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Hepatotoxic effects of sildenafil-containing "Tiger King" herbal supplement in a rat model: An *in vitro* and *in vivo* study

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ABSTRACT

Background: The global market for erectile dysfunction (ED) treatments has seen a rise in herbal supplements marketed as natural alternatives to prescription medications. However, many of these products contain undeclared pharmaceutical ingredients, posing significant health risks.

Objective: This study aimed to investigate the chemical composition and potential hepatotoxic effects of "Tiger King," a purported Chinese herbal supplement for sexual enhancement, using both *in vitro* and *in vivo* experiments.

Materials and methods: Chemical analysis of "Tiger King" tablets was performed using thin-layer chromatography and colorimetric tests. Antioxidant activities were evaluated using DPPH and FRAP assays. *In vivo* studies were conducted using male Wistar rats (N=20) divided into four groups: control, clomiphene, low-dose "Tiger King" (5 mg/kg) or TK1, and high-dose "Tiger King" (10 mg/kg) or TK2. Treatments were administered orally for 30 days. Serum testosterone levels, sperm parameters, oxidative stress markers, liver and kidney function tests, and histopathological changes were assessed.

Results: Chemical analysis revealed the presence of sildenafil in "Tiger King" tablets, with no detectable amounts of the claimed herbal ingredients. *In vivo* studies showed significant increases in sperm count and testosterone levels in treated groups. However, oxidative stress markers (MDA, GSH) were significantly altered, and liver function tests (ALT, AST, ALP, bilirubin) were elevated in treatment groups with ALT increased by 17.8% (from 45.0±1.1 to 53.0±1.1 U/L), AST by 12.5% (from 120.0±1.1 to 135.0±1.1 U/L) in the high-dose TK2 group. Histopathological examination revealed mild to moderate changes in liver, kidney, and reproductive organs of treated animals, including hepatic steatosis, renal glomerular congestion, and glandular atrophy in reproductive tissues.

Conclusion: This study provides evidence that "Tiger King" contains undeclared sildenafil and lacks the advertised herbal components. Its use is associated with improved reproductive parameters but also with significant biochemical and histopathological changes which draw attention to potential health risks of adulterated herbal supplements.

Introduction

The global market for erectile dysfunction (ED) treatments has seen a significant surge in the availability of herbal supplements marketed as "natural" alternatives to prescription medications.[1]especially with regard to the treatment of erectile dysfunction (ED These products, often promoted as safer options, have raised concerns among healthcare professionals and regulatory bodies due to the potential presence of undeclared pharmaceutical ingredients and their associated health risks. One such

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product, "Tiger King", a purported Chinese herbal remedy for sexual enhancement, has gained popularity in various countries, including Israel, Australia, Canada, and the United States.[2-4] Recent investigations have found a disturbing trend in the composition of these "herbal" supplements. Numerous reports have detected the presence of phosphodiesterase type 5 (PDE-5) inhibitors and their analogues in products labeled as "100% natural" or "herbal".[5,6] Sildenafil, the active ingredient in Viagra®, and its analogues are frequently found in these supplements, often in varying and potentially dangerous doses.[7,8] This adulteration poses significant health risks, particularly as consumers may be unaware of the actual contents of these products.

The case of "Tiger King" is particularly alarming. Chemical analyses conducted by health authorities in the United States, Israel, Australia, and Canada have consistently revealed the presence of sildenafil in these tablets, with doses ranging from therapeutic levels to potentially harmful amounts exceeding 200 mg per tablet.[9] More concerning is the complete absence of the herbal ingredients listed on the product label, further emphasizing the questionable nature of these supplements. However, and while the efficacy of sildenafil in treating ED is well-established, its safety profile, particularly in uncontrolled doses and in combination with undisclosed ingredients, remains a subject of concern. Recent case reports have suggested a possible link between sildenafil use and hepatotoxicity, a connection not widely recognized or reported in standard prescribing information.[2,10-13] These cases raise important questions about the safety of sildenafil, especially in patients with pre-existing liver conditions or when consumed in unregulated supplements. Similarly, recent studies have demonstrated the importance of rigorous testing methodologies when evaluating reproductive parameters and drug safety.[14]

The potential for hepatotoxicity associated with sildenafil is particularly worrisome given the prevalence of liver disease globally and the tendency of many patients to seek "natural" remedies without medical supervision. The mechanism by which sildenafil might induce liver injury remains unclear, with hypotheses ranging from idiosyncratic reactions to dose-dependent toxicity in susceptible individuals.[13] Despite these concerns, there is a small number of controlled studies investigating the hepatotoxic potential of sildenafil, particularly in the context of adulterated herbal supplements like "Tiger King". In this respect, we hypothesized that the undeclared presence of sildenafil in "Tiger King" supplements may cause health risks, particularly hepatotoxicity, despite potential benefits for sexual function. Therefore, this study aims to address this critical knowledge gap by investigating the potential hepatotoxic effects of "Tiger King" and its primary active ingredient, sildenafil, in a controlled animal model.

Materials and methods:

In vitro studies

Chemical analysis

Thin-layer chromatography was performed using silica gel 60 F254 plates (Merck, Germany). The mobile phase consisted of ethyl acetate:methanol:ammonium hydroxide (85:10:5 v/v/v).[15] Samples were prepared by dissolving one TK tablet in 10 mL of methanol, sonicating for 15 minutes, and filtering through a 0.45 µm membrane filter. Ten microliters of the sample solution and authenticated sildenafil standard (Sigma-Aldrich, USA) were applied to the TLC plate. After development, the plates were visualized under UV light at 254 nm and 366 nm.[16] Sildenafil identification was confirmed by co-chromatography with reference standard (Rf=0.68±0.02) and subsequent phosphomolybdic acid reagent spray followed by heating at 105 °C for 5 minutes. The presence of sildenafil was indicated by a blue spot.[17]

In addition to TLC analysis, colorimetric tests were also performed such as Dragendorff's test (for alkaloids),[18] vanillin-sulfuric acid test (for ginseng saponins),[19] and ninhydrin test (for amino acids and peptides),[20] as well as the visual inspection for synthetic dyes.[21] All tests were performed in triplicate to ensure reproducibility. Standard solutions of sildenafil, tadalafil, yohimbine, ginseng extract, and *Cordyceps* extract were used as positive controls.[22]

The TK tablets were ground into fine powder using a clean mortar and pestle (Figure 1). For *in vitro* experiments, the powder was dissolved in methanol (10 mg/mL), sonicated for 15 minutes, and filtered through a 0.45 µm membrane filter. For chemical analysis, 10 µL of this solution was used for TLC analysis and appropriate dilutions were made for other assays.

Figure 1. *Tiger king tablets from the local market.*

Antioxidant activities

The antioxidant activities of "Tiger King" were evaluated using multiple assays. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay was performed to measure free radical scavenging capacity.[23] The ferric reducing antioxidant power (FRAP) assay was used to assess the supplement's reducing power.[24] To ensure measurement consistency, all assays were performed in duplicate, with ascorbic acid and Trolox as positive controls.

In vivo studies

Animal model and treatment

Male Wistar rats (8-10 weeks old, weighing 200-250 gm) were used for the *in vivo* studies. Animals were housed under standard laboratory conditions (12 hrs light/dark cycle, 22±2 °C, 55±5% humidity) with free access to food and water. Rats were randomly divided into four groups (N=5 per group): control, clomiphene, low-dose "Tiger King" (5 mg/kg), high-dose "Tiger King" (10 mg/kg). Treatments were administered orally once daily for 30 days. For oral administration, TK tablets were ground into fine powder and dissolved in sterile saline (10 mg/mL). The solution was sonicated for 15 minutes and filtered through a 0.45 µm membrane filter. Fresh solutions were prepared daily and administered via oral gavage according to the designated doses for each treatment group. All animal procedures were approved by the Institutional Ethics Committee and conducted in accordance with international guidelines for the care and use of laboratory animals.

Sperm parameters

On day 29, rats were euthanized, and epididymal sperm were collected. Sperm count was determined using a hemocytometer. Sperm motility was assessed using computer-assisted sperm analysis (CASA). Sperm morphology was evaluated by examining Eosin-Nigrosin stained smears under a light microscope, counting at least 200 sperm per sample and classifying them as normal or abnormal based on head, midpiece, and tail morphology.

Biochemical analysis

Blood samples were collected via cardiac puncture, and serum was separated by centrifugation. Liver function was assessed by measuring serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin using commercial kits. Oxidative stress markers, including malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD) activity, were measured in liver homogenates using spectrophotometric methods.

Histopathological changes

Liver and testicular tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E). Slides were examined under a light microscope by a pathologist blinded to the treatment groups. Liver sections were assessed for signs of hepatotoxicity, including steatosis, inflammation, and necrosis. Testicular sections were evaluated for changes in seminiferous tubule morphology, spermatogenesis, and interstitial tissue.

Statistical analysis

All data were analyzed using SPSS version 27 (IBM, USA). Data normality was assessed using Shapiro-Wilk test and the results are presented as mean±SD. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test was used to compare differences between groups. For fertility rate, Chi-square test was employed. *The* $p<0.05$ was considered statistically significant. Graphs were generated using GraphPad Prism 9 (GraphPad Software, USA).

Results

Chemical analysis

Thin-layer chromatography (TLC) and colorimetric tests were performed to detect the presence of key compounds in the Tiger King (TK) supplement. The results are summarized in Table 1. The TLC analysis revealed a prominent spot (Rf=0.68) under UV light at 254 nm, characteristic of sildenafil. This spot also gave a positive reaction with phosphomolybdic acid spray reagent which additionally confirms the presence of sildenafil. No spots corresponding to tadalafil or other common PDE-5 inhibitors were observed under UV light at 366 nm. The Dragendorff's reagent, used to detect alkaloids such as yohimbine, did not produce any orange-brown spots, which indicates the absence of these compounds. Also, the vanillin-sulfuric acid reagent, typically used to detect ginseng saponins, did not produce any characteristic purple spots, which qualitatively means the absence of ginseng in the sample. Moreover, the ninhydrin reagent, used to detect amino acids and peptides found in *Cordyceps*, did not produce any purple spots. The visual inspection under normal and UV light (366 nm) revealed the presence of synthetic dyes, likely used for tablet coloration.

Table 1. Qualitative chemical analysis of Tiger King (TK).

Compound	Method of detection	Result
Sildenafil	TLC + UV (254 nm)	Present
Tadalafil	TLC + UV (366 nm)	Absent
Yohimbine	Dragendorff's reagent	Absent
Ginseng saponins	Vanillin-sulfuric acid reagent	Absent
Cordyceps markers	Ninhydrin reagent	Absent
Synthetic dyes	Visual + UV (366 nm)	Present

Antioxidant activities

The DPPH radical scavenging activity of TK extract exhibited a concentration-dependent response (Figure 2A). At the lowest concentration tested (10 µg/mL), TK showed 15.3±1.2% inhibition, increasing to 84.2±1.9% at the highest concentration (200 µg/mL). In comparison, ascorbic acid (AA), used as a positive control, demonstrated higher activity, 38.5±1.7% inhibition at 10 µg/mL and 98.9±0.5% at 200 µg/mL. The calculated IC_{50} value for TK was approximately 70 µg/mL, while for AA it was below 25 µg/mL.

The ferric reducing antioxidant power (FRAP) of TK extract also showed a concentration-dependent increase (Figure 2B). At 50 µg/mL, TK exhibited a FRAP value of 215.3±7.8 µmol Fe(II)/gm extract, which rose to 789.4±18.3 µmol Fe(II)/gm extract at 200 µg/mL. The standard antioxidant Trolox demonstrated higher reducing power, FRAP values of 456.7±11.3 and 1785.3±28.6 µmol Fe(II)/gm at 50 and 200 µg/mL, respectively.

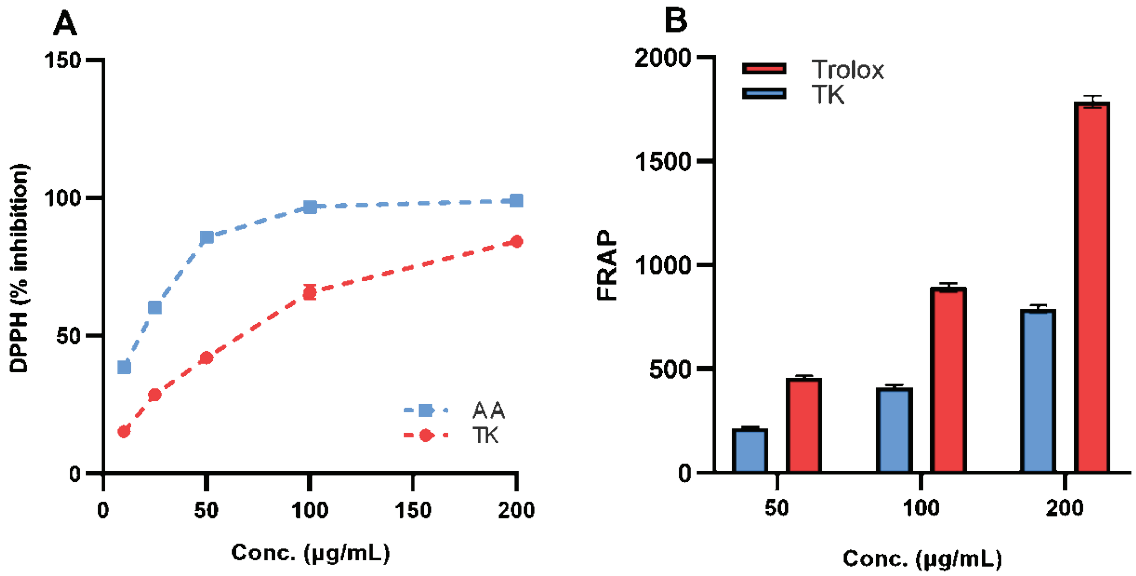

Figure 2. *Antioxidant activity of Tiger King (TK) extract compared to standard antioxidants. A: DPPH radical scavenging activity of TK extract and ascorbic acid (AA), B: FRAP of TK extract compared to Trolox.*

Weight change

Body weight changes in rats were monitored at three time points: day 1, day 14, and day 30, for all treatment groups (Figure 3). At the start of the experiment (day 1), the mean body weights were comparable among groups: control (300.0±1.6 gm), clomiphene (298.8±3.3 gm), TK1 (296.0±1.6 gm), and TK2 (301.0±1.6 gm). By day 14, all groups showed a slight increase in weight: control (305.0±1.6 gm), clomiphene (303.6±3.4 gm), TK1 (300.0± 1.6 gm), and TK2 (306.0±1.7 gm). The thing continued through day 30, with further weight gains observed in all groups: control (310.0±1.6 gm), clomiphene (309.8±3.4 gm), TK1 (306.0±1.7 gm), and TK2 (312.0±1.6 gm). Despite these changes, statistical analysis revealed no significant differences in body weight between the treatment groups and the control group at almost all time points (p>0.05 mostly). These results suggest that neither clomiphene nor the two doses of TK significantly affected body weight gain over the course of the experiment.

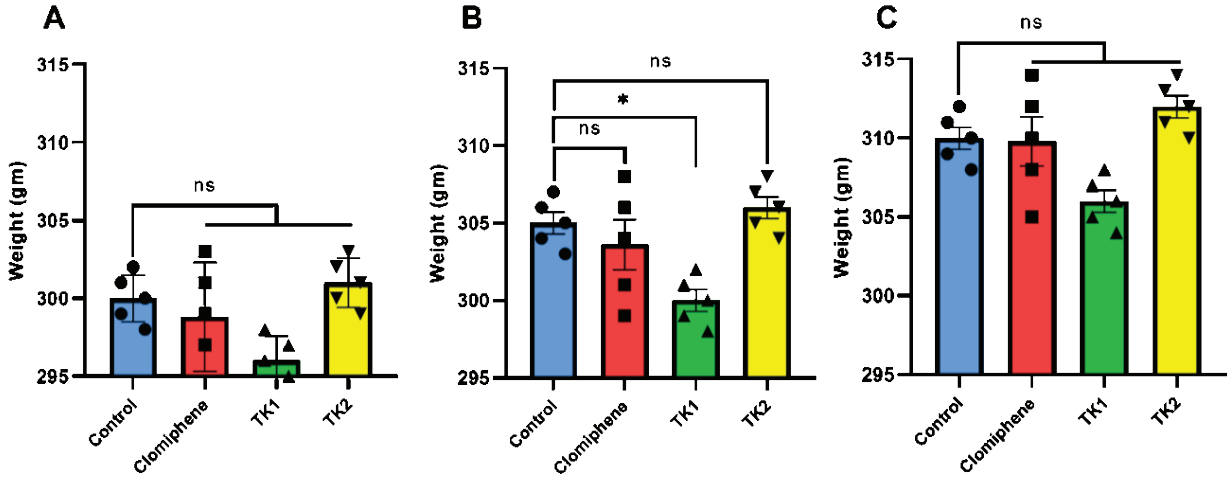

Figure 3. *Body weight changes in rats over the course of the experiment (N=5 per group). A: day 1 initial body weights, B: day 14 body weights, C: day 30 final body weights. Data are presented as mean±SE. No significant differences were observed among groups at almost all time points (p>0.05).*

Sperm parameters

Sperm parameters were affected by the treatments (Figure 4). The mean sperm count in the control group was 65.00±1.58 million/mL. Treatment with clomiphene resulted in the highest sperm count (75.80±1.92 million/mL, p<0.05), showing a significant increase of approximately 16.6% compared to the control. The TK2 group also showed a significant increase in sperm count (73.00±1.58 million/mL, p<0.05), while the TK1 group demonstrated a moderate but still significant elevation (70.00±1.58 million/mL, p<0.05) compared to the control. Interestingly, the clomiphene group showed significantly higher sperm counts compared to TK1 (p<0.05) but not compared to TK2 (p=0.08).

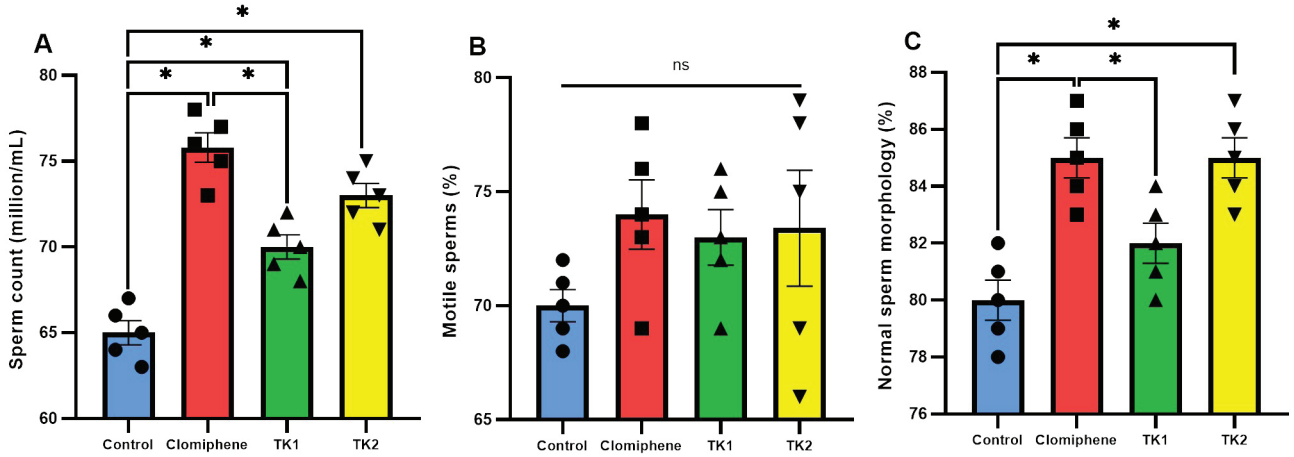

Figure 4. *Sperm parameters in rats after treatment (N=5 per group). A: sperm count (million/mL), B: percentage of motile sperm, C: percentage of sperm with normal morphology. Data are presented as mean±SE. *p<0.05.*

Testosterone levels

Testosterone levels were significantly altered by the treatments (Figure 5). The control group had a mean testosterone level of 450.0±15.81 ng/dL. Both the clomiphene and TK2 groups showed the highest increases in testosterone levels (520.0±15.81 ng/dL for both groups), representing a significant elevation of approximately 15.6% compared to the control. The TK1 group demonstrated a moderate but significant increase (490.0±15.81 ng/dL), showing an 8.9% elevation compared to control levels. The range of testosterone values across groups was consistent, with minimum values of 430.0 ng/dL in the control group and maximum values reaching 540.0 ng/dL in both the clomiphene and TK2 groups.

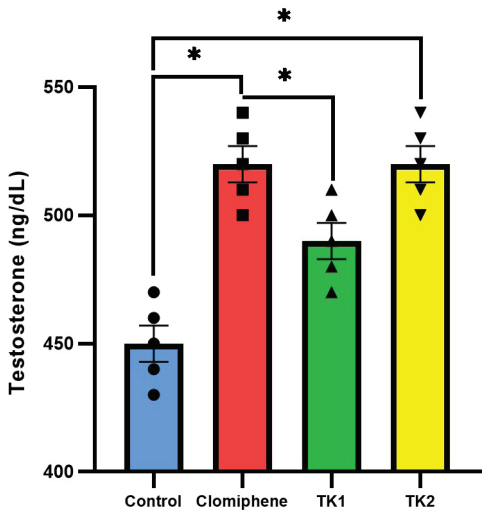

Figure 5. *Serum testosterone levels in rats after treatment (N=5 per group). Data are presented as mean SE. *p<0.05.*

Biochemical parameters

The oxidative stress markers, MDA and GSH, were significantly altered in both serum and liver tissue following treatment (Figure 6). In serum, MDA levels were markedly increased in all treatment groups compared to the control (0.300±0.011 µmol/L), with clomiphene (0.440±0.024 µmol/L, $p<0.05$), TK1 (0.390±0.011 µmol/L, $p<0.05$), and TK2 (0.490±0.011 µmol/L, $p<0.05$) showing progressively higher levels. Conversely, serum GSH levels were significantly decreased in TK1 (2.540±0.093 µmol/L, $p<0.05$) and TK2 (2.060±0.087 µmol/L, $p<0.05$) groups compared to the control (3.240±0.081 µmol/L), whereas the clomiphene group (3.100±0.045 µmol/L) did not differ significantly ($p>0.05$). Furthermore, liver tissue analysis revealed a similar finding. Hepatic MDA levels were significantly elevated in TK1 (1.150±0.092 nmol/mg protein, $p<0.05$) and TK2 (1.474±0.036 nmol/mg protein, $p<0.05$) groups compared to the control (0.796±0.083 nmol/mg protein), while the clomiphene group (0.904±0.035 nmol/mg protein) showed no significant difference ($p>0.05$). In addition, liver GSH levels were significantly reduced in both TK1 (5.380±0.080 µmol/gm tissue, $p<0.05$) and TK2 (4.440±0.108 µmol/gm tissue, $p<0.05$) groups compared to the control (6.780±0.086 µmol/gm tissue), whereas the clomiphene group (6.560±0.087 µmol/gm tissue) again showed no significant change ($p>0.05$).

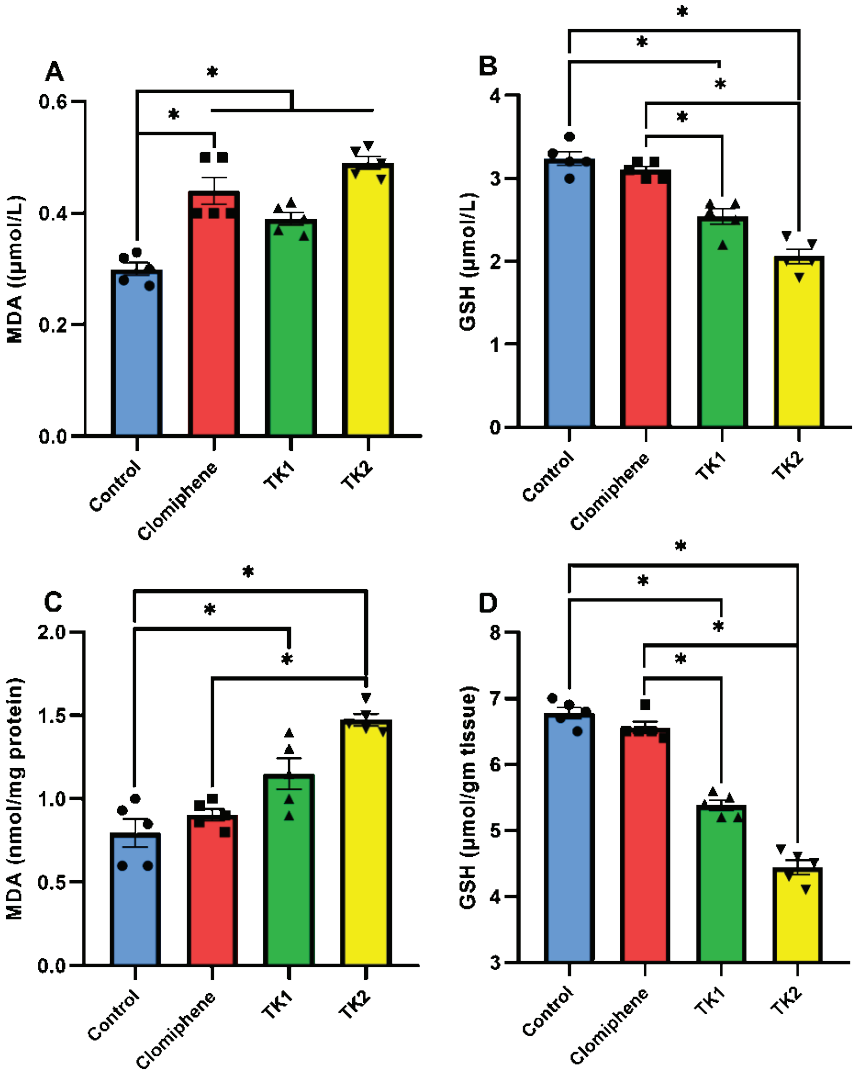

Figure 6. *Oxidative stress markers in serum and liver tissue (N=5 per group). A: serum malondialdehyde (MDA) levels, B: serum glutathione (GSH) levels, C: liver tissue MDA levels, D: liver tissue GSH levels. Data are presented as mean ± SE. *p<0.05.*

Liver function tests revealed significant alterations across treatment groups (Figure 7). Serum bilirubin levels (Figure 7A) were significantly elevated in all treatment groups compared to the control (0.300±0.011 mg/dL), with clomiphene (0.440±0.024 mg/dL, $p<0.05$), TK1 (0.390±0.011 mg/dL, $p<0.05$), and TK2 (0.490±0.011 mg/dL, $p<0.05$) showing progressively higher levels. Similarly, AST levels (Figure 7B) were significantly increased in all treatment groups compared to the control (120.0±1.1 U/L), with clomiphene (131.2±1.2 U/L, $p<0.05$), TK1 (125.0±1.1 U/L, $p<0.05$), and TK2 (135.0±1.1 U/L, $p<0.05$) demonstrating escalating values. ALT levels (Figure 7C) were also significantly elevated in clomiphene (50.0±0.7 U/L, $p<0.05$) and TK2 (53.0±1.1 U/L, $p<0.05$) groups compared to the control (45.0±1.1 U/L), whereas the TK1 group (47.0±1.1 U/L) showed no significant difference ($p>0.05$). Furthermore, ALP levels (Figure 7D) exhibited a similar trend, with significant increases observed in Clomiphene (75.0±0.7 U/L, p<0.05) and TK2 (78.0±1.1 U/L, $p<0.05$) groups compared to the control (70.0±1.1 U/L), while the TK1 group (72.0±1.1 U/L) showed no significant change ($p>0.05$).

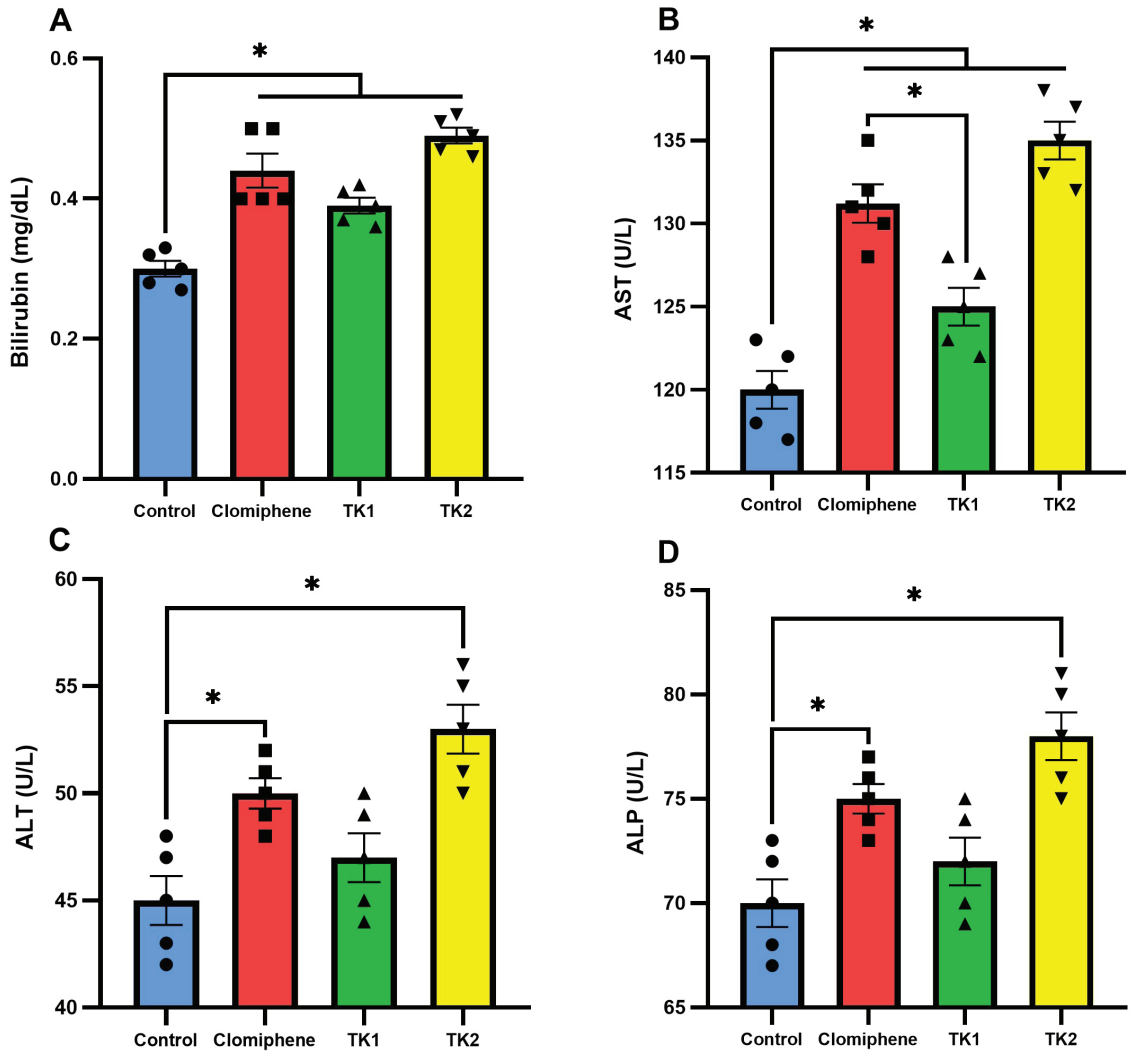

Figure 7. *Liver function tests (LFTs) in rats after treatment (N=5 per group). A: serum bilirubin levels, B: aspartate aminotransferase (AST) activity, C: alanine aminotransferase (ALT) activity, D: alkaline phosphatase (ALP) activity. Data are presented as mean±SD. *p<0.05.*

Renal function tests showed significant alterations as well in the treatment groups (Figure 8). Blood urea nitrogen (BUN) levels (Figure 8A) were significantly elevated in the clomiphene (20.0±0.3 mg/dL, $p<0.05$) and TK2 (21.0±0.4 mg/dL, $p<0.05$) groups compared to the control (18.0±0.4 mg/dL), while the TK1 group (19.0±0.4 mg/dL) showed no significant difference ($p>0.05$). Also, serum creatinine levels (Figure 8B) were significantly increased in the clomiphene (0.900±0.032 mg/dL, $p<0.05$) and TK2 (1.000±0.011 mg/dL, $p<0.05$) groups compared to the control (0.800±0.011 mg/dL), whereas the TK1 group (0.800±0.011 mg/dL) remained unchanged ($p>0.05$). Furthermore, uric acid levels (Figure 8C) exhibited a comparable result, with significant elevations observed in the clomiphene (5.300±0.071 mg/dL, $p<0.05$) and TK2 (5.360±0.068 mg/dL, $p<0.05$) groups compared to the control (5.000±0.071 mg/dL), while the TK1 group (5.100±0.071 mg/dL) showed no significant change ($p>0.05$).

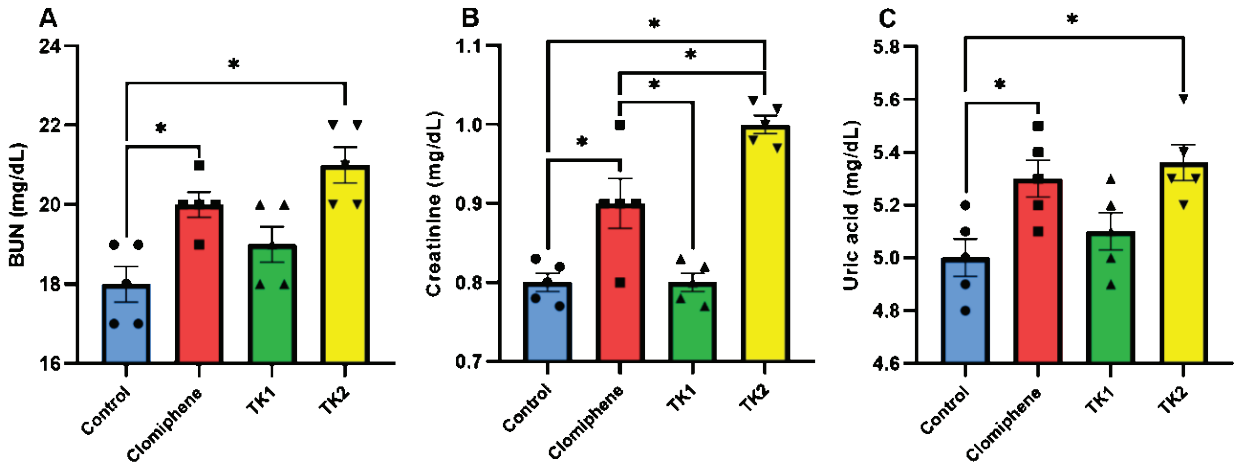

Figure 8. *Renal function tests (RFTs) in rats after treatment (N=5 per group). A: blood urea nitrogen (BUN) levels, B: serum creatinine levels, C: serum uric acid levels. Data are presented as mean±SD. *p<0.05.*

Histological changes

Histopathological examination revealed various changes in the reproductive organs of treatment groups (Figure 9). The testis showed normal size and arrangement of seminiferous tubules in all groups, comparable to the untreated control (Figure 9D). However, mild thickening of testicular interstitial connective tissue was observed in treated groups, although interstitial cells appeared normal. The germinal epithelium showed mild vacuolar degeneration of spermatogenic cells without necrosis in the clomiphene (Figure 9A), TK1 (Figure 9B), and TK2 (Figure 9C) groups. The seminal vesicles maintained normal histological features across all groups, displaying normal glandular fibro-muscular walls, mucosal folds, and glandular crypts with secretion, as exemplified in the normal (Figure 9E) and TK2 (Figure 9F) groups. In contrast, the bulbourethral gland exhibited notable changes in the TK2 group, characterized by moderate glandular atrophy associated with marked atrophy of alveolar cells and significant duct dilatation (Figure 9G, 9H). The control group's bulbourethral gland maintained normal histology (Figure 9I). Importantly, the other treatment groups showed no significant histological changes in the bulbourethral gland compared to the control.

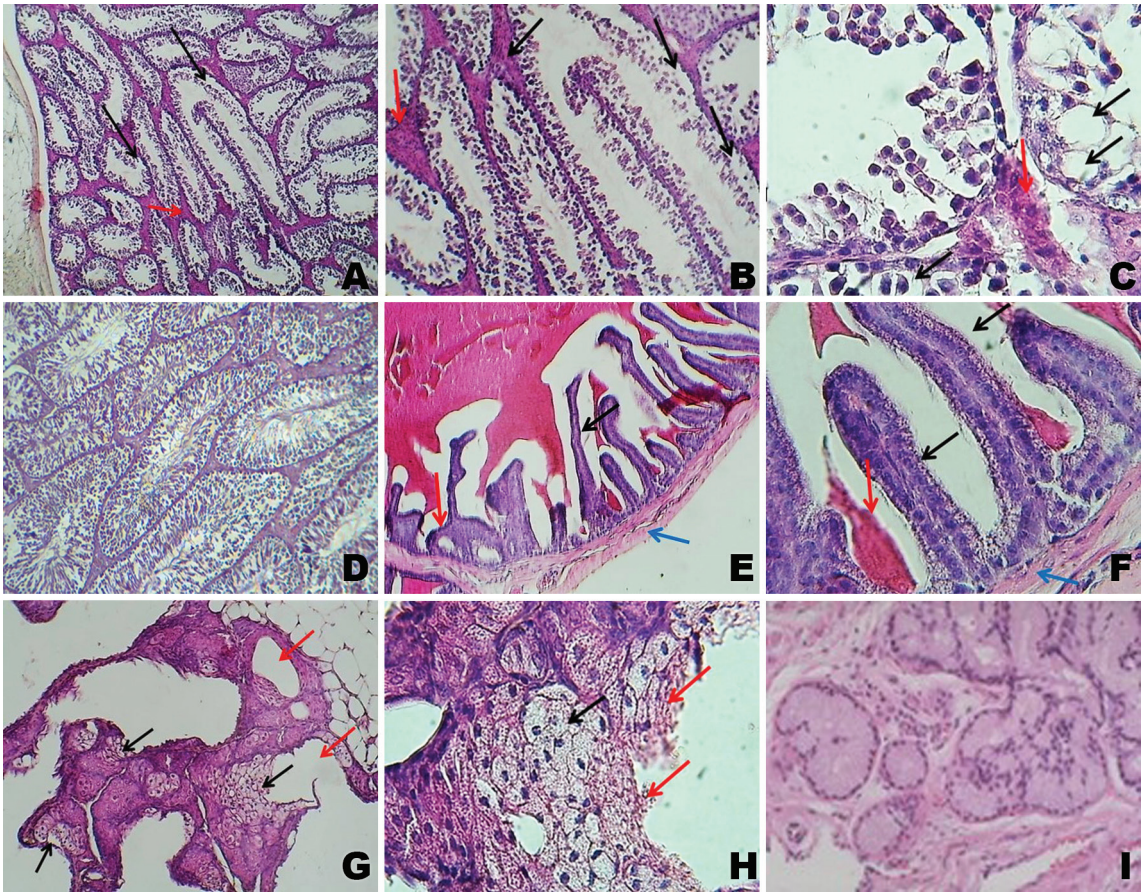

Figure 9. *Histopathological changes in reproductive organs following treatment. (A-D) Testis sections; A: clomiphene, showing normal diameter of seminiferous tubules with mild thickening of testicular interstitial connective tissue (red arrow) and mild vacuolar degeneration of germinal epithelium (black arrow), (H&E stain, 100X), B: TK1 treatment, similar findings as in A (H&E stain, 400X), C: TK2 treatment, showing mild thickening of testicular interstitial connective tissue (red arrow) and mild vacuolar degeneration of germinal epithelium (black arrow), (H&E stain, 400X), D: normal control, showing normal testicular interstitial connective tissue, (H&E stain, 400X). (E-F) Seminal vesicle sections, E: TK2 treatment, showing normal glandular fibro-muscular wall (blue arrow), mucosal folds (red arrow) and glandular crypts (black arrows), (H&E stain, 40X), F: TK2 treatment, showing normal glandular fibro-muscular wall (blue arrow), mucosal folds with normal lining epithelium (black arrow) and secretion (red arrow), (H&E stain, 400X). (G-I) Bulbourethral gland sections; G: TK2 treatment, showing moderate glandular atrophy associated with marked atrophy of alveolar cells (black arrows) and marked dilatation of ducts (red arrows), (H&E stain, 100X), H: TK2 treatment, showing moderate glandular atrophy associated with marked atrophy of alveolar cells (red arrows) and normal alveolar cells (black arrows), (H&E stain, 400X), I: normal control, showing normal bulbourethral gland structure, (H&E stain, 400X).*

The kidneys sections revealed both normal structures and some pathological changes among different regions (Figure 10). The renal medulla exhibited largely normal features, including a normal renal pelvis and intact renal tubules of the duct of Bellini among TK2 group (Figure 10A). Higher magnification of the renal medulla confirmed the presence of normal thick and thin segments of the loop of Henle and collecting tubules (Figure 10B), which appeared consistent with the control group (Figure 10C). In contrast, the renal cortex displayed some alterations. Severe subcapsular hemorrhage was observed, accompanied by congestion of the glomerular tuft (Figure 10D). Upon closer examination, the renal cortex exhibited mild intertubular vascular congestion and congestion of the glomerular tuft, although the renal tubules maintained their normal appearance (Figure 10E). These cortical changes were evident when compared to the control group (Figure 10F).

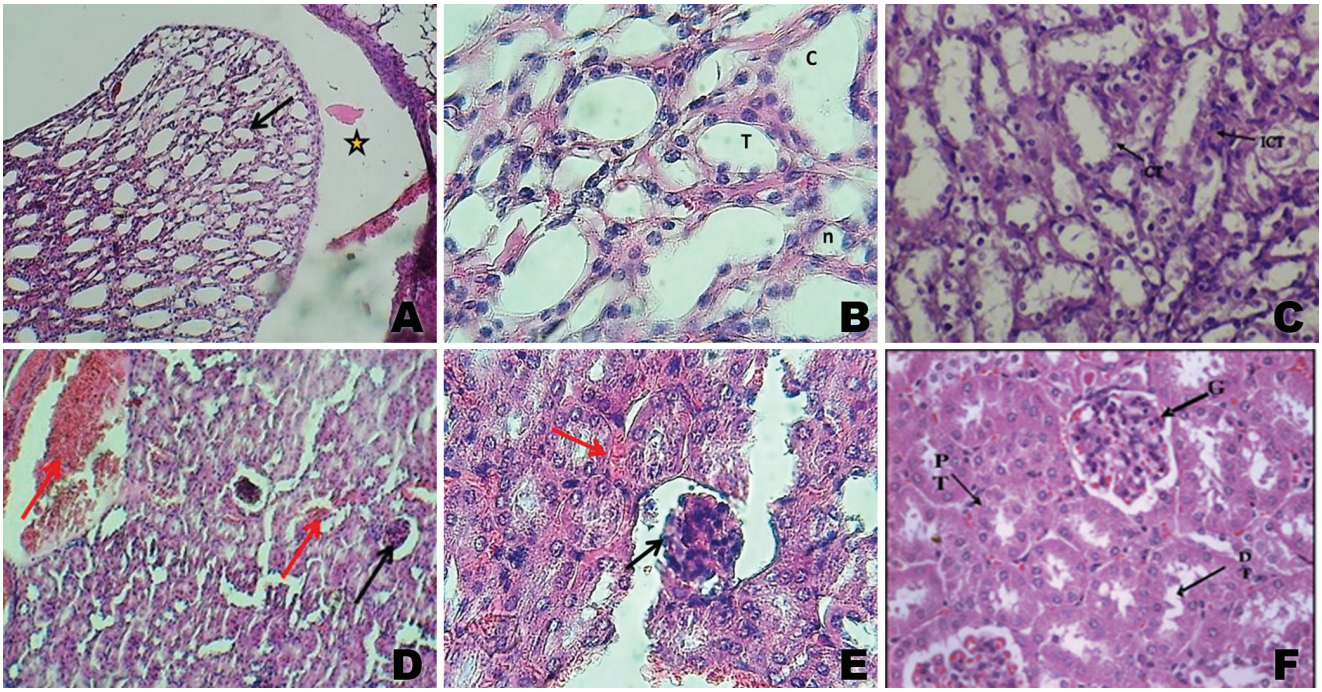

***Figure 10**. Histopathological changes in kidney following treatment. Renal medulla; A: normal renal pelvis (asterisk) and tubules of the duct of Bellini (arrow) in TK2 group, (H&E stain, 100X), B: higher magnification showing normal thick segment (T), thin segment (n) of the loop of Henle and collecting tubules (C) of TK2 group, (H&E stain, 400X), C: control group medulla. Renal cortex; D: severe subcapsular hemorrhage (red arrows) with glomerular tuft congestion (black arrow) of TK2 group, (H&E stain, 100X), E: higher magnification showing mild intertubular vascular congestion (red arrow) and glomerular tuft congestion (black arrow) with normal renal tubules of TK2 group, (H&E stain, 400X), F: control group cortex showing normal proximal tubules (P), distal tubules (T) and normal glomeruli (black arrows), (H&E stain, 100X).*

The liver and heart also revealed various pathological changes (Figure 11). The liver exhibited a spectrum of alterations, ranging from mild to moderate. Hepatic cords were generally normally arranged but showed mild congestion with dilation of the central vein and sinusoidal congestion after TK2 group (Figure 11A). Higher magnification revealed mild steatosis of hepatocytes, occasional necrotic hepatocytes, and sinusoidal dilation with congestion (Figure 11B). More severe changes were also observed, including moderate congestion with central vein dilation, focal necrosis accompanied by mononuclear leukocyte aggregation, marked dilation of portal triad blood vessels with perivascular cuffing, and cellular swelling of hepatocytes in TK2 group (Figures 11C and 11D). These pathological features contrasted sharply with the normal liver architecture observed in the control group (Figure 11E). The heart also displayed several alterations, most marked intrachamber thrombosis (Figure 11F). Also, the myocardium showed moderate vascular congestion, although the myofibers themselves appeared normal (Figures 11G, 11H, and 11I). For low dose TK, no histopathological changes were observed.

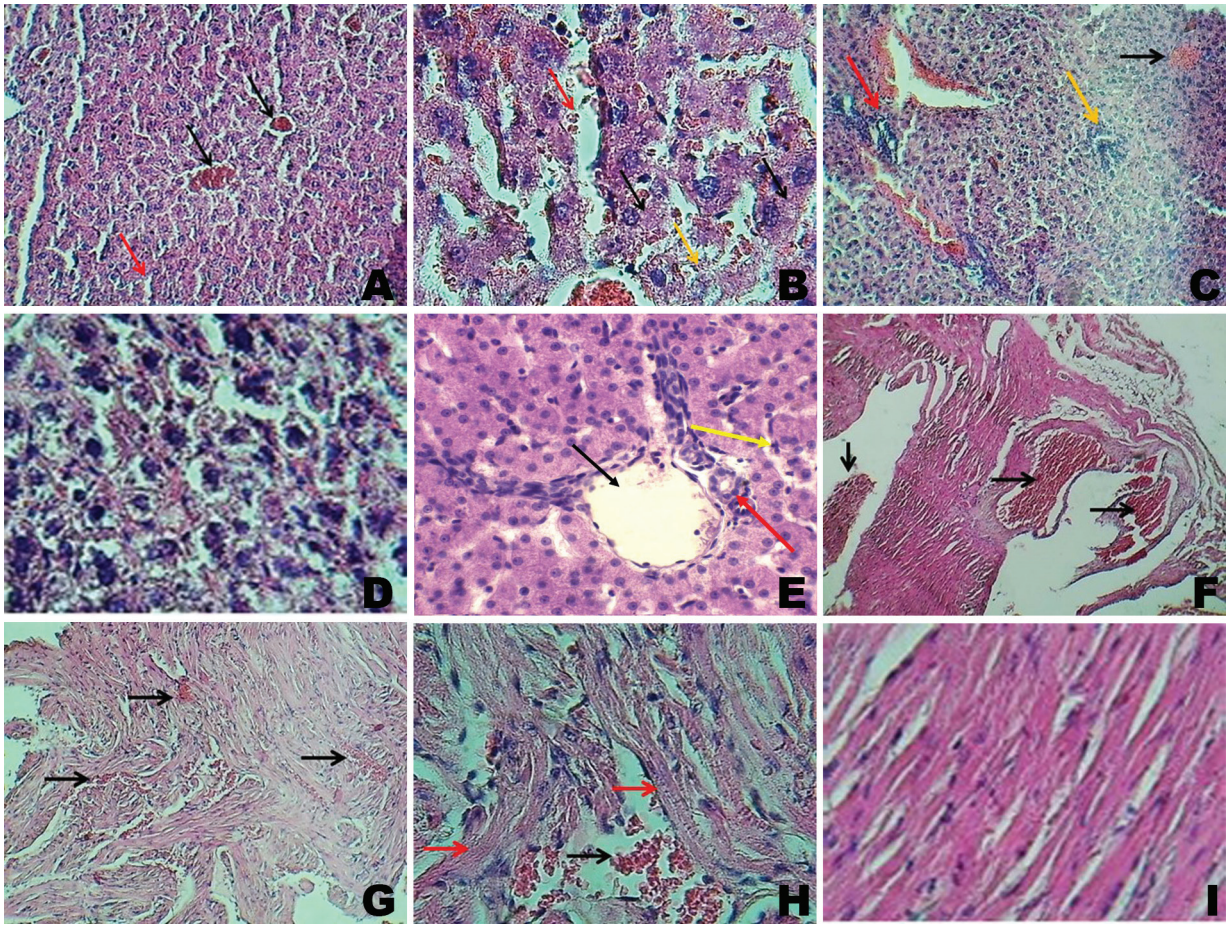

Figure 11: *Histopathological changes in liver and heart following treatment. Liver; A: mild congestion with central vein dilation (black arrows), sinusoidal dilation and congestion (red arrow) in TK2 group, (H&E, 100X), B: mild hepatocyte steatosis (black arrows), necrosis (yellow arrow), sinusoidal dilation and congestion (red arrow) in TK2 group, (H&E, 400X), C: central vein dilation (black arrows), focal necrosis with MNC aggregation (yellow arrow), portal triad vessel dilation with perivascular cuffing (red arrow) in TK2 group, (H&E, 400X), D: Hepatocyte cellular swelling in TK2 group, (H&E, 400X), E: control group showing normal liver architecture: central vein (black arrow), reticular fiber (red arrow), hepatocyte (yellow arrow), portal vein, bile duct, hepatic artery, nucleus, Kupffer cells, sinusoids, binucleated hepatocytes, (H&E, 400X). Heart; F: marked cardiac thrombosis (black arrows) in TK2 group, (H&E, 400X), G: moderate vascular congestion (black arrows) in TK2 group, (H&E, 100X), H: vascular congestion (black arrows) with normal myofibers (red arrows) in TK2 group, (H&E, 400X), I: normal cardiac tissue structure, (H&E, 400X).*

Discussion

This study provides evidence for the potential hepatotoxic effects of the "Tiger King" herbal supplement, which was found to contain undeclared sildenafil. Our findings raise significant concerns about the safety of such adulterated "natural" products and draw attention to the need for stricter regulation and monitoring of dietary supplements marketed for sexual enhancement. The chemical analysis of the "Tiger King" tablets revealed the presence of sildenafil as the primary active ingredient, with no detectable amounts of the herbal components listed on the product label. This finding is consistent with previous reports of adulterated herbal supplements for ED.[5-7,9] The absence of the claimed natural ingredients not only constitutes fraudulent marketing but also poses serious health risks to consumers who may unknowingly ingest pharmaceutical-grade sildenafil without proper medical supervision.

Our *in vitro* studies demonstrated that the "Tiger King" extract influenced some antioxidant properties, as evidenced by the DPPH radical scavenging and FRAP assays. However, these antioxidant effects were significantly lower than those of standard antioxidants like ascorbic acid and Trolox. This suggests that any potential benefits from the antioxidant activity of the supplement are likely reduced by the risks associated with its undeclared pharmaceutical content.

On the other hand, the *in vivo* experiments in rat model revealed several concerning effects of "Tiger King" administration. While we observed improvements in some sperm parameters and increases in serum testosterone levels, these apparent benefits were accompanied by significant adverse effects on multiple organ systems. The histopathological changes observed in the liver, kidneys, and heart are particularly alarming and consistent with potential sildenafil-induced toxicity.

The liver histology showed evidence of steatosis, focal necrosis, and inflammatory cell infiltration, which are indicative of drug-induced liver injury. These findings align with previous case reports of sildenafil-associated hepatotoxicity in humans.[10-12] The mechanism of sildenafil-induced liver injury remains unclear, but our results support the hypothesis that it may involve oxidative stress, as evidenced by the increased levels of MDA and decreased GSH in liver tissue.

While our chemical analysis identified sildenafil as the primary active ingredient without detecting the claimed herbal components, we acknowledge that tablet excipients or trace compounds could potentially contribute to the observed hepatotoxicity. Future studies using more sensitive analytical methods and isolated sildenafil administration would help definitively establish causation.

Renal histopathology revealed glomerular congestion and tubular changes which suggests potential nephrotoxicity. While sildenafil is not typically associated with kidney injury, our findings indicate that the adulterated supplement may have renal effects, possibly due to the presence of undeclared ingredients or contaminants. This underlines the importance of comprehensive safety evaluations for such products. The cardiac histology showed evidence of intrachamber thrombosis and vascular congestion. While sildenafil is generally considered safe for cardiac patients when used as prescribed, our findings raise concerns about potential cardiovascular risks associated with uncontrolled use of adulterated supplements. This is particularly relevant given that many consumers of ED supplements may have underlying cardiovascular conditions.

The reproductive system effects observed in our study were mixed. While we noted improvements in sperm parameters and testosterone levels, consistent with the known effects of sildenafil, we also observed concerning histological changes in the testis and accessory glands. The moderate glandular atrophy and marked duct dilation in the bulbourethral gland are novel findings that require further investigation. These results, together, suggest that long-term use of such supplements may have complex and potentially detrimental effects on male reproductive health. Our findings of improved sperm parameters and increased testosterone levels need mechanistic discussion. While sildenafil is primarily known for PDE5 inhibition in erectile tissue, numerous studies have demonstrated broader reproductive effects. The observed increase in testosterone levels likely results from sildenafil's enhancement of testicular blood flow and nitric oxide signaling, which stimulates Leydig cell function.[25-27] Additionally, PDE-5 inhibition has been shown to improve spermatogenesis through increased cGMP levels in seminiferous tubules.[28] These mechanisms explain how a PDE5 inhibitor like sildenafil can affect both testosterone production and sperm parameters, beyond its classical vasodilatory role in erectile function.

The common availability of adulterated "herbal" supplements like "Tiger King" poses significant risks to consumers who may believe they are taking a natural and safe product. The undeclared presence of sildenafil, often in varying and potentially dangerous doses, can lead to adverse drug interactions, especially in patients with cardiovascular conditions or those taking nitrates.[2,9] The disagreement between the product's labeling and its actual contents draw attention to the urgent need for more stringent quality control and regulatory oversight in the dietary supplement industry. Current regulations in many countries, including the United States, do not require pre-market approval for dietary supplements, making it difficult to prevent adulterated products from reaching consumers.[1,3,4]

In addition to the current study's strengths of combining chemical analysis, *in vitro* assays, and *in vivo* experiments, it also has a limitation as it focused on relatively short-term effects, and longer-term studies are needed to assess the chronic impacts of such supplements.

Conclusion

In conclusion, our study provides evidence for the potential hepatotoxicity and broader systemic effects of the adulterated "Tiger King" herbal supplement in Iraqi market. These findings draw attention to the importance of consumer awareness, healthcare provider vigilance,

and regulatory action to address the risks associated with fraudulent and potentially dangerous sexual enhancement products.

Conflict of interest

The authors declare that they have no known competing interests.

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