



## **The possible protective effect of pumpkin seed oil on dutasteride-induced changes on spleen of adult male albino rats: Histological and immunohistochemical study**

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### **Abstract:**

**Background:** Dutasteride is an anti-androgen used in the treatment of prostatic diseases. Pumpkin seed oil (PSO) is a functional food oil with an antioxidant and immunomodulatory actions. **Aim:** assessing the possible protective effect of PSO on dutasteride-induced changes on spleen of adult male albino rats via histological and immunohistochemical methods. **Methods:** 30 male albino rats 200-250 gm divided into; Control group, Dutasteride low dose (DLD): rats received dutasteride 0.05 ml /day, Dutasteride high dose (DHD): rats received dutasteride 0.15 ml/ day, DLD + PSO: dutasteride low dose group treated with 1.5 ml/kg/day of PSO, DHD + PSO: dutasteride high dose treated with 1.5 ml/kg/day of PSO. Doses were orally for 42 days. **Results:** **DLD &DHD groups (Groups II &III)** showed marked histopathological changes dose dependently. There was dilated blood sinusoids in the subcapsular areas & throughout the parenchyma, disorganization, dilatation& expansion of the red pulp with reduced splenic cords, disorganization & reduction in the size of the white pulp lymphoid follicles having lymphocytes with pyknotic nuclei and vacuolated cytoplasm, macrophages containing apoptotic bodies, dilated central arteriole with detached endothelium and vacuolated wall, and significantly increased collagen mean area%. Also, significantly increased mean number of immune-positive cells for CD68& TGF- $\beta$  while decreased for CD3 immunohistochemical expression. Regarding the DLD+PSO& DHD+PSO groups (Groups

III& IV); marked amelioration of the previously mentioned results was observed.

**Conclusion:** PSO has an ameliorative effect on the histopathological changes on rat spleen induced by dutasteride. So, PSO is beneficial in the treatment of side effects of dutasteride.

**Running title:** Pumpkin seed oil ameliorate dutasteride-induced spleen changes

**Keywords:** Spleen; Dutasteride; Pumpkin Seed Oil; Histopathology

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## 1. Introduction:

Dutasteride is an anti-androgen used in the treatment of different prostatic lesions, besides treatment of male pattern of hair loss. It acts through decreasing the production of dihydrotestosterone (DHT) in different body organs including prostate. This is through selective inhibition of type I and type II of 5 $\alpha$ -reductase enzyme; responsible for the conversion of testosterone into dihydrotestosterone (DHT) [1, 2].

As regards humans, DHT stimulates production of different growth factors like, epidermal growth factor (EGF), keratinocyte growth factor (KGF), as well as insulin-like growth factors (IGFs). These are responsible for cell proliferation and differentiation. Conversely, dutasteride suppresses transforming growth factor- $\beta$  (TGF- $\beta$ ). So, as dutasteride is beneficial for the treatment of different prostatic lesions like benign prostate hyperplasia; it may cause adverse cellular effect on other

body organs due to suppression of DHT actions [3].

Decreased libido, in addition to erectile dysfunction, decreased sperm count, gynecomastia, besides skin changes, cognitive impairment, fatigue, anxiety, as well as depression are also reported as sides effects for dutasteride [4, 5].

Pumpkin is an important vegetable in the traditional medicine that is used by many countries. Pumpkin seed oil (PSO) is a functional food oil of good taste with a hazelnut- like flavor. PSO has an immunomodulatory action, and proved to be useful for the reproductive health and many disease conditions due to its lipoxygenase inhibitory effect [6, 7]. It possesses potent antioxidant effects due to the presence of different constituents. These includes; phytosterols, proteins, polyunsaturated fatty acids, besides different antioxidant vitamins,  $\beta$ -carotenes, and  $\alpha$ -tocopherols [8, 9]. Moreover, it rich in minerals like

potassium, magnesium, and phosphorus. Also, rich in selenium, lutein as well as vanillic and ferulic acid [10]. The antioxidant properties of PSO also reported by different experimental studies on the liver, heart, and reproductive organs [11, 12].

Regarding the previously mentioned data; the aim of the present study is to evaluate the possible protective effect of pumpkin seed oil on dutasteride-induced changes on spleen of adult male albino rats through using histological and immunohistochemical studies.

## **2. Material and methods:**

### **2.1. Drugs**

Avodart (Avodart, galasco, Egypt), soft gelatin capsules filled with an oily and yellowish liquid. Pumpkin seed oil (Imtenan Health Company, Egypt).

### **2.2. Animals**

Thirty adult male albino rats were used (200-250 gm). They were housed at the animal house of Tanta University. They were acclimatized to a hygienic environment with normal room temperature and were fed well-balanced diet and water. The present research was signed with an approval code No.

35779/9/22 from the Institution of Research Ethics Committee, Tanta University.

### **2.3. Experimental groups**

The following groups were used:

**Group 1 (Control group):** 10 rats that were further subdivided into two subgroups; **subgroup a:** 5 rats received no treatments. **Subgroup b:** 5 rats received 1.5 ml/kg/day of PSO and were sacrificed at the duration corresponding to their experimental groups.

**Group 2 (Dutasteride low dose: DLD):** 5 rats received low-dose of dutasteride 0.05 ml (0.04 mg/kg)/day.

**Group 3 (Dutasteride high dose: DHD):** 5 rats received high-dose of dutasteride 0.15 ml (0.12 mg/kg) / day.

**Group 4 (Dutasteride low dose + pumpkin seed oil) (DLD+PSO):** 5 rats received 1.5 ml/kg/day of PSO one hour before low-dose of dutasteride 0.05 ml (0.04 mg/kg)/day.

**Group 5 (Dutasteride high dose + pumpkin seed oil) (DHD+PSO):** 5 rats received 1.5 ml/kg/day of PSO one hour before high-dose of dutasteride 0.15 ml (0.12 mg/kg)/day.

The doses of dutasteride and PSO were administered orally through an intragastric tube for 42 constitutive days and were adjusted according to Mohamad

et al., (2019) [3] and Eraslan et al., (2013) [10] respectively.

At the last day of the experiment, rats were anesthetized by intraperitoneal injection of pentobarbital (40 mg/kg) [13]. Then, spleen samples were dissected out, and washed with saline then, were processed to obtain sections for histological and immunohistochemical evaluation.

## **2.4. Histopathology:**

### **2.4.1.Processing of specimens for light microscopic study [14]:**

- Spleen specimens were fixed in 10% formalin buffered saline. Then, dehydrated and cleared. After embedding, sections of 5 microns were obtained and undergo processing to be stained with Hematoxylin & Eosin (H&E) & Masson's Trichrome.

### **2.4.2.Immunohistochemistry of CD3 (Cluster differentiation 3), CD68 (Cluster differentiation 68), and TGF- $\beta$ 1 (Transforming growth factor- $\beta$ ):**

Splenic sections were undergoing deparaffinization, rehydration and placed at 0.3% hydrogen peroxide/methanol. Then, primary antibodies directed against CD3 (Abcam, UK, 1:100) [15], CD68 (Abcam, UK, 1:200) [16], and TGF- $\beta$ 1 (Abcam, UK, 1:150) [17] were added to sections overnight at 4°C overnight.

Then, the secondary antibody (Vector Labs, UK, 1:100) was added followed by the addition of 3, 3'-diaminobenzidine chromogen and counterstaining with Mayer's hematoxylin. To finish, sections were examined by a light microscope (Olympus company, Japan). Regarding negative control; the previously mentioned steps were repeated but without adding primary antibody. The positive control for CD3& CDE68 was human tonsil, and for TGF- $\beta$ 1 was human breast carcinoma (Abcam, UK).

## **2.5. Morphometry**

A light microscope (Olympus, Japan) with a built-in camera was used to examine and photograph the slides in the Histology and Cell Biology department, Faculty of Medicine, Tanta University. For morphometric analysis, the software "ImageJ" (version 1.48v National Institute of Health, Bethesda, Maryland, USA) was used. Non-overlapping 10 fields for each group were examined at a magnification of 400x to quantitatively evaluate in the followings:

- 1- The mean area% of collagen fibers in Masson's trichrome stained slides.
- 2- The mean number of CD3 +ve cells
- 3- The mean number of CD68 +ve cells
- 4- The mean number of TGF- $\beta$  +ve cells

## **2.6. Statistics**

In order to perform statistical analysis of the present work; data were interpreted and were expressed using by mean  $\pm$  standard deviation (SD). Later, the statistical software program “Graph Pad in Stat” (USA) was used to in order to express the results of the different experimental groups. Through which ANOVA test (analysis of variance) was used and results were considered statistically significant if  $P$  value  $\leq 0.05$  while non-significant if  $P$  value  $\geq 0.05$ .

## **3. Results:**

Regarding animal mortality; there was no animal mortality during all the experimental period.

### **5.1. H&E results**

The examined H&E stained sections of the control groups depicted the normal histological structure of the splenic parenchyma as well as stroma. It was appeared as white pulp, red pulp with a capsule and trabeculae (**Figure 1.A&B**). The white pulp was formed of large rounded or oval masses of lymphoid follicles having a central germinal center. In addition to periarteriolar lymphatic sheath (PALS) that formed of numerous lymphocytes surrounding the central arterioles, and a marginal zone separating

the white pulp from the red pulp and mantle zone (**Figure 1.C&D**). Additionally, the red pulp was composed of splenic cords of lymphocytes, macrophages and blood sinusoids. The latter were lined with endothelial cells (**Figure 1.E**).

As regards the **DLD group (Group II)**; it showed marked histopathological changes. These were in the form of disorganization, expansion of the red pulp and disorganization of the white pulp, besides dilated blood sinusoids in the subcapsular region as well as throughout the splenic parenchyma (**Figure 2.A, B&C**). Furthermore, multiple macrophages and megakaryocytes with their multilobed nuclei were prominent. Regarding the white pulp lymphoid follicles; they were disorganized with lymphocytes having pyknotic nuclei and vacuolated cytoplasm, besides macrophages with its sequestered material. Also, dilated central arteriole with detached endothelial lining and vacuolated wall was encountered. Other lymphoid follicle appeared with germinal center full of either lymphocyte with pyknotic nuclei and vacuolated cytoplasm or macrophage containing apoptotic bodies (**Figure 2.D&E**). For the red pulp, it was formed

of dilated, congested blood sinusoids together with reduced splenic cords, containing macrophages and megakaryocyte (**Figure 2-F&G**).

**As regards DHD group (Group III); marked** reduction in the size of the white pulp and expansion of the red pulp was recorded (**Figure 3.A&B**), in addition to disorganized, atrophied lymphoid follicles with contracted central arterioles that was surrounded by lymphocytes with pyknotic nuclei and vacuolated cytoplasm (**Figure 3.C**). There was also dilatation of the vascular spaces in the subcapsular areas & throughout the parenchyma with depletion of the splenic cords. Besides, many macrophages and megakaryocytes were present within the red pulp (**Figure 3.D&E**)

**The DLD+PSO group (Group IV)** showed marked improvement in the spleen histological structure. There was well organized white & red pulps (**Figure 4.A**). Together with nearly normal lymphoid follicles (**Figure 4.B**). Similarly, many macrophages appeared within the splenic cords separated by blood sinusoids (**Figure 4.C**).

**Considering the DHD+PSO group (Group V);** it revealed restoration of the size of white pulp lymphoid follicles and splenic cords as compared to group III

(**Figure 5.A**). Additionally, nearly normal lymphoid follicles appeared except for some lymphocytes that still with pyknotic nuclei and vacuolated cytoplasm (**Figure 5.B**). There was also blood sinusoids within the red pulp that appeared less dilated as compared to group III while, restored splenic cords with many macrophages was encountered (**Figure 5.C**).

## **5.2. Masson's trichrome results**

The control group revealed collagen fibers within the capsule, trabeculae and throughout the parenchyma. As regards the DLD &DHD groups; they showed marked and significantly increased collagen fibers ( $p<0.001$ ) as compared to the control group. In contrast, the DLD+ PSO and DHD+PSO groups appeared with nearly normal collagen fibers with a non-significant difference ( $p>0.05$ ) as compared to the control group respectively (**Table. 1**) (**Figures 6. A-E& 7**).

## **5.3.CD3 immunohistochemical results**

The examined control groups represented many positive cytoplasmic immunoreactions for CD3 in the lymphocytes of PALS and splenic cords. While, at the DLD, DHD & DHD+PSO groups, marked and significant decrease ( $p<0.001$ ) in the number of CD3

immunopositive cells appeared mainly in the splenic cords as compared to the control group. Contrary, the DLD+PSO group revealed nearly normal CD3 expression with a non-significant difference ( $p>0.05$ ) as compared to the control group (**Table. 1**) (**Figures 8. A-E& 9**).

### **5.3. CD68immunohistochemical results**

There was positive cytoplasmic immunoreaction for CD68 in macrophages of splenic cords of the control group. As for DLD & DHD groups; marked and significantly increased CD68 immuno-positive macrophages was encountered especially at the white pulp and splenic cords ( $p<0.001$ ) as compared to the control group. In contrast, the DLD+PSO & DHD+PSO groups showed decreased

number of CD68 immuno-positive macrophages in the splenic cords with a non -significant difference ( $p>0.05$ ) when compared to the control group (**Table. 1**) (**Figures 10. A-E& 11**).

### **5.4. TGF- $\beta$ immunohistochemical results**

Examination of the control groups revealed few cells with positive TGF- $\beta$  cytoplasmic immunoreaction. The DLD & DHD groups showed marked and significant increase ( $p<0.001$ ) in the number of TGF- $\beta$  immune-positive cells in white pulp and splenic cords as compared to the control group. On the other hand, the DLD+PSO & DHD+PSO groups showed marked and significant decrease in the number of TGF- $\beta$  immuno-positive cells (**Table. 1**) (**Figures 12.A-E& 13**).



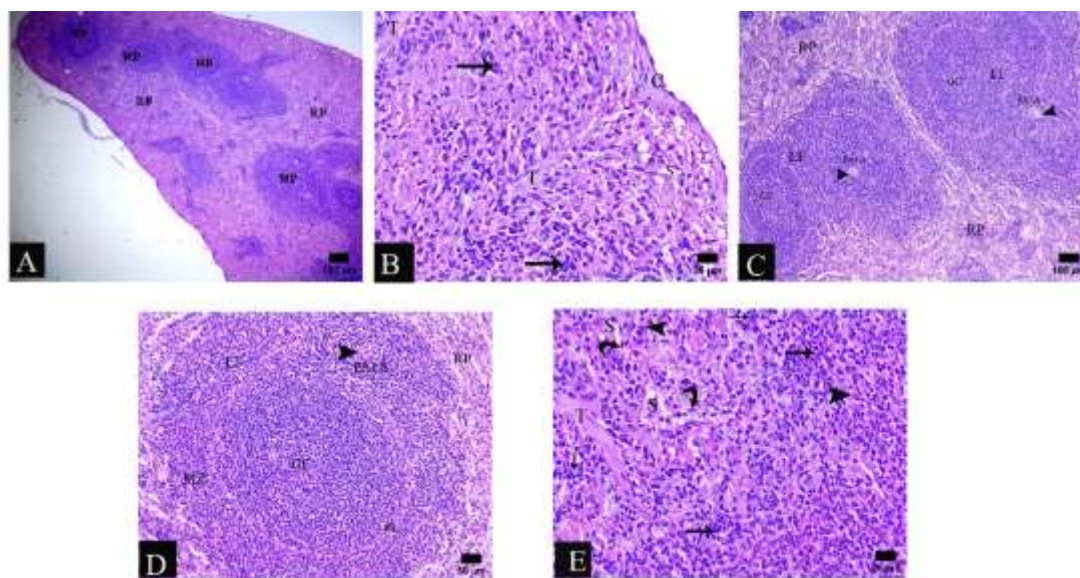
**Table (1): Comparison between the different experimental groups presented as mean±SD**

	<b>Control</b>	<b>DLD</b>	<b>DHD</b>	<b>DLD+PS O</b>	<b>DHD+PSO</b>
<b>Mean area % of collagen</b>	11.78±5.72	28.28±7.99* **	43.67±11.08***	13.36±8.45	13.46±4.17
<b>Mean number of CD3 +ve cells</b>	141.44±14.23	65.22±6.67* **	52.22±4.41* **	131±16.02	93.22±4.60
<b>Mean number of CD68 +ve cells</b>	33.11±5.33	139.44±31.73***	131.67±28.61***	49.22±10.76	58.89±19.97
<b>Mean number of TGF-β+ve cells</b>	9±1.94	139.67±22.90***	160±23.39** *	35.44±9.59*	38.89±11.49*

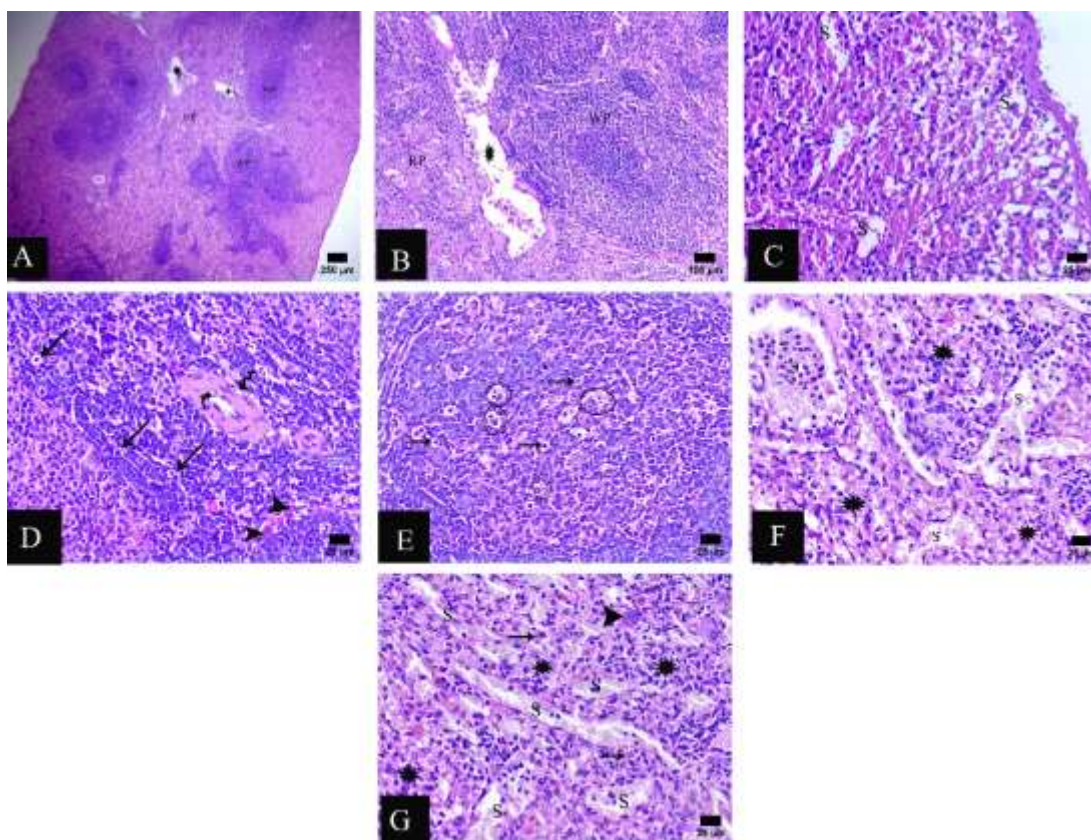
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\*Significant as compared to control

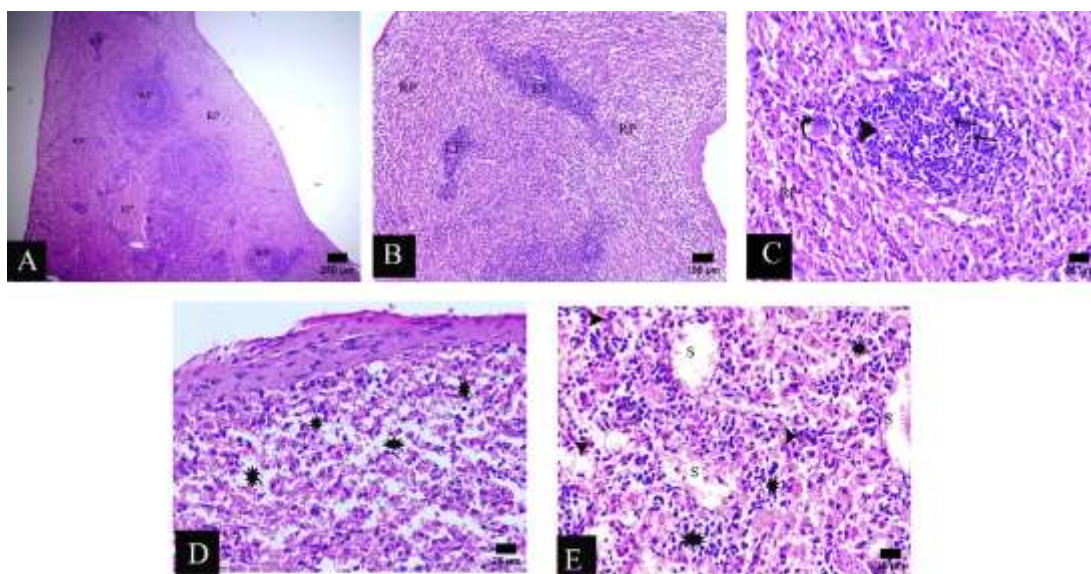




**Figure 1:** photomicrographs of H&E stained rat spleen sections from control group showing the normal histological structure : (A) showing the distribution of white pulp(WP) and red pulp(RP).(B) showing capsule (C), part of trabeculae(T), splenic cords (arrows) and blood sinusoids(S).(C & D)showing white pulp lymphoid follicles(LF) formed of aggregation of lymphocytes with germinal center(GC), mantle zone (m),marginal zone(MZ) separating it from red pulp (RP), central arteriole(arrow head) surrounded by periarterial lymphatic sheath(PALS). (E)showing the red pulp containing splenic cords of cells(arrows), macrophages identified by its sequestered material (arrow heads),parts of trabeculae(T)and blood sinusoides (S)lined by simple endothelium (curved arrows). (H&E A x40 scale bar=250 $\mu$ , B x400 scale bar =25 $\mu$ , C x100 scale bar=100 $\mu$ ,D x200 scale bar = 50 $\mu$  and E x400 scale bar =25 $\mu$ )



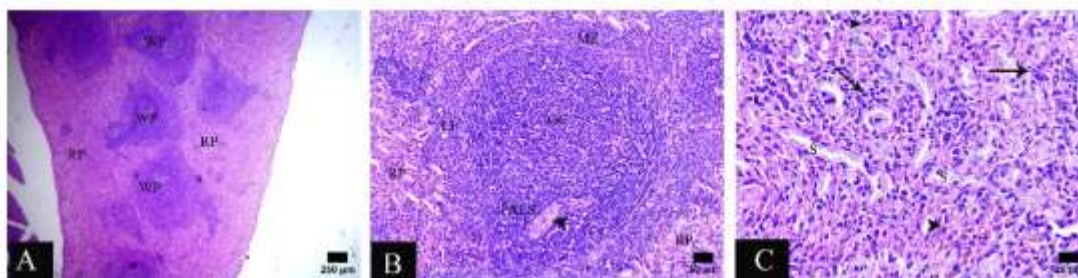
**Figure 2:** photomicrographs of H&E stained rat spleen sections from DLD group II. (A&B) showing dilatation and congestion (stars) within the red pulp (RP) and disturbed white pulp (WP). (C) showing dilatation of subcapsular blood sinusoids (S) and thinning of splenic cords. (D) showing disorganized lymphoid follicle containing lymphocytes with pyknotic nuclei and vacuolated cytoplasm (arrows), macrophages with its sequestered material (arrow heads), dilated central arteriole with detached endothelial lining (curved arrow) and vacuolated wall (wavy arrow). (E) showing abnormal lymphoid follicle which its germinal center is full of either lymphocyte with pyknotic nuclei and vacuolated cytoplasm (arrows) or macrophage containing apoptotic bodies (circles). (F &G) showing the red pulp consists of dilated, congested blood sinusoids (S), reduced splenic cords(stars), containing macrophages (arrows) and megakaryocyte (arrow head). **(H&E A x40 scale bar=250μ, B x100 scale bar =100μ, C,D,E,F&G x400 scale bar=25μ)**



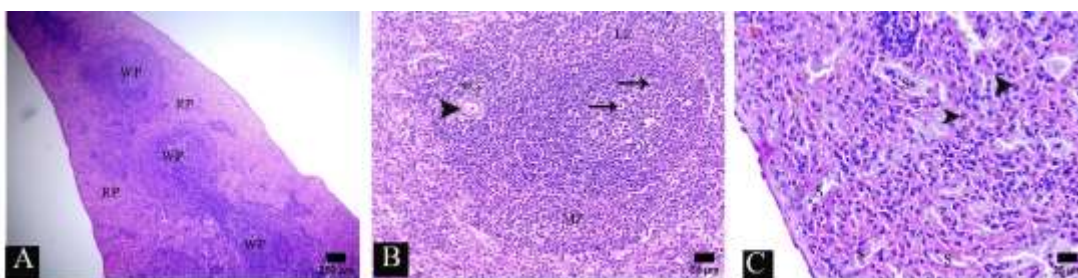
**Figure 3:** photomicrographs of H&E stained rat spleen sections from DHD group III: (A&B) showing marked reduction in the size of white pulp (WP) and expansion of red pulp (RP). (C) showing marked disturbed, atrophied lymphoid follicle containing contracted central arteriole (arrow head), lymphocytes with pyknotic nuclei and vacuolated cytoplasm (arrows) and megakaryocyte also present within the red pulp. (D) showing marked dilatation of subcapsular spaces (stars) and depletion of splenic cords. (E) showing dilated blood sinusoids (S), reduced splenic cords (stars) and macrophages with its sequestered material (arrow heads)

(H&E A x40 scale bar=250 $\mu$ , B x100 scale bar =100 $\mu$ , C,D,E&F x400 scale bar=25 $\mu$ ).

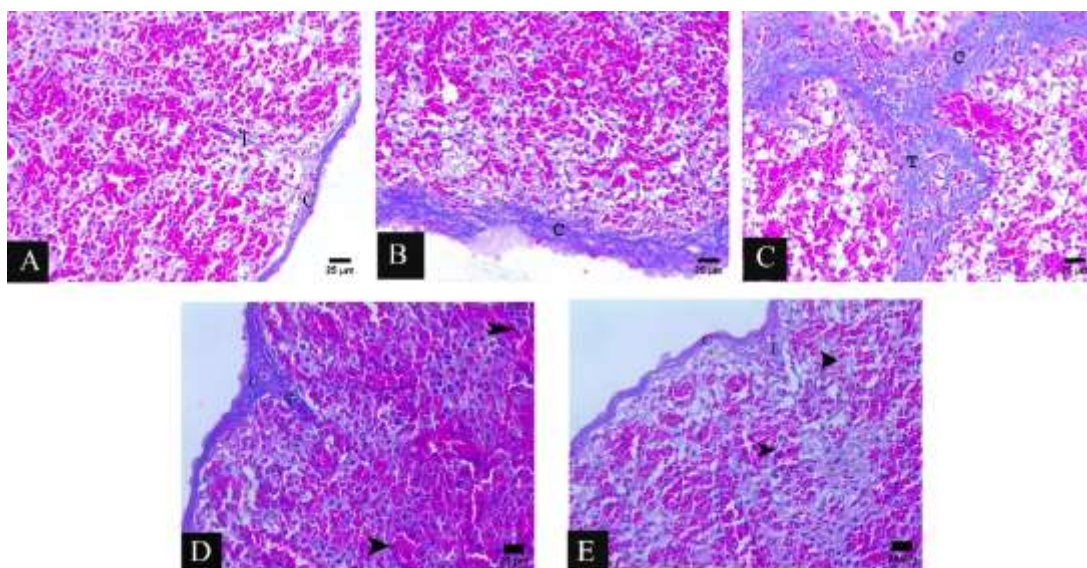




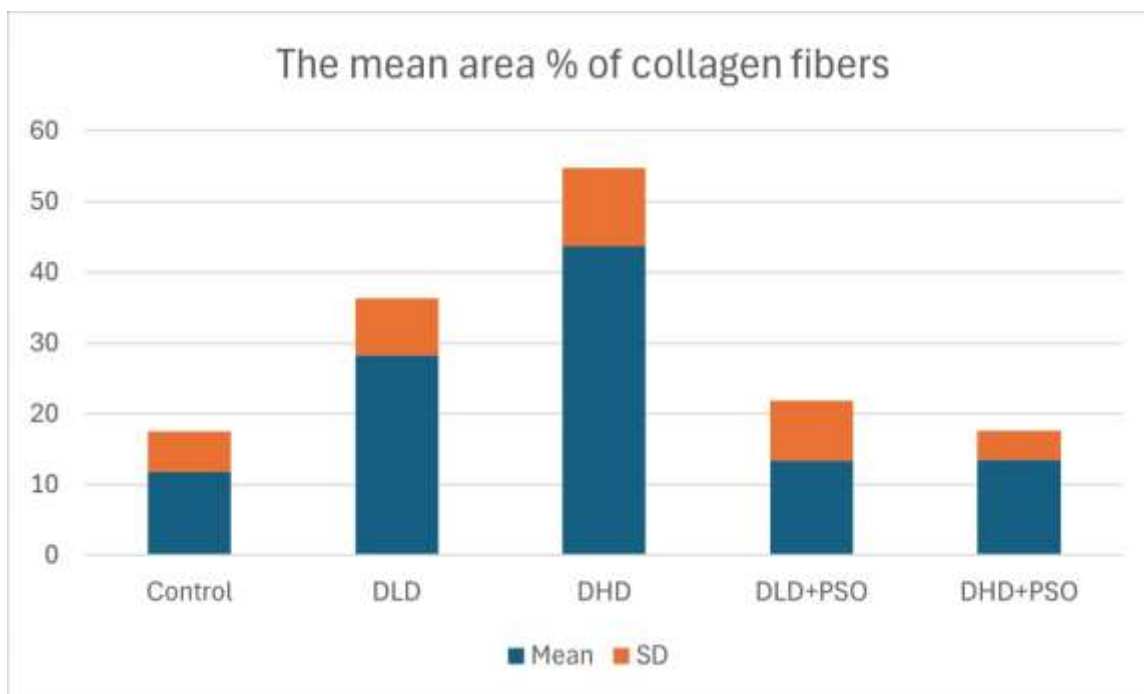
**Figure 4:** photomicrographs of H&E stained rat spleen sections from DLD+PSO group IV:(A) showing restoration of white pulp(WP) and red pulp(RP) distribution to be more or less like the control. (B) showing white pulp lymphoid follicle like the control group. (C) showing the red pulp consists of blood sinusoids (S) and splenic cords(arrows) with many macrophages (arrow heads). (H&E A x40 scale bar=250μ, B x200 scale bar= 50 ,C x400 scale bar=25μ.)



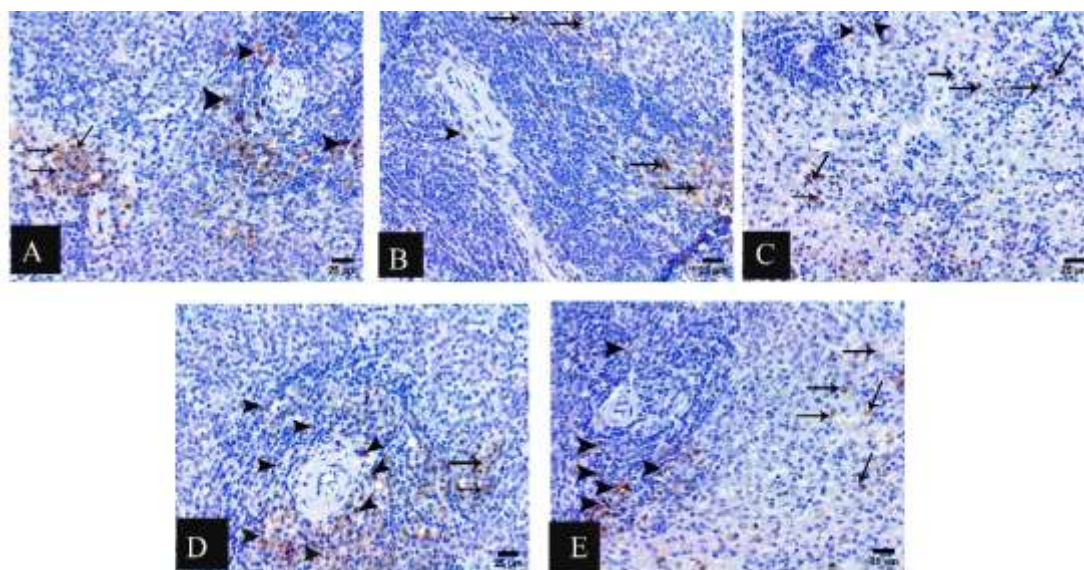
**Figure 5:** photomicrographs of H&E stained rat spleen sections from DHD+PSO group V: (A)showing restoration of the size of white pulp (WP) as compared to group III. (B) showing well organized lymphoid follicle except some lymphocytes appear with pyknotic nuclei and vacuolated cytoplasm (arrows). (C) showing slightly dilated blood sinusoids , splenic cords with many macrophages (arrow heads) . (H&E A x40 scale bar=250μ, B x200 scale bar= 50 ,C x400 scale bar=25μ.)



**Figure 6:** photomicrographs of masson trichrome stained rat spleen sections from the studied groups:( A) showing the distribution of collagen with the capsule(C) and trabeculae(T) in control group. (B) showing collagen fibers within the capsule in DLD group. (C) showing apparent increase in collagen fibers, thick capsule and trabeculae in DHD group. (D &E) showing collagen fibers in capsule(C) and trabeculae(T) in DLD+PSO and DHD+PSO groups respectively. (**Masson trichrome, A, B, C, D and E x400, scale bar = 25µ**)

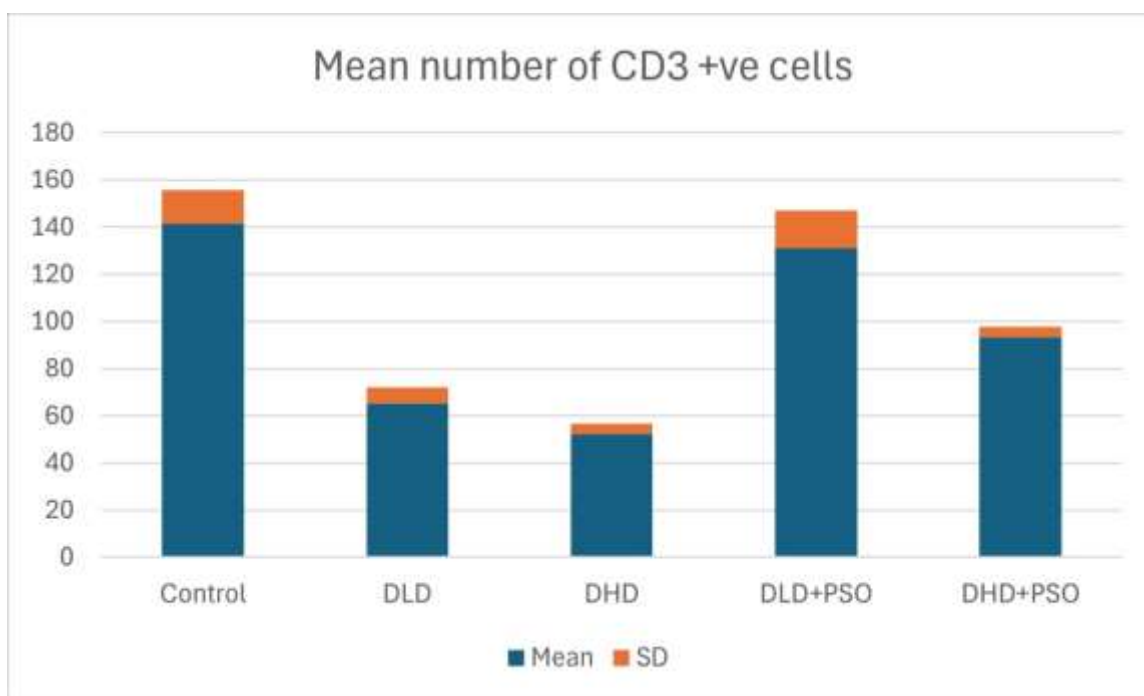


**Figure 7.:** The mean area % of collagen fibers. Values exposed as means  $\pm$  standard deviation. DLD (dutasteride low dose); DHD (dutasteride high dose); DLD+PSO (dutasteride low dose+ pumpkin seed oil); and DHD+PSO (dutasteride high dose+ pumpkin seed oil) as compared to control respectively.

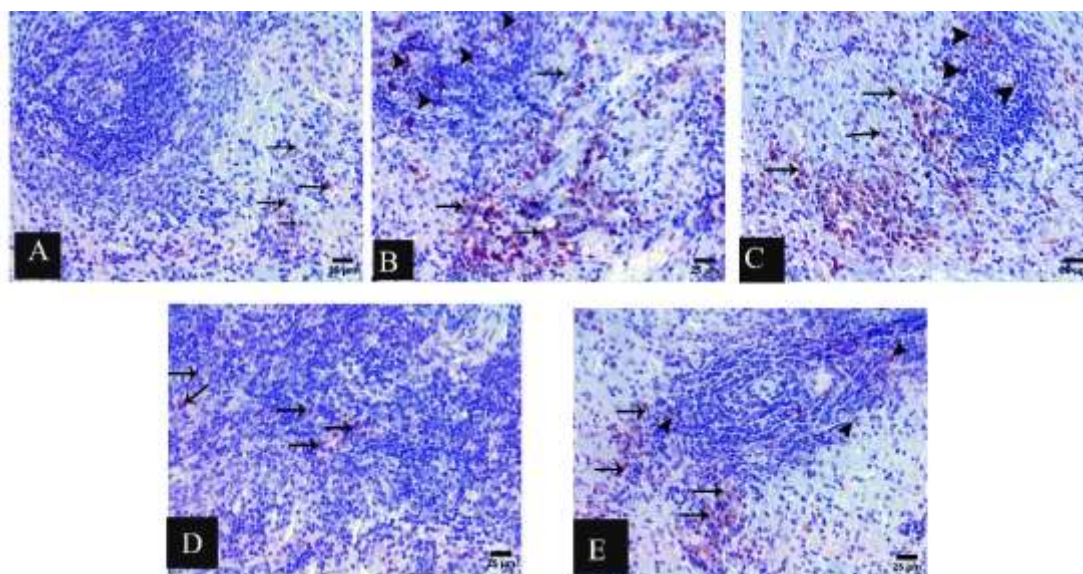


**Figure 8:** photomicrographs of CD3 immuno-stained rat spleen sections from the studied groups:( A) showing many CD3 immuno-positive cells in PALS (arrow heads) and splenic cords (arrows) in control group. (B) DLD group showing few CD3 immuno-positive cells in PALS (arrow heads) and some cells in splenic cords(arrows). (C) DHD group showing marked decrease in the CD3 immuno- positive cells either in PALS (arrow heads) or in splenic cords (arrows) as compared to control. (D&E) showing CD3 immuno- positive cells in PALS (arrow heads) and splenic cords (arrows) in in DLD+PSO and DHD+PSO groups respectively to be more or less like control. **(CD3 immunostaining, A, B, C, D and E x400, scale bar = 25µ)**

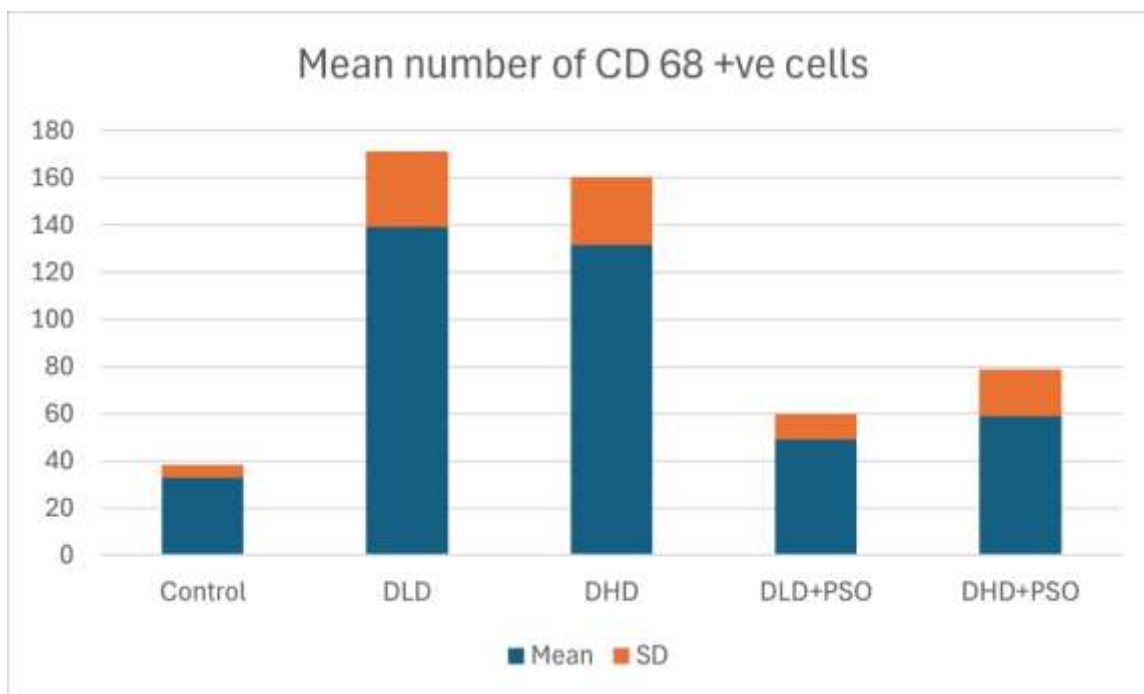




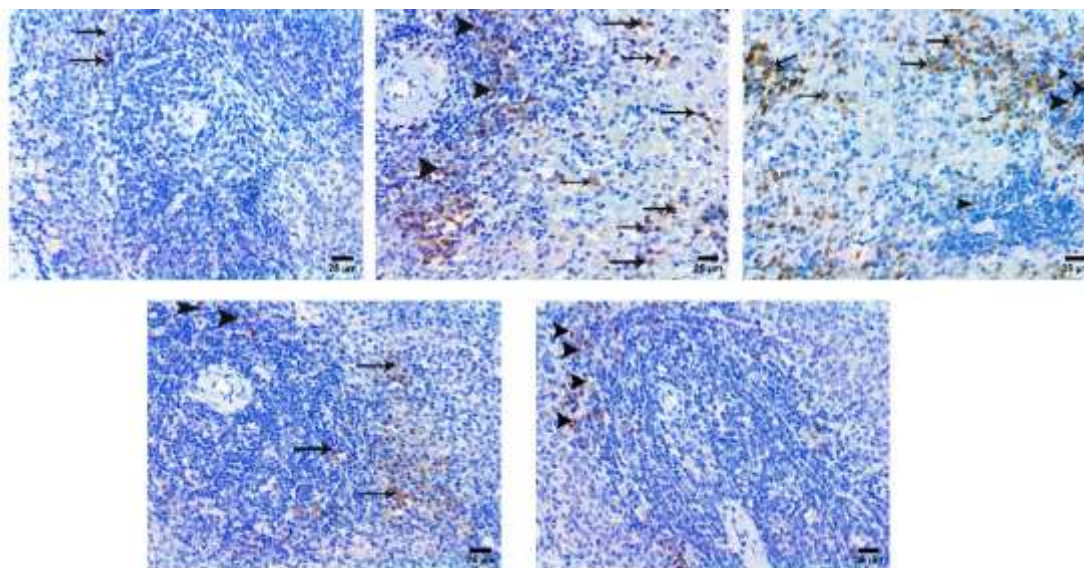
**Figure 9.** The mean number of CD3 +ve cells. Values indicated as means  $\pm$  standard deviation. DLD (dutasteride low dose); DHD (dutasteride high dose); DLD+PSO (dutasteride low dose+ pumpkin seed oil); and DHD+PSO (dutasteride high dose+ pumpkin seed oil) in comparison to control respectively.



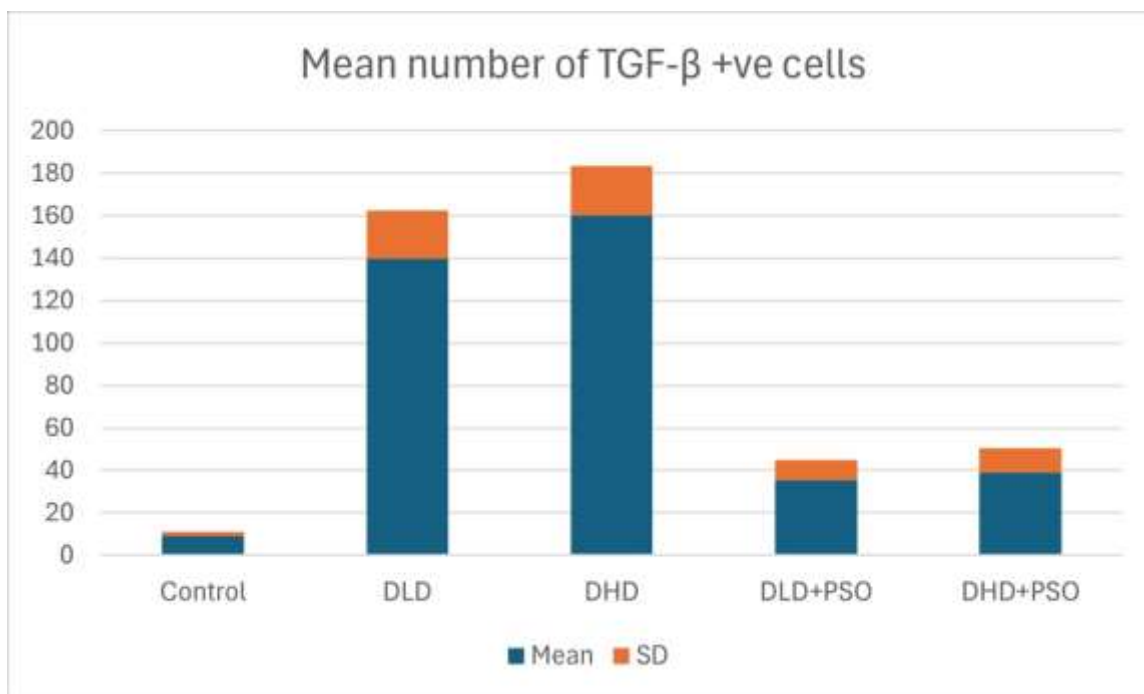
**Figure 10:** photomicrographs of CD68 immuno-stained rat spleen sections from the studied groups:( A) showing CD68 immuno-positive macrophages in splenic cords (arrows) in control group. (B&C) group showing many CD68 immuno- positive macrophages in white pulp (arrow heads) and in splenic cords(arrows) in DLD and DHD groups respectively. (D) showing CD68 immuno- positive macrophages in splenic cords (arrows) in DLD+PSO group to be more or less like control. (E) showing some CD68 immuno- positive macrophages in white pulp (arrow heads) and mainly in splenic cords (arrows) in DHD+PSO group. (CD68 immunostaining, A, B, C, D and E x400, scale bar = 25µ)



**Figure 11.** The mean number of CD68 +ve cells. Values presented as means  $\pm$  standard deviation. DLD (dutasteride low dose); DHD (dutasteride high dose); DLD+PSO (dutasteride low dose+ pumpkin seed oil); and DHD+PSO (dutasteride high dose+ pumpkin seed oil) as compared to control respectively.



**Figure 12:** photomicrographs of TFG- $\beta$  immuno-stained rat spleen sections from the studied groups:( A) control group showing few TFG- $\beta$  immuno-positive cells in splenic cords (arrows). (B &C) DLD and DHD groups respectively showing many TFG- $\beta$  immuno- positive cells in white pulp (arrow heads) and in splenic cords(arrows). (D&E) DLD+PSO and DHD+PSO groups respectively showing some TFG- $\beta$  immuno- positive cells in white pulp (arrow heads) and in splenic cords(arrows). (TFG- $\beta$  immunostaining, A, B, C, D and E x400, scale bar = 25 $\mu$ )



**Figure 13.** The mean number of TGF- $\beta$ +ve cells. Values showed as means  $\pm$  standard deviation. DLD (dutasteride low dose); DHD (dutasteride high dose); DLD+PSO (dutasteride low dose+ pumpkin seed oil); and DHD+PSO (dutasteride high dose+ pumpkin seed oil) compared to control respectively.

#### 4. Discussion:

Dutasteride is a synthetic 4-azasteroid that inhibits type I and type II 5 $\alpha$ -Reductase isozymes with dihydrotestosterone (DHT) phosphorylation suppression [18, 19]. It is effectively used in the treatment of benign prostatic hyperplasia but with systemic side effects; among them was spleen [20, 21].

The results of the present research revealed that, dutasteride-induced

histopathological changes in the rat spleen, dose dependently. The H&E stained sections revealed red pulp expansion with marked congestion, dilated congested blood vessels, multiple hemosiderin laden macrophages and megakaryocytes with their multilobed nuclei. While, the white pulps appeared disorganized with vacuolated cells and fragmented & pyknotic nuclei even lost marginal zone at high dose. Besides, dilated central arteriole associated with wall vacuolations and detached

endothelium. Also, there were dilated congested subcapsular blood vessels and some megakaryocytes.

Moreover, macrophages with apoptotic bodies discovered at the examined H&E stain of dutasteride groups. These were considered as Tingible body macrophages which were reported by many researches as large cells situated at the splenic germinal centers of secondary lymphoid tissues containing varieties of phagocytic apoptotic cells [22]. These were due to increased numbers of dutasteride induced-apoptotic lymphocytes that were engulfed by macrophages [23].

In the current work, dilated central arteriole in association with wall vacuolations and detached endothelium was detected. Dutasteride is an 5 $\alpha$ -Reductase receptor inhibitor. Its prolonged administration could lead to contractility disorders of the smooth muscle cells with subsequent decreased contractility [20].

The functions of the smooth muscle cells of the blood vessels is to control the flow of blood to the organs and its tone is undergoing regulation by its periodic contraction as well as

relaxation. This disturbed mechanism could lead to disorders of the blood flow to spleen leading to congestion as well as detached endothelium [24]. Moreover, smooth muscle cells contain different contractile proteins like actin and myosin. The contractility disorders of the smooth muscle cells might be associated with disorders of these proteins assembly that may be the cause of appeared vacuolated blood vessel wall of the spleen in the current research [20].

The associated adverse effects of dutasteride on the splenic white pulps in the present work was noticed. These include disorganization and decreased number and size of the lymphatic follicles in addition to lost prominent marginal zone. Dutasteride has an effect on the immune cells of spleen. It could suppress the expression of proinflammatory cytokines which might be mediated through the effect of dutasteride on estrogen receptor  $\beta$ . The latter has an important role in the body immunoprotection. It is inhibited by dutasteride through inhibition of its mRNA expression with subsequent inhibition of the release of IL-1 $\beta$ , and IL-18. So, the anti-inflammatory effect of



dutasteride could induce suppressing effect on splenic lymphatic follicles [25].

Dutasteride treatment is associated with tissue adverse effects due to the inhibition of antioxidant production provoking oxidative tissue damage [26]. This is confirmed by the study made by Olayinka ET & Adewole KE, (2023) [26] who proved the effect of dutasteride on the testicular antioxidant levels. They proved that dutasteride inhibited the production of superoxide dismutase, catalase, Glutathione-S-transferase and acid phosphatase, in addition to reduced levels of ascorbic acid and glutathione. Oxidative stress leads to production and accumulation of reactive oxygen species (ROS) like Superoxide radicals, hydrogen peroxide, and singlet oxygen at splenic tissue with subsequent cell and tissue damage [27, 28]. The affected splenic cells in the present research was seen which is in the form of vacuolations and fragmentation could be due to the harmful effect of ROS on the cell structure. It appeared in the form of lipid peroxidation with defective membrane lipids, defective protein modifications with impaired enzymatic activity, besides DNA lesions with nucleic acid and nuclear affection [29]. Moreover,

oxidative cell damage will activate the apoptotic signaling pathway initiating programmed cell death with apoptotic cell features appeared as dark pyknotic nuclei of lymphocytes [30].

Splenic macrophages have an important role in the preservation of tissue homeostasis as well as inflammation. They are responsible for the clearance of body old and deformed erythrocytes as well as foreign antigens [31]. Hemosiderin laden macrophages is a prominent feature of the results of the present work. This is might due to the destructive effect of dutasteride on the splenic architecture and its lymphatic follicles. It is well known that splenic macrophages have a great role in the metabolism of iron by phagocytosis of damaged and old erythrocytes with recycling of its iron content [32]. On the other hand, the oxidative stress induced by dutasteride is associated with adverse effects on the erythrocytes leading to conformational changes on erythrocyte shao, membrane composition as well as hemoglobin content leading to hemolytic effects with subsequent increased activity of splenic macrophages to get rid of them [30]. Moreover, the affected erythrocytes will initiate the body immune and



inflammatory processes [33]. These previously mentioned data could explain the significant increase in the activity and number of splenic macrophages proved by the significantly increased expression of CD68 at DLD& DHD group of the present work and that was in a dose dependent manner.

In the present work, significantly increased collagen fibers was noticed as at dutasteride groups in a dose dependent manner compared with control. This could occur as a reactive splenic process following its tissue injury and inflammation. Also it is might due to upregulation of TGF- $\beta$ 1 signaling pathway. The latter has an important role in the process of fibrosis which is also proved at the present study by the significantly elevated levels of TGF- $\beta$ 1 immunohistochemical expression. Additionally, TGF- $\beta$ 1 could downregulate collagenase enzyme production besides its stimulated effect on the production of metalloproteinase inhibitors. Consequently, the inhibition of extracellular matrix degradation with net result is fibrosis [34, 35].

Considering the immunohistochemical results of the present study, a significantly decreased

expression of CD3 at dutasteride groups was recorded in comparison to the control group. The body immune system is very important for protecting the body against foreign and toxic substances. Spleen is a large immune organ has an important role in the defense mechanisms because of its lymphocytes and macrophages [15]. It was recorded that dutasteride affect the body immune system through its inhibitory effect on the tissue lymphocytes especially the cytotoxic one. Also it was clear that by the present study that dutasteride affect the splenic lymphatic follicle with disorganization and depletion of the lymphatic follicle. All of which could have adverse effects on the body immunity due to decreased expression as well as CD3 T-lymphocytes numbers [15, 36].

The present research revealed marked improvement of the histopathological and immunohistochemical changes on the splenic structure induced by dutasteride. Pumpkin seed oil (PSO) is extracted from the pumpkin seeds. It possesses many health benefits due to its unsaturated fatty acid especially Omega-3 &6 as well as many nutrients with an antioxidant property [37, 38]. It showed its effects on

the treatment and prevention of diseases like cardiovascular, cerebrovascular and prostatic diseases, because of its lipid content as well as bioactive substances [39]. Recently, PSO requirements and medical applications is increased as a source of functional oil in food, cosmetics, and pharmaceuticals. That's because of its antioxidant property with the presence of many bioactive compound like tocopherols ( $\alpha$ ,  $\beta$ ,  $\gamma$  and  $\delta$ ), minerals, vitamins and carotenoids that act as free radical scavengers [40]. Abdelnour et al., (2023) [41] also added that, essential oils like PSO is very important for the enhancement of immunity, antioxidants activation as well as cell growth and regeneration. The bioactive constituents of PSO found to induce effects on estrogen levels. So, could reverses the inhibitory action of dutasteride on estrogen receptors consequently, improving tissue immunity and the return and regeneration of splenic lymphatic follicles [42, 43].

Tocopherols besides their antioxidant and anti-inflammatory effects play a very important role the regulation of gene transcription besides, they promote cell proliferation and differentiation, cell adhesion, as well as

the body immune response and prevention of DNA damage. In the meantime, B-Carotene; a constituent of PSO is considered as a precursor of vitamin A. the latter is very important for tissue health with an important role in the immune response of the tissues and cells especially spleen [44].

In addition, the powerful antioxidant activity is due to its phenolic compounds' content like ferulic acid, apigenin, quercetin, and vanillic acid. These compounds help removing the oxidative stress associated reactive oxygen species improving tissue and cell health state [45].

The present work showed a significant decrease in collagen fibers of groups DLD+PSO& DSHD+PSO. The antifibrotic action of PSO is proved by El-mehi et al., (2023) [46] during their study on liver fibrosis. They recorded the decreased fibrosis due to inhibition of TGF $\beta$ 1 activity; an inflammatory cytokines responsible for the incidence of tissue fibrosis. This is also proved by the present study through the significantly decreased levels of TGF $\beta$ 1 immunohistochemical reaction of the dutasteride groups treated by PSO.

The present work revealed significant decrease of CD68 immunohistochemical expression. This could be due to the effect of PSO on erythrocytes. As it prevents erythrocyte hemolysis due to its antioxidant actions [47].

## **5. Conclusion:**

From the previously mentioned results; it could be concluded that PSO has ameliorative effect on the histopathological changes on rat spleen induced by dutasteride. So, PSO may be of benefit in the treatment of side effects associated with dutasteride treatment.

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