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Gender-Based Differences in Digoxin Induced Toxicity: A Comprehensive Evaluation of Biochemical Parameters and Gonadal Tissue Histopathology in Rats

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Abstract

Introduction: Although for centuries digoxin was widely used for the treatment of variable heart problems and is still used, it is important to evaluate its effects on reproductive organs.

Aim: The present study is designed to compare the effects of this medication in male and female rats.

Materials and Methods: The study included four groups as follows: 3 groups treated with different doses of digoxin solution for 90 days and the 4th group as control.

Results: The results revealed significant changes in liver enzymes and urea levels in male rats. On the other hand, there were no significant differences in serum troponin and creatinine levels between male and female rats. Histopathological changes showed varying degrees of suppression of spermatogenesis along with emptying of epididymis tubules, while the ovaries revealed small non-active corpora lutea without Graafian follicles in comparison to control groups.

Conclusion: The study conclude that male rats appear to be affected more than female rats and digoxin hurts biochemical markers as well as gonadal tissue on both genders.

Keywords: Digoxin, Troponin, Biochemical parameters, Gonadal histopathology.

1. Introduction

Digoxin is a commonly prescribed medication for the treatment of various cardiovascular conditions (1). One area of concern is the impact of digoxin on gonadal tissue, which plays a crucial role in reproductive function and overall health. Digoxin toxicity occurs because the differences between therapeutic dose and toxic dose is very small through blocking the sodium/potassium ATPase (NKA) pump in cardiac tissue and other organs (2). Some factors that are associated with increased digoxin toxicity the gender, age, and disease state, in which several studies mentioned that male gender experiences more toxicity than females (3, 4). In the case of digoxin toxicity, the gender differences in its impact on the reproductive system and fertility in rats have not been well documented. This study is designed to fill existing knowledge gaps regarding the effects of digoxin on gonadal tissue in male and female rats and to elucidate gender response to digoxin toxicity.

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Keywords: Digoxin, Gonadal Tissue, rats, heart problem

2. Materials and methods

2.1. Experimental Animals

Forty-eight adult male and female rats weighing 170 ± 40g, 12 weeks old were used in the study. The animals were kept for acclimatization for two weeks at the laboratory animal house, where they were housed as six rats in each cage, and the male rats were separated from female rats under standard conditions 12 hours light on, mechanical ventilation, ad libitum, tap water and the room temperature 24 ± 2^{o}C.

2.2. Digoxin Solution

Reference tablets of digoxin, England origin were obtained from private pharmacy 0.25mg (therapeutic dose) of digoxin. These tablets were dissolved in distilled water to prepare a digoxin solution.

2.3. Experiment design

The study included 24 male and 24 female rats which were divided into four groups; each group was composed of six rats of each gender that were dosed daily with oral digoxin solution for 90 days as follows:

High Dose Group: dosed 6mg /kg digoxin orally. Intermediate Dose Group: dosed 3mg /kg digoxin orally. Low Dose Group: dosed 1.5mg /kg digoxin orally. Control Group: treated with distilled water.

2.4. Collection of blood samples

On day 90th of the experiments, all animals were sacrificed and blood samples were collected from vena cava, then serum was obtained from blood samples by centrifugation at 5000 rpm for 15 minutes and stored in an Eppendorf tube at -20^{O}C for laboratory analysis.

2.5. Biochemical parameters measurements

Serum troponin concentration was measured using Ichroma^{TM} Tn-I kit (Boditech Med Incorporated) by fluorescence immunoassay (5). Liver function tests including AST and ALT activity and kidney function tests involving urea and creatinine concentrations were measured by U.V assay according to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) in an automatic analyzer ACCENT-200 II GEN (6).

2.6. Gonadal tissue histopathology

Gonadal tissue including testes and ovary was collected from all sacrificed animals and preserved in 10% formalin for histopathological evaluation (7).

2.7. Statistical analysis

Data were expressed as mean ± standard deviation and analyzed statistically using the Microsoft Program SPSS version 11. Specific group differences were determined using least significant differences (LSD).

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3. Results

3.1. Biochemical parameters

The biochemical parameters were found different within the group between male and female rats including a significant (P<0.05) increase in serum urea, AST and ALT enzyme levels in male rats in comparison to female rats. However, other parameters involving troponin and creatinine showed no significant (P>0.05) differences between male and female rats as shown in the table.

Table Shows the effects of digoxin on biochemical parameters in male and female rats. (Mean ± SD).

Parameters	Male rats	Female rats	LSD
Troponin	1.29 ± 0.42 a	1.23 ± 0.34 a	0.18
AST	236.41 ± 73.4 a	205.20 ± 48.8 b	10.08
ALT	122.62 ± 56.1 a	79.91 ± 28.4 b	26.1
Urea	68.04 ± 14.7 a	57.62 ± 8.42 b	6.46
Creatinine	0.94 ± 0.16 a	0.86 ± 0.14 a	0.11

*Values expressed in the red small letters mean significant differences (P<0.05) level.

3.2. Histopathological changes of testes

The testes of the control group showed normal spermatogenesis in seminiferous tubules as in Figure (1). The treated groups showed marked suppression of spermatogenesis in seminiferous tubules and no spermatozoa were seen as in figures (2 & 3) associated with vacuolation of spermatogonia and primary spermatocytes as shown in figure (4). It is also noted that the epididymis tubules appeared to be empty as a result of suppression of spermatogenesis as in Figure (5).

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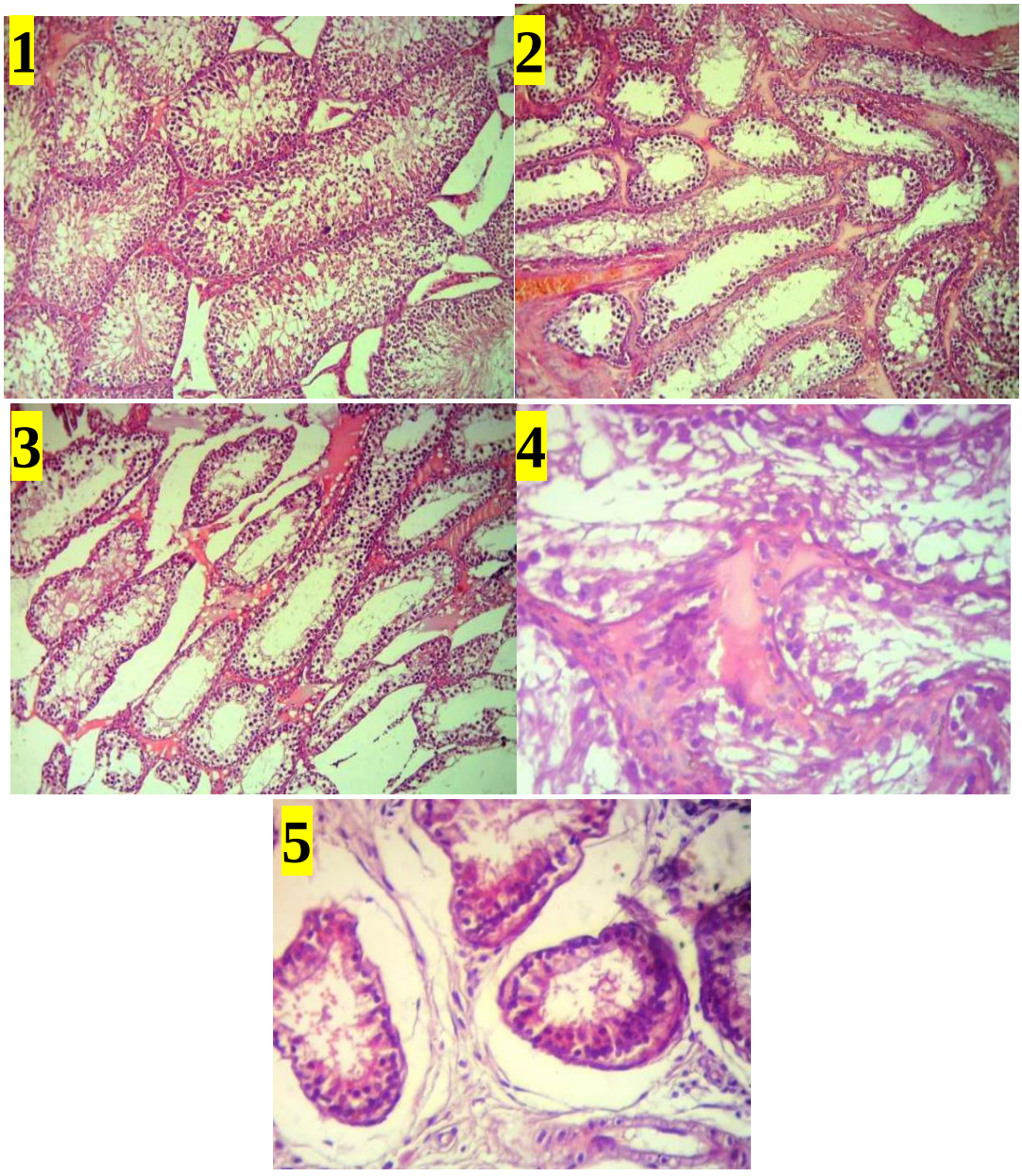

Figure (1): Testes of control rats showed normal spermatogenesis in seminiferous tubules. Figures (2 & 3): Testes of rats treated with 6mg/kg, 3mg/kg digoxin showed severe suppression of spermatogenesis in seminiferous tubules respectively. Figure (4): Testes of rat treated with 1.5mg/kg digoxin showed marked suppression of spermatogenesis associated with vacuolation of spermatogonia and primary spermatocyte. Figure (5): Testes of rat treated with 3mg/kg digoxin showed empty tubules of epididymis associated with suppression of spermatogenesis. H&E stain 100X.

3.3. Histopathological changes of ovary:

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The ovary of control rats showed large corpora lutea and dilated secondary follicles, Graafian follicle and normal structure as in figure (6 & 7). All treated groups show small nonactive corpora lutea and secondary follicles only without graafian follicles as in Figure (8, 9 & 10).

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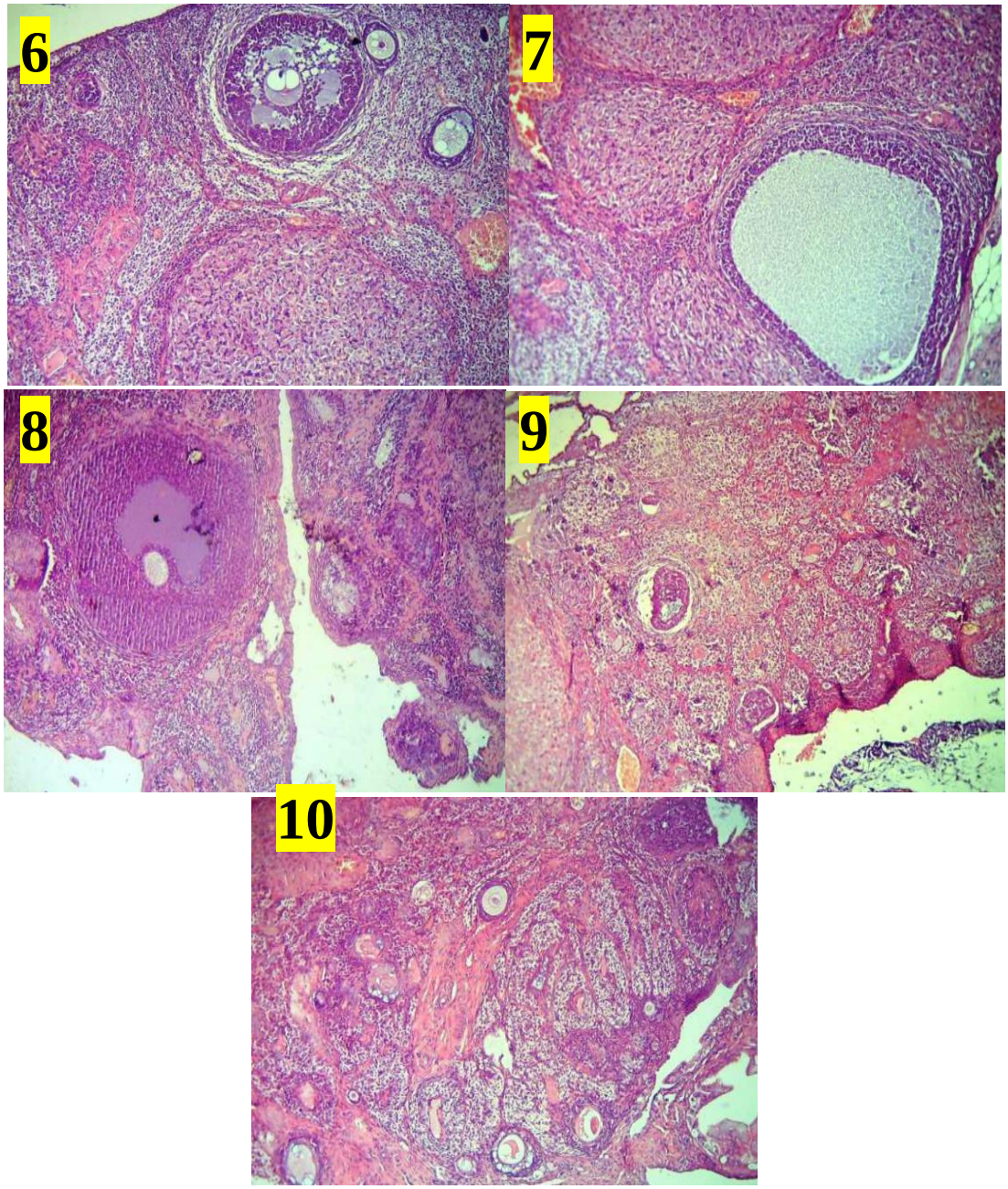

Figure (6 & 7): Ovary of control rat showed large corpora lutea and dilated secondary follicles. Figure (8, 9 & 10): Ovary of rat treated with 6mg/kg, 3mg/kg and 1.5mg/kg digoxin showed corpora lutea and secondary follicles. H&E stain

4. **Discussion**

The current study showed that there was a significant increase in serum urea, AST and ALT level in male when compared to female rats, this indicates that different doses of

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digoxin have more toxic effect on male rat in comparison with female rat by affecting liver and kidney functions. This result is in line with (8, 9) who found that patient with digoxin concentration out of therapeutic level was almost male, this may be related to the changes in activity of P-glycoprotein in which digoxin is a substrate leading to their high digitalis bioavailability and more toxicity (10). Similarly, digoxin administration in albino rats causes an elevation of all biochemical parameters studied along with a significant reduction in total protein, albumin and globulin protein suggesting liver damage (11).

Whitbeck *et al*., (12) reported that there was no gender interaction with digoxin therapy, in which both men and women experienced significantly increased overall mortality with digoxin use.

On the other hand, the present study didn't show any significant differences in serum troponin and creatinine levels between male and female rats within the groups. this may be related to those parameters requiring a longer duration of treatment with digoxin to display changes between male and female rats. This result does not match with (13) who reported that male rats had a 10-fold greater serum cardiac troponin than age-matched female rats. Testicular tissue appeared to be severely affected by high dose digoxin in which the treated groups showed marked suppression of spermatogenesis in seminiferous tubules associated with vacuolation of spermatogonia and primary spermatocytes. Therefore, no spermatozoa were observed in seminiferous tubules and the epididymal tubules appeared to be empty. This result may be related to the toxic effect of high digoxin dose on testicular tissue and may be a result of the oxidative stress that led to tissue injury; digoxin act directly on testes to decrease testosterone production by inhibiting of cAMP production and cytochrome P450scc activity which considered the rate-limiting enzyme of the pathway leading to testosterone biosynthesis (1).

Blanco *et al*., (14) found that blockage of Na-K/ATPase α4 isoform that is present in male gonadal tissues, especially in spermatozoa which is essential for ionic homeostasis of the germ cells of the testes leads to impairment of spermatogenesis, germ cells development and interfere with male fertility (15). These findings support the histopathological changes of testes in this study associated with digoxin treatment.

Several studies have shown that digoxin hurts sexual hormones and causes an increase in serum estrogen along with a reduction in serum luteinizing and testosterone hormone levels; these changes can cause atrophy of Leydig cells and impairment of it is development with suppression of testosterone production (16). However, other studies report the impact of digoxin on sperm motility that harms fertilizing capacity (17).

The present study observed that all treated groups showed small nonactive secondary follicles and corpora lutea only without any Graafian follicles, which may be related to

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either ovarian tissue defect or hormonal deficiency (18). This result may be related to the toxic doses of digoxin in the present study which may disturb ovarian tissue germ cells for which there was neither a primary follicle seen nor a secondary follicle and Graafian follicle developed.

5. Conclusion

It has been noted that digoxin may hurt fertility by affecting both gonadal tissues of laboratory rats and this may be related to it is effect either on sexual hormonal level or blocking of NKA channel that presents in germinal cells of gonads thus affecting ionic homeostasis of testes and ovary tissues, on the other hands, the biochemical parameters also affected by digoxin administration in both genders but the male rats being with more prominent negative effect than female rats.

Conflict of Interest.

None declared.

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