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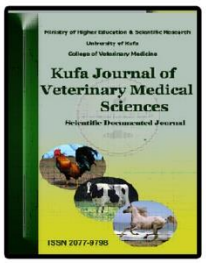

Study the toxicity of digoxin on liver and their transaminase enzymes level in rat

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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. Digoxin is an important cause of poisoning. It is prescribed and widely used to patients with heart failure. Digoxin toxicity can emerge during long-term therapy as well as after an overdose and because it is metabolized and eliminated by liver. Therefore, the study is designed to determine the toxic effects of digoxin on liver histologically and biochemically by measuring liver transaminase enzymes 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 (1.5mg/kg), Intermediate dose (G3) dosed with (3mg/kg) and High dose (G4) dosed with (6mg/kg) of 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 parameter of rat. Result reveals histopathological changes as sever periportal fibrosis, bile duct proliferation and aggregation of mononuclear cells specially with high (G4) group. There is a significant ($P \leq 0.05$) increase in serum AST & ALT level in high (G4) group when compared with other study groups. We conclude that high dose digoxin has a toxic effect on liver tissue.

دراسة سمية الديجوكسين على الكبد و مستوى انزيماته الناقلة للأمين في الجرذان

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الخلاصة:

اجريت الدراسة الحالية في كلية الطب البيطري جامعة البصرة في الفترة الممتدة من 2016/10/22 الى 2017/1/22. الديجوكسين هو سبب مهم للتسمم. وهو يوصف ويستخدم على نطاق واسع للمرضى الذين يعانون من قصور القلب. يمكن أن تظهر السمية للديجوكسين خلال العلاج على المدى الطويل وكذلك بعد جرعة زائدة ولأنه يطرح من الجسم بواسطة الكبد. لذلك، تم تصميم الدراسة للإبلاغ عن الآثار السامة للديجوكسين على الكبد نسيجيا وكيميائيا بيولوجيا عن طريق قياس مستوى انزيمات الكبد الناقلة للأمين في المصل. الجرعة السمية العالية لعلاج الديجوكسين تم تحديدها باستخدام جرذين مختبريين

وتجريعهما عن طريق الفم لمحلول الديجوكسين ابتداءا من 1ملغم لحد 30 ملغم وظهور التأثير السمي للديجوكسين وتم اعتبارها الجرعة السمية العالية.
شملت الدراسة 48 جرذا مختبريا بالغا (24جرذ ذكرا بالغا و 24جرذ انثى باكرا) وقسمت الى اربعة مجاميع حيث شملت كل مجموعة 6 جرذان من الذكور ومثلها من الاناث, وجرعت بجرع مختلفة من عقار الديجوكسين لمدة 90 يوما وكما مبين ادناه:
G1 مجموعة السيطرة: عوملت بالماء المقطر خلال فترة التجربة.
G2 مجموعة الجرعة القليلة: عوملت ب 1.5 ملغ/كغم وزن الجسم من الديجوكسين.
G3 مجموعة الجرعة المتوسطة: عوملت ب 3ملغ/كغم وزن الجسم من الديجوكسين.
G4 مجموعة الجرعة العالية: عوملت ب 6ملغ/كغم وزن الجسم من الديجوكسين, تعادل 5% من تركيز الجرعة السمية العالية.
اظهرت نتائج الفحص النسيجي للكبد انحلال وتليف في المنطقة الفصية الكبدية القريبة من الوريد المركزي, تكاثر القناة الصفراوية وتجمع الخلايا الالتهابية كما بينت ايضا ارتفاع ($P\leq0.05$) معنوي في مستوى كل من انزيم AST وALT في مصل الجرذان المختبرية المعاملة بالجرعة العالية من الديجوكسين مقارنة مع المجاميع الاخرى ومجموعة السيطرة. نستنتج من ذلك ان الجرعة العالية من عقار الديجوكسين لها تأثير سمي على نسيج الكبد

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 is metabolized, eliminated by hepatocytes and has narrow therapeutic range (**5**). Therefore, our study is designed to determine the toxic effects of long term use, high dose administration of digoxin on liver and transaminase enzymes.

Materials and Methods

Forty-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 (**6**), 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 transaminase (AST & ALT) enzymes level detection.

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) activity were measured by U.V assay according to International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) without pyridoxal phosphate activation, through using reagents in automatic analyzer (Mindray) ACCENT-200 and ACCENT-200 II GEN (**7**).

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 liver of control rats show normal architecture of hepatocytes as in figure (1).

All treated groups show different histopathological changes and the severity differ among groups according to the dose of digoxin administered, ranging from minimal periportal fibrosis as in figure (2) to severe periportal fibrosis and septal fibrosis as in figure (3). The hepatocytes associated with vaccuolation, centrilobular enlargement and dilation of liver sinusoid as in figures (4,5). The liver tissue also showed bile duct proliferation, congestion of portal vein and aggregation of mononuclear cells showed in figure (6).

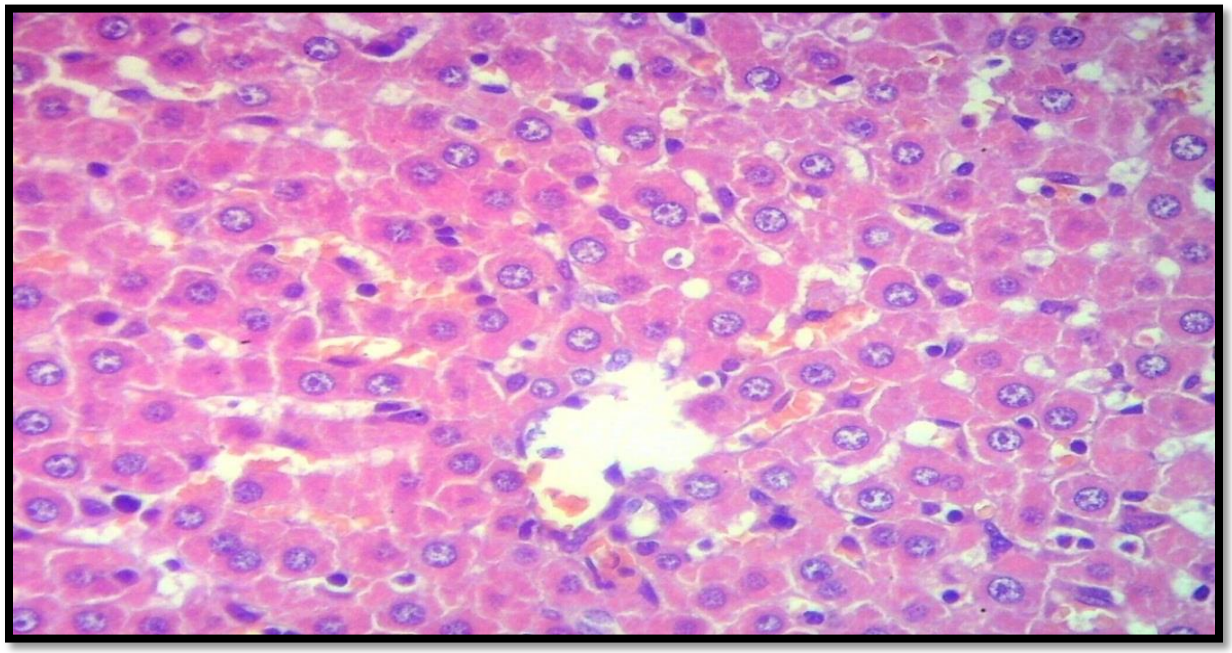

Figure (1): Liver tissue of rat untreated control shows normal hepatocytes. H&E stain 400X.

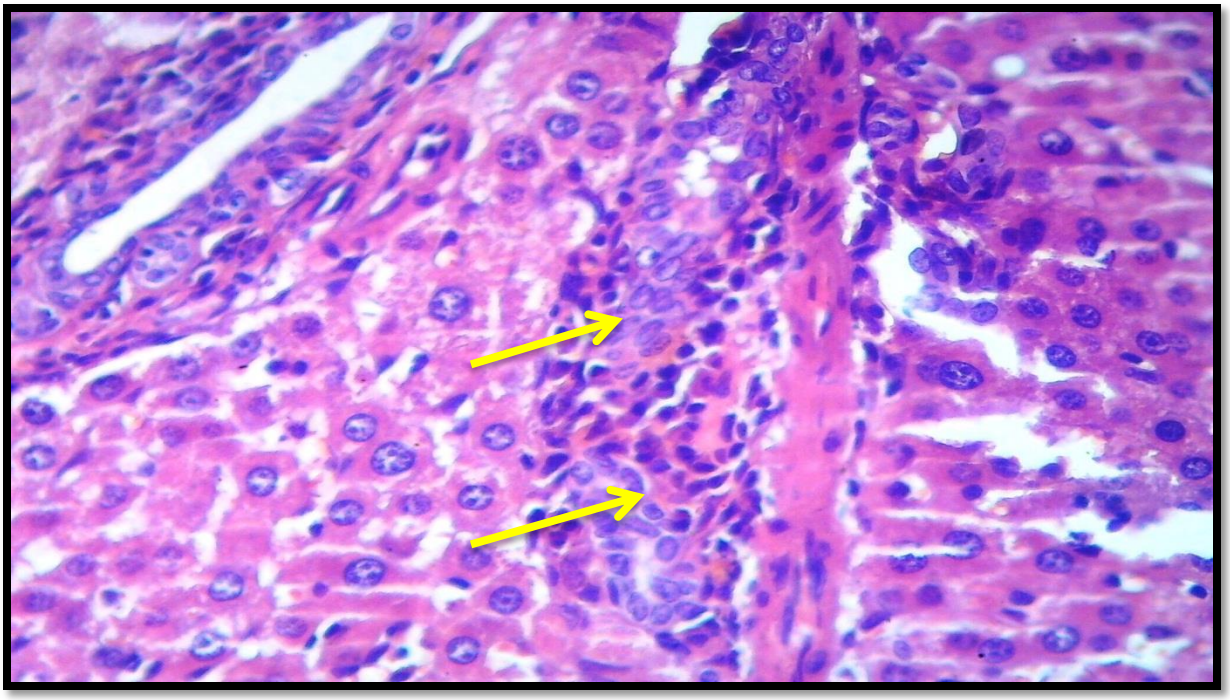

Figure (2): Liver tissue of rat treated with digoxin shows periportal fibrosis and aggregate of mononuclear cells. H&E stain 400X.

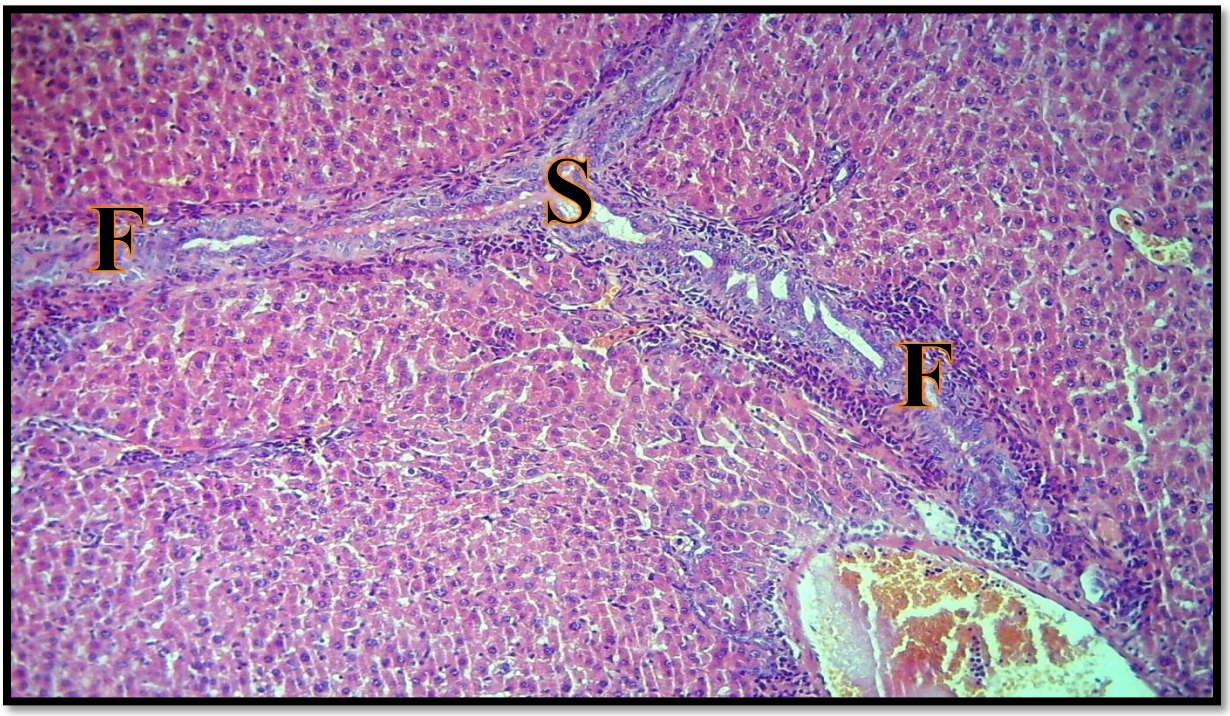

Figure (3): Liver tissue of rat treated with digoxin shows septal fibrosis (S) and periportal fibrosis (F). H&E stain 100X.

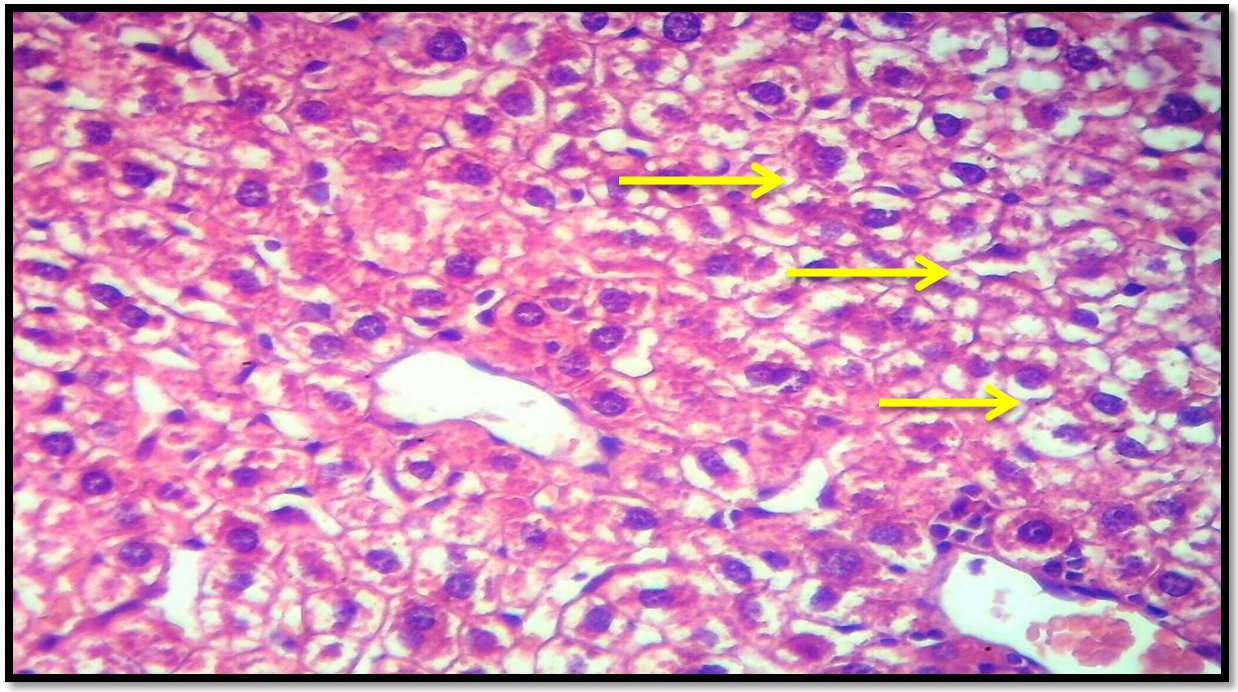

Figure (4): Liver tissue of rat treated with digoxin shows diffuse vacuolation of hepatocytes. H&E stain 400X.

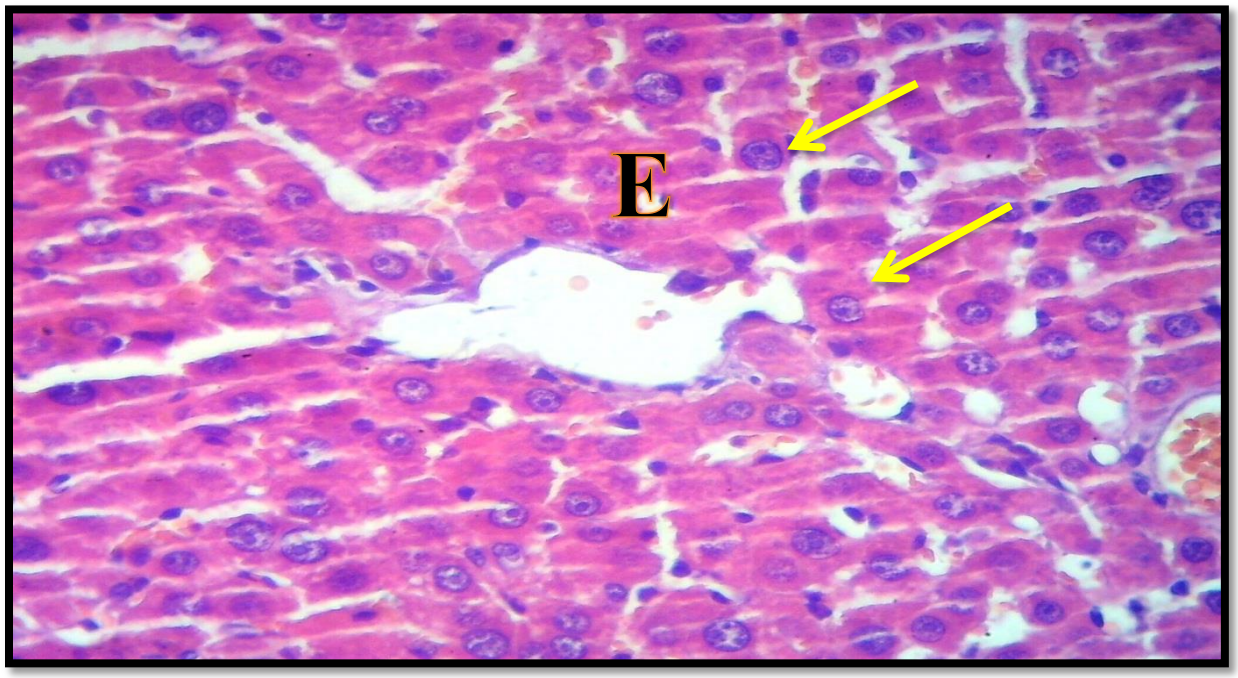

Figure (5): Liver tissue of rat treated with digoxin shows enlarged centrilobular hepatocytes (E). H&E stain 400X.

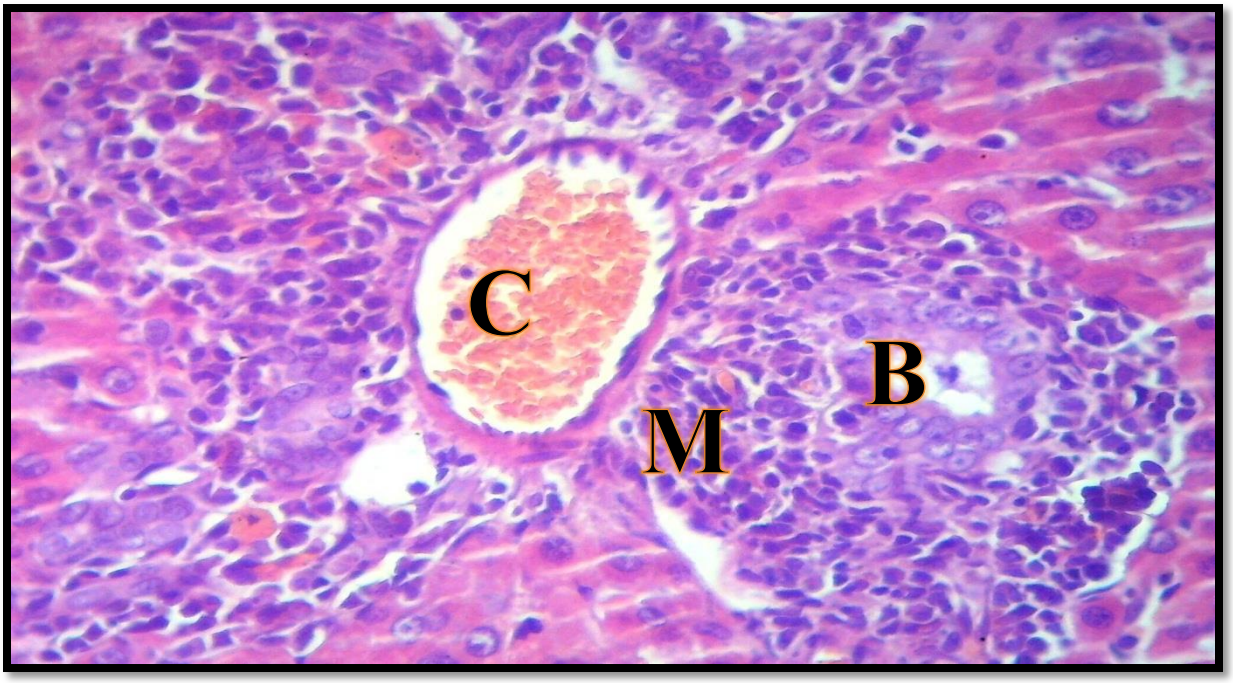

Figure (6): Liver tissue of rat treated with digoxin shows congestion of portal vein (C), periportal aggregates of mononuclear cells (M) and bile duct proliferation (B). H&E stain 400X.

Biochemical study

Effect of digoxin on serum AST & ALT enzymes level

The table below showed a significant (P≤0.05) increase of serum AST and ALT level in all treated groups when compared with Control group. As well as, show a significant (P≤0.05) increase of serum AST and ALT level in High group (G4) when compared with Intermediate (G3) and Low groups (G2). On the other hand there is a significant (P≤0.05) increase of serum AST & ALT enzymes level in Intermediate group (G3) when compared with Low group (G2).

Table shows the effect of digoxin on AST and ALT enzymes level. N = 12 (Mean ± SD).

Parameter / Groups	AST mean	ALT mean
Control (G1)	**147.25 ± 10.7 d**	**53.83 ± 7.86 d**
Low (G2)	**194.33 ± 34.2 c**	**78.16 ± 15.5 c**

Intermediate (G3)	**243.75 ± 12.1 b**	**115.16 ± 46.1 b**
High (G4)	**297.91 ± 46.4 a**	**157.91 ± 32.8 a**
LSD	**47.08**	**24.3**

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

Discussion

The present study shows several histopathological changes in liver tissue ranges from enlargement of centrilobular hepatocytes as showed in figure (5) to diffuse vacuolation of hepatocytes as in figure (4), this result is in agreement with **(8,9)** they report the administration of oleanderin cardiac glycoside cause hydropic degeneration in the hepatocytes and in other field scattered necrosis of liver cells as a toxic effect of high dose oleanderin glycoside, this change occur as an adaptation of hepatocytes to compete for metabolism of high dose of digoxin.

On the other hand, the study showed the toxicity effects of digoxin on the liver cells presented as degeneration, necrosis, minimal to sever periportal fibrosis and septal fibrosis as showed in figure (2&3), this result is in line with **(10)** who repot the administration of alcoholic solution of oleanderin glycoside associated with mild damage to sever necrosis of hepatocytes and dilation of liver sinusoid due to the toxic effect of glycoside on hepatocytes that lead to their degeneration and necrosis. This may be due to exhaustion of the capacity of centrilobular hepatocytes to metabolized high dose of digoxin and this result is matching with **(11)**.

Figure (6) show other hepatic pathological changes like central vein congestion, bile duct proliferation and aggregation of mononuclear cells, these changes is corresponding with **(12)** who state that liver cells suffering from focal necrosis and cholangiohepatitis with mononuclear cells infiltration and mild bile duct hyperplasia due to high dose of cardiac glycoside administration.

High dose (G4) has the most severe damage and toxic effect of digoxin on liver histologically presented as hepatic necrosis, sever periportal fibrosis and septal fibrosis with accumulation of mononuclear cells when compared with other study groups, this observation is in line with **(8,13)** they state that the damage was higher and massive with high dose and it is less in other groups when compared with control group.

The present study showed in the table above a significant (P≤0.05) increase in serum level of AST & ALT enzymes in high dose (G4) when compared with other study groups (G3, G2 & G1), this result is in line with **(6,14)** they found that digoxin or oleanderin cardiac glycoside administration associated with a significant increase in serum level of AST and ALT enzyme when compared with control group. This may be due to the damage and hydrolysis in the hepatocytes that occur as a toxic effect of digoxin on liver cells directly and this is corresponding with **(15)** who found a significant elevation of AST enzyme level in case of acute and chronic hepatitis and other diseases of liver tissues.

The table also showed a significant ($P \leq 0.05$) increase in serum level of AST and ALT enzymes level in (G3) when compared with (G2), as well as a significant ($P \leq 0.05$) increase in these enzymes level in (G2) when compared with control group (G1), this results may be due to the dose of digoxin used in each group and also depends on the level of damage that occur to the hepatocytes and this is in line with **(8)** who found the damage was most sever in the high dose group and less effect was in the low dose group when compared to control group.

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