TOXICOPATHOLOGICAL AND BIOCHEMICAL STUDY OF MULTIPLE DOSES DIGOXIN IN SPRAGUE DAWLEY RATS

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(Received 1 October 2017 ,Accepted 21 November 2017)

Key words: MTD, Digoxin,Glomeruli.
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ABSTRACT

The current study was conducted at the college of veterinary medicine, university of basrah in the periods extended from 22/10/2016 to 22/1/2017. The present study is designed to report the toxic effects of digoxin on kidney histologically and biochemically by measuring kidney parameters urea and creatinine level in the serum. Maximum toxic dose determined by using 2 rats dosed orally until clinical signs of toxicity became prominent at 30mg for each rat and considered as MTD. The chronic toxicity study was carried out on 48 adult rats divided into 4 groups. Control (G1) receive distilled water, Low dose (G2) dosed with 1/20 MTD(1.5mg/kg) digoxin, Intermediate dose (G3) dosed with 1/10 MTD(3mg/kg) digoxin and High dose (G4) dosed with 1/5 MTD(6mg/kg) digoxin by oral gavage for 90 days. At the end of experiments all animals were sacrificed and blood sample were collected for estimation of biochemical parameters of rat. Result reveals histopathological changes presented as dilation/vaccuolation of renal tubules and atrophy of glomeruli. There is a significant ($P \leq 0.05$) increase in serum urea and creatinine level in high dose (G4) group when compared with other study group. We conclude that high dose digoxin has toxic effect on renal tissue and lead to their damage.

INTRODUCTION

Cardiac glycosides are an important cause of poisoning, toxicity can occur during long-term treatment as well as after an overdose. Digoxin is the most common cardio

tonic medications still in use around the world (1). Digoxin is a purified cardiac glycoside extracted from the leaves of the foxglove plant (*Digitalis purpurea*) (2). Digoxin increases intracellular calcium in myocardial cells indirectly, by inhibiting the sodium–potassium pump in the cell membrane. Increased intracellular calcium increases cardiac contractility (3,4). Because digoxin elimination is mainly by renal clearance and is prolonged in patients with renal impairment (4). Therefore, our study is designed to determine the toxic effects of long term use, high dose administration of digoxin on kidney and their parameters.

MATERIALS AND METHODS

Fourty-eight adult rats (24 male and 24 female rats) weighing (170 ± 40g) were used in the study and divided into 4 groups including, High dose (G4), Intermediate dose (G3), Low dose (G2) and Control (G1) group. Each group consists of 12 rats (6male and 6 female rats) and dosed with digoxin as follows, High dose group receive (6mg/kg) digoxin body weight of rat, that is 5% MTD (5), Intermediate dose group receive (3mg/kg) digoxin body weight of rat, that is 1/2 High dose, Low dose group receive (1.5mg/kg) digoxin body weight of rats, that is 1/2 Intermediate dose and the Control group receive distilled water. After 90 days study, all animals were sacrificed and blood sample were collected and centrifuged at 3000rpm for 15min. and the serum collected in eppendorf tube and stored at -20C^{o} for laboratory analysis of serum urea and creatinine level detection.

Measurement of serum urea and creatinine level:

The urea level was measured by ultra violet assay and the creatinine level was measured by UV assay (Modified Jaffe' method) through the reagents used in automatic analyzer (Mindray) ACCENT-200 and ACCENT-200 II GEN (6).

Statistical analysis

Data were expressed as mean ± standard deviation and analyzed statistically using the Microsoft Program SPSS version 11. Statistical analysis of data was performed on the basis of Two-Way Analysis of Variance (ANOVA) using a significant level of ($P<0.05$). Specific group differences were determined using least significant differences (LSD).

RESULTS

Histopathological study

The kidney of control rat show normal sub capsular cortical tubules, normal glomeruli and tubules in inner cortex as showed in figure (1). All treated groups of the study show several kidney changes including dilation/ vaccuolation of sub capsular cortical tubules as in figure (2) and dilation/ vaccuolation of tubules of inner cortex in figure (3). The glomeruli some time appear to be atrophied and in other field show vacculation of mesangial cells as in figure (2&4). Finally, there is peri renal adipose tissue, congestion and focus of mononuclear cells present showed in figure (5&6). These changes were more severe in high dose group than other groups.

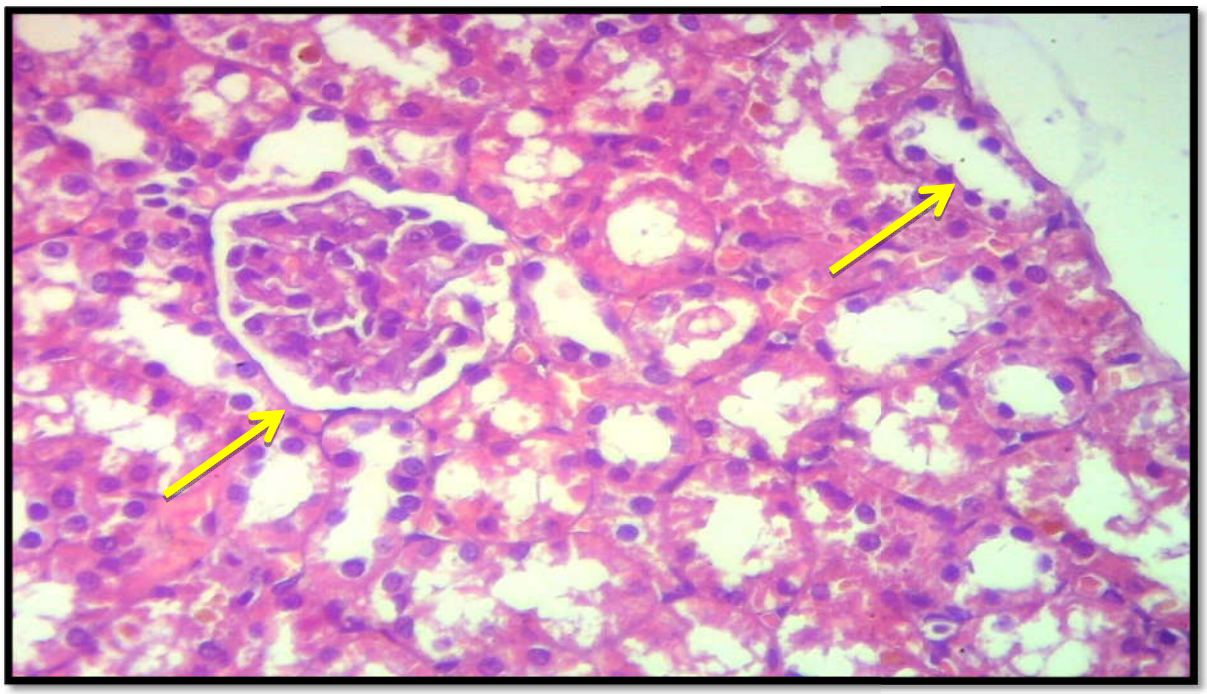

Figure (1): Kidney tissue of rat untreated control shows normal sub capsular cortical tubules and normal glomeruli. H&E stain 40X.

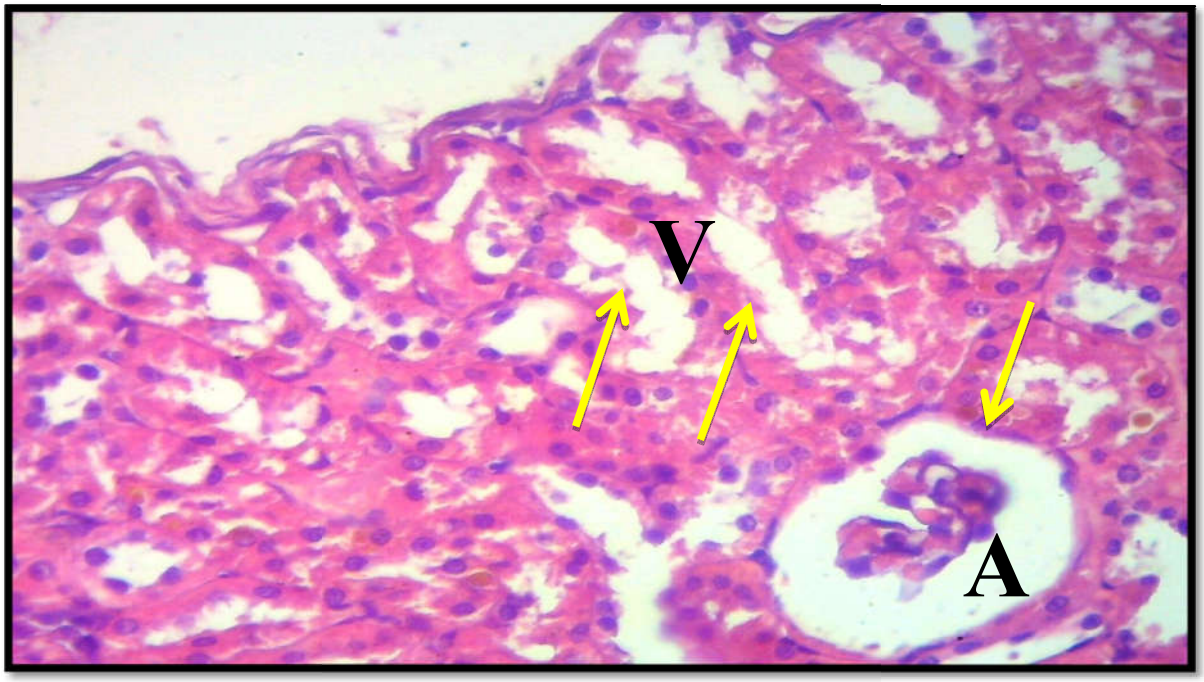

Figure (2): Kidney tissue of rat treated digoxin shows dilated/vacuolated sub capsular cortical tubules (V) and atrophic glomerulus (A). H&E stain 40X.

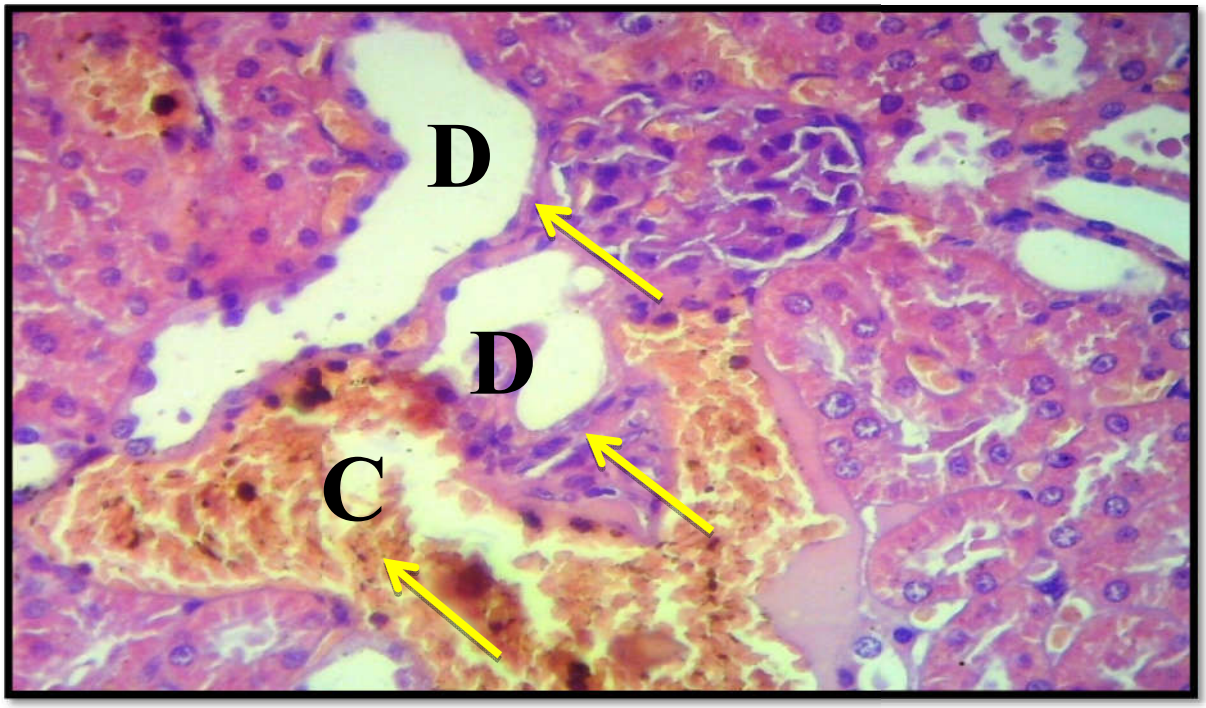

Figure (3): Kidney tissue of rat treated with digoxin shows dilation of inner cotical tubules (D) and renal congestion (C). H&E stain 40X.

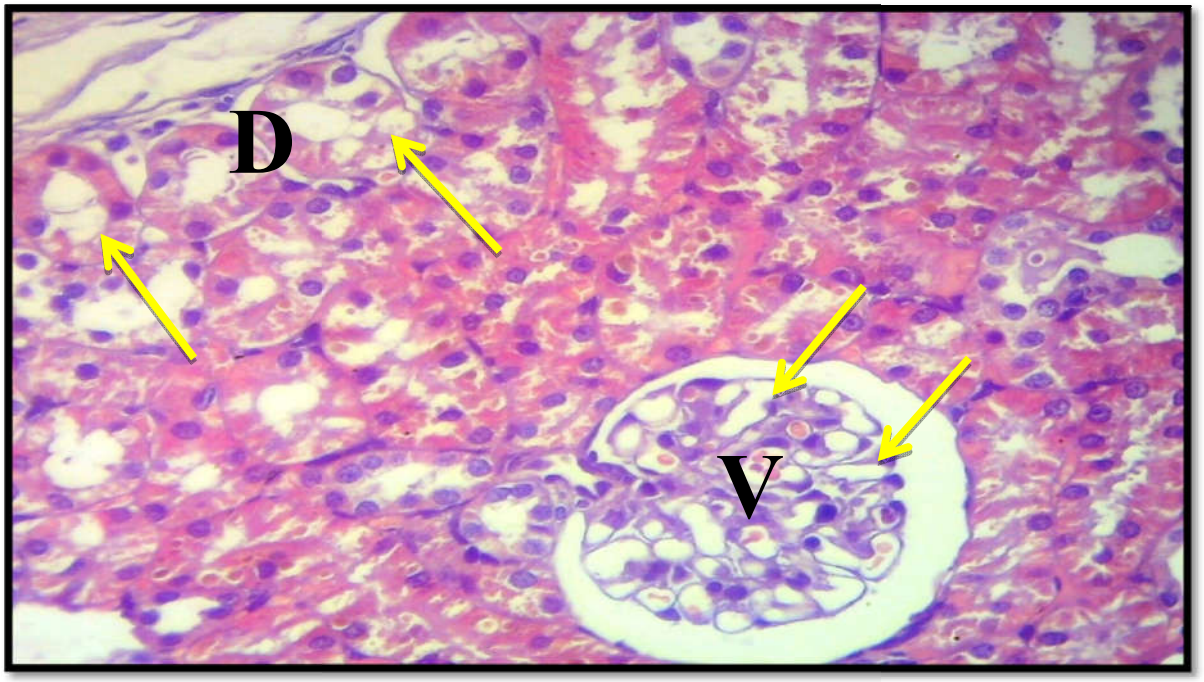

Figure (4): Kidney tissue of rat treated with digoxin shows dilated sub capsularcortical tubules (D) and vacuolation of mesangial cells of glomeruli (V). H&E stain 40X.

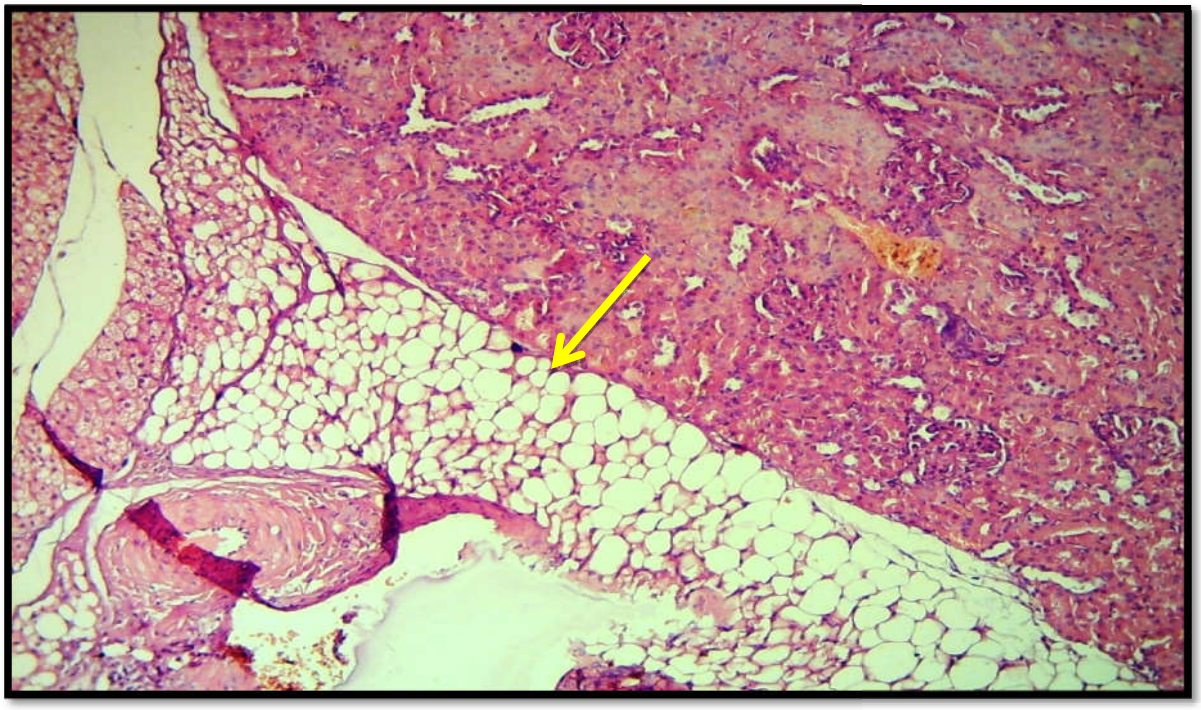

Figure (5): Kidney tissue of rat treated with digoxin shows peri renal adipose tissue. H&E stain 10X.

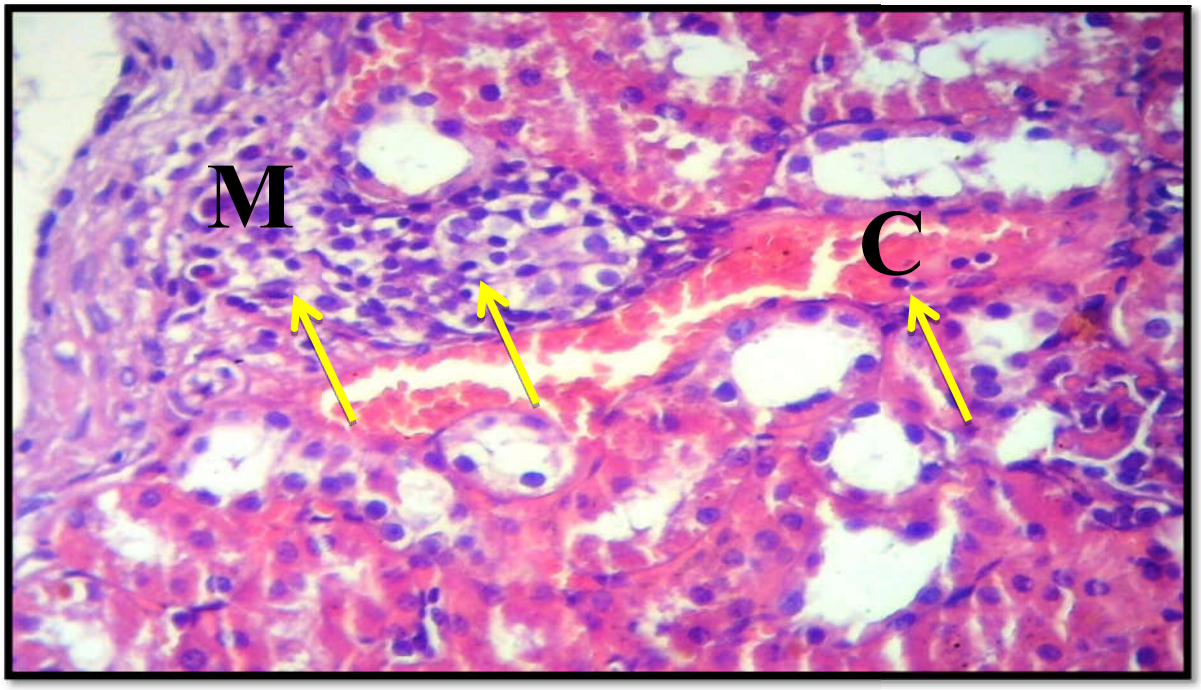

Figure (6): Kidney tissue of rat treated with digoxin shows focus of mononuclear cells (M) and renal congestion (C). H&E stain 40X.

Effect of digoxin on serum urea and creatinine level

The biochemical results showed a significant (P≤0.05) increase in serum urea level of High group (G4) when compared with other study groups. As well as, Intermediate (G3) showed a significant (P≤0.05) increase of serum urea level in comparison with Low (G2) and Control (G1), while no significant differences between Low and Control group. On the other hand the table also shows a significant (P≤0.05) increase in serum creatinine level in High group when compared with other study groups. Intermediate and Low groups showed a significant (P≤0.05) increase in creatinine level when compared with Control group, while on significant differences between Intermediate and Low groups in creatinine level.

Table1: Shows the effect of digoxin on serum urea and creatinine level. N = 12 (Mean ± SD).

Parameter / Groups	Urea	Creatinine
Control (G1)	52.75 ± 5.27 c	0.78 ± 0.10 c
Low (G2)	56.16 ± 7.62 c	0.88 ± 0.09 b
Intermediate (G3)	65.33 ± 10.4 b	0.89 ± 0.11 b
High (G4)	77.08 ± 11.7 a	1.05 ± 0.17 a
LSD	9.16	0.10

Values expressed in the small letters mean significant differences at (P≤0.05) level.

DISCUSSION

The elimination of digoxin metabolites from the body occur primarily in the kidney through renal tubules (4). High dose of digoxin will cause severe damage to renal tubules and effect on their function because the elimination of digoxin is proportional to the glomerular filtration rate and thus to creatinine clearance (7).

The present study shows several changes related to the renal tubules in the inner cortex, in sub capsular region associated with dilation and vaccuolation of tubular cells as in figure (2&3) this result is corresponding with (8) and (9) they report that administration of digoxin like oleander glycoside associated with acute cellular degeneration and swelling of renal tubules especially with proximal tubules this may be due to the toxic effect of high dose digoxin on renal tubules lead to their dilation, vaccuolation and with times to degeneration and necrosis of tubules this is in line with

(10) and (11) they report renal tubular necrosis with digoxin like oleander glycoside toxicity.

The toxic effect of digoxin does not effect on renal tubules only but the toxicity associated with glomerular damage ranging from atrophy as presented in figure (2) to vaccuolation of mesangial cells of glomeruli as in figure (4) this result is in line with (8) who report dilation of bowman capsule and shrinkage of glomeruli as a direct toxic effect of oleanderin glycoside this may be due to damage to the mesangial cells of glomeruli lead to their atrophy and shrinkage as presented in this study.

Other renal changes as presented in figures (3,5&6) shows renal congestion, peri renal adipose tissue formation and also show aggregation of mononuclear cells in renal tissue respectively, these changes are matched with (12) who report with high dose of digoxin like oleander glycoside use has toxic effect on renal tubules presented as coagulative necrosis and interstitial nephritis associated with hyperemia and mononuclear cell infiltration this may be due to the inflammation of damaged renal tubules caused by the toxicity of digoxin on renal tissue.

The present study show a significant ($P \leq 0.05$) increase in serum urea and creatinine levels of high (G4) when compared with other study groups, this result is in line with (13) who report that toxicity of digoxin associated with increase in serum level of urea and creatinine as a result of damage that occur in renal tissue by high dose digoxin and that proved in this study histologically as in figure (2,3 and 4).

The study also show a significant ($P \leq 0.05$) increase in serum urea level of (G3) when compared with (G2 and G1). As well as, there is a significant ($P \leq 0.05$) increase in serum creatinine level of (G2) when compared with control (G1) this result is in agreement with (12) and (14) both report that elevation of serum urea and creatinine level associated with renal impairment and with increasing the dose of digoxin the damage will be more sever associated with higher levels of urea and creatinine in the serum, this result is in line with (8).

دراسة سمية مرضية و كيموحياتية لجرع مختلفة من الديجوكسين في جرذان Sprague Dawley

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الخلاصة

اجريت الدراسة الحالية في كلية الطب البيطري جامعة البصرة في الفترة الممتدة من ٢٠١٦/١٠/٢٢ الى ٢٠١٧/١/٢٢. صممت الدراسة الحالية لبحث التأثير السمي لعقار الديجوكسينعلى الكلية نسيجيا و كيموحياتيا بواسطة قياس مستوى كل من اليوريا و الكرياتينين في مصل الجرذان المختبرية. الجرعة السمية العالية لعلاج الديجوكسين تم تحديدها باستخدام جرذين مختبريين وتجريعهما عن طريق الفم لمحلول الديجوكسينابتداءا من 1ملغم لحد ٣٠ ملغم وظهور التأثير السمي للديجوكسين وتم اعتبارها الجرعة السمية العالية.

شملت الدراسة ٤٨ جرذا مختبريا بالغا (٢٤جرذ ذكرا بالغا و ٢٤جرذ انثى باكرا) وقسمت الى اربعة مجاميع حيث شملت كل مجموعة ٦ جرذان من الذكور ومثلها من الاناث، وجرعت بجرع مختلفة من عقار الديجوكسين لمدة ٩٠ يوما وكما مبين ادناه:

G1 مجموعة السيطرة: عوملت بالماء المقطر خلال فترة التجربة.

G2مجموعة الجرعة القليلة: عوملت ب ١.٥ ملغ/كغم وزن الجسم من الديجوكسين.

G3مجموعة الجرعة المتوسطة: عوملت ب ٣ملغ/كغم وزن الجسم من الديجوكسين.

G4مجموعة الجرعة العالية: عوملت ب ٦ملغ/كغم وزن الجسم من الديجوكسين، تعادل ٥% من تركيز الجرعة السمية العالية.

أظهرت نتائج الفحص النسيجي للكليتين توسع وتفجي في بطانة النبيبات الكلوية مع ضمورالكبيبةالكلوية و كما بينت أيضا ارتفاع ($P\leq0.05$) معنوي في مستوى كل من اليوريا و الكرياتينين في مصل الجرذان المختبرية المعاملة بالجرعة العالية من الديجوكسين مقارنة مع المجاميع الاخرى ومجموعة السيطرة. نستنتج من ذلك أن الجرعة العالية من عقار الديجوكسين لها تأثير سمي على نسيج الكلية ويؤدي الى تلفها.

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