

## Study The Toxic Effect Of Different Doses Of Duprost On Spleen Of Albino Mice.

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**Abstract:** The intent of this task was to examine the toxic effect of different dosages of Duprost (dutasteride) on the spleen of mice.

**Methods:** Twenty four albino mice were purchased and fractioned into three parts: control (3 mice), acute group (12 mice) and chronic group (9 mice). The acute group was subdivided to 4 groups, each of which contained 3mice. Every group was given a lonely oral dose of the following doses: subgroup1 dosed with 0.25ml (0.5 mg/kg of Duprost, subgroup2 0.15ml (0.12mg/kg) dose, subgroup3 0.1 ml (0.08mg/kg) dose and subgroup4 0.05ml (0.04mg/kg) dose of Duprost for 24 hours. Whereas the chronic group was subdivided into 3 subgroups and each set was given a daily dose of (0.15ml, 0.1ml, and 0.05 ml of Duprost respectively) for 42 days. After the mentioned periods, the mice of all groups were sacrificed and the spleen of each animal was removed, processed, sectioned and stained for histological analysis.

**Results:** In acute group, all mice that dosed with (0.25 ml) dose passed after 15 minutes of dosing. The histological analysis of spleen in residual mice of acute subgroups showed marked congestion and edema. In comparison with chronic subgroups in which a depletion of white pulp had been noticed in addition to the absence of follicles and decreased size of the periarterial lymphatic sheaths(PALS), irregular bands of fibrosis and early collagen deposition causing distortion of the splenic contour. Moreover, there was an expansion of red pulp with increased cellularity and size of the red pulp. And the separation of splenic cords by irregularly shaped wide sinusoids.

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### I. Introduction:

Benign prostatic hyperplasia (BPH) is a common cause of difficult lower urinary tract symptoms. It is a form of extension of the prostate which is common among aging men, with a rate of 90 % by the age of 85 [1]. This disease is a progressive condition, with growth in prostate size accompanied by lower urinary tract symptoms that can result in long-term complications and need for enlarged prostate-related surgery [2]. Current pharmacologic treatment options include alpha-blockers (like: alfuzosin, doxazosin, tamsulosin, and terazosin) and 5-alpha-reductase inhibitors (5ARIs) (include finasteride and dutasteride) [3].

Recently, Duprost (active ingredient: Dutasteride 0.5mg, type: 5 $\alpha$ -reductase inhibitor / anti-androgenic) is used to treat BPH. This remedy helps to shrink the prostate and reduce the risk of urinary retention caused by restricted urine flow as the prostate gland becomes enlarged and presses against the urethra which helps urine to flow more easily, prevents urine accumulation in the bladder and restores bladder control [4]. This drug is also used to treat male pattern hair loss by increasing hair growth and preventing further hair loss from all areas of the scalp, including the front [5]. The active component, Dutasteride inhibits the action of both forms (type I and type II) of the enzyme 5 $\alpha$ -reductase that convert the male hormone testosterone to dihydrotestosterone (DHT) in the skin and the liver [6]. DHT is the androgen primarily responsible for development and growth of the prostate gland and also causes BPH. The action of Dutasteride is to reduce the levels of DHT in the blood, so that prostate growth is no longer stimulated in men with BPH allowing the enlarged prostate to shrink [7]. In humans, DHT may induce the production of epidermal growth factor (EGF), keratinocyte growth factor (KGF), and insulin-like growth factors (IGFs)—all of which stimulate cellular proliferation. Similarly, DHT suppresses the capacity of transforming growth factor- $\beta$  (TGF- $\beta$ ) to induce apoptosis of prostatic epithelial cells. In prostate cancer, cell proliferation and apoptosis dysregulation leads to an imbalance between cell division and cell death, which collectively contribute to tumorigenesis and tumor progression [8].

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The most commonly reported side effects when taking this drug include decreased libido, erectile dysfunction, sexual anhedonia, decreased sperm count, gynecomastia, skin changes, cognitive impairment, fatigue, anxiety, depression, and suicidal ideation. [9]. Although, the effect of this brand on spleen is not studied according to available data.

The aim of this study is to examine the histological changes in the spleen of mice dosed orally with different doses and concentrations of Duprost.

## **II. Materials and methods:**

### **1. Experimental Animals:**

Twenty four, 6-week-old male mice, weight (20-25gm) were purchased from National Center for Drug Control and Research in Baghdad, and kept under standardized environmental conditions; constant temperature, moisture and with a 12-hr. light regime without stress factors. Mice were allowed to take laboratory food and water.

### **2. Design of the study:**

Mice were randomized into three groups, and treatment was carried out according to the following classification:

- A. Group I:** Control Group; contained 3 mice, received standardized lab food and water without treatment.
- B. Group II:** Acute group; contained 12 mice, segregated into 4 subgroups of 3 mice\subgroup, each was given a lonely oral dose of the following doses:
  - Subgroup1: dosed orally with 0.25ml (0.5mg\kg) of drug.
  - Subgroup2: dosed orally with 0.15ml (0.12mg\kg) of drug.
  - Subgroup3: dosed orally with 0.1 ml (0.08mg\kg) of drug
  - Subgroup 4: dosed orally with 0.05ml (0.04mg\kg) of drug.

All mice of subgroup 1 died after 15 minutes of dosing. After 24 hours of dosing, the residual mice of group II were euthanized; spleens were carefully removed and fixed in 10% buffered formaldehyde solution. Then, the fixed biopsies were embedded in paraffin and cut into 5µm slices. The slices were mounted on glass slides and stained with hematoxylin and eosin for histological analysis. The images were examined under light microscope.

- C. Group III:** Chronic group; contained 9 mice, divided into 3 subgroups of 3 animals per each, and received one daily oral dose of following doses:
  - **Subgroup1:** dosed orally with 0.15ml (0.12mg\kg) of drug per day, one of them died after the fifth dose while the second died after 21 days of dosing.
  - **Subgroup2:** dosed orally with 0.1 ml (0.08mg\kg)of drug per day.
  - **Subgroup3:** dosed orally with 0.05ml (0.04mg\kg) of drug per day.

During the dosing period, all mice were observed behaviorally and morphologically, and notes were recorded. After 42 days of dosing, the residual mice were sacrificed and the spleens were taken for histological study, the same as acute group.

## **III. Results:**

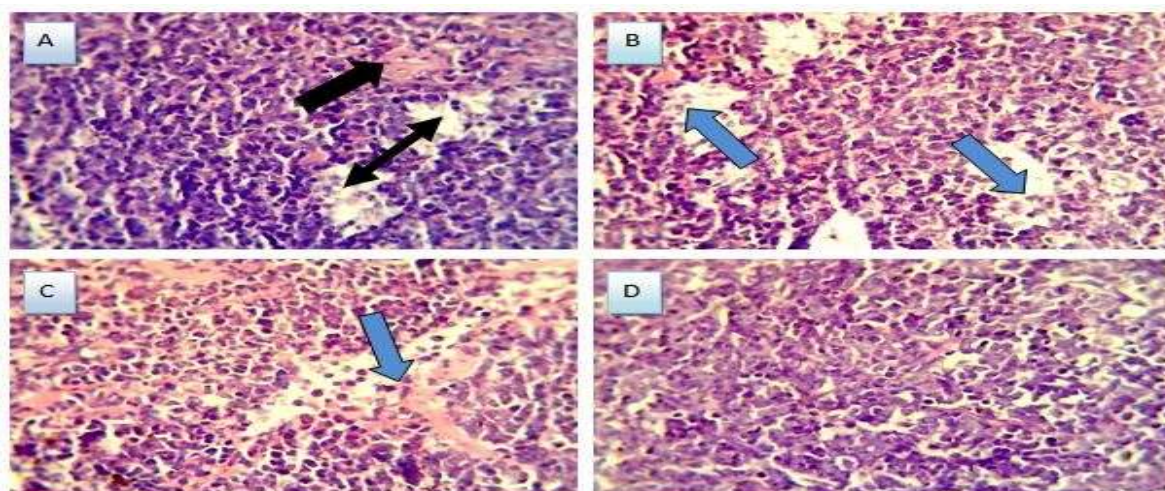
### **1. Histological changes of spleen in acute Group:**

#### **a) Effect of 0.05ml dose of Duprost:**

The section of spleen obtained from mice dosed with 0.05 ml of duprost(dutasteride) after 24hrs, showed marked edema and congestion(wide arrow) in addition to apoptosis of lymphocytes in white pulp(thin arrows) [Figures 1, A].

#### **b) Effect of 0.15ml and 0.1ml dose of Duprost:**

The section of spleen obtained from mice dosed with 0.1 ml and 0.15 ml of duprost (dutasteride) after 24hrs, showed excessive distension of sinuses within the red pulp by erythrocytes [Figures 1, B&C], compared to spleen section from control animal which showed normal structure [Figures 1, D].



**Figure 1:** A – C: Spleen of 6-week-old male albino mice, after 24 hours from single oral dose of Dutasteride (Duprost) [0.05ml (0.04mg/kg), 0.1 ml (0.08mg/kg), 0.15 ml (0.12mg/kg) respectively], shows marked edema and congestion (wide black arrow), apoptosis of lymphocytes in white pulp (thin black arrows) excessive distension of sinuses within the red pulp by erythrocytes (blue arrows). (H&E, 40x). D: Spleen from a 6-week-old male mice control on the same study, demonstrates normal dec pulp forms the bulk of the splenic parenchyma. (H&E 40x)

## 2. Histological changes of spleen in chronic subgroups:

### a) Effect of 0.05 ml dose of Duprost:

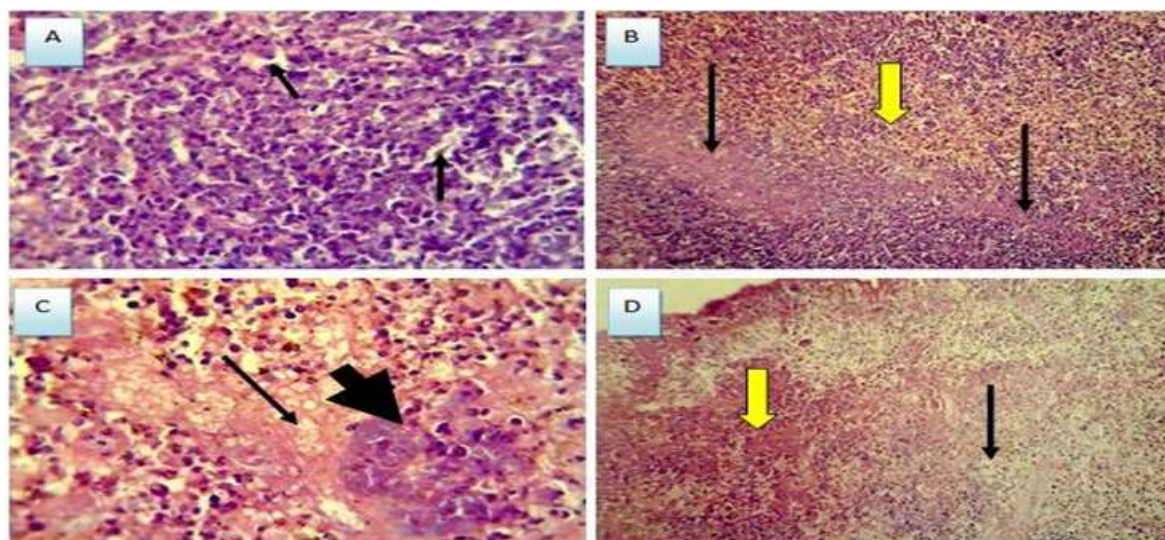
The section of spleen obtained from mice dosed with (0.05 ml) of duprost after 42 days, showed a depletion of the white pulp [Figure 2, A, B, thin arrows]. Moreover, there was an absence of follicles and decreased size of the periarterial lymphatic sheaths (PALS). The PALS has a reduced number of lymphocytes [Figure 2, B, wide yellow arrow].

### b) Effect of 0.1ml dose of Duprost:

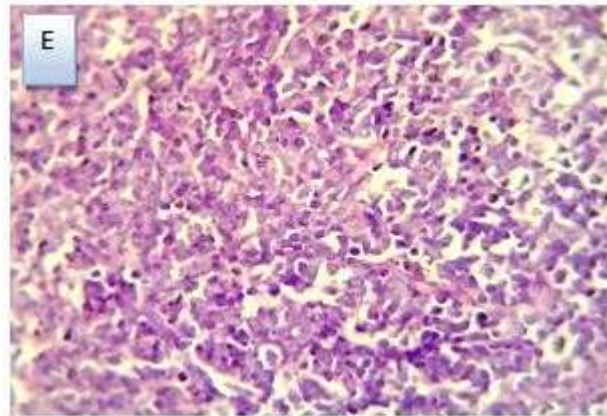
The section of spleen obtained from mice dosed with (0.1 ml) of duprost for 42 days, showed irregular bands of fibrosis and early collagen deposition causing distortion of the splenic contour (Thin black arrow) [Figure 2, C]. Additionally, in some areas of fibrosis [Figure 2, C, wide black arrow] there was a deposition of pigment consistent with hemosiderin.

### c) Effect of 0.15 ml dose of Duprost:

The section of spleen obtained from mice dosed with (0.15 ml) of duprost for 42 days, showed that the red pulp appeared to be expanded, plus the cellularity and size of the red pulp were increased [Figure 2, D yellow arrow]. And the splenic cords are separated by irregularly shaped wide sinusoids [Figure 2, D black arrow]. While spleen section from control animal which showed normal structure [Figure 2, E].







**Figure 2 :A – C:** Spleen of a 6-week-old male albino mice, from a chronic study, on one daily oral dose of Dutasteride (Duprost) (0.04mg/kg), (0.08mg/kg) and (0.12mg/kg) respectively, for 42 days. Sections show a depletion of white pulp. There is an absence of follicles and decreased size of the periarterial lymphatic sheaths (PALS). The PALS has a reduced number of lymphocytes. Irregular bands of fibrosis and early collagen deposition causing distortion of the splenic contour. In some areas of fibrosis there is a deposition of pigment consistent with hemosiderin. The red pulp appear to be expanded, the cellularity and size of the red pulp is increased. The splenic cords are separated by irregularly shaped wide sinusoids. (H&E 40x). **D:** Spleen from a 6-week-old male mice control on the same study, demonstrates white pulp (lymphocytes) surrounding a central arteriole. The red pulp forms the bulk of the splenic parenchyma. (H&E 40x).

#### **IV. Discussion:**

The current study was designed to evaluate the toxic effects of different doses of a new brand; Duprost on the spleen of adult male mice. Although the efficacy of this treatment in inhibiting tumor growth has been studied extensively, we believe that we are the first to determine its side effects on spleen.

Our findings showed that this brand caused appetite & weight loss, as well as aggressive behavior in treated mice compared to the control animals. These results agree in some way with those of previous study by (Alexander et al., 2013) who stated that final body weights and genitourinary tract weights as percentage of body weights were significantly decreased in the Pre- and Post-dutasteride groups compared with the control [10]. As well, other study found that dutasteride administration at doses of 2.5 and 5 mg/kg body weight to 8-week-old male Sprague–Dawley rats for 2 weeks led to a significant decrease in their body weights [11].

As well, the histological analysis of the treated spleens in animals of both acute and chronic groups showed pathological changes especially in high doses (0.08mg/kg and 0.12mg/kg) when compared with normal tissues of control animals. High doses cause depletion of the white pulp, absence of follicles and decreased size of the periarterial lymphatic sheaths (PALS) and prominent fibrosis causing distortion of the splenic contour. These results may due to anticancer properties of active ingredient (dutasteride), as it is well known that the anticancer drug kills cancer cells and, at the same time can harm /impair the normal cells causing some unpleasant side effects [12, 13].

There are clear limitations to this study. We were unable to make further comparisons due to the scarcity of references or previous works in this aspect. This limited the interpretation of the results.

**Conclusion:** Our results may provide histological evidence of spleen toxicity caused by Duprost. This can be used to consider administration of the lowest possible doses of this drug in order to improve its' toxic effects. Further studies are necessary to investigate the pathological effects of this drug on other body organs.

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