Ameliorating Effect of Damiana (Turnera Diffusa) Extract against Trenbolone-induced Nephrotoxicity and Hepatotoxicity in Male Albino Rats

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KEYWORDS
Trenbolone;
Hepatotoxicity;
Nephrotoxicity;
Oxidative stress;
Damiana (Turnera Diffusa) extract.

ABSTRACT

Anabolic steroids abuse has become one of the escalating issues in global public health as they can result in severe irreversible organ damage. Current study aimed to investigate the potential protective role of damiana extract in altering trenbolone induced- nephrotoxicity and hepatotoxicity in rats. Fifty adult male albino rats were classified into five groups. Group I acted as a control group; group II received damiana extract (80 mg/kg body weight (BW) orally daily) for 4 weeks; group III received trenbolone (10 mg/Kg BW intramuscular injections once weekly) for 4 weeks; group IV received trenbolone and damiana together for 4 weeks; group V received trenbolone for 4 weeks then damiana for another 4 weeks. Liver and kidney specimens were studied with hematoxylin and eosin. Trenbolone prompted significant increases in water and food intake, relative BW, relative weights of kidney and liver, serum creatinine, blood urea, serum sodium, potassium, chloride levels, liver enzymes, alkaline phosphatase and total bilirubin. In contrast, significant decreases in calcium level and serum albumin were recorded in trenbolone group. Liver and kidney tissues revealed a significant increase in malondialdehyde and significant reductions in reduced glutathione, catalase and superoxide dismutase. Also, trenbolone caused marked inflammation and degeneration in liver and kidney tissues. Meanwhile, administration of damiana improved significantly the above-mentioned toxic effects of trenbolone. Trenbolone induced oxidative-nephrotoxic and -hepatotoxic effects in rats that evidently improved via the efficient antioxidant properties of damiana. This highlights the possibility of using damiana extract as a protective agent against trenbolone toxicity.

Introduction

Anabolic androgenic steroids (AAS) are testosterone synthetic cholesterol derivatives that have been therapeutically used in catabolic chronic cases, hypogonadism and breast cancer (Mahmoud and Halloull, 2023). Due to their ability to increase muscle protein synthesis, AAS have widespread and growing illicit use predominantly in young men and athletes as image and performance enhancing drugs (Tay Wee Teck and McCann, 2018; Sharma et al., 2022). Furthermore, AAS are often present in over-the-counter dietary supplements without any declaration in the ingredients' list (Solimini, et al., 2017). Unfortunately, online shopping has made these pharmaceuticals more readily available illegally without a medical prescription besides, the fact that consumers are frequently ignorant of the risks associated with taking these drugs (Yonis et al., 2021).

Anabolic androgenic steroids are classified as a Schedule III substance. Such medications include Stanozolol, Ultima-Tren E, Trenbolone, Mibolerone, Boldenone, Winsol, Trenorol, Rexogin Androxine,

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Rexobol and Neurabol (El-Moghazy et al., 2012; Tousson et al., 2012; Solimini et al., 2017).

Abuse of AAS can result in serious health risks such as hypogonadism, diminished fertility, cardiovascular diseases, central nervous system, metabolic and musculoskeletal disorders, as well as behavioral and psychiatric disturbances. In addition, they are reported to cause severe irreversible organ damage mainly liver and kidney. Hepatic adenoma, cholestasis, peliosis hepatis and hepatocellular carcinoma are concerns related to AAS hepatotoxicity (Tousson et al., 2016; Solimini, et al., 2017; Abd-Elraouf and Araby 2022). Furthermore, AAS abuse is associated with disturbed renal functions as well as acute and chronic renal damage (Mahmoud and Halloull, 2023).

Oxidative stress and enhanced reactive oxygen species generation is one of the main suggested mechanisms that explain AAS induced toxic effects (Mahmoud and Halloull, 2023). Hence, the usage of a potent antioxidant has been anticipated as a rational therapy against trenbolone toxicity.

Damiana (Turnera diffusa) is a member of the Turneraceae family of plants. It is a tiny shrub which grows up in the tropical and subtropical areas of the United States. It was utilized by the Maya in the past to treat a variety of diseases (Tousson et al., 2020). Damiana extract has been used as an antioxidant and scavenger of active free radicals through the properties of its polyphenolic compounds. The leaves of Damiana contain a minimum of twenty composites (1, 8-cineole, P-cymene, -pinene, -copaene thymol, and calamene), as well as tannins, damianin, -sitosterol, flavonoids, arbutin gonzalitosin and tetraphyllin B (Sultana et al., 2020; Hasan et al., 2023).

This work was designed to investigate the nephrotoxicity and hepatotoxicity of the anabolic androgenic steroid; trenbolone in adult male albino rats and to examine any possible impacts of Damiana (*Turnera diffusa*) extract in altering the trenbolone induced oxidative-nephrotoxicity and -hepatotoxicity.

Material and Methods:

Chemicals and reagents

Trenbolone

Trenbolone 200 (Trenbolone Enanthate) vial was attained from Dragon Pharma Laboratories, Co., (S.A. Mexico). Oily solution is contained in each vial (200mg /ml vehicle).

Damiana's aqueous extracts production

Damiana extract was prepared after Tousson et al. (2020) where the dried leaves of damiana were powdered, immersed in 37 ˚C boiling water for twenty-four hours, then removed and stored in the dark at -30 ˚C until needed.

Experimental animals

Concerning this study, 50 healthy male albino rats were used, each weighing 200-220 g and being about 14 to 16 weeks old. They were brought from the animal house of Alpha Center in Tanta, Egypt. The animals were kept in cages with wire mesh throughout the research with unrestricted access to food and water. Rats were subjected to equal cycles of light and darkness (12 hour per cycle) while the ambient temperature hovered around 22-24 °C. Rats were closely watched throughout the study phase. Weekly notes were made on body weight, fluid intake, and food intake.

Ethical considerations:

Tanta Faculty of Medicine-Ethical Committee approved the methods that were followed in order to accomplish the current study (approval code: 36264PR2783/7/23).

Experimental design

Fifty rats were divided into five groups after two weeks of acclimatization.

Group I: Control group; where rats were given distilled water orally.

Group II: Damiana group; aqueous solution of damiana extract was administered to rats orally via gastric gavage (80 mg/kg body weight/daily) for 4 weeks (Estrada-Reyes et al., 2009).

Group III: Trenbolone group; rats were intramuscularly injected with trenbolone (10 mg/Kg body weight once weekly) for 4 weeks (Saddick, 2021).

Group IV: Co-treated group (trenbolone + damiana); rats received intramuscular injections of trenbolone together with oral damiana extract for 4 weeks (in the previously mentioned doses in groups II and III).

Group V: Post-treated group (trenbolone then damiana); rats were intramuscularly injected with trenbolone for 4 weeks and then treated with damiana orally for another 4 weeks (in the previously mentioned doses in groups II and III).

Samples collection:

Rats from each group were euthanized with aesthetic ether at the appropriate time and underwent a full necropsy following a 10- to 12- hour fast.

Tissue preparation: The livers and kidneys were immediately removed and tissue samples from them were divided into two parts. The first part was cleaned, and homogenized in saline before being centrifuged for seven minutes at 4000 rpm, the resultant suspension was then gathered and kept at -80 ^{o}C for subsequent analysis of tissues oxidative stress and antioxidant markers. After obtaining the second portion of the liver and kidney tissue samples, they were promptly fixed by immersing them in a 10% buffered formalin solution and letting them sit for 24 to 48 hours. After that, the specimens were cleaned, dried, and embedded in paraffin. Using a rotary microtome (Litz), serial slices with a thickness of 5 µm were cut and stained with eosin and haematoxylin (Bancroft and Gamble, 2008).

Samples of blood were drawn from each rat's inferior vena cava, which were subsequently put into glass tubes without heparin. Separating the blood serum required 15 minutes of centrifugation at 3000 rpm. The gathered serum was kept in storage at -18^{o}C for biochemical assays.

A. Biochemical assays

- *Liver enzymes and function tests:*

  A commercial kit from Randox (Egypt) was used to measure the activity of the enzymes aspartate transaminase (AST) and alanine transaminase (ALT) via the Rietman and Frankel (1957) technique. Following the methodology of Belfield and Goldberg (1971), alkaline phosphatase (ALP) activity was determined using a commercial kit from France's BioMérieux Co. Balistreri and Shaw (1987) used the colorimetric Diazo technique to determine the concentration of serum total bilirubin. Serum albumin was determined using spectrophotometric analysis and commercial diagnostic kits provided by Diamond (Egypt).

- *Kidney function tests and serum electrolytes estimation*

  Blood urea and serum creatinine were measured in accordance with Patton and Crouch (1977). Commercial kits of Indian Sensa-core electrolyte were used to assess the levels of potassium, calcium, sodium, and chloride ions in accordance with the method proposed by Abd Eldaim et al. (2019).

B. Oxidative stress and antioxidant markers in kidney and liver tissues

- *Malondialdehyde (MDA)*

  Assessing the malondialdehyde (MDA) levels in tissue homogenates was performed via using Biodiagnostic kits, Egypt and according to Mesbah et al. (2004).

- *Reduced glutathione (GSH)*

  GSH concentration was calculated after tissue homogenates were treated with 5.5′-dithiobis (2-nitrobenzoic acid), according to Habig et al. (1974) and expressed as µmol GSH/ g tissue.

- *Catalase (CAT)*

  Catalase enzyme converts H_2O_2 into water. Spectrophotometry at 240 nm was used to measure the activity of CAT by calculating the rate at which H_2O_2 was degraded according to the method of Aebi (1984).

- *Superoxide Dismutase (SOD)*

  According to Misra and Fridovich (1972), the best method for determining SOD activity is to block adrenaline from being converted to adrenochrome in an alkaline media. This process is significantly slowed down when SOD is present.

Statistical Assessment

Data were reported, tabulated and statistically analyzed using GraphPad Prism (GraphPad Prism, Inc., La Jolla, CA, USA). Values were conveyed as means±SE. To define the significance of difference between the studied groups, one-way ANOVA and Dunnett's test were employed. Significance was adopted at $p<0.05$. [a] and [b] were significant deviations after control and trenbolone groups, respectively.

Results:

The present study illustrated that no statistically significant differences were detected between the mean values of water intake, food intake, relative body weight (RBW) and relative weights of kidney (RKW) and liver (RLW) in control group and damiana group. In contrast, there were statistically significant elevations of their means in group III (trenbolone) and group V (post-treated) as compared to control group ($p<0.05$). Meanwhile, group IV (co-treated) revealed no significant differences between the mean values of all previously mentioned parameters when compared to control group as shown in table (1).

Treatment of trenbolone with damiana in group IV (co-treated) and group V (post-treated) revealed significant improvements in all parameters, however, results reported in group IV exhibited superior ameliorative effects over that observed in group V. As illustrated in table (1), both groups IV and V showed statistically significant decreases in the means of water intake, food intake, RBW and RKW as compared to trenbolone group ($p<0.05$). Despite, there was marked decrease in the means of relative liver weight in both groups IV and V when compared to trenbolone group, but this decrease was statistically significant only in group IV (co-treated).

Table (1): Comparison of water intake, food intake, relative body weights and relative weights (g/100 g body weight) of kidney and liver between the studied groups (n=50

Variables	Group I (Control; n=10)	Group II (Damiana; n=10)	Group III (Trenbolone; n=10)	Group IV (Co-treated; n=10)	Group V (Post-treated; n=10)
Water intake (ml/rat/day)	$31.9^{b} \pm 2.35$	$32.9^{b} \pm 2.29$	$44.8^{a} \pm 2.71$	$34.0^{b} \pm 2.25$	$39.5^{ab} \pm 2.41$
Food intake (g/rat/day)	$12.9^{b} \pm 0.88$	$13.2^{b} \pm 1.05$	$23.7^{a} \pm 1.55$	$15.1^{b} \pm 1.02$	$19.8^{ab} \pm 0.91$
RBW (g/100 g)	$27.0^{b} \pm 1.41$	$25.3^{b} \pm 1.45$	$46.8^{a} \pm 1.89$	$30.3^{b} \pm 2.06$	$39.5^{ab} \pm 2.24$
RKW (g/100 g)	$3.42^{b} \pm 0.18$	$2.40^{b} \pm 0.21$	$4.85^{a} \pm 0.25$	$3.52^{b} \pm 0.21$	$3.89^{ab} \pm 0.31$
RLW (g/100 g)	$0.52^{b} \pm 0.04$	$0.52^{b} \pm 0.03$	$0.59^{a} \pm 0.04$	$0.54^{b} \pm 0.03$	$0.57^{a} \pm 0.04$

n: number, ml: milliliter, g: gram, RBW: relative body weight, RKW: relative kidney weight, RLW: relative liver weight. Data were expressed as mean ± SE of 10 observations. [a] Significant difference from the control group at $p < 0.05$, [b] Significant difference from the trenbolone group at $p < 0.05$. (Relative organ weight= Organ weight/ Body weight x 100)

Concerning renal function tests and serum electrolytes (sodium, potassium, calcium and chloride), table (2) demonstrated that no significant differences were detected between the means of these parameters in group I (control) and group II (damiana). In contrast, significant elevations in the means of serum creatinine, blood urea, sodium, potassium, chloride and a significant depletion in the mean of calcium were recorded in group III (trenbolone) as compared to control and damiana groups. Meanwhile, these levels were improved significantly on treatment of trenbolone with damiana in both groups IV (co-treated) and V (post-treated) with best results for group IV (co-treated).

Table (2): Comparison of renal function tests and serum electrolytes between the studied groups (n=50)

Variables	Group I (Control; n=10)	Group II (Damiana; n=10)	Group III (Trenbolone; n=10)	Group IV (Co-treated; n=10)	Group V (Post-treated; n=10)
Creatinine (mg/dl)	$0.68^{b} \pm 0.04$	$0.64^{b} \pm 0.03$	$1.12^{a} \pm 0.04$	$0.83^{ab} \pm 0.04$	$0.99^{ab} \pm 0.05$
Urea (mg/dl)	$28.9^{b} \pm 1.54$	$27.5^{b} \pm 1.24$	$44.6^{a} \pm 1.88$	$36.5^{ab} \pm 2.02$	$37.5^{ab} \pm 1.35$
Na^{+} (mmol/l)	$135.9^{b} \pm 8.92$	$136.1^{b} \pm 7.56$	$148.5^{a} \pm 10.79$	$138.1^{b} \pm 5.29$	$141.0^{ab} \pm 7.55$
K^{+} (mmol/l)	$4.22^{b} \pm 0.35$	$4.07^{b} \pm 0.31$	$5.29^{a} \pm 0.26$	$4.84^{ab} \pm 0.30$	$5.06^{ab} \pm 0.24$
Ca^{++}(mmol/l)	$1.24^{b} \pm 0.10$	$1.25^{b} \pm 0.14$	$1.17^{a} \pm 0.09$	$1.23^{b} \pm 0.14$	$1.20^{ab} \pm 0.11$
Cl^{-} (mmol/l)	$101.8^{b} \pm 4.77$	$100.8^{b} \pm 6.54$	$119.5^{a} \pm 8.91$	$108.0^{ab} \pm 6.05$	$113.0^{ab} \pm 8.52$

n: number, mg/dl: milligram per deciliter, mmol/l: millimoles per liter, Na^{+}: sodium, K^{+}: potassium, Ca^{++}: calcium, Cl^{-}: chloride. Data were expressed as mean ± SE of 10 observations. [a] Significant difference from the control group at $p < 0.05$, [b] Significant difference from the trenbolone group at $p < 0.05$.

Similarly, table (3) revealed that no significant differences were detected between group I (control) and group II (damiana) regarding hepatotoxicity markers; ALT, AST, ALP, serum albumin and total bilirubin. However, rats in group III showed a statistically significant decrease in the mean of serum albumin and significant elevations in the mean values of ALT, AST, ALP and bilirubin when compared to control and damiana groups ($p<0.05$). On the contrary, rats of the protected groups IV and V demonstrated a significant elevation in the mean of serum albumin and significant depletions in the means of ALT, AST, ALP and bilirubin as compared to trenbolone group ($p<0.05$); however, better results were recorded in group IV (Table 3).

Table (3): Comparison of liver function tests between the studied groups (n= 50)

Variables	Group I (Control; n=10)	Group II (Damiana; n=10)	Group III (Trenbolone; n=10)	Group IV (Co-treated; n=10)	Group V (Post-treated; n=10)
ALT (U/L)	$21.9^{b} \pm 1.17$	$18.4^{b} \pm 1.05$	$76.0^{a} \pm 2.31$	$24.0^{b} \pm 1.47$	$62.4^{ab} \pm 3.83$
AST (U/L)	$35.0^{b} \pm 1.45$	$32.2^{b} \pm 2.25$	$87.5^{a} \pm 3.68$	$39.5^{ab} \pm 2.70$	$59.2^{ab} \pm 2.56$
ALP (U/L)	$89.5^{b} \pm 6.9$	$83.0^{b} \pm 6.2$	$151.5^{a} \pm 10.7$	$91.4^{b} \pm 7.1$	$125.0^{ab} \pm 9.0$
Albumin (mg/dl)	$3.99^{b} \pm 0.19$	$4.05^{b} \pm 0.221$	$3.15^{a} \pm 0.18$	$4.01^{b} \pm 0.24$	$3.50^{ab} \pm 0.15$
Total Bilirubin (mg/dL)	$0.79^{b} \pm 0.16$	$0.70^{b} \pm 0.17$	$2.26^{a} \pm 0.16$	$0.98^{b} \pm 0.18$	$1.84^{ab} \pm 0.21$

n: number, ALT: alanine aminotransferase, AST: aspartate aminotransferase, ALP: Alkaline phosphatase, U/L: unit per liter, mg/dl: milligram per deciliter. Data are expressed as mean ± SE of 10 observations. [a] Significant difference from the control group at $p < 0.05$, [b] Significant difference from the trenbolone group at $p < 0.05$.

Regarding oxidative stress parameters in liver and kidney tissues, the present study demonstrated that no statistically significant differences were detected between liver and kidney MDA, GSH, catalase and SOD activities in group I (control) and group II (damiana). While, group III (trenbolone) showed significant elevations in liver and kidney MDA together with significant decreases in liver and kidney GSH, catalase and SOD when compared to control and damiana groups ($p<0.05$). Unlike the results of group IV, group V showed significant differences in all parameters as compared to control group ($p<0.05$; Table 4).

Current study illustrated that treatment of trenbolone with damiana in both groups IV and V ameliorated the oxidative stress reported in trenbolone group. As demonstrated in table (4), group IV (co-treated) revealed significant decreases in liver and kidney MDA and significant elevations in liver and kidney GSH, catalase and SOD concentrations as compared to trenbolone group ($p<0.05$). While, in group V (post-treated), there were significant decreases in both liver and kidney MDA and significant elevations in liver GSH, catalase and SOD concentrations, as well as, a significant elevation in kidney catalase activity when compared to trenbolone group ($p<0.05$). Despite the mean values of kidney GSH and SOD in group V were elevated than that recorded in trenbolone group but these elevations were not statistically significant ($p>0.05$; Table 4).

Table (4): Comparison of liver and kidney tissues oxidative stress parameters in the studied groups (n= 50)

	Variables	Group I (Control; n=10)	Group II (Damiana; n=10)	Group III (Trenbolone; n=10)	Group IV (Co-treated; n=10)	Group V (Post-treated; n=10)
Liver	**MDA (nmol/g tissue)**	$31.6^{b} \pm 1.28$	$29.5^{b} \pm 1.52$	$75.0^{a} \pm 3.19$	$34.2^{b} \pm 2.07$	$59.5^{ab} \pm 3.35$
	GSH (µmol/mg protein)	$3.61^{b} \pm 0.21$	$3.69^{b} \pm 0.24$	$1.25^{a} \pm 0.18$	$3.53^{b} \pm 0.31$	$2.98^{ab} \pm 0.26$
	CAT (U/mg protein)	$44.2^{b} \pm 3.09$	$45.5^{b} \pm 2.61$	$28.5^{a} \pm 2.50$	$41.9^{b} \pm 3.6$	$36.0^{ab} \pm 2.81$
	SOD (U/mg protein)	$49.4^{b} \pm 2.57$	$47.2^{b} \pm 2.84$	$33.0^{a} \pm 2.21$	$44.5^{b} \pm 2.80$	$38.2^{ab} \pm 2.06$
Kidney	**MDA (nmol/g tissue)**	$49.6^{b} \pm 2.25$	$45.2^{b} \pm 2.71$	$68.0^{a} \pm 3.45$	$51.0^{b} \pm 3.07$	$61.8^{ab} \pm 2.91$
	GSH (µmol/mg protein)	$3.05^{b} \pm 0.17$	$3.16^{b} \pm 0.22$	$0.994^{a} \pm 0.11$	$2.72^{b} \pm 0.18$	$1.25^{a} \pm 0.13$
	CAT (U/mg protein)	$49.1^{b} \pm 2.25$	$52.5^{b} \pm 3.30$	$31.7^{a} \pm 2.82$	$46.0^{b} \pm 3.62$	$36.5^{ab} \pm 2.35$
	SOD (U/mg protein)	$53.0^{b} \pm 3.46$	$52.8^{b} \pm 3.65$	$39.5^{a} \pm 2.61$	$49.9^{b} \pm 2.90$	$41.5^{a} \pm 3.02$

n: number, MDA: malondialdehyde; GSH: reduced glutathione; CAT: catalase; SOD: superoxide dismutase. nmol/g: nanomoles per gram; µmol/mg: micromole per milligram; U/mg: unit per milligram. Data are expressed as mean ± SE of 10 observations. [a] Significant difference from the control group at $p < 0.05$, [b] Significant difference from the trenbolone group at $p < 0.05$.

In the present study, examining H- & E-stained liver sections from male rats in the control and damiana groups revealed normal hepatocyte structure with polygonal hepatocytes featuring prominent round nuclei, eosinophilic cytoplasm, and sparsely spaced hepatic sinusoids assembled in-between the hepatic cords with finely arranged Kupffer cells (Figures 1A & 1B). However, liver sections in trenbolone group (group III) demonstrated severe hepatotoxicity evidenced by marked inflammatory cells, hepatic cords degeneration, karyomegaly and pyknotic nuclei denoting apoptosis, moderate fibrosis, and congested blood sinusoids (Figures 1C & 1D). Meanwhile, the administration of damiana in treated groups (groups IV and V) showed marked improvement. Liver sections in post-treated group (group V; trenbolone then damiana) revealed mild degenerated hepatocytes, and moderate inflammatory cells (Figure 1E). Whereas, liver sections in co-treated group (group IV; trenbolone + damiana) revealed dramatic improvement of the hepatocytes with only mild inflammatory cells (Figure 1F).

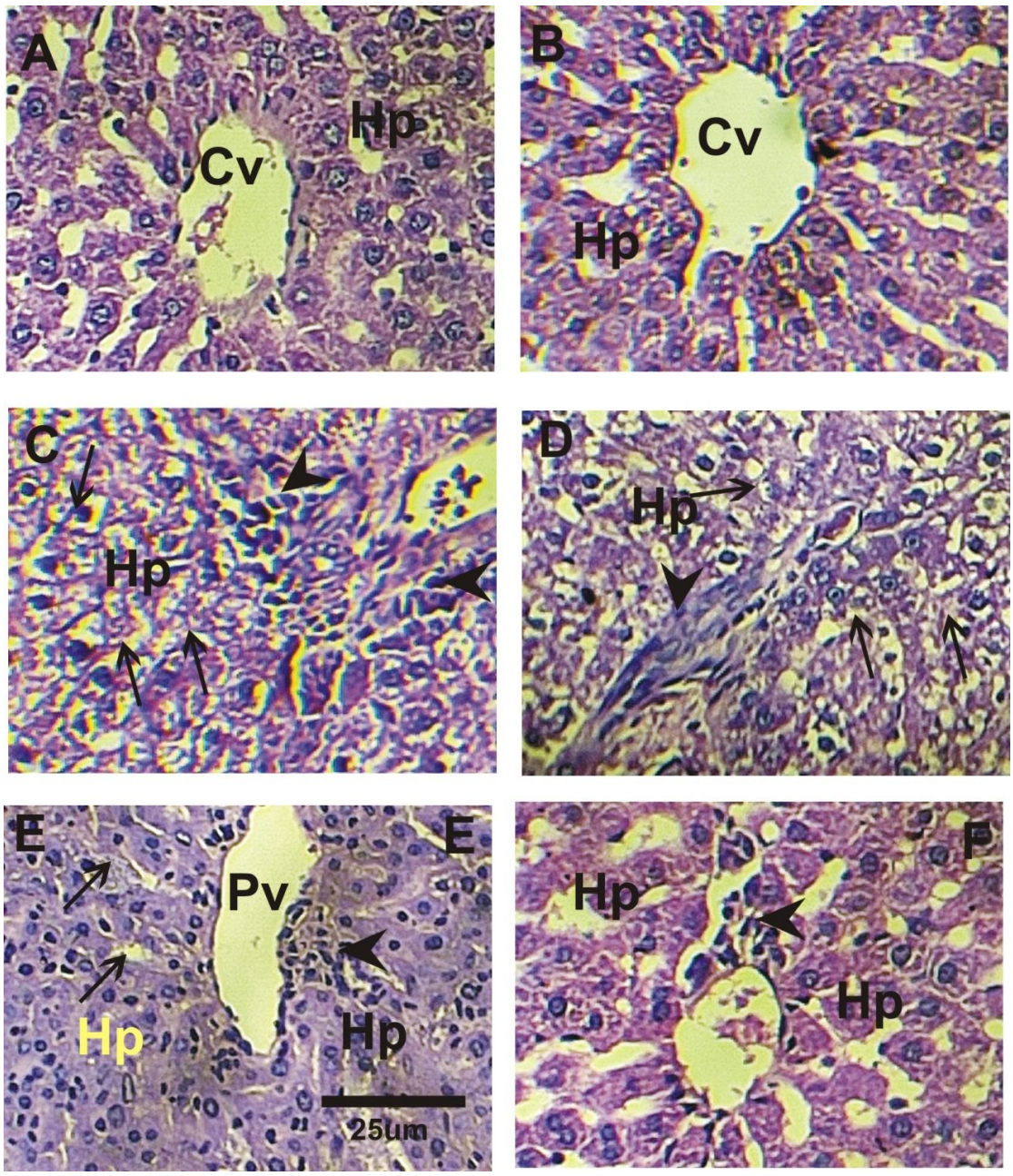

Fig. (1): Hematoxylin- & Eosin-stained liver sections in the different groups. **A & B:** Liver sections in control (group I) and damiana (group II) groups showing normal hepatocytes (Hp), and central vein (CV). **C & D:** Liver section in trenbolone group (group III) revealed hepatotoxicity manifested by marked inflammatory cells (arrowheads), degeneration in hepatic cords in addition to karyomegaly and pyknotic nuclei indicative of apoptosis, and moderate fibrosis (arrows). **E:** Liver sections in post-treated group (group V; trenbolone then damiana) revealed mild degenerated hepatocytes (arrows), and moderate inflammatory cells (arrowheads). **F:** Liver sections in co-treated group (group IV; trenbolone + damiana) revealed very good improvement of the hepatocytes with only mild inflammatory cells (arrowheads). MP= 250X

Similarly, the histological examination of the kidney sections in both control and damiana groups illustrated the normal structures of the renal cortex that consisted of renal corpuscles, proximal and distal convoluted tubules (Figures 2A & 2B). Conversely, kidney sections in trenbolone group (group III) revealed a variety of pathological alterations in glomeruli and renal tubules including significant renal tissues degeneration, marked glomerular atrophy. Moreover, mesangial stroma with leucocytic infiltrations were seen in the interstitium, and the majority of the glomeruli appeared to have lost their attachments (Figures 2C & 2D). Meanwhile, kidney sections in post-treated group (group V; trenbolone then damiana) revealed mild leucocytic infiltrations and moderate degenerated renal tubules (Figure 2E). In contrast to trenbolone group, kidney sections from the co-treated group (group IV; trenbolone + damiana) showed marked improvement and arrangement in the kidney histological structure (Figure 2F).

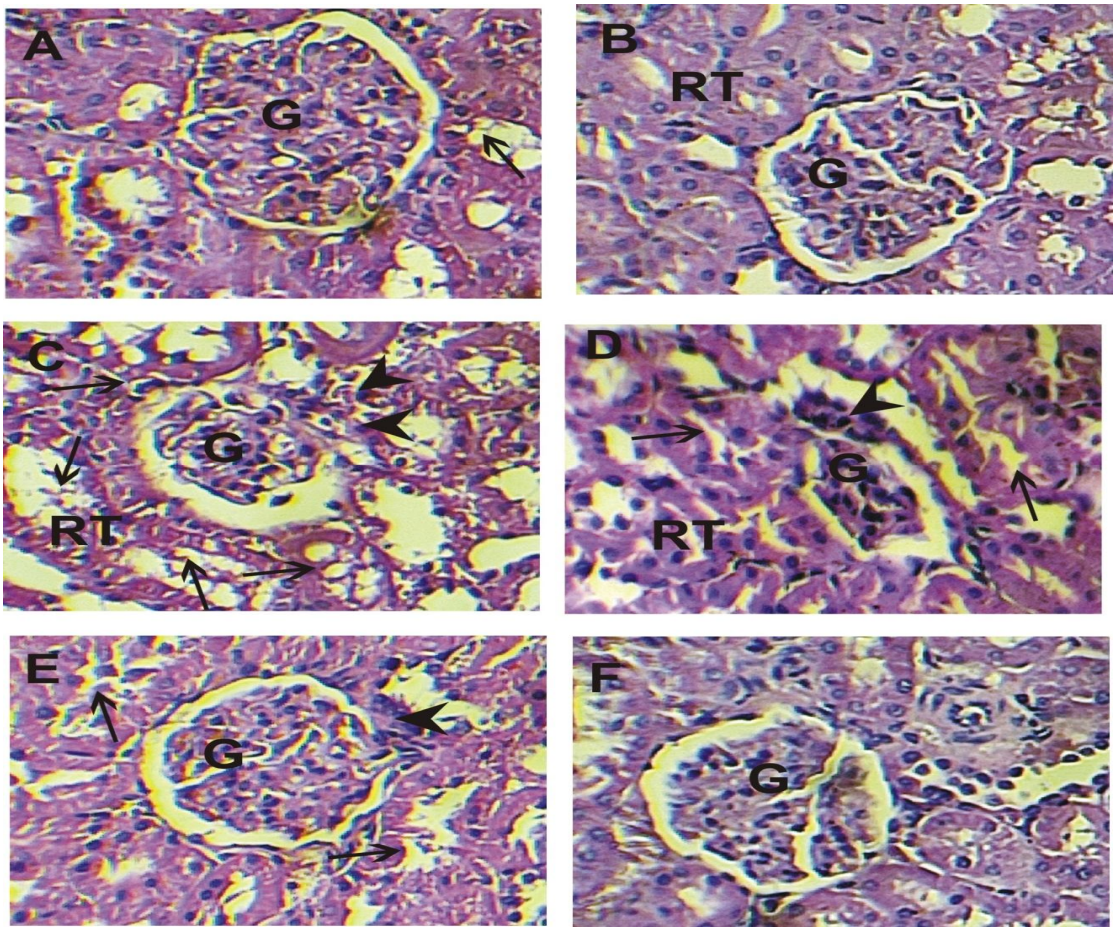

Fig. (2): Hematoxylin- & Eosin-stained kidney sections in the different groups. **A&B:** Kidney sections in control and damiana groups showing normal structure of glomeruli (G) and renal tubules (RT). **C&D:** Kidney sections in trenbolone group (group III) revealed marked damage and degeneration to the renal tissues (arrows), marked glomerular atrophy (G), and leucocytic infiltrations (arrow heads). **E:** Kidney sections in post-treated group (group V; trenbolone then damiana) demonstrated mild leucocytic infiltrations (arrow heads) and moderate degenerated renal tubules. **F**: Kidney sections in co-treated group (group IV; trenbolone + damiana) revealed mild leucocytic infiltrations (arrow heads) and mild degenerated renal tubules. MP=250X.

Discussion

Trenbolone recently becomes one of the commonly used anabolic androgenic steroids (AAS) by body builders and athletes for its ability to increase muscle protein synthesis. However, studies in the literature illustrated that AAS misuse can be associated with organs toxicity and disfunctions. AAS-induced toxicities are thought to be caused by a number of processes, including oxidative damage (Solimini et al., 2017; Islam et al., 2022; Sharma et al., 2022; Mahmoud and Halloull, 2023). Thus, the aim of current study was to investigate any potential amending properties of damiana on AAS; trenbolone- induced liver and kidney toxicities and oxidative stress in rats.

Current results demonstrated that intramuscular injection of the anabolic steroid-trenbolone to male rats revealed significant elevations in the mean values of water intake, food intake, relative body weight of rats and relative weights of liver and kidney as compared to control group.

These findings supported those of El-Moghazy et al. (2012) and Mohammed et al. (2016), who investigated the effects of AAS-boldenone injections in male rabbits and reported significant enhancement of body weight as well as total protein concentrations of their studied animals. Also, Olivares et al. (2014) discovered that fluid intake and body weight of male adult rats were significantly increased when an anabolic steroid was administered throughout their adolescent phase. Furthermore, Holt et al. (1990) stated that anabolic steroid- methenolone enanthate induced elevation in the rate of weight gain and kidney weight in the growing rat. On the other hand, Oda and El-Ashmawy (2012) detected that boldenone did not significantly affect the body weight or growth in weight of male rabbits.

In addition, this study detected that treatment of trenbolone with damiana extract in co-treated and post-treated groups decreased the significant elevations in water intake, food intake, relative body weight of rats and relative weights of liver and kidney as compared to trenbolone group. These findings were in agreement with Lim (2013) who reported that damiana (*Turnera diffusa*) leaves extract induced depletion in the rate of weight gain and kidney weight. Also, Esquivel-Gutiérrez et al. (2019) declared that damiana (*Turnera diffusa*) aqueous extract endorsed body weight loss in healthy rats and decreased weight gain in diabetic ones.

The present study revealed that trenbolone induced renal toxicity where it increased serum creatinine and blood urea level, as well as, it increased serum sodium, potassium, chloride levels and decreased the serum calcium level. These findings were compatible with other studies such as Harrington et al. (2011) who declared that repeated regular use of AAS, whether through oral or parenteral routes, were associated with increased serum urea and creatinine. Furthermore, Tousson et al. (2016) stated that boldenone injection in rabbits caused liver and kidney damage, as well as, Mahmoud and Halloull (2023) who found that administration of nandrolone decanoate produced significant increases in serum urea and creatinine in rats.

This study demonstrated that administration of damiana extract to trenbolone improved trenbolone-induced renal toxic effects. This was in the same line with the findings of Enaibe et al. (2021) who stated that giving mature rats oral doses of damiana extract improved their kidney functions and structure. Also, Hasan et al. (2022) illustrated the role of *Turnera diffusa* supplementation against amitriptyline induced kidney toxicity and DNA damage in male rats. Likewise; the present findings were

coherent with the results of Hasan et al. (2023) who demonstrated the protective role of damiana extract against chlorpyrifos pesticide-induced liver and kidney toxicity in male rats.

The dramatic rise in the usage of AAS has brought the risk of hepatotoxicity to light (Solimini et al., 2017). It is well known that hepatocytes contain abundant amounts of liver enzymes; hence, a rise in these enzymes' plasma levels is a sign of liver cell injury or an increase in the permeability of the hepatic membrane (Gurakar et al., 1994; Yonis et al., 2021).

The current results revealed that trenbolone induced liver toxicity in rats. There were significant elevations in the mean values of AST, ALT, ALP, total bilirubin and a significant decrease in serum albumin in trenbolone group when compared with control one. These findings were comparable with the results of other studies, where, Welder et al. (1995) firstly described AAS- toxic effects in primary rat hepatic cultures, and the hepatotoxic effects of anabolic steroids were documented by Dickerman et al. (1999). Likewise, Urhausen et al. (2003) indicated significant increase in serum ALT and AST levels in athletes abusing anabolic androgenic steroid. Similarly, El-Moghazy et al. (2012) detected hepatic and renal structural and functional changes after boldenone injection in rabbits. Furthermore, Gabr et al. (2009) and Tousson et al. (2016) found that growth promoter boldenone induced liver toxicity by increasing ALT, AST and decreasing albumin levels in rabbits. Similar results were demonstrated by Bond et al. (2016) and Neri et al. (2011) who reported that AAS-abuse induced hepatotoxicity.

Current results showed that treatment of trenbolone with damiana revealed depletions in ALT, AST, ALP, bilirubin levels and an elevation in albumin level as compared to trenbolone group. This agreed with Tousson et al. (2020) who reported that damiana extract improved liver functions after amitriptyline treatment. Also, Rodríguez-Rodríguez et al. (2021) reported that; profibrotic, extracellular matrix, and mitochondrial markers were reduced by *Turnera diffusa* extract in activated human hepatic stellate cells.

Anabolic steroid-induced toxicities have been closely associated with oxidative stress (Mahmoud and Halloull, 2023). Due to this oxidative stress, biological macromolecules, particularly the cell membrane, are destroyed through lipid peroxidation, glutathione exhaustion besides other oxidant processes, leading to cellular damage and death (Mirakbari, 2015; Yousef et al., 2015).

The present study demonstrated that trenbolone increased significantly the mean value of MDA and significantly decreased the means of GSH, catalase and SOD as compared to control group. These findings were in accordance with Frankenfeld et al. (2014) who proposed that nandrolone induced oxidative stress in heart, liver, as well as in kidney of male rats. Also, Dornelles et al. (2017) stated that anabolic steroids injections induced oxidative damage in rats' liver and kidney. Similarly, Kara et al. (2018) reported that anabolic steroids stanozolol administration induced oxidative stress in rat cardiac tissue. The results of this study encouraged using damiana extract as the oxidative stress changes were improved significantly by administration of damiana extract to trenbolone in both groups IV and V. These results were supported by previous studies in the literature that have emphasized the antioxidant effects of damiana extract (Sultana et al., 2020; Tousson et al., 2020; Hasan et al., 2023).

In this study, microscopic examinations of H & E liver sections supported the biochemical findings of hepatotoxicity in the trenbolone group which revealed inflammation, degeneration, fibrosis and risk of apoptosis. Conversely, the H&E liver sections from the post-treated group revealed mild to moderate improvement in hepatic changes. Meanwhile, liver sections from the co-treated group showed marked improvement in hepatic architecture. Likewise, histopathological examination of kidney sections in the trenbolone group showed marked damage and degenerated renal tissues, marked glomerular atrophy and leucocytic infiltrations. However, these lesions were more or less improved after administration of damiana in the protected groups (IV and V) with marked improvement in co-treated group (IV).

These results were in agreement with the renal pathological findings of other studies in the literature (Bento-Silva et al., 2010; Shabir et al., 2015; Ebeye et al., 2016; Mahmoud and Halloull, 2023) who studied the nephrotoxic effects of anabolic steroids. In addition, current results agreed with El-Moghazy et al. (2012) who identified the pathological effects of boldenone in liver and kidney tissues of rabbits. Furthermore, Enaibe et al. (2021), Hasan et al. (2022) and (2023) illustrated the capability of damiana extract to markedly improve the structure and function of liver and kidney after toxicities.

Conclusion

In light of the current study's findings, trenbolone produced nephrotoxicity and hepatotoxicity in rats. These toxic effects could be attributed to trenbolone induced oxidative stress as evidenced by the elevation of malondialdehyde and the marked decline in antioxidants enzymes; glutathione reductase, catalase and superoxide dismutase. While, administration of damiana extract significantly ameliorated these toxic effects because it inhibited the oxidative stress and stimulated production of antioxidant enzymes. Hence, damiana extract can provide a protective role through its antioxidant effects against trenbolone-induced renal and hepatic toxicity. Nonetheless, more clinical trials are advised to clarify its clinical use.

Conflict of Interest: No competing interests to be declared.

Funding: Nil.

Data Availability: The corresponding author can provide the data supporting the study's findings upon request.

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التأثير المحسن لمستخلص الداميانا (Turnera diffusa) ضد التسمم الكلوى و الكبدى الذي يسببه ترينبولون فى ذكور الجرذان البيضاء

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لقد أصبح تعاطي الستيرويدات البناءة إحدى مشكلات الصحة العامة المتنامية عالميا حيث يمكن أن يؤدي الاستخدام الغير القانوني لهذه الأدوية إلى تلف شديد لا يمكن إصلاحه في الأعضاء. لقد أجريت الدراسة الحالية للكشف عن التسمم الكلوي والكبدي الذى يسببه الترينبولون في ذكورالجرذان البيضاء البالغة، وتقييم الدور الوقائي المحتمل لمستخلص داميانا في تعديل هذه التأثيرات السامة الناتجه عن الترينبولون. و تم تصنيف خمسين من ذكور الجرذان البيضاء البالغة إلى خمس مجموعات. المجموعة الأولى استخدمت كمجموعة ضابطة؛ تلقت المجموعة الثانية مستخلص داميانا (٨٠ مجم / كجم من وزن الجسم بالفم عن طريق أنبوب معدة يوميًا) لمدة ٤ أسابيع؛ تلقت المجموعة الثالثة ترينبولون (١٠ مجم / كجم من وزن الجسم بواسطة الحقن العضلي مرة واحدة أسبوعيا) لمدة ٤ أسابيع؛ تلقت المجموعة الرابعة ترينبولون و مستخلص داميانا معًا لمدة ٤ أسابيع (بنفس الجرعات السابقة)؛ بينما تلقت المجموعة الخامسة ترينبولون لمدة ٤ أسابيع ثم عولجت بمستخلص داميانا لمدة ٤ أسابيع أخرى (بنفس الجرعات السابقة). كما تم فحص عينات أنسجة الكبد و الكلى باستخدام الهيماتوكسلين و الأيوسين. و قد أظهرت هذه الدراسة أن حقن الجرذان بالترينبولون أدى إلى زيادات ذات دلالات إحصائية في تناول الماء و الغذاء، ووزن الجسم النسبي، والأوزان النسبية للكلى والكبد، وكذلك زيادات ذات دلالات إحصائية في مستويات كل من اليوريا والكرياتينين و الصوديوم والبوتاسيوم و الكلوريد في الدم، بالاضافة إلى زيادات ذات دلالة احصائية في كل من إنزيمات الكبد والفوسفاتيز القلوي والبيليروبين. في المقابل، تم تسجيل انخفاض ملحوظ إحصائيا في مستوى الكالسيوم و مستوى الألبومين بالدم في مجموعة الترينبولون. وقد أظهرت قياسات الإجهاد التأكسدى فى أنسجة الكبد والكلى عن زيادة ذات دلالة إحصائية في مستوى المالونديالدهيد مع انخفاض معتد به إحصائيا في الانزيمات جلوتاثيون ريداكتاس وكاتالاز وسوبرأكسيد ديسميوتيز. بينما ساعد استخدام مستخلص داميانا فى تحسن ملحوظ احصائيا فى جميع التأثيرات السامة المذكورة سابقا للترينبولون. كما تسبب ترينبولون في حدوث التهاب ملحوظ و تليف شديد في أنسجة الكبد والكلى. و خلصت هذه الدراسة إلى أن الترينبولون يسبب تسمم كلوي وكبدي يصاحبه إجهاد تأكسدي في الجرذان. ولكن قد تحسن هذا التسمم بشكل ملحوظ عند استخدام مستخلص داميانا والذى يمتلك خصائص فعالة كمضاد أكسده مما يسلط الضوء على إمكانية استخدام مستخلص داميانا كعامل وقائي ضد التسمم بالترينبولون.