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Original Article

Protective Effect of Testosterone against Gentamicin's Toxicity in Adult Male Rabbits

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Abstract

Background: Gentamicin is an effective aminoglycoside antibiotic widely used in clinical practice; however, its therapeutic application is often limited by adverse effects, particularly liver and kidney toxicity. Experimental evidence suggests that sex hormones may influence susceptibility to gentamicin-induced organ damage. Testosterone, in particular, has been proposed to exert a protective effect against such toxicity. **Objective:** This study evaluated the biochemical and histopathological changes in the liver and kidneys of adult male rabbits treated with gentamicin and testosterone, administered either alone or in combination. **Methods:** Twenty adult male rabbits were randomly assigned to four groups: a control group, a gentamicin-treated group (40 mg/kg body weight), a testosterone-treated group (15 mg/kg body weight), and a group receiving testosterone followed by gentamicin. Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea, and creatinine were measured, and liver and kidney tissues were examined histologically. **Results:** Rabbits treated with gentamicin alone showed marked increases in serum ALT, AST, urea, and creatinine levels, indicating significant hepatic and renal injury. Administration of testosterone alone resulted in lower enzyme and metabolite levels compared with the gentamicin-treated group, suggesting limited organ stress. Co-administration of testosterone with gentamicin significantly reduced ALT and AST levels relative to gentamicin treatment alone, indicating partial hepatoprotection. However, urea and creatinine levels remained elevated, suggesting that the testosterone dose used was insufficient to prevent gentamicin-induced renal damage. Histopathological findings supported the biochemical results, with evident structural alterations in liver and kidney tissues following gentamicin exposure. **Conclusion:** Gentamicin induces pronounced hepatic and renal toxicity in adult male rabbits. Testosterone exerts organ-specific protective effects, providing partial protection to the liver but limited benefit to the kidneys under the conditions of this study.

Keywords: Gentamicin; Testosterone; Liver enzymes; Urea; Creatinine; Rabbits

1. Introduction:

Gentamicin (GM) is a widely prescribed aminoglycoside antibiotic that remains an important option for the treatment of severe infections caused by Gram-positive and Gram-negative aerobic bacteria. Despite its effectiveness, the clinical use of gentamicin is often limited by dose-dependent toxicity, particularly affecting the kidneys and liver [1, 2]. Gentamicin-induced nephrotoxicity is commonly characterized by acute tubular necrosis, elevated serum creatinine, and increased blood urea levels, while hepatotoxicity is reflected by increased serum transaminases and histological alterations in hepatic tissue [2, 3].

The mechanisms underlying gentamicin toxicity are complex and involve the generation of reactive oxygen species (ROS), oxidative stress, inflammation, mitochondrial dysfunction, and activation of apoptotic pathways [4, 5]. The kidneys are especially vulnerable due to the accumulation of gentamicin within proximal tubular epithelial cells through receptor-mediated endocytosis. This accumulation disrupts cellular metabolism and antioxidant defenses, leading to progressive tissue injury. In the liver, gentamicin-induced oxidative damage can compromise hepatocyte membrane integrity and promote inflammatory responses [4, 6]. Recent evidence indicates that susceptibility to gentamicin-induced organ damage may be influenced by sex hormones. Experimental studies suggest that hormonal status plays an important role in modulating renal and hepatic responses to toxic insults [4, 6]. Testosterone, the primary male sex hormone, has been reported to exert protective effects against certain forms of drug-induced nephrotoxicity, although its role appears to be dose-

dependent and tissue-specific. Conversely, gentamicin itself has been shown to disrupt endocrine function by impairing Leydig cell activity and inhibiting steroidogenic enzymes, leading to reduced endogenous testosterone levels [7-9]. These interactions highlight the potential importance of hormonal modulation in determining the severity of gentamicin-induced toxicity.

Testosterone and its synthetic derivatives, collectively referred to as anabolic-androgenic steroids, are used clinically to treat conditions such as hypogonadism, delayed puberty, and osteoporosis. They are also widely misused to enhance muscle mass and physical performance [10, 11]. Physiologically, testosterone plays a central role in male sexual development, spermatogenesis, metabolic regulation, and behavior [12, 13]. While therapeutic use of testosterone addresses conditions such as hypogonadism, osteoporosis, and delayed puberty [14-16], misuse or high doses can induce adverse effects, including hepatotoxicity, cardiac hypertrophy, testicular atrophy, and behavioral disturbances [17-19].

At the cellular level, testosterone has been shown to influence oxidative balance by modulating antioxidant enzyme activity and reducing lipid peroxidation. Through androgen receptor-mediated pathways, testosterone may also affect renal tubular function and hepatocyte metabolism, potentially contributing to tissue protection under certain conditions [4, 5]. Recent clinical and experimental studies have suggested that testosterone therapy may reduce the risk of acute kidney injury and improve systemic outcomes in specific disease contexts, further supporting its potential protective role [20]. Given the dual role of gentamicin as a potent antibiotic and a nephrotoxic/hepatotoxic agent, and considering testosterone's potential protective mechanisms, it is important to further clarify the nature of this interaction [21].

Given the widespread use of gentamicin and the growing evidence of hormone-dependent modulation of drug toxicity, a clearer understanding of the interaction between gentamicin and testosterone is needed. In particular, there is limited experimental information on how testosterone influences gentamicin-induced hepatic and renal injury in animal models. Therefore, the present study aimed to evaluate the biochemical and histopathological effects of testosterone on gentamicin-induced liver and kidney damage in adult male rabbits. By examining changes in liver enzymes, renal function markers, and tissue architecture, this study seeks to clarify whether testosterone confers protective effects against gentamicin toxicity and whether such effects differ between organs.

2. Materials and Methods

2.1 Chemicals

Testosterone hormone was obtained from Ibn Hayyan Pharmacy (Sana'a, Yemen). The commercial preparation used was Testoki® testosterone (Sanzyme Company), supplied as ampoules containing testosterone undecanoate (250 mg/mL), equivalent to 157.9 mg/mL testosterone.

Gentamicin sulfate was purchased from Sam Pharmacy (Sana'a, Yemen) and manufactured by KRKA. Each ampoule contained 80 mg gentamicin in 2 mL solution. Gentamicin was administered intramuscularly without dilution.

2.2 Experimental Animals

Twenty adult male rabbits weighing between 800 and 1500 g were obtained from a local breed and housed individually at the animal care facility of the Faculty of Applied Science, Thamar University. Animals were maintained under standard laboratory conditions at a constant temperature (25 ± 3 °C) with a 12-hour light/dark cycle. Rabbits had free access to food and water throughout the study. All animals were allowed to acclimatize for four weeks prior to the start of the experiment.

2.3 Experiment Design

Rabbits were randomly divided into four groups, with five animals per group, as follows:

- **Group I, GI (Control):** Rabbits received 1 mL of distilled water.
- **Group II, GII (Gentamicin):** Rabbits were administered gentamicin at a dose of 40 mg/kg body weight intramuscularly.
- **Group III, GIII (Testosterone):** Rabbits were administered testosterone at a dose of 15 mg/kg body weight intramuscularly.
- **Group IV, GIV (Testosterone + Gentamicin):** Rabbits received testosterone (15 mg/kg body weight) followed 30 minutes later by gentamicin (40 mg/kg body weight), both administered intramuscularly.

Rabbits were administered testosterone and gentamicin intramuscularly (IM) for a period of 5 days a week for a duration of one treatment [22].

2.4 Blood Collection

At the end of the experimental period, rabbits were fasted for approximately 10 hours. Animals were euthanized by slaughtering, and blood samples were collected by cardiac puncture into non-heparinized tubes. Samples were centrifuged at 3500 rpm for 5 minutes, and serum was separated and stored at 4 °C for enzyme assays [23]. Biochemical analyses were performed at the laboratories of Dhamar General Hospital.

2.5 Biochemical Analysis

Serum samples were used to assess liver function markers, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), as well as renal function parameters (urea and creatinine). Measurements were performed using standard ELISA-based techniques according to the manufacturer's instructions.

2.6 Histological Examination

At autopsy, liver and kidney specimens were collected from all animals, rinsed with normal saline, and fixed in 10% formalin for 24 hours. Tissues were dehydrated in graded ethanol, cleared in xylene, embedded in paraffin, and sectioned at 5 μm thickness. Sections were stained with hematoxylin and eosin and examined under a light microscope [24]. Histological changes were documented and photographed using a digital imaging system. All procedures followed standard histological techniques [25].

2.7 Statistical Analysis

Data are presented as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) with SPSS version 22, followed by post hoc multiple range tests. Differences were considered statistically significant at $p < 0.05$.

3. Results:

3.1 Biochemical Analysis of Liver Function Tests (ALT and AST)

Table 1 shows a significant increase in the mean serum levels of ALT and AST in both the gentamicin (GII) and testosterone (GIII) treated rabbit groups compared with the control group. Treatment with testosterone followed by gentamicin (GIV) produced a significant decrease in serum ALT and AST levels compared to the gentamicin (GII) treated group, but levels remained significantly higher than those of the testosterone (GIII) treated group.

Table 1: Effect of testosterone against the toxicity of gentamicin serum levels of ALT and AST in male rabbits

Group	ALT (U/L)	AST (U/L)
Control (GI)	72.40 ± 3.36	30.60 ± 5.41
Gentamicin-treated group (GII)	102.0 ± 6.48 ^{a**}	44.40 ± 4.56 ^{a***}
Testosterone-treated group (GIII)	85.80 ± 24.71 ^{a*}	37.80 ± 2.16 ^{a*}
Testosterone + Gentamicin treated group (GIV)	95.20 ± 7.59 ^{b*}	40.20 ± 3.96 ^{b**}

Data presented Mean ± SD values in each column were compared by one-way ANOVA followed by Post hoc multiple range test. Values with the same superscript letters are not significantly different, whereas those with different superscript letters are significantly different. *$p < 0.05$; **$p < 0.01$; and *** $p< 0.001$. ^{a} superscript letter indicates the significant differences between treatment groups and the control group; ^{b} superscript letter indicates the significant differences between treatment group no GIV and the GII treatment group.
GI: 1 mL of distilled water, GII: 40 mg/kg bw Gentamicin, GIII: 15 mg/kg bw testosterone, & GIV: 15 mg/kg bw Testosterone + 40 mg/kg bw Gentamicin.

3.2 Renal Function Tests (Urea and Creatinine)

Table 2 indicates a significant elevation in mean serum urea and creatinine levels following treatment with gentamicin (GII) and testosterone (GIII) compared to the control group. However, treatment

with testosterone followed by gentamicin (GIV) showed a significant increase in urea and creatinine levels compared to both GII and GIII.

Table 2: Effect of testosterone against the toxicity of gentamicin on serum levels of Urea and creatinine in male rabbits.

Groups	Urea mg\dl	Creatinine mg\dl
Control (GI)	22.40 ± 1.94	0.48 ± 0.08
Gentamicin-treated group (GII)	35.60 ± 5.59^{a**}	0.68 ± 0.10 ^{a*}
Testosterone-treated group (GIII)	28.00 ± 2.54^{a*}	0.56 ± 0.11 ^{a*}
Testosterone + Gentamicin treated group (GIV)	42.00 ± 7.68^{b***}	0.82 ± 0.13 ^{b**}

Data presented Mean ± SD values in each column were compared by one-way ANOVA followed by Post hoc multiple range test. Values with the same superscript letters are not significantly different, whereas those with different superscript letters are significantly different. *p < 0.05; **p < 0.01; and *** p< 0.001. ^{a}superscript letter indicates the significant differences between treatment groups and the control group, ^{b}superscript letter indicates the significant differences between treatment group no GIV and the GII treatment group.
GI: 1 mL distilled water, GII: 40 mg/kg bw Gentamicin, GIII: 15 mg /kg bw Testosterone, & GIV: 15 mg/kg bw Testosterone + 40 mg/kg bw Gentamicin.

3.3 Liver Histology:

Histological examination of the liver in the control group revealed a normal architecture with a normal central vein, hepatocytes, and sinusoids (Figure 1A). gentamicin-treated rabbits (40 mg/kg bw) exhibited hydropic changes and congestion (Figure 1B). The testosterone-treated group (15 mg /kg bw) showed hydropic changes (Figure 1C). The liver of rabbits treated with testosterone followed by gentamicin exhibited hydropic changes, inflammatory cell infiltration, and congestion (Figure 1D).

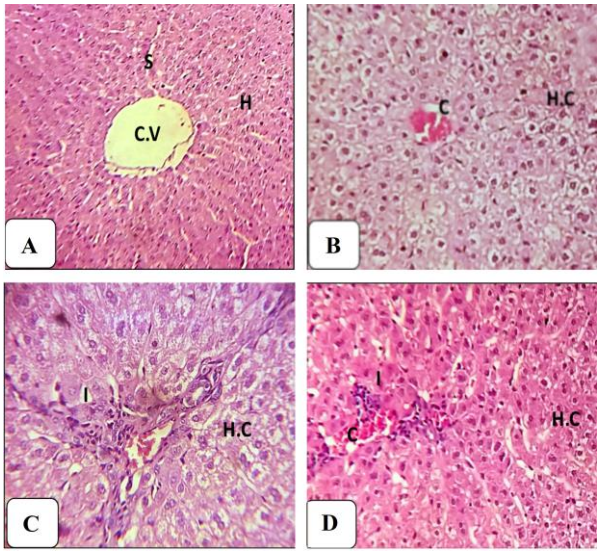

Figure 1: Photograph of rabbits liver sections exhibited **A:** liver section of control group **(GI)** reveal normal central vein (C.V), normal hepatocytes (H) and normal sinusoids (S), **B:** liver section of gentamicin treated rabbits **(GII)** shows hydropic changes (H.C) and congestion (C), **C:** liver section of testosterone treated rabbits **(GIII)** shows hydropic changes (HC) **D:** liver section of testosterone and gentamicin treated rabbits **(GIV)** shows hydropic changes (HC), inflammatory cells infiltration (I) and congestion (C). H & E (X 400).

3.4 Kidney Histology

The control group showed normal glomeruli and normal tubules (Figure 2A). Gentamicin-treated rabbits (GII) demonstrated tubular necrosis, glomerular degeneration, and tubular vacuolation (Figure 2B). The testosterone group (GIII) exhibited tubular necrosis and vacuolation (Figure 2C). The combination treatment group (GIV) showed glomerular degeneration, mild tubular necrosis, and vacuolation, indicating partial structural preservation (Figure 2D).

4. Discussion

Gentamicin administration in GII significantly elevated serum ALT, AST, urea, and creatinine levels compared to the control group, reflecting marked hepatic and renal dysfunction. It is well-known that gentamicin induces renal tubular necrosis, elevates plasma creatinine, and increases blood urea nitrogen, primarily via the generation of reactive oxygen species (ROS) and subsequent oxidative stress, which corresponds with previous findings that associate gentamicin toxicity with oxidative stress, lipid peroxidation, and mitochondrial impairment [2, 3, 26]. Besides, the biochemical disruptions align with previous studies demonstrating the hormone-dependent susceptibility of renal tissue to nephrotoxic insults[6]. Gentamicin nephrotoxicity is mainly attributed to its accumulation within proximal tubular cells via megalin- and cubilin-mediated endocytosis, which disrupts mitochondrial integrity and triggers apoptotic signaling pathways [4, 6]. Similarly, its hepatotoxicity has been linked to oxidative injury, membrane instability, and inflammatory responses within hepatocytes, which might explain the significant rise in serum transaminases observed in the current study. The gentamicin group has been reported to show hepatocellular hydropic degeneration and sinusoidal congestion. Also, the findings of the present study corroborate previous studies that reported structural disorganization due to aminoglycoside-induced oxidative damage [7, 8].

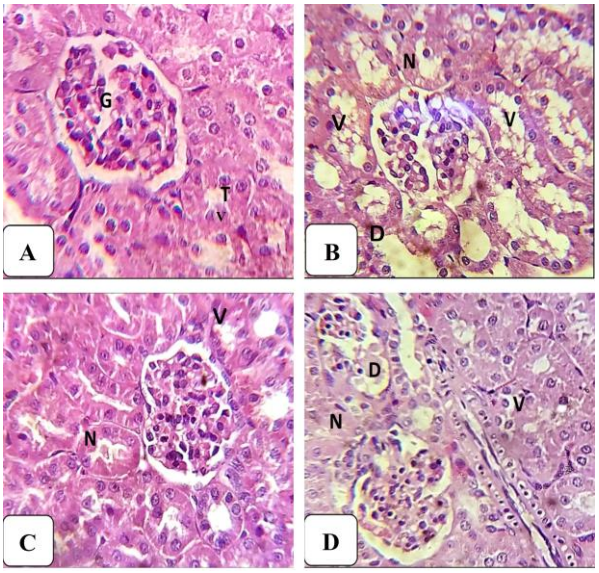

Figure 2: Photograph of rabbits kidney's cross sections exhibit **A**: kidney's cross section of control group **(GI)** shows normal glomerulus (G) and normal tubules (T), **B**: kidney's cross section of gentamicin treated rabbits **(GII)** shows tubular necrosis (N), glomerular degeneration (D) and tubular vacuolation (V) **C**: kidney's section of testosterone treated rabbits **(GIII)** shows tubular necrosis (N) and vacuolization (V), **D**: kidney's cross section of testosterone and gentamicin treated rabbits **(GIV)** shows glomerular degeneration (D), tubular necrosis (N) and tubular vacuolation (V). H & E (X 400).

In testosterone-treated rabbits (GIII), slight elevations in ALT, AST, urea, and creatinine were observed compared with the control group, suggesting mild metabolic stress. Some studies suggested that higher doses of testosterone might induce oxidative and metabolic stress on these organs, causing renal and hepatic damage [17, 19]. However, the changes in the present study were not severe and indicate that testosterone at the administered dose does not cause marked toxicity. Histological examination in testosterone-treated rabbits revealed preserved renal tubular architecture and reduced hepatocyte degeneration compared to the gentamicin treatment group, confirming the absence of major tissue damage.

In this study, when testosterone was administered prior to gentamicin (GIV), biochemical and histopathological findings revealed a mixed response. Hepatic enzyme levels (ALT and AST) were significantly lower than those in the gentamicin-treated group (GII), indicating a partial hepatoprotective effect of testosterone. This effect might be mediated by its antioxidant properties, which enhance the activity of enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), thereby reducing lipid peroxidation and cellular injury in the liver [5]. Histologically, liver sections in GIV showed reduced hepatocellular degeneration, less inflammatory infiltration, and better preservation of lobular architecture compared to GII. Regarding hepatic protection, testosterone has been reported to attenuate ALT and AST elevation, reduce oxidative damage, and preserve hepatocellular architecture in toxic injury models. These effects were attributed partly to its antioxidant properties and its ability to stabilize mitochondrial function and limit ROS generation

[27]. However, the inability of testosterone to effectively protect hepatic and renal tissues against gentamicin-induced toxicity in the present study might be attributed to several interrelated mechanisms. Gentamicin generates intense oxidative stress that markedly suppresses key antioxidant enzymes (GPx, CAT, and SOD), exceeding the compensatory antioxidant potential of testosterone. Moreover, testosterone has been reported to upregulate megalin expression in proximal tubular cells, thereby enhancing gentamicin uptake and aggravating nephrotoxicity [4]. In addition, differences in tissue-specific receptor expression and metabolic capacity may explain the organ-dependent response observed; the liver, with its higher regenerative and detoxifying capacity, exhibited partial protection, whereas the kidney remained highly vulnerable to oxidative and mitochondrial injury. Hence, the overall protective action of testosterone appears insufficient under the current experimental conditions.

In renal tissues, testosterone pre-treatment failed to confer protection and even appeared to aggravate kidney injury. Urea and creatinine levels in GIV were significantly higher than those in both GII and GIII, indicating incomplete or insufficient nephroprotection. This observation might be due to testosterone-induced upregulation of megalin receptors in proximal tubular cells, which could facilitate greater gentamicin uptake and thereby enhance its nephrotoxic potential [4]. Besides, a recent study by Althunibat *et al.* [26] reported that gentamicin accumulates preferentially in the proximal renal tubules, where it disrupts mitochondrial integrity, enhances lipid peroxidation, and suppresses the endogenous antioxidant system, particularly GPx, CAT, and SOD, leading *to severe* marked architectural distortion of the renal tissue. On the other hand, our findings disagreed with those of Sekula *et al.* [6], Soljacic *et al.* [28], and Patil *et al.* [29], who reported that testosterone showed variable protective effects against chemically induced renal and liver injury through enhancement of endogenous antioxidant capacity. Thus, this might be due to the fact that testosterone failed to prevent or alleviate gentamicin's nephrotoxicity, suggesting that the severity of oxidative stress and the marked suppression of key antioxidant enzymes exceeded its protective capacity. Additionally, the antioxidant capacity of the administered testosterone dose might not have been sufficient to counteract the high oxidative burden induced by gentamicin. These findings may be due to a complex interaction between testosterone and gentamicin, in which testosterone exerts beneficial effects on hepatic tissues but fails to provide adequate renal protection at the current dosage. Protective action appears to be dose-dependent and organ-specific, reflecting differences in receptor expression, metabolic capacity, and oxidative vulnerability between the liver and kidney.

5. Conclusion

This study demonstrates that gentamicin induces significant hepatic and renal toxicity in adult male rabbits. Testosterone administration provided partial protection against gentamicin-induced liver injury, as evidenced by reduced serum transaminase levels and improved histological features. However, testosterone did not prevent renal dysfunction or structural kidney damage. These findings suggest that testosterone exerts organ-specific effects and may offer limited hepatoprotection without conferring comparable renal benefits. Further studies are needed to explore optimal dosing, treatment duration, and underlying mechanisms to define better the therapeutic potential and limitations of testosterone in drug-induced toxicity.

Ethical Approval

The study protocol was approved by the Animal Ethics Committee of the Department of Biological Sciences, Faculty of Science, Sana'a University (ethical code: BAHSS101).

Data Availability

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions:

FSM: Provided supervision, critical revision, and expert advice throughout the study, contributed to data curation and formal analysis, and drafted the first version of the manuscript. **NMAH**: Verified the data, contributed significant manuscript revision, and finalized the manuscript. **EASA** and **FAMQ** reviewed the draft of the manuscript for publication. **MAAQ, AA-S, AA-K, EA-K,** and **OA-A:** carried out experimental work, collected, analyzed the data, and performed data curation, formal analysis, and biochemical findings interpretation.

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