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Long-term treatment with finasteride induces apoptosis and pathological changes in female mice

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Abstract

Androgenetic alopecia is the most common type of alopecia, and it affects humans of both genders. Finasteride is a type II selective 5α-reductase inhibitor that is administered orally to treat androgenetic alopecia and benign prostatic hyperplasia in human males. However, its effect on the vital organs of females is unknown. This study was designed to investigate the effects of finasteride on the vital organs such as liver, kidney, and heart of female mice. To study the prospective effects of finasteride, female mice were orally administered two doses of finasteride (0.5 and 1.5 mg/kg) once daily for 35 days, and serum levels of various biochemical parameters and histopathology of various organs were examined. The results showed that serum levels of alkaline phosphatase were significantly increased by both high- and low-dose finasteride, whereas cholesterol was significantly increased by the high dose only. Creatine kinase was significantly increased by the high and low doses, whereas glucose was significantly decreased by both doses. Histopathological analysis and DNA damage assays showed that finasteride has adverse effects within both the short and the long periods in female mice. In addition, the proapoptotic genes *Bax* and *caspase-3* were significantly increased by high dose finasteride, whereas the antiapoptotic gene *Bcl-2* was significantly decreased by the low and high doses. In conclusion, finasteride is not currently approved for therapeutic use in females, and the findings in this study suggest caution in any future consideration of such use.

Keywords

Finasteride, androgenetic alopecia, metabolic modulation, apoptosis, female

Introduction

Alopecia is a condition in which hair is lost from some body parts, especially the head.[1] Androgenetic alopecia is the most common noncitric alopecia, and it affects both men and women. Many people are very concerned about alopecia, and the desire to treat this health condition is growing. Alopecia leads to the progressive miniaturization of hair follicles, with a distinctive distribution in genetically disposed men and women.[2] The onset of androgenetic alopecia is usually gradual, and the condition slowly develops with age, as do the frequency and severity. Various genetic and environmental factors cause androgenetic alopecia.[3–4] The current treatments for androgenetic alopecia are inadequate. One drug that is used to treat alopecia is finasteride (4-azaandrost-1-ene-17-carboxamide, *N*-(1,1-dimethylethyl)-3-oxo-; 5α,17β), which has an empirical formula of $C_{23}H_{36}N_2O_2$ and a molecular weight of 372.55 Da. Finasteride

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is a white crystalline powder that is freely soluble in various solvents, such as chloroform and low-percentage alcohol. However, it is nearly insoluble in water and has a melting point near 250°C. Finasteride has no affinity for the androgen receptor and has no androgenic, estrogenic, anti-androgenic, or progestational effects.[5] Finasteride is a competitive and specific inhibitor of type II 5α-reductase, and inhibition of type II 5α-reductase blocks the peripheral conversion of testosterone to dihydrotestosterone (DHT), resulting in significant decreases in serum and tissue DHT levels.[6]However, administration of finasteride may have adverse effects due to its impact on the sex hormone ratio, which are generally referred to as sexual side effects,[7] including low libido, problems achieving erections and orgasms, and abnormal ejaculation,[8] as well as decreased semen parameters and fertilization problems.[9] Salvarci and Istanbulluoglu[10] demonstrated that finasteride causes sperm DNA damage, and Collodel et al.[11] reported that finasteride alters sperm morphology, leading to necrosis and elevates the frequency of diploidy and sex chromosome disomy.

Soni et al.[12] reported that the main proteins levels related to endoplasmic reticulum (ER) stress were alerted by finasteride administration (5 mg/kg per day for 3 months) in male rats. In addition, the authors also found apoptosis in the testis was induced after finasteride administration. ER stress is bidirectional, and unfolded proteins aggregation in the ER lumen is sufficient to trigger the generation of reactive oxygen species (ROS).[13] Indeed, excessive and prolonged stresses lead cells to initiate programmed cell death in male.[14] However, the therapeutic effect of finasteride was found to be sex-dependent, and its uses in female has been limited due to a lack of research examining adverse effects in female population,[15] due to which finasteride is not approved for female. Hence, this study aimed to examine whether finasteride treatment of female mice has an impact on vital organ morphohistology and whether the pathological alterations caused by finasteride treatment differ for short-term and long-term treatments.

Methods

Chemicals and animals

Finasteride and hematoxylin and eosin (H&E) stain were purchased from Sigma-Aldrich (St Louis, Missouri, USA). Other analytical reagents were purchased from resident sources. Female Swiss albino mice were acquired from the Central Animal Laboratory of the College of Science at King Saud University (Saudi Arabia). The mice were nearly the same age (7–9 weeks old) and weighed 28–30 g.

Experimental design

Female Swiss albino mice were housed in humidity and temperature-controlled (22 $\pm$ 2°C) ventilated polypropylene cages under sterile conditions under a 12-h light/12-h dark cycle and were provided a standard laboratory rodent diet. The study protocol was approved by the local review board of the authors' institution. Animal care and the experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Institutional Animal Ethics Committee (IAEC) of King Saud University, Saudi Arabia.

Based on the median lethal dose value of finasteride at 72 h, we selected 2 doses, 0.5 mg/kg/day as the low dose and 1.5 mg/kg/day as the high dose, and a study was conducted to compare the toxicity of finasteride at these 2 doses for 35 days. A preliminary study was conducted to explore any sign of toxicity, and the results showed no signs of toxicity, behavioral changes, or mortality in mice administered these two doses. Thus, the animals were divided into the following three groups (5 mice each): group I, which received a normal diet (normal control group); group II, which was administered the low dose of finasteride (0.5 mg/kg per day) for 35 consecutive days + the normal diet (low-dose group); and group III, which was administered the high dose of finasteride (1.5 mg/kg per day) for 35 consecutive days + normal diet (high-dose group).

At the end of the experiment, the mice were anesthetized, their livers, kidneys, spleens, stomachs, and hearts were removed for histopathology, and blood was obtained for biochemical assessments.

Estimation of biochemical parameters

Blood was obtained from the retro-orbital plexus of the mice. The blood samples were allowed to clot for 15 min at room temperature, and then centrifuged at 2500 r/min for 10 min to separate the serum. The obtained serum was then used to measure different biochemical parameters.

Alkaline phosphatase levels. The effect of the different doses of finasteride on serum levels of alkaline phosphatase (ALP) was determined by measuring the ALP

levels in finasteride-treated and untreated mice using Randox kit (Crumlin, UK).

Cholesterol levels. The effect of the different doses of finasteride on cholesterol levels was determined by the method of Izawa et al.[16] using Randox kits (Crumlin, UK).

Table 1. Sequences of specific forward and reverse primers of target genes were used in *qRT-PCR*.

Gene symbols	Primers sequences
GAPDH	5′-GGTCATCCCAGAGCTGAACG-3′
	5′-TTGCTGTTGAAGTCGCAGGA-3′
BAX	5′-TCCCCCCGAGAGGTCTTTT-3′
	5′-CGGCCCCAGTTGAAGTTG-3′
BCL2	5′-GTCCCGCCTCTTCACCTTTCAG-3′
	5′-GATTCTGGTGTTTCCCCGTTGG-3′
Caspase-3	5′-CCTCAGAGAGACATTCATGG-3′
	5′-GCAGTAGTCGCCTCTGAAGA-3′

GAPDH: glyceraldehyde 3-phosphate dehydrogenase; PCR: polymerase chain reaction.

Creatine kinase levels. The effect of different doses of finasteride on creatine kinase levels was determined using the Vitro Scient (UV/Kinetic; Hannover, Germany) kit for quantitative measurement in serum samples.[17]

Glucose levels. The effect of finasteride on glucose levels was determined by measuring glucose according to the method of Trinder[18] with Randox kits (Crumlin, UK).

Assessment of DNA strand breakage

A modified alkaline single-cell gel electrophoresis method was performed as described by Ali and Dixit.[19] A Komet-5.5 image system (Kinetic Imaging, Ltd., Nottingham, UK) attached to a fluorescent microscope (Leica, Wetzlar, Germany) equipped with suitable filters was used for the analysis. Percent tailed DNA was selected as the parameter to determine DNA damage.

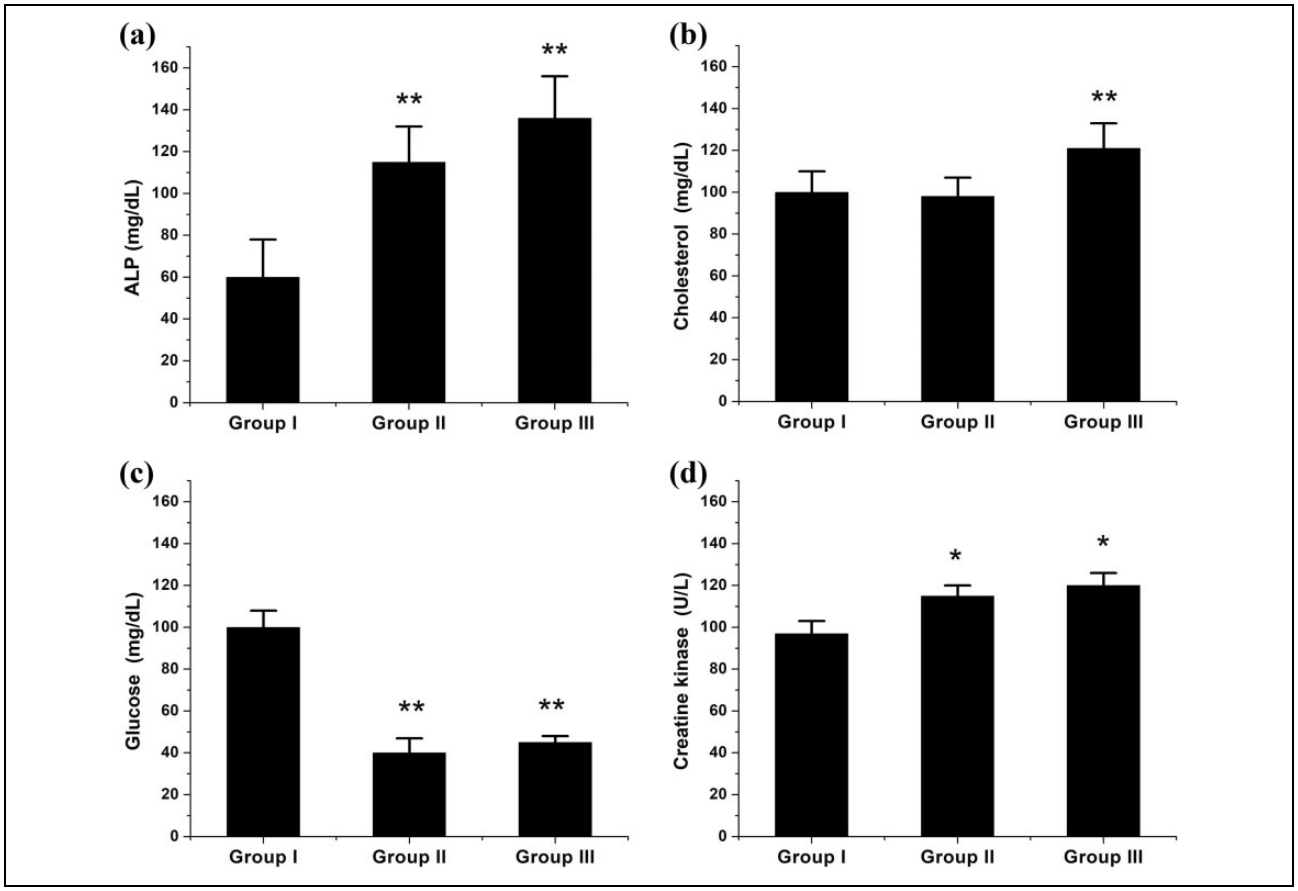

Figure 1. Biochemical analyses of sera samples obtained from mice in the finasteride-treated and control groups. Group I, control; group II, finasteride low-dose (0.5 mg/kg per day), and group III, finasteride high-dose (1.5 mg/kg/day). All values are expressed as mean ± SE, $n = 5$. *$p < 0.01$, **$p < 0.001$. SE: standard error.

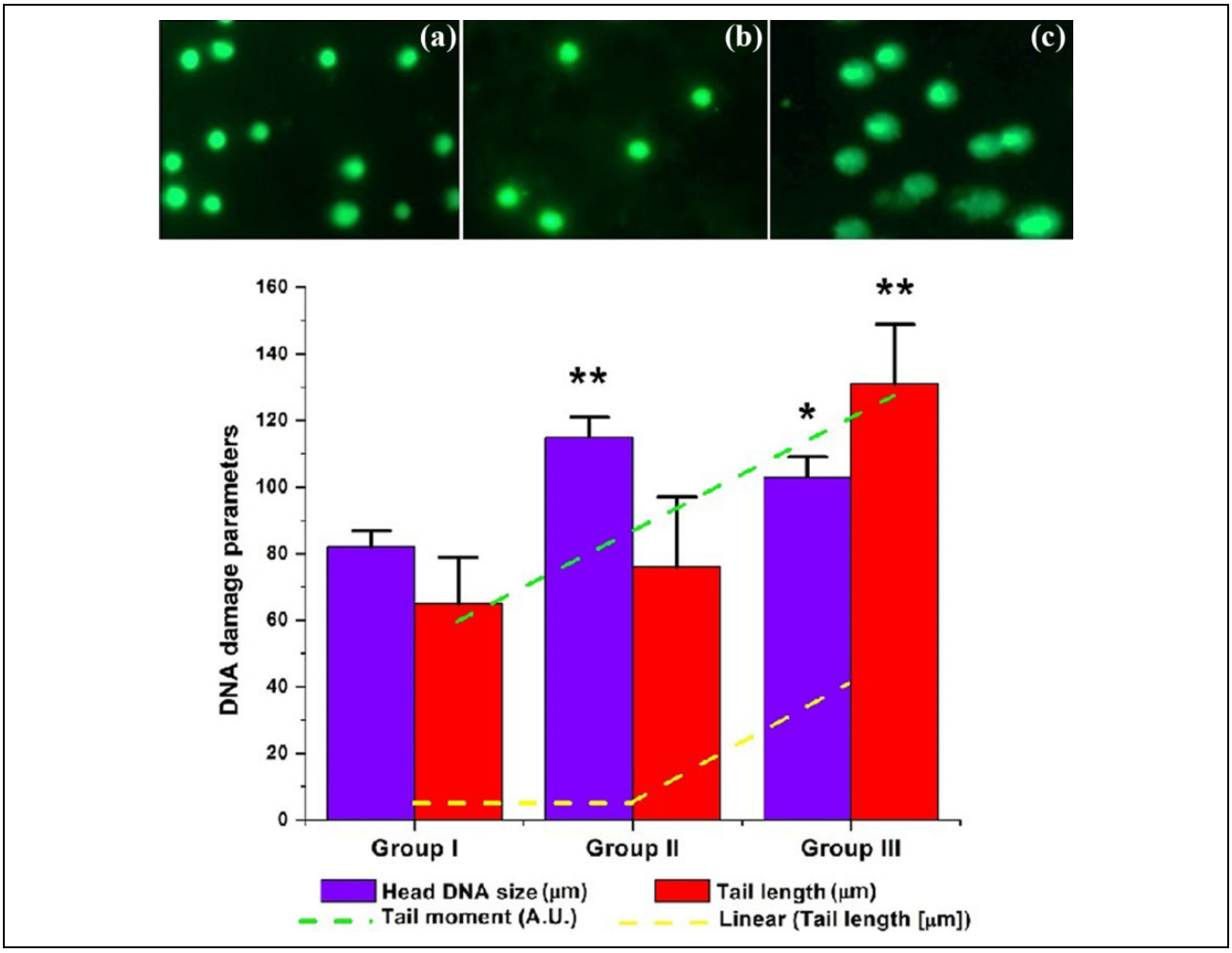

Figure 2. Finasteride-induced DNA damage in lymphocytes from female Swiss albino mice in the (a) control (normal diet), (b) low-dose finasteride D (0.5 mg/kg/day), and (c) high-dose finasteride (1.5 mg/kg/day) groups. (d) Quantified data for DNA head size, tail length, and moment. All values are represented as mean ± SE, $n = 5$. SE: standard error.

Histopathology

Fresh portions of the liver, kidney, spleen, stomach, and heart from each mouse were rapidly fixed with buffered formalin and desiccated with a graded series of methanol. Paraffin-embedded sections (4–5 μm) were stained with H&E for conventional histological staining according to the method of Carleton et al.[20] Stained sections of organs from the mice in the control and drug-treated groups were observed and photographed using an optical microscope equipped with a digital camera (BX51; Olympus, Japan) to determine any structural differences in the hepatocytes and sinusoids as well as evidence of deterioration, necrosis, fatty changes, and portal fibrosis.

Determination of apoptotic genes

At the end of the experiment, 50 mg of liver tissue was taken from five mice in each group and immediately placed in liquid nitrogen. The liver tissues were ground into a homogenized powder with a mortar and pestle. The homogenized powder was immediately placed in tubes and stored at −80°C until analysis.

Total RNA was extracted from the powdered liver tissues with TRIzol reagent (5 mL; Invitrogen Life Technologies, Carlsbad, California, USA), according to the manufacturer's instructions, and eluted in 200 μL of RNase-free water. The extracted mRNA was reverse transcribed into complementary DNA (cDNA) using the QuantiTect Reverse Transcription kit (Qiagen, Limburg, Netherlands). An aliquot of each cDNA sample was used as a template for real-time polymerase chain reaction (PCR).

PCR (25 μL reaction volume) was conducted using specific forward and reverse primers (synthesized by Invitrogen, San Diego, California, USA) to determine the levels of *Bax*, *Bcl-2*, and *caspase-3* relative to

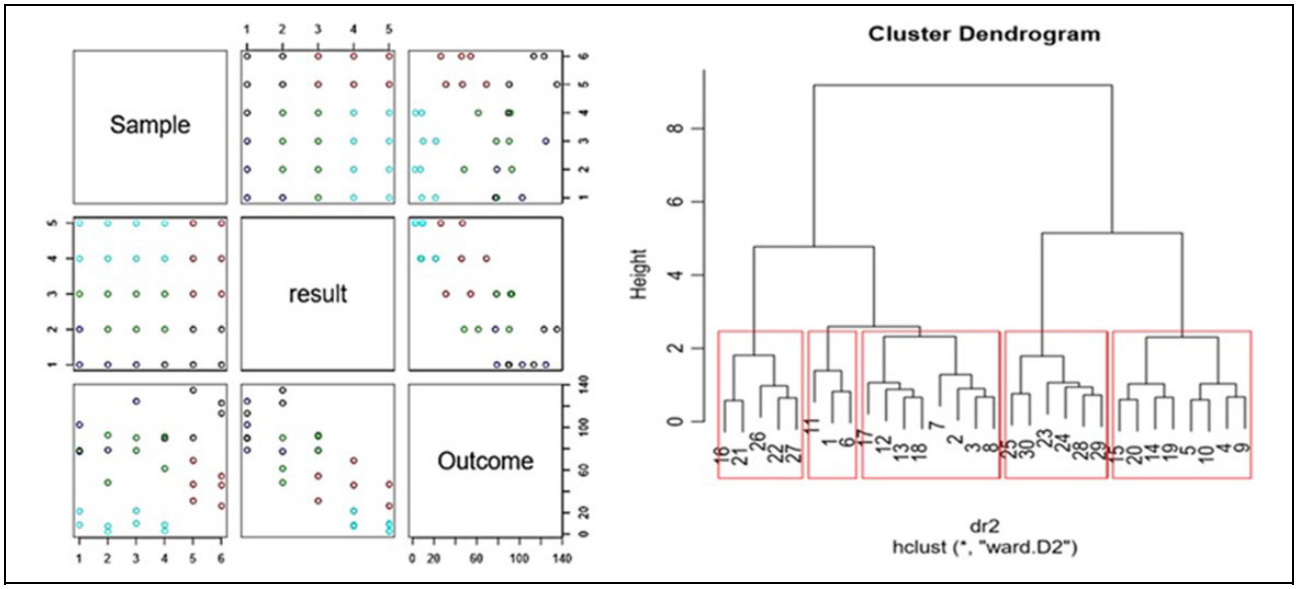

Figure 3. (a) The cluster plot and (b) dendrogram. The between sum of square divided by total sum of square of the *k*-mean model for $k = 5$ was 85.2%.

glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), which is a housekeeping (internal control) gene that was included as a control. The PCR was performed using the SYBR Green PCR kit, and the amplifications were repeated in triplicate with a real-time PCR instrument (7500; Applied Biosystems, Grand Island, New York, USA). Results were analyzed by the $2^{-\Delta\Delta CT}$ method. The sequences of the primers used are listed in Table 1.

Statistical analysis

All presented data are expressed as mean ± standard error (SE). Group differences were analyzed by one-way analysis of variance followed by an unpaired Student's *t* test. Numerical data were analyzed with SPSS 16.0 statistical software (Chicago, Illinois, USA). The significance level was set at $p \leq 0.05$, $p \leq 0.01$, and $p \leq 0.001$.

Results

Effects of finasteride on serum biochemical parameters

A significant elevation in ALP was observed in the low-dose and high-dose finasteride groups ($p \leq 0.001$) when compared to that in the control group. A significant increase in cholesterol in the high-dose finasteride group was observed ($p \leq 0.001$), whereas no significant difference ($p \geq 0.05$, Figure 1) was observed in the low-dose finasteride group when compared to that in the control group. In contrast, a significant reduction in glucose levels was observed in the low and high dose finasteride groups ($p \leq 0.001$). In addition, a marked increase was observed in the concentration of creatine kinase in both the low- and high-dose finasteride groups ($p \leq 0.01$).

Effects of finasteride on DNA damage

The comet assay revealed that finasteride induced substantial DNA damage in lymphocytes from mice in the low dose ($p \leq 0.01$) and high dose ($p \leq 0.001$) groups when compared to the control group (Figure 2). In addition, tail length was significantly longer in the drug-treated groups than that in the normal control group (group I). A nonsignificant alteration in the head size of the DNA was observed in the treated groups (Figure 2(d)). In contrast, a significant increase in the length and moment of the DNA tail was observed in the low-dose ($p \leq 0.05$) and high-dose ($p \leq 0.001$) groups when compared to that in the control group.

We also used "k-mean cluster analysis" to identify clusters. The plot of the *k*-mean clusters of variables, with $k = 5$, is shown in Figure 3 (kmr2). The colors of each point depend on membership in the cluster. The clusters in the figure were assigned to five colors (clusters). The *k*-mean algorithm was able to separate the data into a number of clusters, depending on the value of *k*. In this case, there were very few misclassifications in the clustering. The between sum of the

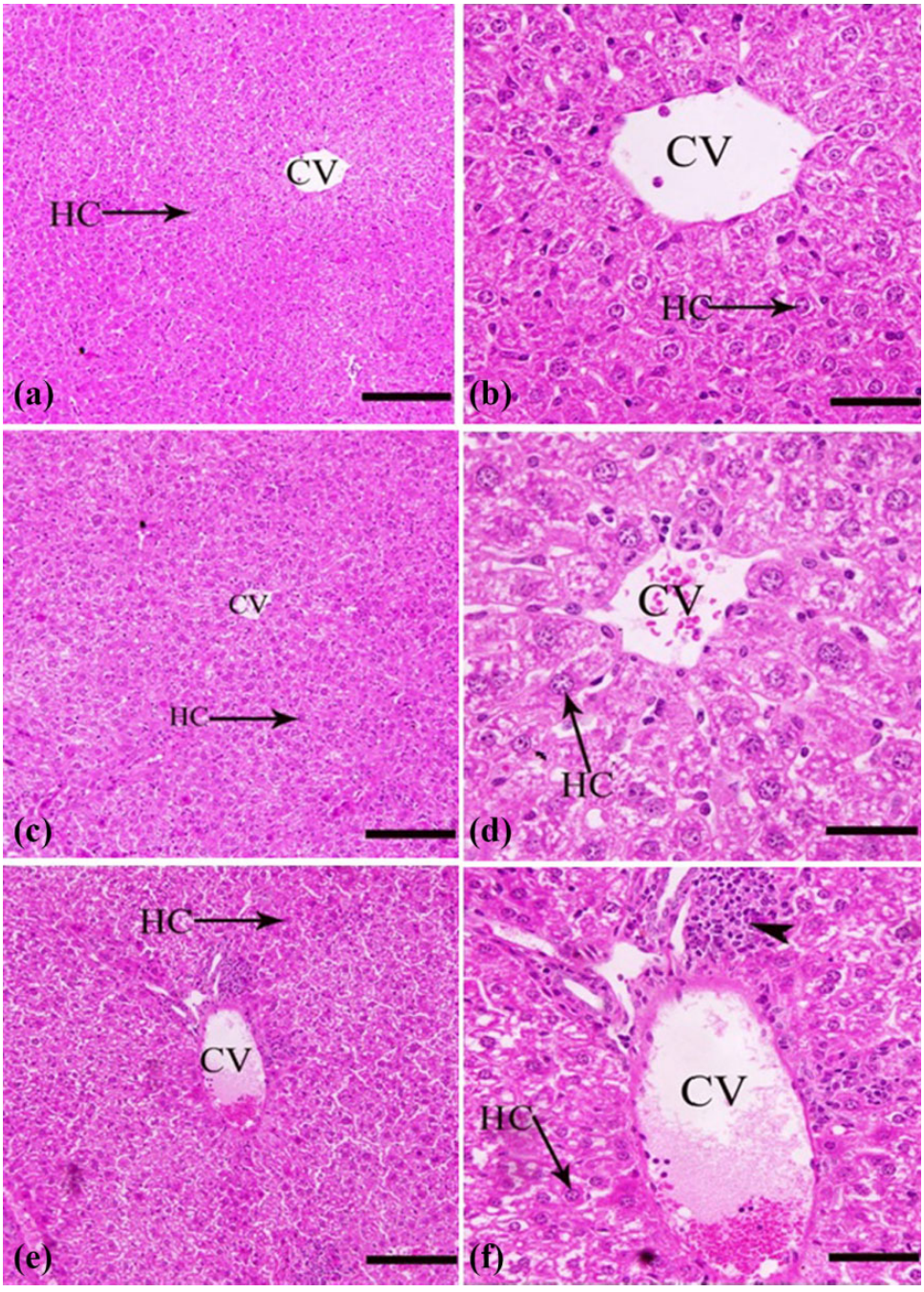

Figure 4. Liver sections (H&E stained) showing hepatic cells (HC), ventral vein (CV), and inflammatory cells (arrowheads) in the (a, b) control, (c, d) low dose, and (e, f) high dose finasteride groups of mice. H&E: hematoxylin and eosin.

square divided by total sum of the squares of the k-mean model for $k = 5$ was 85.2%.

Effect of finasteride on the histological appearance of tissues

Figures 4, 5, 6, 7, and 8 show the histological alterations observed in the low- and high-dose finasteride groups as compared to the control group in the liver, kidney, spleen, stomach, and heart tissues, respectively. Histological variations were observed in the liver tissue of the mice in the drug-treated groups (groups II and III); leaky membranes were observed in damaged liver cells along with slight steatosis, fat droplets, and lipid accumulation, especially in the high dose finasteride group (Figure 4). In the kidney,

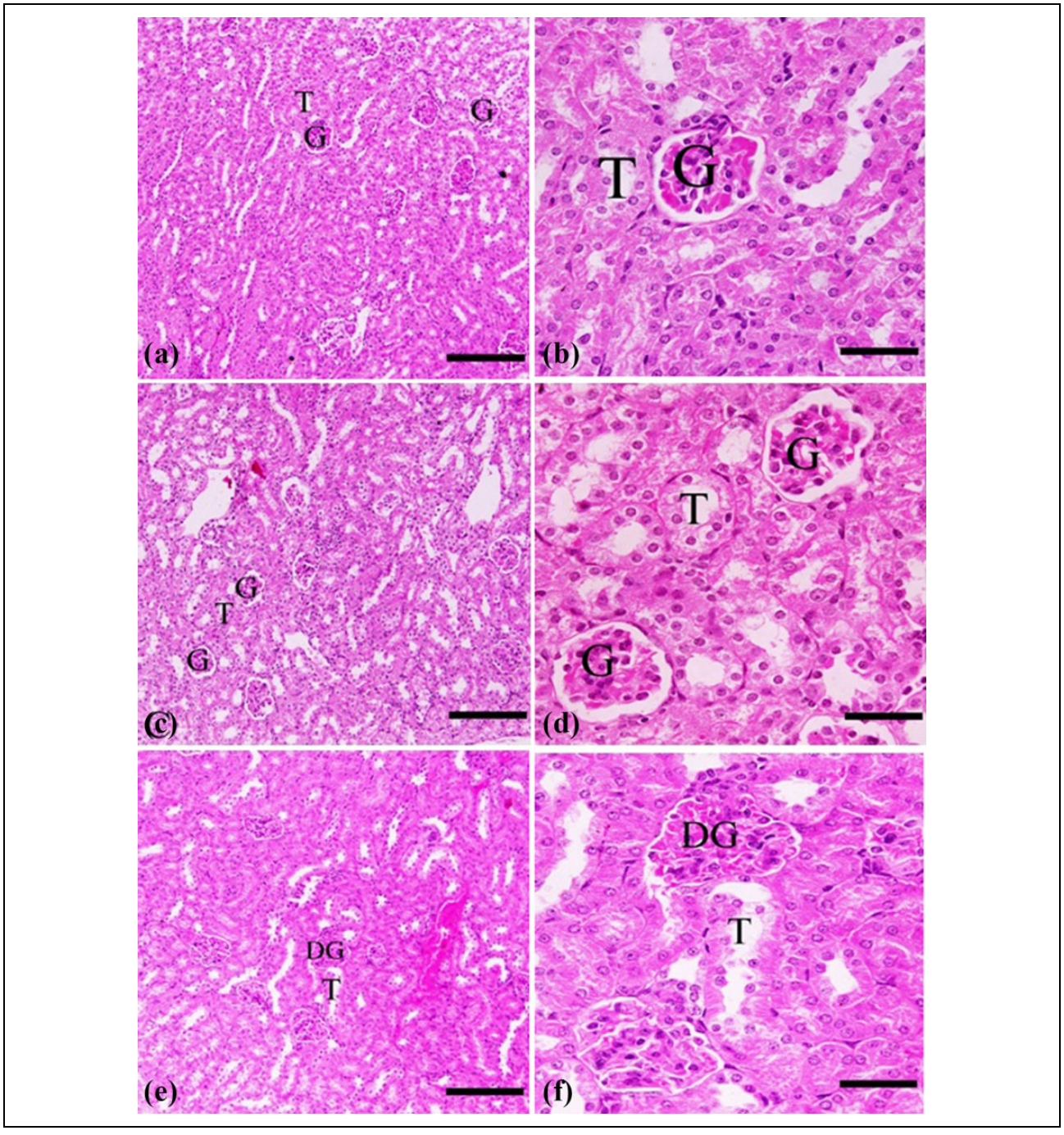

Figure 5. Kidney sections (H&E stained) showing the glomerulus (G), renal tubules (T), and degenerated glomerulus (DG) in the (a, b) control, (c, d) low dose, and (e, f) high dose groups of mice. H&E: hematoxylin and eosin.

finasteride at the high dose caused edema, medullary congestion, glomerular swelling, epithelial shedding in tubular structures, and pyknotic nuclei (Figure 5). In the spleen, white bulbs started to fuse together, especially in the high-dose group, and the capsule of the spleen appeared to be thinner in the high-dose group than in the control group (Figure 6). In the stomach, there was no evidence for any pathological alterations at either dose (Figure 7). In the heart, many inflammatory cells were observed, and the blood vessels started to become congested in mice that were administered the high dose of finasteride (Figure 8).

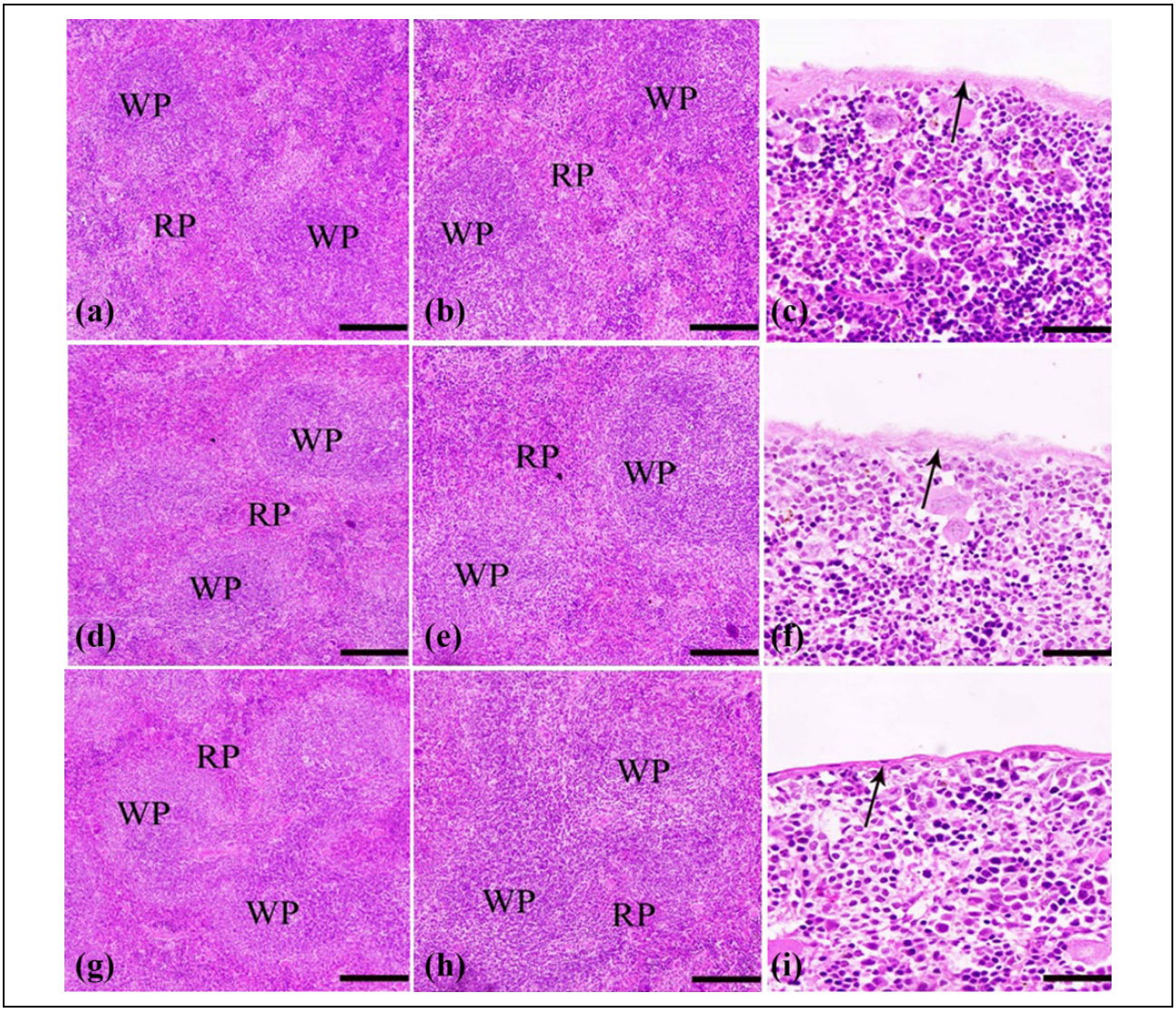

Figure 6. Spleen sections (H&E stained) showing white pulp (WP), red pulp (RP), and the spleen capsule (arrows). In the high-dose group (G), the WP is starting to fuse together, and the spleen capsule appears thinner in high-dose group than in the control group. (a to c) Control group, (d to f) low-dose group, and (f to h) high-dose group. H&E: hematoxylin and eosin.

Effects of finasteride on apoptosis

The mRNA expression of apoptosis-related genes in the liver of finasteride-treated and control mice was assessed by qRT-PCR, and the expression of selected programmed cell death-related genes is shown in Figure 9. *Bax* expression was not affected by the low or high doses of finasteride, and there was only a slight to no increase in apoptosis in the livers of the mice in the finasteride-treated groups when compared to that in the control group (group I). Caspase-3 activation was markedly increased in the livers of mice treated with high dose finasteride when compared to the levels in mice from other the groups ($p < 0.01$). In contrast, significant downregulation of the *Bcl-2* gene was observed in the low- and high-dose finasteride groups ($p < 0.05$) when compared to that in the control group (group I).

Discussion

Finasteride is a therapeutic pharmacologic product with hair growth-promoting activity. The effects of finasteride for the treatment of androgenetic alopecia are generally well tolerated, and finasteride has been shown to yield significant improvements in hair growth in males when administered orally or topically to the scalp.[21] However, the side effects of finasteride

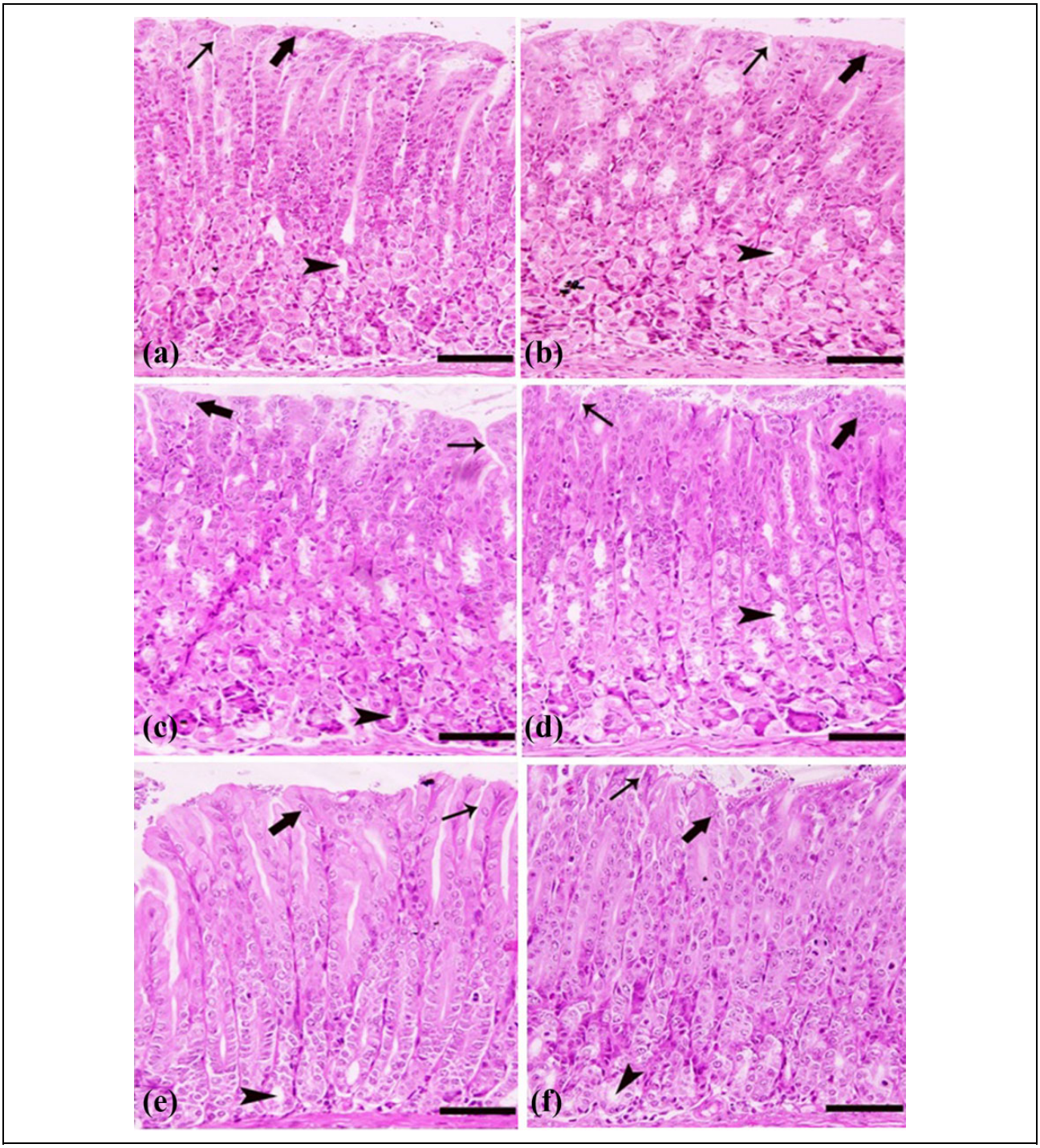

Figure 7. Stomach sections (H&E stained) showing gastric pits (thin arrows), the gastric gland (arrowheads), and surface mucous cells (thick arrows) in the (a, b) control, (c, d) low dose, and (e, f) high-dose groups of mice. H&E: hematoxylin and eosin.

on the vital organs of females has not been well studied.

Several previous studies noted that oral administration of finasteride, even the 1 mg dose used to treat androgenic hair loss, significantly reduces serum DHT levels, which may lead to complications, such as increased cholesterol levels and testosterone levels and an altered lipid profile due to the elevation in testosterone concentration.[22,23] These previous findings are consistent with the results of the current

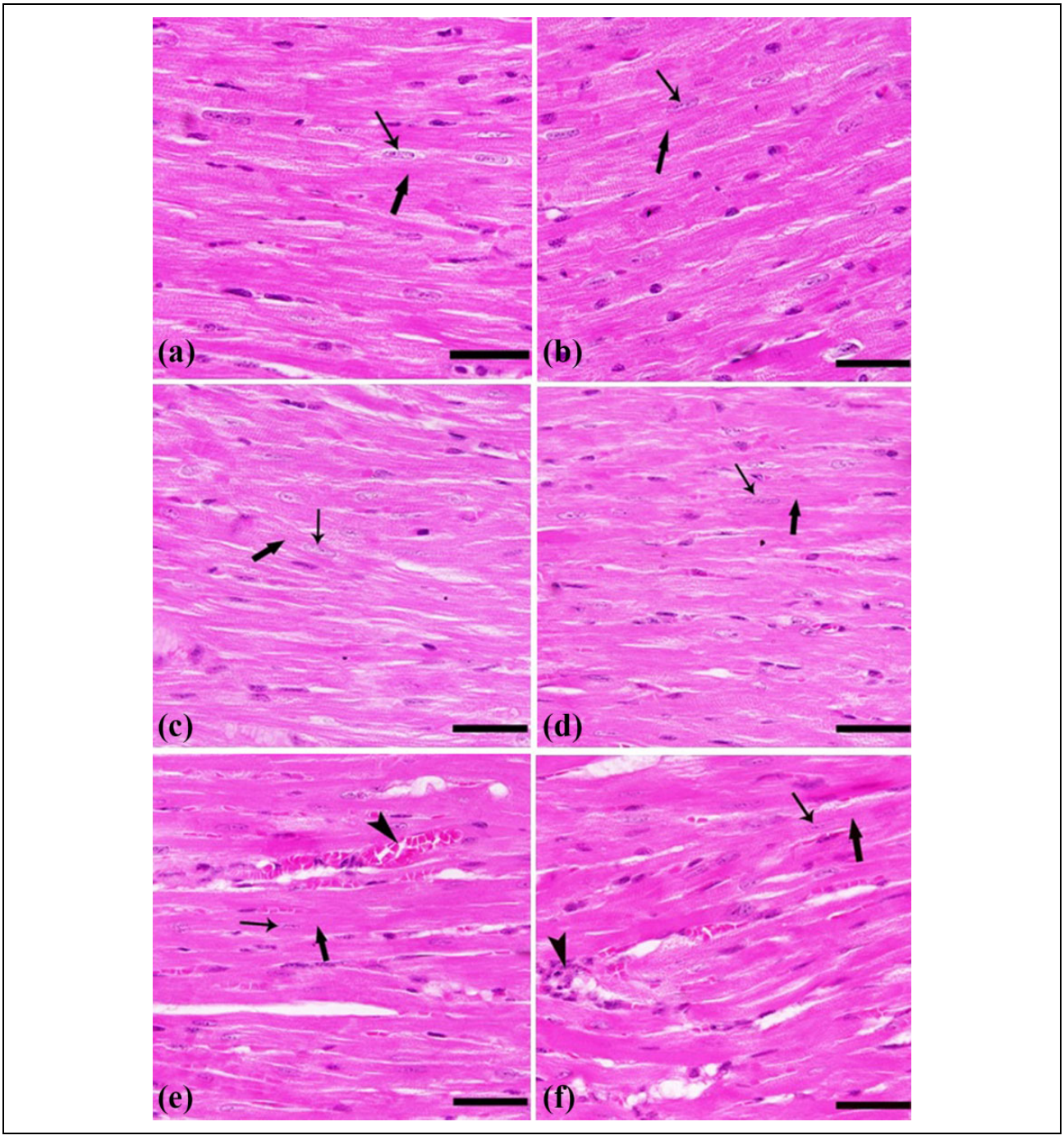

Figure 8. Heart sections (H&E stained) showing the myocardium (thick arrows) and nuclei (thin arrows) as well as inflammatory cells (in (f), arrowhead) and congested blood vessels (in (e), arrowhead) in the (a, b) control, (c, d) low dose, and (e, f) high-dose groups of mice. H&E: hematoxylin and eosin.

study. Previous studies have also shown that there is a direct relationship between finasteride and creatine kinase, and creatine kinase levels were decreased after finasteride was discontinued.[24,25]

A reduction in glucose was observed in the low and high dose finasteride groups, due to the effect of testosterone on glucose concentrations, as higher testosterone levels are associated with increased insulin resistance, leading to decreased glucose levels.[22,26] Moreover, a significant increase in ALP was observed when compared to the control group. These results were confirmed by histological analysis, demonstrating the adverse effects of finasteride. Histological alterations were also observed in the liver.

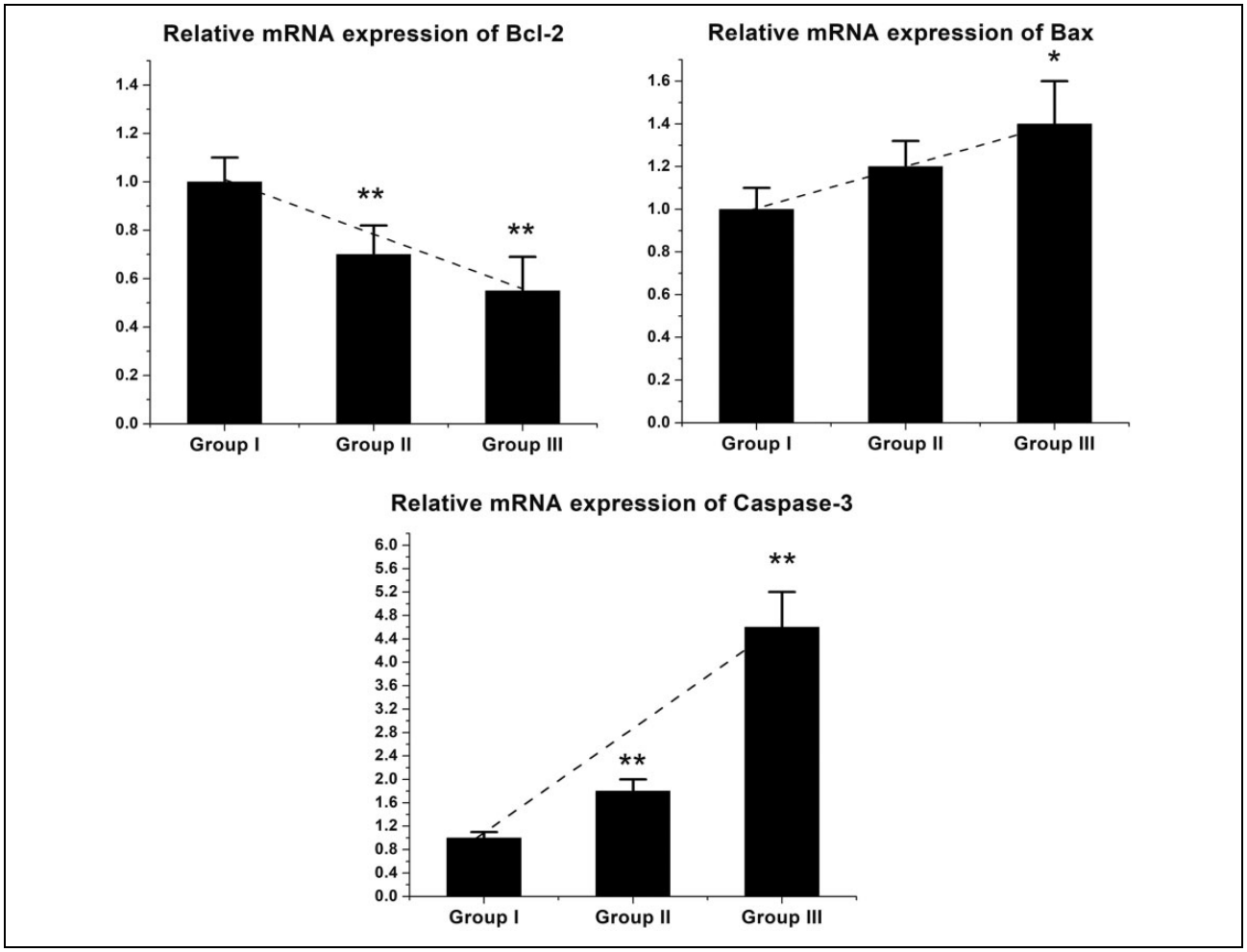

Figure 9. Hepatic mRNA expression of apoptotic-related genes in female Swiss albino mice. Bcl-2, Bax, and caspase-3 gene expression was assessed by qRT-PCR. Expression was normalized to *GAPDH*, a housekeeping gene, and relative expression in the treated groups is reported compared to that in the control group, $n = 5$. Data are presented as mean $\pm$ SD of three independent experiments. Group I, control; group II, finasteride low-dose (0.5 mg/kg/day), and group III, finasteride high-dose (1.5 mg/kg/day). *$p < 0.05$, **$p < 0.01$. PCR: polymerase chain reaction; GAPDH: glyceraldehyde 3-phosphate dehydrogenase.

Compared with the normal control group, various histological changes were noted in the liver tissues of mice in the finasteride-treated groups (groups II and III), such as leaky membranes on damaged liver cells. In fact, liver is the most important organ in the body, performing up to 500 functions. It metabolizes most ingested substances and detoxifies toxic molecules.[14] Nowadays, it is recognized that quality of human life can be markedly affected in many chronic liver ailments through the impact of systemic features such as fatigue, cognitive impairment, and autonomic dysfunction typically evident by vasomotor disorder and sleep disturbance.[27]

The comet assay showed that finasteride administration induced substantial DNA damage in lymphocytes. The tail lengths in the drug-treated groups were significantly longer than in the normal control group (group I). These results are in accordance with those of Tu and Zini,[28] who showed a significant reduction in the DNA fragmentation index (DFI) within 3 months of finasteride cessation and continued improvement, which suggested a causal link between finasteride and sperm DNA damage. The authors hypothesized that low-dose finasteride may have a negative influence on sperm DNA integrity, resulting in increased pregnancy losses. Moreover, Amory et al.[29] reported a dose–response relationship between finasteride and finasteride-induced genotoxicity, in which they found that high-dose (5 mg) finasteride has been shown to have a reversible

negative effect on semen parameters greater than low-dose (1 mg). Furthermore, finasteride leads to altered oestrogen/testosterone balance. Elevated oestrogen/testosterone ratio in a male may lead to genotoxicity, mutagenicity and has transforming and carcinogenic potential.[30]

Soni et al.[12] also showed that reduced fertility and increased ER stress and apoptotic markers (procaspase-3 and cleaved caspase-3) caused by finasteride administration were triggered in a ROS-dependent manner. Serga[31] suggested that ROS could contribute to the deleterious effects of an anti-androgen such as finasteride.

In conclusion, our results indicate that long-term treatment with finasteride at high dose as well as the low dose, which is typically not well accepted by females as it has side effects, led to pathological alterations in vital organs that are associated with metabolic modulations and apoptosis in female Swiss albino mice. However, as an alternative, body surface area adjustment would suggest the doses (0.5 and 1.5 mg/kg/day) used in the present study are the approximate equivalent of 2.5 and 7.5 mg oral doses for humans, both above the approved dose of 1 mg/day for androgenic alopecia.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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