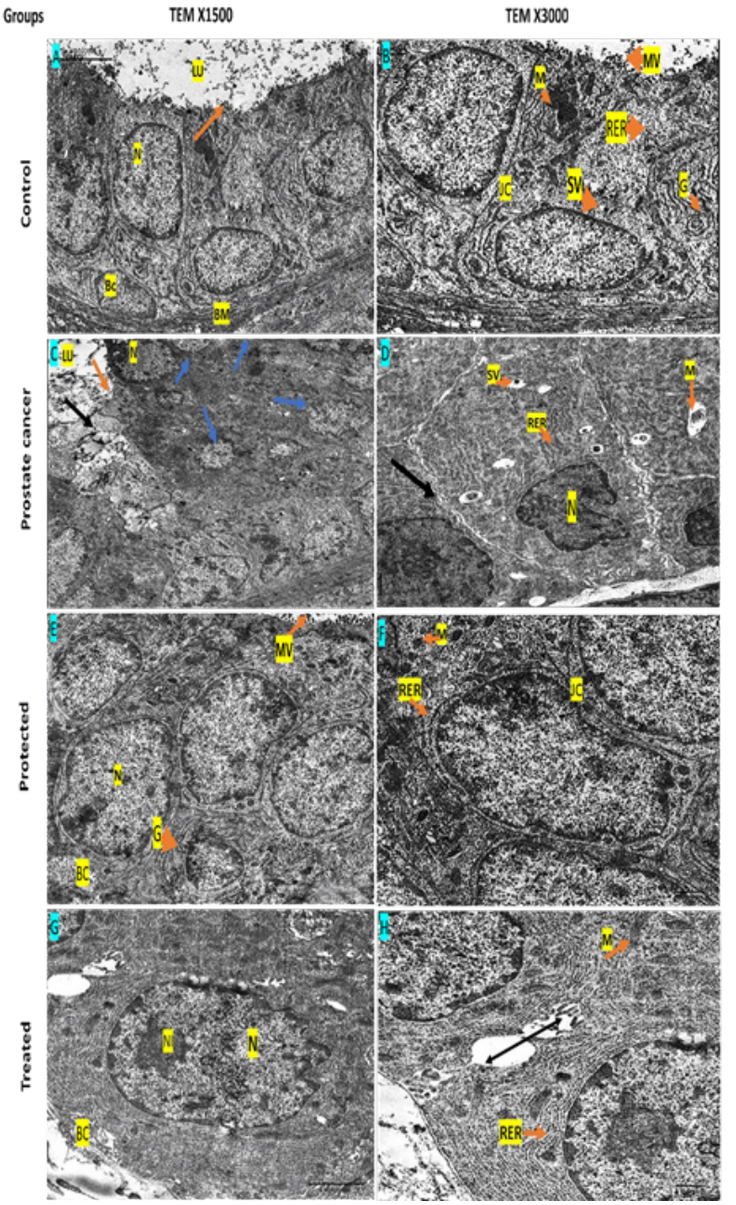


Fig. 6 (A-D): Transmission electron micrographs of the ventral prostate sections in all experimental groups: Control group showing portion of acinus composed of columnar epithelial cells with basal euchromatic nucleus (N). Basal cells are intermingled within epithelial cells (BC). The acinus encircling lumen (LU) contained homogeneous secretion. The basement membrane (BM) is regular (A). The cytoplasm contains normal shape and distribution of mitochondria (M), well-developed Golgi apparatus (G) and many rough endoplasmic reticulum (RER). Moderate number of secretory vesicles (SV) and many microvilli (MV) covered the surface epithelium are noticed. The Junctional complex (JC) between the cells is present (B).

In PCa group, the cells are destroyed with dissolved cytoplasm (black arrow). Many fragmented bodies are noticed in the lumen (LU). The acinus showing an area of epithelial stratification (blue arrows) (C). The lining epithelium showing irregular, hyperchromatic and indented nucleus (N), many secretory vesicles (SV), and dilated rough endoplasmic reticulum (RER). Separation between some cells is present (black arrow) (D).

Protected group showing the lining epithelium that restored its structural organization. The columnar cells have basally located nuclei (N), normal basal cells (BC) and well-developed Golgi apparatus (G). Microvilli (MV) are detected on the surface epithelium (E). The cytoplasm has abundant RER, few mitochondria (M) and intact junctional complex (JC) (F).

Treated group showing acinus with hyperchromatic nucleus (N) with prominent nucleolus (Nl) and basal cell with degenerated nucleus (BC) (G). Randomly distributed mitochondria (M) with pale matrix, dilated RER and massive intercellular separation are noticed (black arrow) (H).

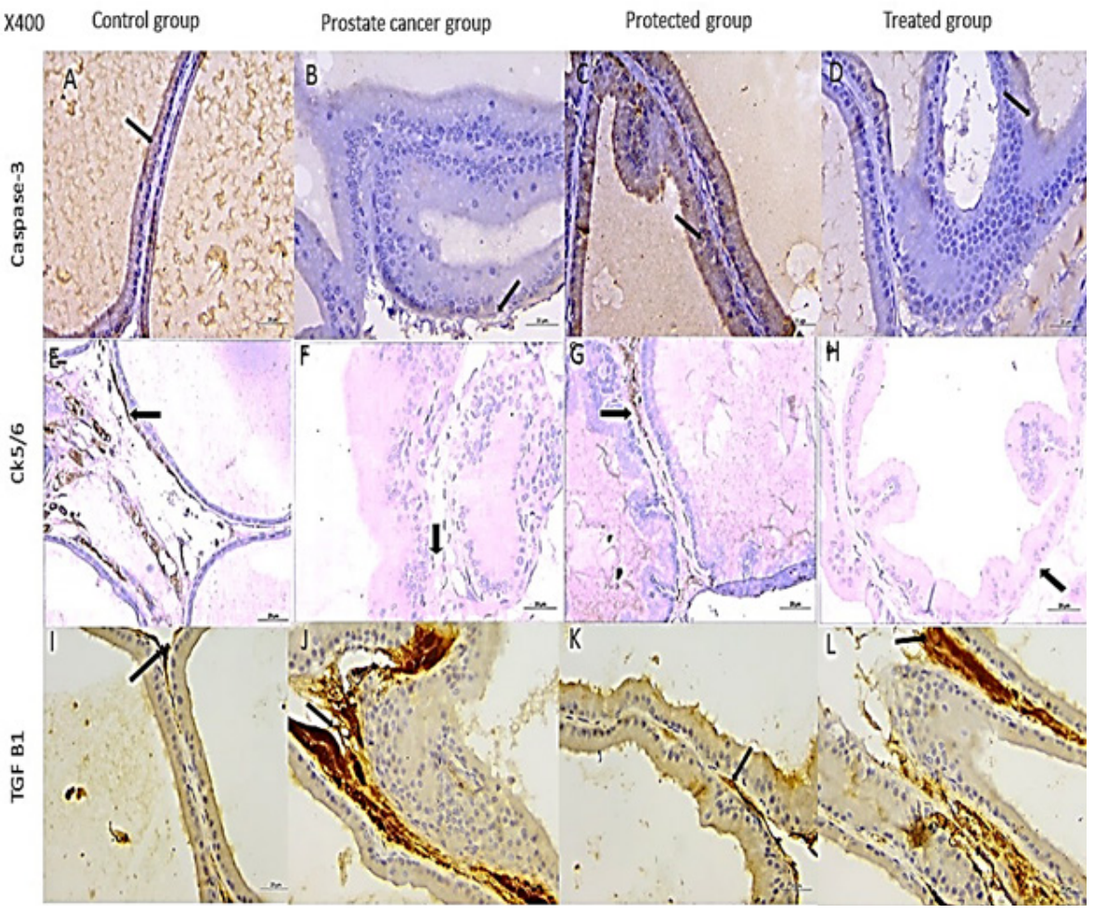


Fig. 7: Photomicrographs of Caspase-3, CK5/6 and TGF-B1 immuno-stains (x400): Caspase-3 (A-D): Strong positive immuno-staining in the control group (arrow) (A). Negative reaction in PCa group (arrow) (B). Moderate positive reaction in the protected group (arrow) (C). Negative reaction in the treated group (arrow) (D).
CK5/6 (E-H): Positive immuno-staining in the control group (arrow) (E). Negative reaction in PCa group (arrow) (F). Moderate positive reaction in the protected group (arrow) (G). Negative reaction in the treated group (arrow) (H).
TGF B1 (I-L): Mild positive immuno-staining in the control group (arrow) (I). Intense positive immuno-staining in the PCa group (arrow) (J). Mild positive reaction in the protected group (arrow) (K). Strong positive reaction in the treated group (arrow) (L).

Table 1: Mean initial and final body weight of all rats (in grams)

Group/Subgroup	Initial weight (gm.) $\bar{X}$±SD	Final weight (gm.) $\bar{X}$±SD
Control (Group I)	187.46±2.47	198.09±2.81*
Avocado seeds extract (Group II)	183.44±4.01	186.10±2.35*
Prostate cancer (Group III)	187.59±2.03	123±6.46**
Protected (Group IV)	183..73±2.19	182.22±2.75
Treated (Group V)	184.76±2.47	120.73±2.60**

Foote notes:
$\bar{X}$= the mean value. SD= the standard deviation. gm. = grams.
* Significant increase (p <0.05) from the initial body weight.
** Significant decrease (p <0.001) from the initial body weight.

Table 2: Mean ventral prostate weight (gm.) and mean serum prostatic specific antigen (PSA) level of all rats (ng/ml)

Group/Subgroup	Prostate weight (mg.) $\bar{X}$±SD	PSA (ng/ml) $\bar{X}$±SD
Control (Group I)	0.180±0.023	1.96±0.603
Avocado extract (Group II)	0.185±0.005	1.85±0.778
Prostate cancer (Group III)	0.704±0.124**	51.19±18.47**
Protected (Group IV)	0.358±0.043♦♦ ◦◦	23.98±6.90♦♦ ◦◦
Treated (Group V)	0.683±0.087**	44.74±14.15**

Foote notes:
$\bar{X}$= the mean value. SD= the standard deviation. mg. = milligrams. ng/ml: nanogram/milliliter.
** Significant increase (p <0.001) from the control group.
♦♦ Significant decrease (p <0.001) from the prostate cancer and treated groups.
◦◦ Significant decrease (p <0.001) from the treated group.

Table 3: Mean tissue level of Interlukein-8 (IL-8) (pg/ml), Tumor Necrosis Factor- α (TNF-α) (ng/l) and Dihydrotestosterone (DHT) (pg/ml)

Group/Subgroup	IL-8 (pg/ml) $\bar{X}$±SD	TNF- α (ng/l) $\bar{X}$±SD	DHT (pg/ml). $\bar{X}$±SD
Control (Group I)	5.02±0.301	11.97±0.467	194.80±10.40
Avocado extract (Group II)	4.69±0.242	11.35±0.462	192.10±7.51
Prostate cancer (Group III)	26.41±0.872**	73.11±4.53**	597.50±51.16**
Protected (Group IV)	15.001±0.306♦♦ ◦◦	29.42±4.53♦♦ ◦◦	304.70±19.98♦♦ ◦◦
Treated (Group V)	23.36±1.47**	66.14±4.36**	519±72.97**

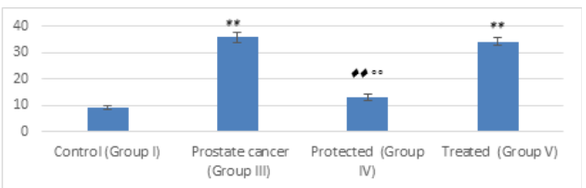
Foote notes:
$\bar{X}$= the mean value. SD= the standard deviation. Pg/ ml. = picogram/milliliter. ng/l: nanogram/liter.
** Significant increase (p <0.001) from the control group.
♦♦ Significant decrease (p <0.001) from the prostate cancer and treated groups.
◦◦ Significant decrease (p <0.001) from the treated group.

Table 4: Mean tissue level of Antioxidant (CAT) activity (µmol/mg.) and lipid peroxidation (MDA) concentration (nmol/mg.) of all rats

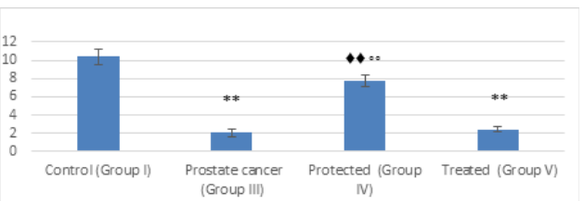
Group/Subgroup	CAT activity (µmol/mg.) $\bar{X}$±SD	MDA level (nmol/g.) $\bar{X}$±SD
Control (Group I)	50.70±2.26	30.41±1.62
Avocado extract (Group II)	50.40±1.84	29.35±2.81
Prostate cancer (Group III)	14.43±1.05**	71.55±2.12**
Protected (Group IV)	34.66±1.50 ◦◦♦♦	44.74±1.85 ◦◦♦♦
Treated (Group V)	17.66±1.50**	69.49±1.0**

Foote notes:

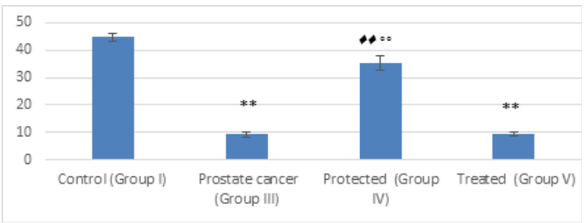
$\bar{X}$= the mean value. SD = the standard deviation. mg. =milligram. µmol = micro mole nmol = nano mole
** Significant increase (p <0.001) from the control group.
♦♦ Significant decrease (p <0.001) from the prostate cancer and treated groups.
◦◦ Significant decrease (p <0.001) from the treated group.



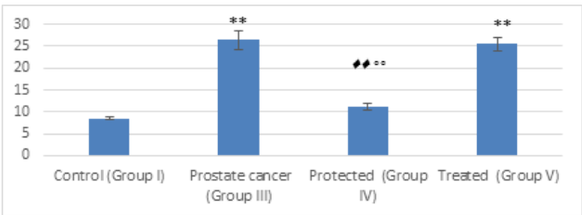
Histogram 1: Mean initial and final body weight of all rats (in grams)



Histogram 2: Mean ventral prostate weight (gm.) and mean serum prostatic specific antigen (PSA) level of all rats (ng/ml)



Histogram 3: Mean tissue level of Interlukein-8 (IL-8) (pg/ml), Tumor Necrosis Factor- α (TNF-α) (ng/l) and Dihydrotestosterone (DHT) (pg/ml)



Histogram 4: Mean tissue level of Antioxidant (CAT) activity (µmol/mg.) and lipid peroxidation (MDA) concentration (nmol/mg.) of all rats

DISCUSSION

The incidence of prostate cancer is increasing worldwide. several attempts were done to convert it to a preventable disease. Avocado seed is a natural substance and was proved to have anti-inflammatory and anti-cancerous effects[17]. So, this study was performed to demonstrate the role of avocado seed aqueous extract whether protective or therapeutic agent against prostate cancer.

Rat was the animal of choice for induction of prostate cancer in this study. The rat was first used as a model of prostate cancer in 1937[18]. Prostate carcinogenesis in rats and men is controlled by similar molecular mechanisms, making it a valuable model for this study[1].

Samples from ventral prostate lobes were selected as it is the largest lobe, constituting approximately half of the mass of all the prostatic tissue, and is most easily separated from the rest of the prostate[19].

In the present study, the elevated serum PSA levels more than 20 ng/ml judged the occurrence of prostate cancer; this was in accordance with[2] who proved that PSA is a reliable marker for prostate cancer. Prostate cancer was also supported by the highly significant reduction in the final body weight of this group when compared to the initial one. A significant reduction in the body weight may be due to the formation of hydroxylated bases of DNA[20].

In the present study: the PCa group revealed a significant increase in the prostate weight. Administration of testosterone significantly increased the prostate weight in prostate cancer induced rats when compared to controlled rats.[21] postulated that an increase in the ventral prostatic weight were due to induction of fibromuscular tissue and the massive multiplication of the prostate epithelium caused by testosterone administration.

However, using avocado seeds aqueous extract in protected group decrease the ventral prostate weight significantly. The decrease in the prostate weight was explained by the decreases in the epithelial hyperproliferation, stromal collagen fibers and dihydrotestosterone tissue level.

Furthermore, the PCa group showed a significant increase in the tissue levels of TNF-α and IL-8. This explained by the sever inflammation and congestion happened in the prostate tissue. This was in accordance with[22,23] who postulated the role of TNF-α and IL-8 in the progression of PCa as an important inflammatory cytokine.

However, there was a significant decrease in PSA, TNF-α and IL8 levels in the protected group. This might suggest the anti-cancer and anti-inflammatory actions of aqueous extract of avocado seeds.[24] reported that avocado has significant anti-inflammatory properties. Phenolic compounds found in avocado were responsible to reduce inflammation, and platelet aggregation. Also,[25] the potential anticancer effect of the avocado seeds was due to presence of carotenoids, and phenolics compounds[24].

Furthermore, the prostate cancer induced group showed a significant increase in the tissue levels of dihydrotestosterone (DHT). DHT is the most active androgen; it stimulates prostate cancer cells to grow[26]. This was in accordance with[27] who reported that prostate cancer is caused by DHT, a metabolite obtained from the conversion of testosterone by 5α-reductase enzyme.

However, there was a significant decrease in the DHT level in the protected group. The decrease in the DHT explained by the marked decrease that happened in the epithelial and stromal growth when avocado seeds extract was used. This might suggest the anti-5 alpha reductase inhibitor of aqueous extract of avocado seeds.[28] reported that plants contained polyphenols had anti-5 alpha reductase activity that led to a significant reduction in DHT concentrations.

In the present work, comparing with the control group, the prostate cancer group showed marked increase in the peroxidative activity by a significant increase in the MDA level and showed marked decrease in the antioxidant activity by a significant decrease in the CAT level. This explained the hyperplasia occurred in the prostate tissue. This was in accordance with[29] who stated that there were significant alterations in oxidant-antioxidant balance in prostate cancer group and reported strong evidence between the oxidative stress and the pathogenesis of prostate cancer.

However, there was such regulation of the oxidative stress markers and increase in the antioxidant capacity caused by avocado seeds extract in the protected group by a significant increase in the activity of CAT enzyme and a significant decrease in the MDA level. This was in accordance with[30] who reported that avocado extracts have great antioxidant capacity through their high phenolic compounds[31] also postulated that the oxygenated carotenoids are the essential antioxidants extracted from the avocado extracts.

In the present study, PCa was further evidenced by the massive pathological changes affecting the ventral prostate. The acini were with irregular growth and distribution. Epithelial proliferation resulted in very long papillary projections. The acini contained abundant secretions. There was also hyperpigmented and irregular nuclei, and nucleolar hypertrophy. Hyperplasia of the epithelium was further evidenced in this study by the transmission electron microscopic results that showed stratifications in their lining epithelial cells. This was in accordance with[32] who observed hyperproliferative epithelium with characteristic foci of prostatic intraepithelial neoplasia (PIN).

However, the protected group showed improvement of the disrupted prostate histological patterns when aqueous extract of avocado seeds used as a protective agent against prostate cancer. The irregularity of the acini decreased dramatically. The acini also showed few papillary projections. The lining epithelium of the acini showed atrophic changes. The basement membranes were regular and intact in most of the acini. The interstitial stroma showed mild inflammatory infiltration. This suggested the anti-proliferative effects of avocado seeds.[33] reported that avocado seed extract has significant inhibition on both human lung A549 and human gastric BGC823 cancer cell proliferation.

In the current study, the prostate cancer group showed fibrosis in fibro-muscular stroma in between the acini, this was evidenced by the highly significant increase of the percentage of collagen fiber deposition in the latter regions. This was explained by the increased DHT level in the prostate tissue. Testosterone stimulates fibromuscular tissue to be developed aggressively[32].

However, there was a significant decrease in the collagen fiber deposition in fibro-muscular stroma of the protected group when compared to the prostate cancer induced one. This might suggest the anti-fibrotic action of aqueous extract of avocado seeds. This could be explained as avocado seeds extract caused regression of inflammation with subsequent regressed fibrosis[34].

In the present work, the prostate cancer induced group showed a marked reduction of immunoreaction for CK5/6 stain which might prove absence of basal cells. Basal cells defect was also noticed by electron microscopes in this study. This agreed with[35] who postulated that the diagnosis of prostate malignancy is often based on the absence of basal cells. Also,[36] reported that focal loss or attenuation of the basal cell layer promotes prostate cancer.

In the present work, prostate cancer induced group showed strong immuno-staining for TGF-ß1. This was agreed with[34] who mentioned that TGF-ß1 levels were much higher in prostate adenocarcinomas. TGF-β1 is an important factor in tumor genesis. It is a potent modulator of cell proliferation, differentiation, angiogenesis, and the immune system[37].

However, the protected group showed positive immunoreaction for CK5/6 and mild positive immunoreaction for TGF-ß1 stains. This may suggest the anticancer effect of avocado seeds extract. This was agreed with[31] who reported that avocado seeds extracts exhibited anticancer activities and demonstrated a significant inhibition of hepatocellular carcinoma and colon cancer[38] also concluded that avocado considered a very favorable source in treating cancers.

In the current study, prostate cancer induced group showed a highly significant decrease in the Caspase-3 positively stained surface area when compared to the control group. Caspases are expressed in normal prostate secretory epithelial cells[39]. This was agreed with[32] who observed the down regulation of caspase-3 in the prostate cancer tissue compared to control so, dysregulation of apoptosis represents an important mechanism of prostate carcinogenesis as Caspases are essential for induction of apoptosis. This explains the epithelial hyperproliferation that happened in the prostate cancer.

However, the protected group showed a significant increase in the Caspase-3 positively stained surface area when compared to the prostate cancer induced group. This could suggest the apoptotic effect of aqueous extract of avocado seeds. This was in accordance with[40] who postulated that phytochemicals extracted from avocado seeds have an amazing effect in cell cycle arrest and growth inhibition of cancer cells through stimulation of apoptosis.

On the contrary, the treated group did not show any impressive improvement compared with the protected groups. Treated group showed minimal improvement of the histopathological results. There was a significant increase in the ventral prostate weight. There is also a significant increase in the PSA plasma level and (IL-8, TNF-α and DHT) tissue levels. MDA level was significantly increased while CAT level was significantly decreased. CK5/6 and Caspase-3 showed a significant decrease in the immuno-positive surface area. However, TGF-ß1 showed a significant increase in the immuno-positive surface area when compared to the protected group. This was in accordance with[41] who suggested that the cancer preventive effect of avocado extract is through inhibition of tumor progression, rather than reversing existing malignant changes induced by carcinogen. Also, this is consistent with the findings by[42] in cancer prevention studies who found that inhibition tumor formation occurred when agents were applied before and during carcinogens treatment, but these agents had no effect when applied to already established lesions.

CONCLUSION

This study revealed the chemo-preventive (protecting role), anti-inflammatory, anti-fibrotic and antioxidant proprieties of avocado seeds aqueous against prostate cancer, but it is useless when used alone as a treatment for induced prostate cancer.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Nascimento-Gonçalves E, Faustino-Rocha AI, Seixas F, Ginja M, Colaço B, Ferreira R, Fardilha M and Oliveira PA: Modelling human prostate cancer: Rat models. Life sciences. (2018) 203:210-24.
2. Saritha S, Prakash T, and Ghatamaneni, DJ: Effect of Taraxerol on Testosterone Induced Prostate Cancer in Albino Wistar Rats Clin Exp Pharmacol. (2018) 4: 90-96.
3. Fong LY, Jing R, Smalley KJ, Wang ZX, Tacciolim C, Fan S, Chen H, Alder H, Huebner K, Farber JL, Fiehn O and Croce CM: Human-like hyperplastic prostate with low ZIP1 induced solely by Zn deficiency in rats. Proc Natl Acad Sci U S A. (2018) (47): 20-115.
4. Chhabra G, Singh CK, Ndiaye MA, Fedorowicz S, Molot A and Ahmad N: Prostate cancer chemoprevention by natural agents: Clinical evidence and potential implications. Cancer letters. (2018) 37(1): 52-57.
5. Sung YM, He G and Fischer SM: "Lack of Expression of the EP2 but not EP3 Receptor for Prostaglandin E2 Results in Suppression of Skin Tumor Development". Cancer Res. (2005) 65: 9304–9311.
6. Salazar-López NJ, Domínguez-Avila JA, Yahia EM, Belmonte-Herrera BH, Wall-Medrano A, Montalvo-González E and González-Aguilar G A: Avocado fruit and by-products as potential sources of bioactive compounds. Food Research International. (2020) 138: 109774.
7. Hamouda AF, Sameeh MY, Randa M and Shrourou RM: Effect of Avocado (Persea Americana), Cabbage (Brassica Oleracea) and Ginger (Zingiber Officinale) on Rat Liver and Thyroid Injuries Induced by CCl4 (Carbon Tetrachloride) Journal of Pharmacy and Pharmacology. (2016) 4:108-118.
8. Widiyastuti Y, Pratiwi R, Riyanto S and Wahyuono S: Cytotoxic activity and apoptosis induction of avocado Persea americana Mill. seed extract on MCF-7 cancer cell line. Indonesian Journal of Biotechnology. (2018) 23(2): 61-67.
9. Dabas D, Elias RJ, Ziegler GR and Lambert JD: In *Vitro* Antioxidant and Cancer Inhibitory Activity of a Colored Avocado Seed Extract. International journal of food science. (2019) 2019.
10. Ejiofor CN, Ikechukwu E, Ezeagu, Moses-Ayoola, Peter U and Achukkwu: Histopathological effect of aqueous extract of Persea Americana seed on alloxan-induced diabetic rats, journal of Pharmacognosy and Phytochemistry. (2018) 20(5): 31-36.
11. Heibashy IA, El-Nahla AM and Saleh YA: Protective and Therapeutic Effect of Green Tea (Camellia sinesis) against Rat Prostate Cancer. Bull. Egypt. Soc. Physiol. Sci. (2008) 28 (2): 112-116.
12. Bosland MC: Testosterone treatment is a potent tumor promoter for the rat prostate. Endocrinology. (2014) 155(12): 4629–4633.
13. Ibrahim AY, El-Newary SA, Youness ER, Ibrahim AM and El Kashak WA: Protective and therapeutic effect of Vitex agnus-castus against prostate cancer in rat. J App Pharm Sci. 7. (2017) (12): 133-143.
14. Bahey NG, Soliman GM, El-Deeb TA and El-Drieny EA: Influence of insulin and testosterone on diabetic rat ventral prostate: Histological, morphometric and immunohistochemical study. Journal of Microscopy and Ultrastructure. (2014) 2(3), 151-160.
15. Ekins RH: Free and bound testosterone. Ann ClinBiochem. (1990) 27: 91–4.
16. Cai H, Zhang G, Yan Z and Shang X: The Effect of Xialiqi Capsule on Testosterone-Induced Benign Prostatic Hyperplasia in Rats. Evidence-Based Complementary and Alternative Medicine (2018) 34(4): 82-87.
17. Duarte PF, Chaves MA, Borges CD and Mendonca CRB: Avocado: characteristics, health benefits and uses. Ciencia Rural. (2016) 46(4): 747-754.
18. Moore RA and Melchionna RH: Production of tumors of the prostate of the white rat with 1: 2-benzpyrene. The American Journal of Cancer. (1937) 30(4): 731-741.
19. Hayashi N, Sugimura Y, Kawamura J, Donjacour AA and Cunha GR: Morphological and functional heterogeneity in the rat prostatic gland. Biology of reproduction. (1991) 45(2), 308-321.
20. Bartsch H and Nair J: Potential role of lipid peroxidation derived DNA damage in human colon carcinogenesis: studies on exocyclic base adduct as stable oxidative stress markers. Cancer Detect Prev. (2002) 26:308–312.
21. Banudevi S, Elumalai P, Arunkumar R, Senthilkumar K, Gunadharini DN, Sharmila G and Arunakaran J: Chemopreventive effects of zinc on prostate carcinogenesis induced by N-methyl-N-nitrosourea and testosterone in adult male Sprague-Dawley rats. Journal of cancer research and clinical oncology. (2011) 137(4): 677-686.
22. Elberry A, Mufti S, Al-Maghrabi J, Ghareib S, Mosli H, El-Halawany A and Abdel-Sattar E: The protective effect of Sophora japonica on prostatic hypertrophy and inflammation in rat. Inflammopharmacology. (2020) 28: 1525-1536.
23. Ma L, Zhao J, Li T, He Y, Wang J, Xie L and Li S: Association between tumor necrosis factor-alpha gene polymorphisms and prostate cancer risk: a meta-analysis. Diagnostic pathology. (2014) 9(1): 1-11.

24. Bhuyan DJ, Alsherbiny MA, Perera S, Low M, Basu A, Devi OA and Papoutsis K: The odyssey of bioactive compounds in avocado (Persea americana) and their health benefits. Antioxidants. (2019) 8(10): 426.

25. Dreher ML and Davenport AJ: Hass avocado composition and potential health effects: Critical reviews in food science and nutrition. (2013) Jan 1;53(7):738-50.

26. Stanbrough M, Bubley GJ, Ross K, Golub TR, Rubin MA, Penning TM, Febbo PG and Balk SP: Increased expression of genes converting adrenal androgens to testosterone in androgen-independent prostate cancer. Cancer research. (2006) 5:2815-25.

27. Saritha S and Prakash T: Comprehensive assignments of extraction, isolation and characterization of taraxerol from bark annona reticulata L. and chemopreventive e ȝect on human prostate cancer cell lines (lncap and pc-3). J Carcinog Mutagen. (2018) 9: 313.

28. Azizi A, Mumin NH and Shafqat N: Phytochemicals with Anti 5-alpha-reductase Activity: A Prospective for Prostate Cancer Treatment. F1000Research. (2021) 10: 221.

29. Sharmila G, Bhat FA, Arunkumar R, Elumalai P, Singh PR, Senthilkumar K and Arunakaran J: Chemopreventive effect of quercetin, a natural dietary flavonoid on prostate cancer in in *vivo* model. Clinical nutrition. (2014) 33(4):718-726.

30. Boyadzhieva S, Georgieva S and Angelov G: Optimization of the extraction of natural antioxidants from avocado seeds. Bulg. Chem. Commun. (2018) 50:80-84.

31. Alkhalaf MI, Alansari WS, Ibrahim EA and ELhalwagy ME: Anti-oxidant, anti-inflammatory and anti-cancer activities of avocado (Persea americana) fruit and seed extract. Journal of King Saud University-Science. (2019) 31(4): 1358-1362.

32. Ummanni R, Lehnigk U, Zimmermann U, Woenckhaus C, Walther R and Giebel J: Immunohistochemical expression of caspase-1 and-9, uncleaved caspase-3 and-6, cleaved caspase-3 and-6 as well as Bcl-2 in benign epithelium and cancer of the prostate. Experimental and therapeutic medicine. (2010) 1(1): 47-52.

33. Vo TS and Le PU: Free radical scavenging and anti-proliferative activities of avocado (Persea americana Mill.) seed extract. Asian Pacific Journal of Tropical Biomedicine. (2019) 9(3): 91.

34. Steiner MS, Zhou Z-Z, Tonb DC and Barrack ER: Expression of transforming growth factor-b1 in prostate cancer. Endocrinology. (1994) 13:2240

35. Weinstein MH, Signoretti S and Loda M: Diagnostic utility of immunohistochemical staining for p63, a sensitive marker of prostatic basal cells. Modern pathology. (2002) 15(12):1302-1308.

36. Wang M, Nagle RB, Knudsen BS, Rogers GC and Cress AE: A basal cell defect promotes budding of prostatic intraepithelial neoplasia. Journal of cell science. (2017) 130(1): 104-110.

37. Mantel PY and Schmidt-Weber CB: Transforming growth factor-beta: recent advances on its role in immune tolerance. Suppression and Regulation of Immune Responses. (2010) 303-338.

38. Barakat, H.S., El-Hallouty, S., and Salem, D., (2013). Effect of the water extracts of avocado fruit and cherimoya leaf on four human cancer cell lines and vicia faba root tip cells. J. Agric. Sci. 5(7), 245.

39. O'Neill AJ, Boran SA, O'Keane C, Coffey RN, Hegarty NJ, Hegarty P and Watson RWG: Caspase 3 expression in benign prostatic hyperplasia and prostate carcinoma. The Prostate. (2001) 47(3): 183-188.

40. D'Ambrosio SM, Han C, Pan L, Kinghorn AD and Ding H: Aliphatic acetogenin constituents of avocado fruits inhibit human oral cancer cell proliferation by targeting the EGFR/RAS/RAF/MEK/ERK1/2 pathway. Biochem. Biophys. Res. Commun. (2011) 409 (3): 465-469.

41. Ding H, Casto B, Deng Y, Grill K, Duan W and Zhang N: Avocado Extract Inhibits 7, 12-Dimethylbenz [a] anthracene (DMBA)-Induced Carci-nogenesis in Hamster Cheek Pouches. Med Chem S. (2014) 1; 2161-0444.

42. Casto BC, Knobloch TJ, Galioto RL, Yu Z, Accurso BT and Warner BM: Chemoprevention of oral cancer by lyophilized strawberries. Anticancer research. (2013) 33(11): 4757-4766.

الملخص العربى

الدورالوقائي والعلاجي المحتمل للمستخلص المائي لبذور الأفوكادوعلى سرطان البروستاتا المستحث تجريبيا في ذكور الجرذان البيضاء البالغة

نهي محمد عبد العزيز، احمد سعيد ذو الفقار، نيفين محمد الشريف، مروه عبد الصمد الغلام

قسم التشريح وعلم الأجنة - كلية الطب - جامعة المنوفية – مصر

مقدمة: يعد سرطان البروستاتا هو ثاني أكثر السرطانات شيوعًا لدى الرجال، ويصيب حوالي ١,١ مليون رجل في جميع أنحاء العالم، وهو السبب الرئيسي الخامس للوفاة المرتبطة بالسرطان لدى الرجال. حوالي ١ رجل في ٣٩ يموتون من سرطان البروستاتا.

الغرض من البحث: يعد سرطان البروستاتا هو ثاني أكثر السرطانات شيوعًا لدى الرجال، و هناك العديد من المنتجات الطبيعية والعوامل الغذائية لها خصائص واقية كيميائية. لذلك هذا العمل قام بدراسة الدور الوقائي و العلاجي المحتمل لمستخلص مائي من بذور الأفوكادو على سرطان غدة البروستات

خطة البحث: ٨٠ جرذ من الذكور البالغة تم تقسيمهم الي اربع مجموعات: المجموعه الاولى (الضابطه): مكونة من ١٠ فئران. المجموعه الثانية (مجموعة سرطان البروستاتا المستحث تجريبيا) : مكونة من ٢٠ جرذ بعد اربع اسابيع من حث سرطان البروستاتا تجريبيا تم التضحية بهم.المجموعه الثالثه(المجموعة المحمية): مكونة من عشرين جرذ تم اعطاؤها مستخلص الافوكادو المائي مرة واحد يومياً بجرعة ٥٠٠ مجم لكل كجم لمدة ١٠ اسابيع ثم تم حث سرطان البروستاتا تجريبيا. المجموعه الرابعه (المجموعة المعالجة): مكونة من ٢٠ جرذ تم حث سرطان البروستاتا ثم اعطاؤها مستخلص الافوكادو المائي مرة واحد يومياً بجرعة ٥٠٠ مجم لكل كجم لمدة ١٠ اسابيع.

نتائج البحث: طبقاً لكل من نتائج الدراسات الهستولوجية والصبغات المناعية وعمل قياسات عن طريق جهاز تحليل الصورة بالكمبيوتر، تم التوصل إلى أن المستخلص المائي لبذور الافوكادو له دور فعال فى الوقاية من سرطان البروستاتا.

التوصيات: تم استخلاص ما يلى من البحث:

سرطان البروستاتا مرض يمكن الوقاية منه لذلك كان استخدام مستخلص بذور الافوكادو له دور كمادة وقائية من سرطان الروستاتا، لكنة لا يمكن إستخدامه كعلاج. كما يوصي أيضاً البحث بإجراء دراسات أخرى على الدور الوقائي و العلاجي المحتمل لبذور الافوكادو لأعضاء الجسم الأخري وكذلك البحث عن مواد أخرى جديدة تعطي أملاً واعداً لتأثير المنتجات الطبيعية في الوقاية من سرطان البروستاتا.