# Biochemical and Histopathological evaluation of prostatic tissue under effect of Pterostilbene in benign prostatic hyperplasia rat model

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## Abstract:

**Background:** Benign prostatic hyperplasia [BPH] is the urologic condition that affects elderly men the most frequently Benign prostatic hyperplasia. Benign prostatic hyperplasia must be distinguished from lower urinary tract symptoms and benign prostatic enlargement. which refers to an enlarged prostate, benign prostatic hyperplasia is a purely histological term the development, maintenance, and secretory activity of the prostate and other sex-accessory tissues are stimulated by the presence of certain hormones and growth factors. the pathophysiology of Benign prostatic hyperplasia is significantly influenced by the activity of the enzyme 5α-reductase. It's important to remember that 5-αreductase is responsible for creating Dihydrotestosterone a stronger androgen. Pterostilbene Mostly found in blueberries and grapes and pterostilbene substance with a number of biological properties including anticancer properties. pterostilbene is a lipid-soluble molecule that exists in both cis and trans forms with the latter being more prevalent. The conventional medication for Benign prostatic hyperplasia utilized in this trial was finasteride which inhibits the 5α-reductase enzyme and lowers the amount of Dihydrotestosterone.

**Methods:** Forty-eight male rats were divided into six groups; the control group consisted of eight rats who received subcutaneous injections of oil vehicle for a period of 42 days. The induction group consisted of eight rats who received subcutaneous injections of testosterone propionate for a period of fourteen days. The finasteride group consisted of eight rats who received finasteride 0.44 mg/kg by oral gavage for a period of twenty-eight days following the induction of Benign prostatic hyperplasia and Pterostilbene 200 group included 8 rats were given pterostilbene 200mg/kg by oral gavage for 28 days after 14 days of Benign prostatic hyperplasia induction. pterostilbene 100 group included 8 rats were given a pterostilbene 100mg/kg per day kg by oral gavage for 28 days after 14 days of induction Benign prostatic hyperplasia dose and the resveratrol group included 8 rats were given a resveratrol 100mg/kg per day kg by oral gavage for 28 days after 14 days of induction Benign prostatic hyperplasia After twenty-eight days.

**Results:** Histological section of prostate Pterostilbene 200 were similar those in control negative revealed numerous variable sizes alveoli that filled with homogenous eosinophilic secretion, had normal epithelial and stromal tissue.

**Conclusion:** Pterostilbene have a potent anti-proliferative effect by decrease the hyperplastic nodules for prostate and return epithelial cell to normal and have a very good scavenging activity for free radical [very good as antioxidant] in compare with Vitamin c and resveratrol.

**Aim of study:** evaluate the effect of Pterostilbene as Anti proliferative on Benign prostatic hyperplasia and assess the antioxidant activity for Pterostilbene by DPPH Assay.

**Key words:**Benign prostatic hyperplasia, lower urinary tract symptoms,5α-reductase enzyme, Dihydrotestosterone, pterostilbene

## التقييم الكيميوحيوي والنسيجي لأنسجة البروستات تحت تأثير التيروستلبين لتضخم البروستاتا الحميد المستحث في ذكور الجرذان

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**الخلاصة:**
تضخم البروستاتا الحميد هو حالة مرضية تصيب المسالك البولية لدى الرجال المسنين بشكل شائع. يجب تمييز تضخم البروستاتا الحميد عن أعراض المسالك البولية السفلية. يعد تضخم البروستاتا الحميد هو مصطلح نسيجي بحت، حيث يتصف بأنه تطور ونشاط إفرازي للبروستاتا من خلال تحفيز الأنسجة الإضافية بوجود بعض الهرمونات وعوامل النمو. تتأثر الفسيولوجيا المرضية لـ تضخم البروستاتا الحميد بشكل كبير بنشاط إنزيم الفا الاختزالي الخامس ومن المهم أن نتذكر ان هذا الانزيم هو مسؤول عن تكوين هرمون دايهايدروتستيرون، وهو أقوى أندروجين. التيروستلبين يوجد غالبًا في التوت الأزرق والعنب وهو عبارة عن مادة نباتية لها عدد من الخصائص البيولوجية، بما في ذلك الخصائص المضادة للسرطان. وهو مادة قابلة للذوبان في الدهون موجود في شكلين سس وترانس، مع كون الأخير أكثر انتشارًا. وكان هنالك الدواء التقليدي لـ تضخم البروستاتا الحميد والمستخدم أيضا في هذه التجربة هو الفيناستراید، والذي يثبط إنزيم الفا الاختزالي الخامس ويقلل من كمية هورمون الدايهايدروتستستيرون.
**طرائق العمل:** تم على أساس ذلك تقسيم ثمانية وأربعين من ذكور الجرذان إلى ست مجاميع. تألفت المجموعة الضابطة من ثمانية جرذان تلقوا حقنًا تحت الجلد لمركبة زيتية لمدة ٤٢ يومًا .حيث تتكون المجموعة التعريفية من ثمانية جرذان تلقوا حقنًا تحت الجلد من بروبيونات التستوستيرون لمدة أربعة عشر يومًا .اما مجموعة الفيناستراید تألفت من ثمانية جرذان تلقوا الفيناستراید ٠,٤٤ ملغم / كغم بالتزقيم الفموي لمدة ثمانية وعشرين يومًا بعد تحريض تضخم البروستاتا الحميد.اما مجموعة التيروستلبين ٢٠٠ التي تضمنت ٨ جرذان تم إعطاؤهم التيروستلبين ٢٠٠ مجم / كجم عن طريق تزقيم فموي لمدة ٢٨ يومًا بعد ١٤ يومًا من تحريض تضخم البروستاتا الحميد .و تم إعطاء مجموعة التيروستلبين ١٠٠ التي تضم ٨ جرذان تيروستلبين ١٠٠ مجم / كجم يوميًا كجم عن طريق تزقيم فموي لمدة ٢٨ يومًا بعد ١٤ أيام من جرعة التحريضية . اما مجموعة الريسفيراترول التي اشتملت على ٨ جرذان تم إعطاؤهم الريسفيراترول ١٠٠ مجم / كجم يوميًا عن طريق الحقن الفموي لمدة ٢٨ يومًا بعد ١٤ يومًا من تضخم البروستاتا الحميد .
**النتائج:** كان المقطع النسيجي للبروستاتا لمجموعة التيروستلبين ٢٠٠ متشابهًا مع تلك الموجودة في المجموعة الضابطة التي كشفت عن العديد من الحويصلات ذات الأحجام المتغيرة المملوءة بإفراز حمضي متجانس وكان لها نسيج طلائي وسدي طبيعي.
**الاستنتاجات:** يمتلك التيروستيلبين تأثيرًا قويًا مضادًا لفرط التكاثر عن طريق تقليل تنسج العقيدات المفرطة للبروستاتا وإعادة الخلايا الظهارية إلى وضعها الطبيعي ولها نشاط قوي كمضاد للتأكسد من خلال إزالة جيدة جدًا للجذور الحرة مقارنة بفيتامين سي والريسفيراترول.
**هدف الدراسة:** تقييم تأثير التيروستلبين كمضاد لفرط التكاثر على تضخم البروستاتا الحميد وتقييم نشاطه كمضاد لأكسدة.

**الكلمات المفتاحية:** تضخم البروستاتا الحميد، أعراض المسالك البولية السفلية، إنزيم الفا المختزل الخامس، دايهيدروتستوستيرون، التيروستيلبين.

## Introduction

The most frequent urologic illness in older men is benign prostatic hyperplasia [BPH]. is a strictly histological term that must be separated from benign prostatic enlargement [BPE], which refers to an enlarged prostate, and lower urinary tract symptoms [LUTS][1]. In senior men, urodynamic abnormalities in the lower urinary tract, such as benign prostatic blockage and detrusor over activity or underactivity, are the most common causes of LUTS. the presence of specific hormones and growth factors stimulates the development, maintenance, and secretory function of the prostate, as well as other sex-accessory tissues[2].

The activity of the enzyme 5α-reductase is significant in the pathophysiology of BPH. It's vital to note that 5α-reductase is in charge of producing DHT, a more strong

androgen[3]. Inflammation and apoptosis are essential regulators of cell proliferation and tissue homeostasis, with apoptotic machinery abnormalities associated to benign prostatic hyperplasia[4].

Pterostilbene[4-[[E]-2-[3,5-Dimetho-xyphenyl] ethenyl]phenol] is a natural plant product that can be found mostly in blueberries and grapes, Pterostilbene has a variety of biological activities, including anticancer actions[5].

Pterostilbene is a lipid-soluble molecule that comes in two forms: cis and trans, with the trans form being the most common[6].

Pterostilbene was shown to be pharmacologically safe because no organ-specific or systemic toxicity was seen[7]. Pterostilbene is a natural analog of resveratrol [3,5,40-trihydroxystilbene], however it has ten times the antifungal activity of resveratrol, Furthermore, PTE has a higher lipophilicity and potential for cellular absorption than resveratrol, which has three hydroxyl groups[8].

Anti-inflammation, antiobesity, antiox-idant, cholesterol lowering, Analgesia, antiaging, antidiabetic and neuroprotective properties are all exhibited by PTE[9]. Pterostilbene's pharmacological actions are frequently reported to be stronger in vitro and/or in vivo than resveratrol's, despite the structural and general bioactivity similarities between the two[10].

## Methodology

### In vitro free radical scavenging activity for pterostilbene [DPPH assay]:

The antioxidant activity of PTE was measured using the 1,1-diphenyl-2-picrylhydrazyl [DPPH] free radical scavenging activity. With the use of a chemical balance with a minimum limit, 3.94 mg of DPPH was measured, in order to make a 0.1 mM solution of DPPH, it was dissolved in 100 ml of ethanol, next dissolved in 1 ml of DMSO. Obtaining [3.125,6.25,12.5, 25, 50, 100, 200, and 400] milligrams of each via serial dilution, in a 96-well plate, 100 microliters of DPPH and 100 microliters of each compound were combined, after 30 minutes of incubation in the dark, absorbance was measured, all concentrations were repeated twice. Vitamin C was also utilized as a strong scavenger for free radical and similar to that resveratrol's potential as an antioxidant, DMSO was utilized as a negative control[11,12].Pterostilbene was pure powder from sigma Aldrich com. Prepared with DMSO solvent 10% [30mg PTE diluted in 1 ml DMSO] to make stock solution 6.66mg/ml give to rats by oral gavage[12,13].Pterostilbene [PTE] 100mg stock solution preparation: The dose of PTE was 100mg/kg/day depend on treatment of PTE to reduce Oxidative stress in experimental rats[14,15].Resveratrol [RES] 100mg stock solution preparation: The dose of Resveratrol was 1g/ day per human[60kg][18].the Rat dose of Resveratrol its prepared by dividing 1g/60kg/day to give per kg and then multiply with conversion factor 6.2 [FDA], the final dose for Rat was 100mg /kg/day. Resveratrol was pure powder by Fluorochem, diluted with DMSO solvent 10% [16mg RES diluted in 1ml DMSO] and prepared stock solution 6.25 mg/ml given by oral gavage[19].

### In vivo laboratory animal study

Forty-eight Adult male Wister Rats 250-300 gram were purchased from Iraqi center for genetics and cancer research.

Animals were kept in normal room temperature approximately 21℃ with sustained at dark/light cycle with four rats in each cage and a strict pathogen-free environment, all rodents were grown on a 12-hour dark/light cycle. with maintenance of free food and water access.

Laboratory animals were separated into sex groups as showed in table (1) below.

**Table (1): Experimental groups**

| Study Groups | Rats No. | Treatment type with duration |
|---|---|---|
| Control group | eight | Subcutaneous injection of olive oil vehicle for 42 days |
| Induction group | eight | BPH Induction 14 days+ oil vehicale 28days. |
| Finasteride group | eight | BPH Induction 14 days+ finasteride 28days. |
| Pterostilbene 100 group | eight | BPH Induction 14 days pterostilbine100mg/kg 28days. |
| Pterostilbene 200 group | eight | BPH Induction 14 days pterostilbine200mg/kg 28days. |
| Resveratrol 100 group | eight | BPH Induction 14 days Resveratrol 100mg/kg 28days. |

Note: Where I.P =Intraperitoneal, BPH= Benign prostatic Hyperplasia.

**The First group [Control Group]** consists of eight rats who received a 42-day subcutaneous injection of 0.5 ml of vehicle olive oil.

**The Second group [Induction group]** consist of 8 rats, and the Induction group received a subcutaneous injection of testosterone propionate at a dosage of [4 mg/kg/day] for [14days] to inducing benign prostatic hyperplasia. After that The Induction group was also given a subcutaneous injection of 0.5 ml of vehicle olive oil every day for a total of 28 days[20].

**The Third group [Finasteride group]** The conventional treatment group, which consists of 8 rats, received a subcutaneous injection of testosterone propionate with a daily dosage of 4 mg/kg for 14 days to cause BPH. then, for 28 days, rats in this group received oral gavage doses of Finasteride 0.44 mg/kg depend on conversion equation from human dose[5mg daily] to rat dose[21,22].

**The Fourth group [Pterostilbene 100 group]** Eight rats from the PTE 100mg/kg group were administered testosterone propionate 4mg/kg subcutaneously for 14 days before receiving PTE 100mg/kg orally by gavage for 28-day period[23].

**The Fifth group [Pterostilbene 200 group]** the testosterone propionate [4 mg/kg] subcutaneous injection was given to 8 rats in the pterostilbene [PTE] 200mg/kg group for 14 days in order to develop BPH. followed with 28 days of oral gavage administration of PTE at 200 mg/kg[24].

**The Sixth group [Resveratrol 100 group]** consisting of 8 rats, group receiving resveratrol 100mg/kg. BPH is produced by administering testosterone propionate at a dose of 4 mg/kg daily for 14 days, then 28 days oral gavage of 100 mg/kg of resveratrol[20].put this group for evaluate effect for resveratrol on benign prostatic hyperplasia in compare with superior analog [Pterostilbene][26].

**Sample collection:**

The rats were given intraperitoneal doses of 50 mg/kg ketamine and 5 mg/kg xylazine at the conclusion of the study to anesthetized[27].

Rats' abdominal cavities were opened with forceps and scissors on day 42, serum samples were collected by direct cardiac puncture using special jell tubes, and in order to remove and preserve the prostate for tissue histopathology, small amount of prostate tissues was preserved in 10% buffered neutral formalin to create paraffin-embedded blocks for the study.

**Prostate specific antigen [PSA] Elisa kit:**
A microplate has been pre-coated with an antibody that is specific for PSA. The immobilized antibody binds any PSA that may be present after standards and samples were pipetted into the wells. A PSA-specific biotin-conjugated antibody was added to the wells after any unbound compounds have been removed. Horseradish peroxidase [HRP] with avidin conjugation is then added to the wells after washing. A substrate solution is then added to the wells after a wash to get rid of any unbound avidin-enzyme reagent, and color develops in proportion to how much PSA was bound in the first stage. In order to measure the color's intensity, the color's development is paused[28].

**Assessment of histopathological tissue samples**:

**A- Tissue samples fixation:**
All of the samples were promptly fixed in 10% buffered neutral formalin, with a 24-hour room-temperature fixing period. Neutral formalin buffered at 10% was made as follows:
100 ml of 40 % formalin, 900 ml of distil water, A four-gram dose of sodium dihydrogen phosphate [monobasic], Six and a half grams of dibasic sodium phosphate [anhydrous].

**B- Dehydration:**
After fixation, tissue samples were run through the next protocol. Following are the grades of ethyl alcohol in which samples were submerged:
Ethyl alcohol, fifty percent [50,70,80,90,100 %] 2 hours for all concentration.

**C- Embedding:**
Blocks of the samples were labeled and embedded in molten paraffin wax that had been heated to [60-65] C for 1-2 hours.

**D- Sectioning:**
The paraffin blocks were cut into slices that were 4 m thick, glued on a standard slide, and then stained.

**E- Trichrome staining:**
Connective tissue [collagen fibers] are seen histologically in tissue slices using the Trichrome stain [connective tissue stain], the distasteful explanation:

| | |
|---|---|
| [Collagen] | [Blue] |
| [Muscle Fiber] | [Red] |
| [Nuclei] | [Black/Blue] |

The staining assessment process was carried out in accordance with the steps that are shown in [appendix 1][29][30][31].

**Statistical Analysis:**
Statistical analysis was preformed utilizing SPSS to analyze the findings, each result's value was represented as a mean plus standard deviation [standard deviation].
The post hoc test was utilized, and a significant result was defined as a less than 0.05 in the *P*-value. *P* value higher than 0.05 was regarded as no significant difference, In the current investigation, Pearson correlation analysis was also conducted to see whether there were any relationships between the biomarker levels in BPH. The strength and direction of the linear association between the biomarkers are classified as per the value of the Pearson correlation coefficient [r].

## Results

***In vitro* free radical scavenging activity for pterostilbene [DPPH Assay]:**
Using a free radical scavenging assay called 1,1-diphenyl-2-picrylhydrazyl [DPPH], the PTE antioxidant activity was assessed. A quick, colorimetric assay is the DPPH assay frequently employed for examining the antioxidant capability of tested compounds. DPPH scavenging activity and reducing power were evaluated using a series of concentrations [3.125-400mg/ml] and the results were compared to those of the reference drug [Ascorbic acid and resveratrol], in current study make this is assay [in vitro study] to evaluate the antioxidant effect of Pterostilbene in compare with stronger antioxidant [Ascorbic acid] and with

resveratrol as antioxidant in same analog and stilbene group.

With an increase in the content of the Pterostilbene or Ascorbic acid and resveratrol, the scavenging activity and reducing power activity of the PTE and reference medication were both significantly enhanced.

In the following tables demonstrates that the serial concentrations [3.125,6.25 ,12.5,25,50,100,200,400] mg tested compounds free radical scavenging ability compares favorably to the reference standards Ascorbic acid and Resveratrol.

**Table (2): Duplicate stock solution for Vitamin C, Resveratrol & Pterostilbene [serial concentrations tested compounds free radical scavenging ability]**

| Concentration | Ascorbic acid | Resveratrol | Pterostilbene |
|---|---|---|---|
| 3.125 | 3 | 12 | 39 |
| 6.25 | 25 | 41 | 45 |
| 12.5 | 41 | 57 | 56 |
| 25 | 51 | 62 | 58 |
| 50 | 63 | 68 | 59 |
| 100 | 70.4 | 75 | 65 |
| 200 | 81 | 88 | 73 |
| 400 | 90 | 93.4 | 86 |

Dose response curve for scavenging activity for Pterostlibene[PTE], Vitamin c, resveratrol 400 milligram.

Y = % of DPPH scavenging activity.

X= concentration.

The IC50 was calculated by the logarithmic regression equation by considering y is 50 %.

Y= ax + b

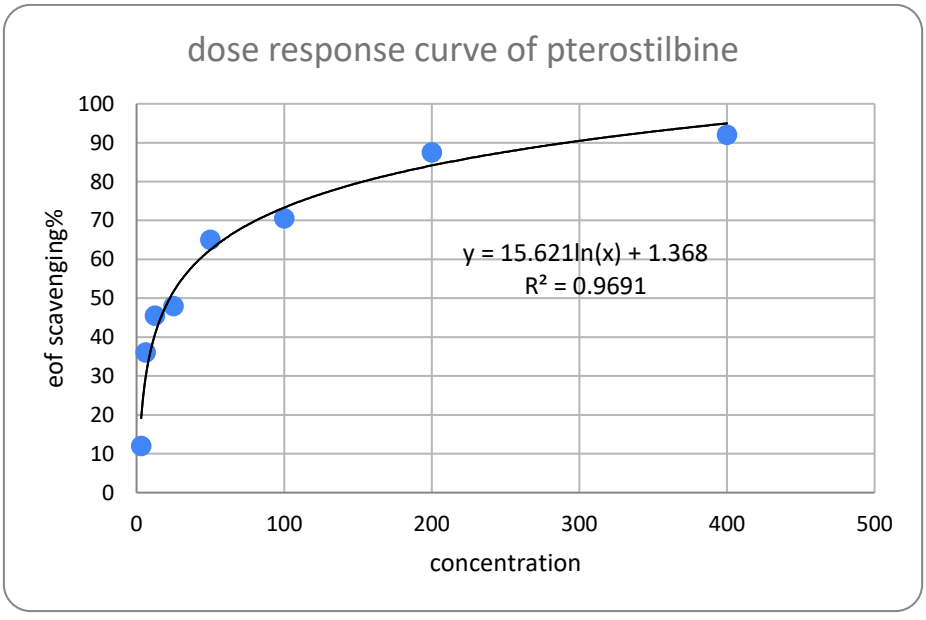



**Figure (1): Dose response curve of pterostilbene [milligram]**

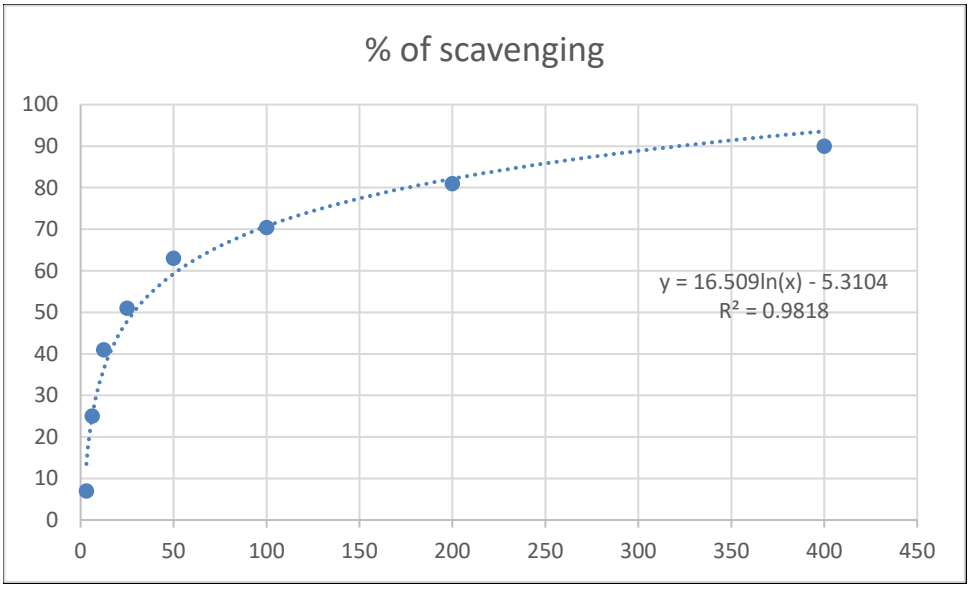



**Figure (2): Dose response curve of Vit. C [milligram]**

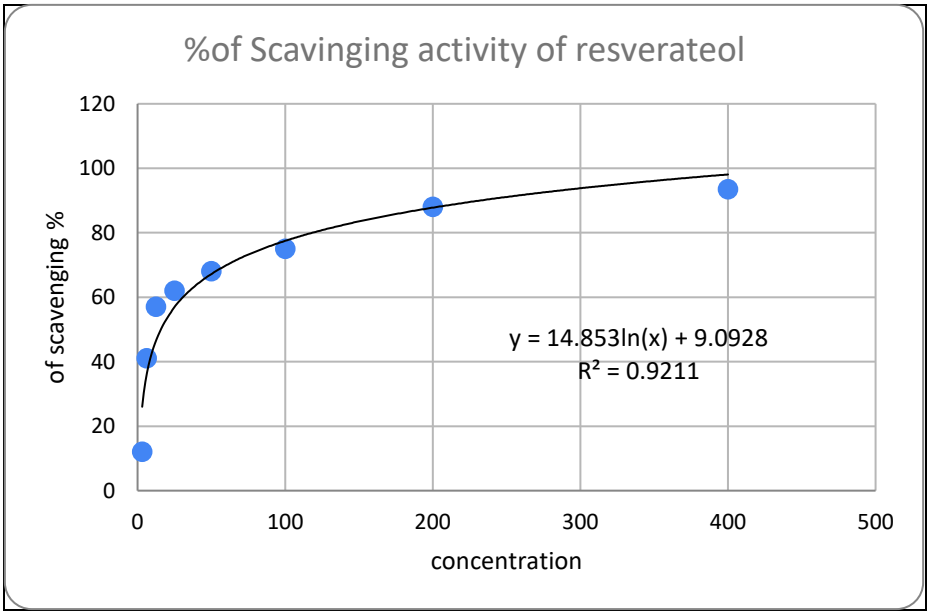



**Figure (3): Dose response curve of resverateol [milligram]**

Additionally, the results showed that the PTE and the reference drug's [ascorbic acid and Resveratrol] respective IC50 values for the DPPH scavenging activity were 10.70 mg /ml, 9 mg/ml, 10.66 mg/ml.

**Serum level of Prostate Specific Antigen [PSA] in studied groups:**

The mean prostate specific antigen [PSA] serum level of Induction group= [0.808±0.081] ng/ml, was obtained significant increase in compared with [control, PTE 200, Finasteride, PTE 100 and resveratrol] groups [*P<0.05*].

The mean serum level of PSA for control group = [0.276±0.026] ng/ml, showed significant decrease in compare with induction group [*P<0.05*]. while had significant difference compared with [Finasteride and PTE 200, PTE 100 and resveratrol] groups [*P <0.05*]. as shown in table (3)

**Table (3): Prostate specific antigen serum level for all studied groups.**

| Study groups | Mean PSA serum level ng/ml± SD |
|---|---|
| Control group | 0.276±0.026 [a] |
| Induction group | 0.808±0.081 [b] |
| Finasteride group | 0.359±0.050 [c] |
| Pterostilbene 200 group | 0.417±0.030 [c] |
| Pterostilbene 100 group | 0.546±0.047 [d] |
| Resveratrol group | 0.537±0.068 [d] |

Data represents Mean ± Standard deviation [SD].
Different lowercase letters indicate significant differences between groups [$p<0.05$]. post hoc test was utilized.

The mean serum level for PSA of finasteride group = [0.359±0.050] ng/ml, was observed significantly reduce compared to induction group [$P<0.05$]. PSA serum level for Finasteride group shown no significant difference in compared with PTE 200 group, p value= [0.293]. while PSA serum level had significant lower compared with [control, PTE 100, and resveratrol] groups [$P<0.05$].

The mean serum level for PSA of PTE 200 group = [417±0.030] ng/ml, was obtained significant depletion in PSA compared with Induction group [$P<0.05$]. in addition, the serum level PSA of PTE 200group recorded a significant difference in compare with [control, PTE100, resveratrol] groups *[$P\leq0.05$]*. Conversely, The PSA serum level of PTE 200 group had no significant difference in compare with Finasteride group [p value =0.293].

The mean serum level of PSA for PTE 100 group = [0.546±0.047] ng/ml, was indicated significant decrease in compare with induction group [$P<0.05$].

At same time PSA serum level for PTE 100 group had significant rise compared with [control, Finasteride and PTE200] groups [$P<0.05$]. Contrarily, the PSA of PTE 100 group was obtained no significant reduction compared with resveratrol group [p value =0.999].

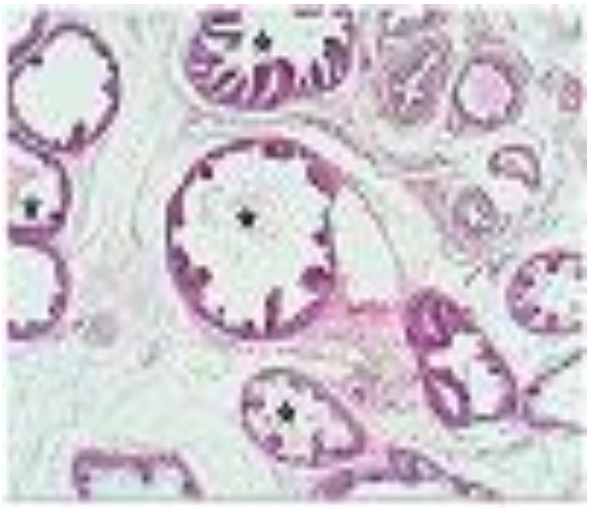
The mean serum level of PSA for Resveratrol group = [0.537±0.068] ng/ml, PSA serum level recorded significant increase compared with Control, Finasteride and PTE 200] groups [$P<0.05$]. Conversely, the Resveratrol PSA serum level group had no significant difference in compare with PTE 100 group [p value =0.999]. as shown in figure (3).

**Histopathological examination of prostate tissue:**

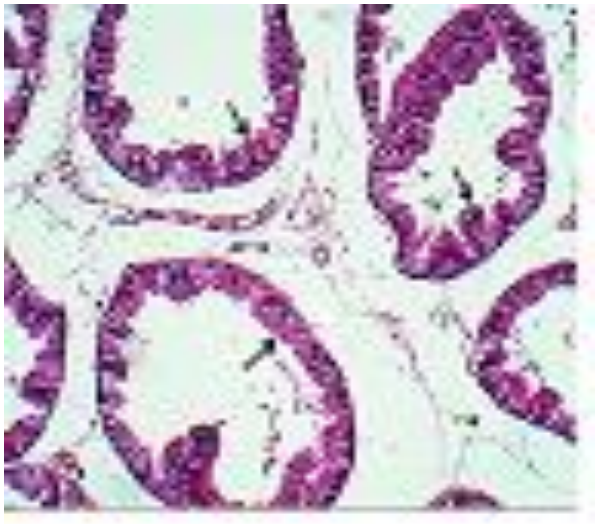
**Control group:**

The prostate is compound lobular gland that surrounded by a thin connective capsule. Each lobe is composed groups of slight small sizes individual glands called [alveoli] in addition to series of ducts system that opened into the urethra. The prostate lobules are separated from each other by the stromal loose connective tissue.

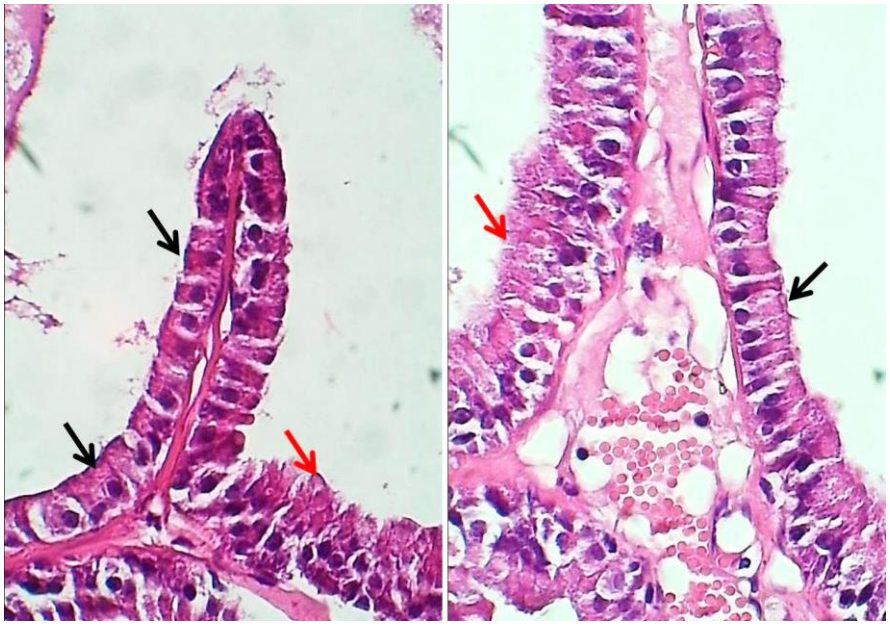
The glands composed two types of cells: luminal secretory cells and basal cells. Secretory cells were columnar to cuboidal types. Basal cells were small, flat cells that situated at the basement membrane figure (4- A, B &C).



**Figure[4A]: Histological section of prostate [control] showed: Variable sizes of alveoli [Asterisks], inter alveolar loose connective tissue [C], & duct [d]. H&E stain.40x**



**Figure[4B]: Histological section of prostatic alveoli [control] showed: alveolar epithelial [Arrows] & inter alveolar loose connective tissue [C]. H&E stain.100x.**

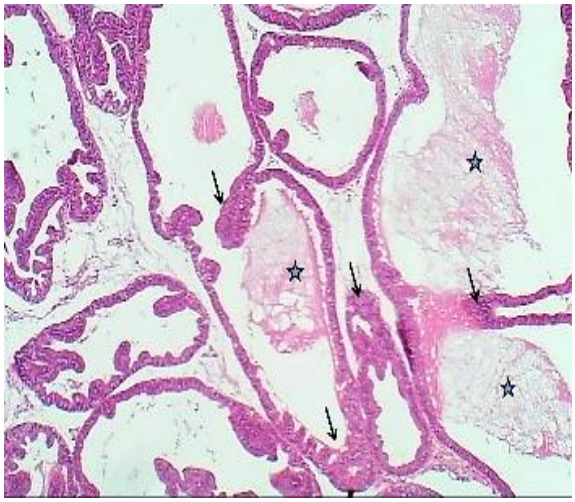


**Figure[4C]: Histological section of alveolus [control] showed: alveolar epithelial during resting phase [Black arrows] & during secretory phase [Red arrows]. H&E stain.400x.**

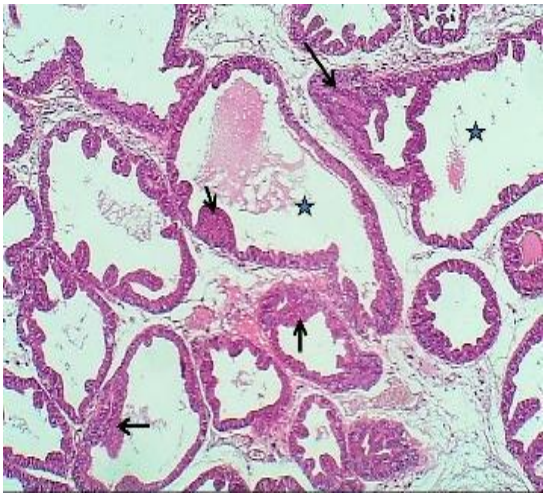
**Induction Group:**

The prostatic lobules revealed numerous hypertrophied alveoli that filled with homogenous eosinophilic secretion [figure [3.30A&B]. The alveoli revealed numerous luminal epithelial hyperplasia [thickening] that associated with increased in the luminal epithelial cell's proliferation figure [3.30C]. Epithelial basal cells also revealed marked hyperplasia and stromal invasion, both types of cells proliferation led to an increase in a population of cells

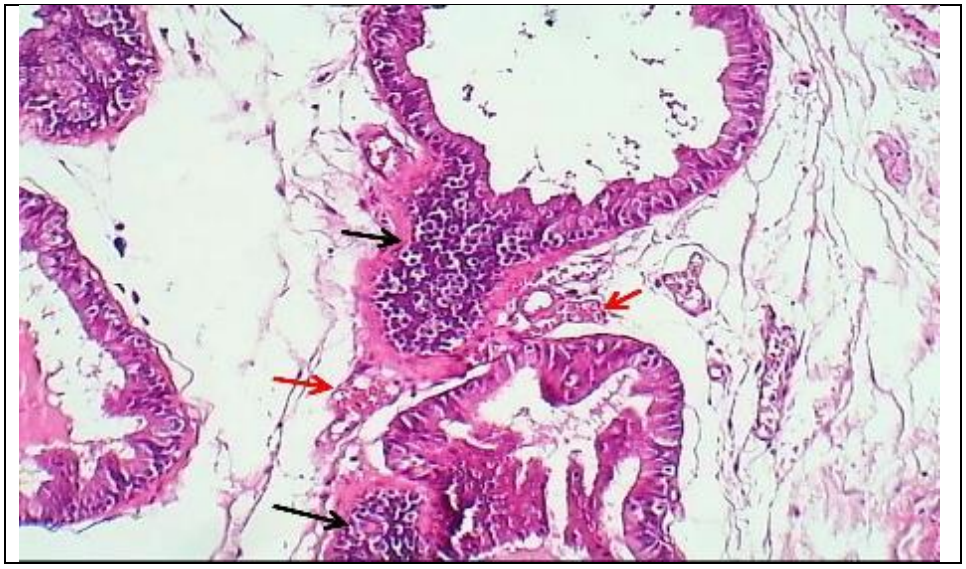
and increase of secretory epithelial luminal cells that shows mucinous secretions which displace the epithelium resulting in clear cytoplasm and small pyknotic nuclei. Apoptotic bodies were the characteristic figures of epithelial proliferation. As shown in figure (5-A, B, C)



**Figure[5A]: Histological section of prostate [Induction] showed: enlarge alveoli [hypertrophied] filled with eosinophilic secretions [Asterisks], numerous intra epithelial hyperplasia [arrows]. H&E stain.40x.**



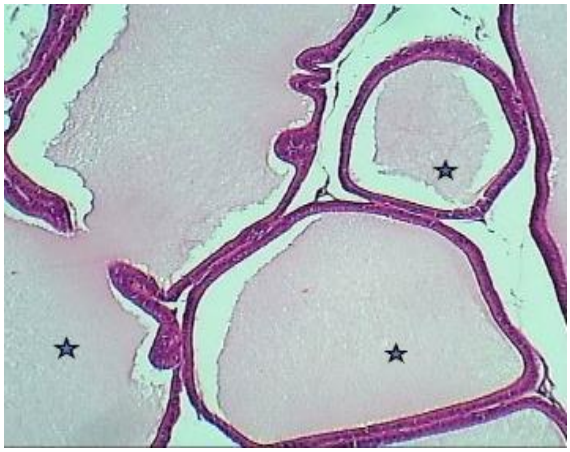
**Figure[5B]: Histological section of prostate [induction] showed: enlarge alveoli [hypertrophied] filled with homogenous eosinophilic secretions [Asterisks], numerous intra epithelial hyperplasia [arrows]. H&E stain.100x.**



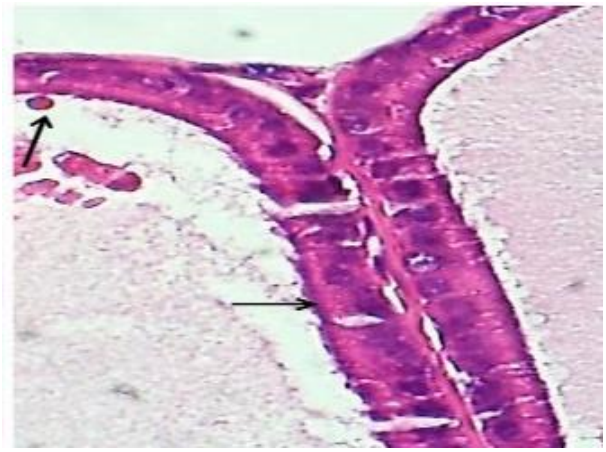
**Figure(5C): Histological section of prostate [Induction] showed: basal cells hyperplasia [Black arrows], stromal vascular congestion [Red arrows]. H&E stain.100x.**

**Finasteride group:**

The prostatic lobules revealed few hypertrophied alveoli. The alveoli revealed normal columnar epithelial cells, normal basal cells and stromal tissue, as shown in figures [6-A&B]



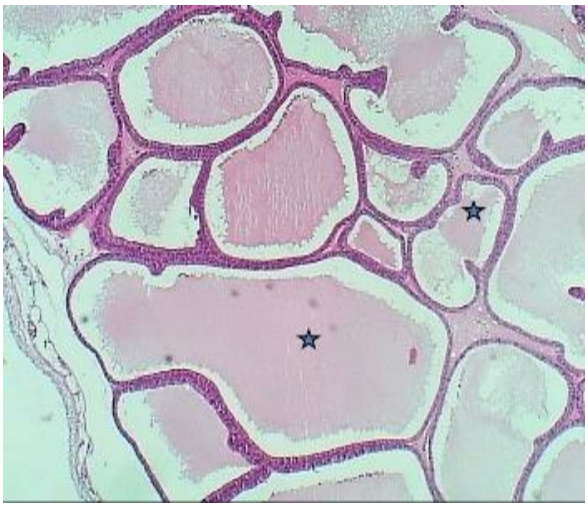
**Figure(6A): Histological section of prostate [Finasteride] large sizes alveoli filled with secretion [asterisks], has normal epithelial cells [arrows]. stain. 100x.**



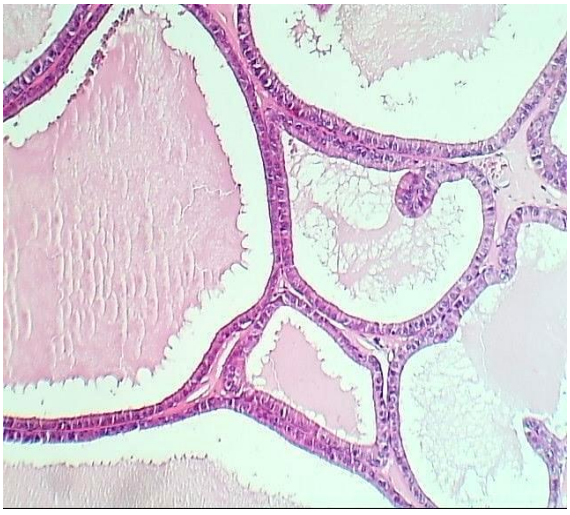
**Figure[6B]: Histological section of prostate [Finasteride] large sizes alveoli filled with secretion [asterisks], & normal epithelial cells [arrows]. H&E stain.40x.**

**PTE 200mg/kg group:**

Histological section of prostate [PTE 200 mg/kg] were similar those in control negative revealed numerous variable sizes alveoli that filled with homogenous eosinophilic secretion, had normal epithelial and stromal tissue, as shown in figures [7A&B].



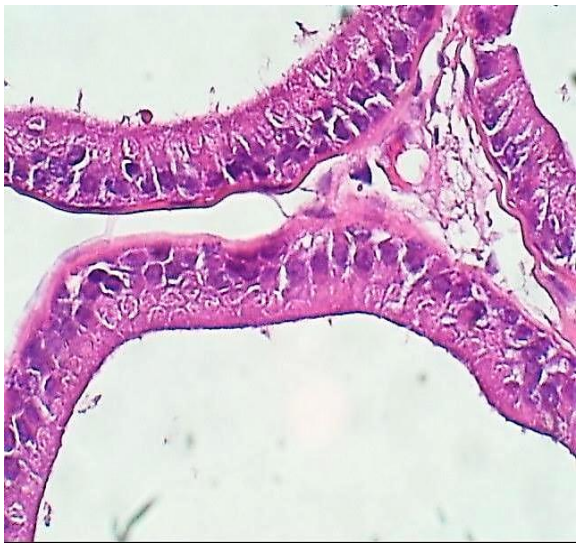
**Figure(7A): Histological section of prostate [PTE 200mg/kg] variable sizes alveoli filled with secretion [asterisks] has normal epithelial cells and stromal tissue. H&Estain.40x**



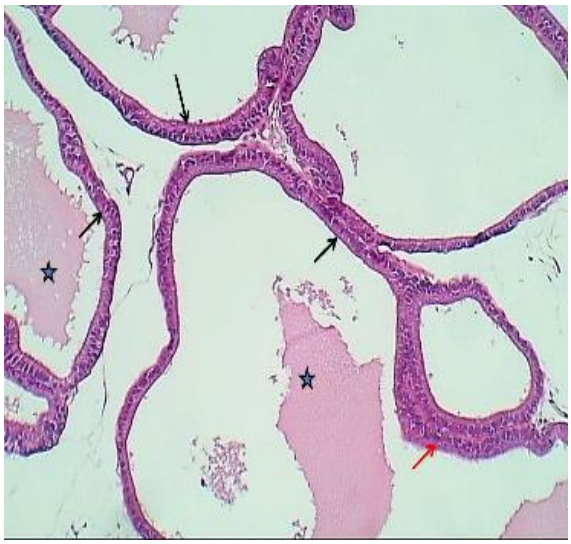
**Figure[7B]: Histological section of prostate [PTE 200mg/kg] variable sizes alveoli filled with secretions, has normal epithelial cells and stromal tissue. H&E stain.100x**

**PTE 100mg/kg group:**

Histological section of prostate [PTE 100] revealed numerous large sizes alveoli that filled with homogenous eosinophilic secretion, had normal epithelial cells and showed mild epithelial hyperplasia, the epithelial cells showed no figures of apoptotic bodies, as shown in figures [8-A, B].



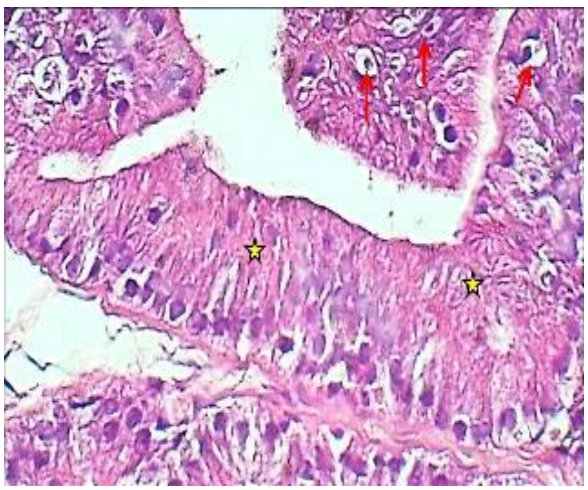
**Figure(8A): Histological section of prostate [PTE100mg/kg] showed: mild luminal epithelial hyperplasia. H&E stain.100x.**



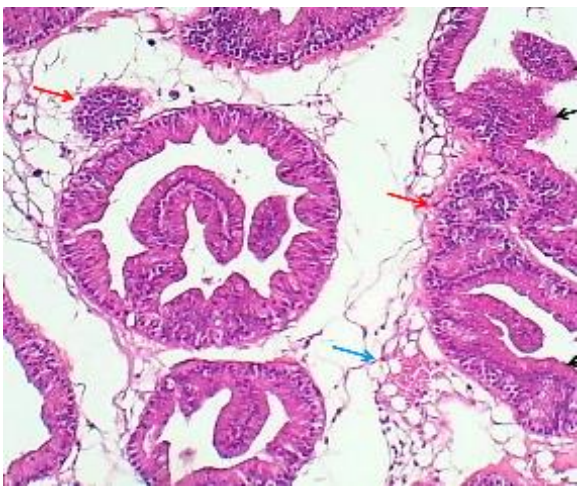
**Figure(8B): Histological section of prostate [PTE100mg/kg] large sizes alveoli filled with secretion [asterisks] has normal epithelial cells [Black arrows] & mild epithelial hyperplasia [red arrow]. H&E stain.40x.**

**Resveratrol group:**

The prostatic lobules revealed few hypertrophied alveoli figure[9-A]. The alveoli revealed moderate luminal epithelial hyperplasia with basal cells hyperplasia and stromal invasion figure[9-B]. The epithelial cells revealed marked increase in height that associated with increase production of secretion with numerous apoptotic bodies.



**Figure(9A): Histological section of prostate [resveratrol] shows: increase epithelial height associated with secretory activities [asterisks] and apoptotic bodies [Red arrows]. H&E stain.100x**



**Figure(9B): Histological section of prostate [resveratrol] shows: intra epithelial luminal epithelial hyperplasia [Black arrows] and stromal invasion by basal cells [Red arrows]. H&E stain. 40x.**

## Discussion

In biopsy and surgical specimens from elderly with BPH, histologically proven prostatic inflammation was often observed and is allegedly present in 43-77% of sample [28–30]. In comparison to individuals without chronic inflammation, those with BPH have bigger prostate volumes, are more likely to experience severe LUTS, are more likely to experience acute urine retention, and respond less well to standard medical treatment [31–33]. Inflammation may trigger the production of cytokines and increase the concentration of growth factors, which will cause prostatic cells to proliferate abnormally [35].

Free radical scavenging activity of pterostilbene

The current study found that the IC50 values for the antioxidant effects of [vitamin C, PTE, and resveratrol] were [9, 10.70, and 10.66 mg/ml, respectively]. Comparing PTE to resveratrol and Vitamin C, it demonstrated a greater proportion of free radical scavenging activity, the free radicals that PTE scavenges include PTE lessens oxidative stress and the generation

of reactive oxygen species [ROS], such as superoxide anion [O2] and hydrogen peroxide [H2O2] [34–36]. which are connected to the onset and pathophysiology of several disease processes, Additionally, the anti-oxidant properties of polyphenolic compounds and flavonoids are greatly enhanced by the presence of a hydroxyl group, which transfers hydrogen to other molecules in the structure, Therefore, the greater the anti-oxidant activity, the greater the number of hydroxyl groups [39]. Additionally, Pterostilbene treatment of numerous cell lines resulted in enhanced production of the antioxidants catalase, total glutathione [GSH], glutathione peroxidase [GPx], and superoxide dismutase [SOD], as well as a decrease in malondialdehyde levels [MDA], modify cellular oxidative activity, which may be key in Pterostilbene-mediated cell death [40]. The antioxidant activity of prostate cancer cells was similarly altered by PTE therapy, indicating a probable connection between the processes of oxidation and apoptosis, this result supports earlier research on the anti-oxidant properties of PTE due to the presence of phenolic components like resveratrol and high quantities of vitamin C [41].

The effect of pterostilbene on Blood serum level of prostate specific antigen [PSA] biomarker

In current study, the prostate specific antigen serum levels were significantly elevated for the induction group compared to control group this finding in line with fact that state highest level of PSA produce from tissue of prostate in Benign prostatic hyperplasia [42,43].

The PTE 200mg/kg group obtained significantly reduced levels of PSA serum level by comparing with Induction group after induced by testosterone propionate S/C. results optained with current study agreed with recently studies that found the pterostilbene have anti- proliferative effect and enhance of apoptosis [44]. By increasing p21 expression and promoting p53 expression, pterostilbene stopped cell cycle progression during the G1 phase, keeping tight control over proliferation, Additionally, PTE reduced prostate specific antigen [PSA][38].

In current work administration of finasteride after giving testosterone propionate showed significant reduction in prostate specific antigen PSA compared with induction group, on other hand had no significant reduction in compare with control, these results consistent with previous studies that appear depletion in level of PSA after controlling with finasteride [40–42].

The Pterostilbine 100 group in present study also showed significant depletion in the PSA level in compare to induction group inject with TP S/C, in addition showed significant increase comparing

with Finasteride, on other hand obtained no significant reduction in compare to PTE 200 group.

Administration of resveratrol group in current experiments after induce BPH by giving S/C testosterone propionate for Wister rat had significant increase compared with [Control, Finasteride and PTE 200] groups in PSA levels further more showed a significant decrease in PSA serum level in compare with Induction group, these finding contradicted with previous studies [13,43].

The histopathological changes of prostate in studied groups

The current study's histopathological findings supported the biochemical findings, in which the stromal and epithelial cells of the prostate tissue were significantly adversely affected by testosterone propionate, Dihydrotesto-sterone [DHT], an androgen produced when testosterone is converted into its metabolite by the enzyme 5-reductase, appears to be the primary hormonal inducer of stromal and glandular growth in males. Due to its larger affinity for ARs than testosterone [which is three times more] and adrenal androgen [which has 15–30 times greater affinity for the receptor], DHT is a more powerful androgen than testosterone, to boost the transcription of androgen-dependent genes and eventually drive protein synthesis, the hormone receptor interacts to particular DNA-binding sites in the nucleus [44,45].

The presented study was considered to be first study for Pterostilbene administration for BPH, Histopathological outcomes in the PTE group[treated group] revealed evidently, the lumen area was smaller when PTE was administered, the amount of epithelial thickening decreased, and in a dose-dependent manner, there were also fewer papillary fronds and a greater lumen area, Recent research indicates that PTE inhibits proliferation and induces apoptosis in a variety of malignancies to provide its anticancer effect[46].

The finasteride group had a not good decrease in proliferation When compared to the induction group, after the giving of finasteride. This outcome was in contrast to a prior study that discovered finasteride greatly reduced hypertrophy brought on by T.P, moderate reduction in the thickness of the epithelial layer and hyperplasia, few hypertrophied alveoli were seen in the prostatic lobules, along with normal columnar epithelial cells, normal basal cells, and stromal tissue. along with minor reduction in inflammatory cells [46,47].

The biochemical findings in the current investigation were supported by the histological findings, the previous study showed that resveratrol significantly improved the structure of prostate tissue, there were only a few hypertrophied alveoli seen in the prostatic lobules. The alveoli showed mild luminal epithelial hyperplasia together with hyperplasia of basal cells and stromal invasion. The epithelial cells had a noticeable rise in height, which was linked to an increase in secretion and a number of apoptotic [48,49].

Regarding histopathological finding, the histology of BPH resected tissues, inflammatory cells and pro inflammatory cytokines such interferon-mRNA, interleukins [IL-2, IL-4, IL-6, IL-7, IL-8, IL-15, IL-17], and tumor necrosis factor-alpha [α] have been found [50]. Additionally, it has been discovered that the interstitium and surrounding epithelial glands of BPH contain elevated amounts of inflammatory cells. Increases in pro inflammatory cytokines occur along with the infiltration of inflammatory cells in BPH, The BPH epithelial cells enhanced expression of both TNF-α receptor types [51]. The enzyme 5-alpha-reductase converts nearly 90% of testosterone to DHT, the primary tissue androgen involved in the formation of the adult prostate [52]. The androgen receptor is active when DHT and the remaining testosterone attach to it. The androgen receptor then travels to the nucleus where it binds to androgen-responsive regions in

the DNA of prostate cells, finally causing proliferation [53]. Oncogenic transcription factors can be activated by the pro inflammatory cytokine TNF-α, Proliferation, anti-apoptotic activity, and inflammatory response are further accelerated by these factors, TNF- plays a crucial role in the prostate's inflammatory and cancer-promoting pathways, according to epidemiologic and molecular findings [50].

In the tissues of BPH patients, the activity of proteins like glutathione peroxidase and/or catalase that reduce oxidative stress and oxidative DNA damage has diminished. In line with similar human studies, elderly rats' prostates have lower glutathione peroxidase gene expression [51]. The Malondialdeyde, a byproduct of oxidative damage, had higher plasma levels in BPH patients Since plasma levels of malondialdehyde drop after surgically removing BPH tissue, some of the elevated levels of malondialdehyde are caused by hyperplastic [52].

## Conclusion

The pterostilbene have good effect on benign prostatic hyperplasia in reducing the prostate specific antigen [PSA] level and have a bigger antioxidant activity than resveratrol in DPPH assay and suppress the proliferation in BPH.

**Acknowledgment**

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https://www.uomustansiriyah.edu.iq/

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