Toxicity of different doses of alcoholic extract for *Citrullus Colonocynthis* fruits in mice

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Abstract

The present study was designed to evaluate the toxic effect alcoholic extract for *Citrullus Coloncynthis* fruits in mice. Four treatment groups, each consist of 6 mice divided according to daily treatment with alcoholic extract for *Citrullus Colonocynthis* fruits to T_1, T_2, T_3 and Crepresenting dosing orally with 30, 60,120 mg/kg respectively the fourth group act as control and treated with distilled water in 30 day. The result showed significant ($P<0.01$) elevation in their serum clinical enzyme Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) positively proportional with the dose of the plant extract. Yellow of spots was noticed in the liver in T_2 and T_3 groups at the end of experiment. All treated group T1,T2,T3 show no gross lesion on kidney compares control group. Toxic signs were also noticed increase proportionally with the dose of treated group including: depression, change in stool color and increase in respiration with decrease in weight. Histopathological changes shows the lesion which increased proportionally with the dose that lesion as inflammation and necrosis appear more in high dose T3= (120 mg/kg) in both liver and kidney in addition for the presence of multiple cast in urinary tubules and less at the other doses. The results of this study reveal that the alcoholic extract for *Citrullus Colonocynthis* fruits toxic effect in dose T3= (120 mg/kg) and less effect in T2= (60 mg/kg). Because of the popularity of this plant in traditional medicine for treatment of some diseases such as diabetes mellitus therefore must careful when uses.

التأثير السمي لجرع مختلفة للمستخلص الكحولي لثمار الحنظل في الفئران

فرح رسول جعفر
كلية الطب البيطري/ جامعة بغداد

الخلاصة

صممت الدراسة لمعرفة التأثير السمي للمستخلص الكحولي لثمار الحنظل في الفئران. استعملت في هذه التجربة أربع مجاميع إذ احتوت كل مجموعة 6 فئران وجرعت فموياً وقسمت حسب نوع المعاملة اليومية للمستخلص الكحولي لثمار الحنظل إلىC,T3,T2,T1 المتمثلة بالجرع الآتية: 30-60-120 ملغم/ كغم من وزن الجسم على التوالي والمجموعة الرابعة سيطرة إذ أعطيت الماء المقطر خلال 30 يوماً. أظهرت النتائج ارتفاعاً معنوياً في مستوى ($P<0.01$) خميرتي اسبارتيت امينو ترانسفيريز والالنين امينوترانسفيريز في مجموعتي T_3وT_2 مقارنة مع السيطرة تناسبت طردياً مع جرع المستخلص. تناولت الدراسة أيضاً الآفات العيانية للكبد والكلية في نهاية مدة التجريع إذ شملت وجود مناطق صفراء اللون على الكبد خاصة في المجموعتين T2و T_3 مقارنة لمجموعة السيطرة. إذ لم تظهر أي تغيرات في الآفة العيانية للكلية في جميع المجاميع المعاملة T3,T2,T1 مقارنة بمجموعة السيطرة. شوهدت العلامات بشكل زيادة تناسبت طردياً مع الجرعة ومدة التعرض إذ شملت: خمول، قلة في الوزن، تغير لون البراز، زيادة في التنفس. كما لوحظت التغيرات النسيجية المرضية التي سببت آلافه زيادة التي تناسبت شدتها مع الجرعة إذ أظهرت شدة في الجرعة العالية T3=120 ملغم/كغم حدوث التهاب شديد ونخر في كل من الكبد والكلية إضافة إلى وجود أعداد من القوالب في داخل النبيبات بينما كانت التغيرات اقل شدة في الجرع

الأخرى. كما أظهرت نتائج الدراسة بان المستخلص الكحولي لثمار الحنظل له تأثيراً سمياً في الجرعة T3=120 ملغم/ كغم واقل تأثيراً في جرعة T2= 60 ملغم/ كغم وبسبب رواج استعمال النبات في الطب الشعبي لعلاج بعض الأمراض مثل داء السكري لذا يجب الحذر عند استعماله.

Introduction

Citrullus Colocynthis (CCT) (Cucurbitaceae), commonly known as "bitter apple", "colosynth", "handal" comely known as bitter apple, are bitter, cooling cathartic (1). Is one of the native to dry area of North Africa, being common through out the Sahara, area of Morocco, Egypt, Sudan and middle east countries, plant that grows naturally in the western Iraqi which is used in traditional medicine. The fruits CCT contains active substance such as saponins, alkaloids and glycosides (2) *Citrullus colocynthis* had a beneficial effect on improving the glycemic profile without severe adverse effects in type II diabetic patients (3). It used as purgative, anathematic, antipyretic, carminative, cures tumors, leucoderma, asthma, jaundice, enlargement of spleen, tuberculous glands of the neck, elephantiases and ulcers, also reported that fresh fruit and seeds are eaten as an laxative and removing kidney stones (4,5). It possess cardiac depressant and smooth muscle relaxant effects and cytotoxic activities (6,7) and used locally for stimulation to hair growth (8) and its cucrbitacin content had an anticancer effects and anti-hepatotoxic activity (9,10). Many of the modern purgative pills contain the solid extract of *colocynth* in small doses it is expectorants, so root is useful in cough and asthmatic attacks in children, jaundice, urinary disease, rheumatism and for abdominal enlargement (11) and have inhibitory effect for prostaglandin formation. This effect was accompanied by significant induction of COX-2 protein expression (12). It is used in folk medicine as an abortifacient (13) some reports of its side effects which can limited its use as a traditional remedy. For instance, some report about its carcinogenic effects (14), there are also reports on sheep death after consuming The Plant (15). Regarding reproductive System, it was reported to induce infertility in both sexes (16,17). The histoarchitecture of testes is shown to undergo degenerative changes of somniferous epithelium, prevention of Spermatogenesis at the secondary spermatocyte stage and cytolysis Induces antiandrogenic and reversible infertility in male albinorat (16) in female rats, anti implantation activity was reported (17) the objective of this study was, to evaluate the toxic effects in mice after treatment with various doses of alcoholic extract for *Citrullus colocynthis*.

Materials and Methods

Fresh fruits of *Citrullus Colocynthis* were purchased from the local market and certified at the Iraq National Herbarium in abo Grebe, Fresh fruits were dried at the room temperature and powdered then extracted by 70% ethanol using magnetic stirrer for 72 hours at 50^{o}C then the extract was filtered and evaporated for drying rotary evaporated at 45^{o}C under reduced pressure (18). The resulting extract was collected (yield: 4%). Animals were divided for four equal groups T_1, T_2, T_3 and C, the first three groups were treated groups. While C was control group, the doses of this experiment were 30, 60, 120 mg/kg B. W. and the animals received 0.1ml/10g B. W. at concentration 3 mg/ml, 6 mg/ml and 12 mg/ml respectively for treated groups while the control group received 0.1ml/10g B.W. distill water. All groups received treatment daily for 30 days.

- **Animals:** Twenty five albino Swiss mice weighing 25-30g of either sex were used. The animals were grouped and kept in cage housed at standard condition of light and ventilation and have freely access to standard rodent diet (commercial feed pellets) and tap water. The animal was kept for a week for adaptation.
- **Experimental Design:** Four mice groups (T_1, T_2, T_3 and C) were used, each group consist of 6 mice given orally different doses alcoholic extract of CCT at 30, 60, 120

mg/kg and distill water respectively in all treated group, the animals were dosed daily for 30 days the dose were prepared as above.

- **Parameter:** At the end of experiment (after 30 days) the following parameters were studied in all groups and then the animals were sacrificed by decapitation for gross and histopathological study:

1. Estimation of serum enzyme activity for AST, ALT (19) by taking the blood from direct the heart after anesthetized mice.
2. Clinical signs development in all groups through the period of experiment (30 day).
3. Gross lesion observation of liver and kidney.
4. Histopathological study: for liver and kidney by taking small section and fixed in 10% formalin solution then embedded in paraffin waxes and stained with Hematoxylin and Eosin stain.

- **Statistics Analysis**: Results were expressed as means ± standard error that subjected to statistical analysis using one or two-way analysis of variance (ANOVA) and LSD. The significance level considered was ($P < 0.01$).

Result and Discussion

ALT, AST level Show Significant ($P<0.01$) Increase at the end of treatment period in T_2 and T_3 groups positively proportional with the dose while T1 show no significant change in ALT and AST in comparison with that of control group show in Table (1).

Table (1) Effect of alcoholic extract of CCT on the enzyme activity (ALT, AST) in different experimental group

Group	AST (I/U) after 30 days	ALT (I/U) after 30 days
C	57-80±0.4 A	9.7±0.99 A
T_1	59.20±1.22 A	11.25±0.46 A
T_2	86.00±0.89 B	29.50±0.64 B
T_3	102.0±0.30 C	36.75±0.85 C

n=6
c=control group
T_1=Treated groups with 30 mg/kg CCT .
T_2= Treated groups with 60 mg/kg CCT.
T_3= Treated groups with 120 mg/kg CCT.
Mean value different letter significant value ($P<0.01$) F-test

In this study, the activity of theses enzymes in serum T_2 and T_3 groups were higher then the control group may reflect hepato cellular damage according proportionally with the increases in CCT doses in mice. The results agree with that reported that administration of *Citrullus Colocynthis* seed extract for 50 days for normal rats resulted insignificant increase in LDH, GSH, ALT, AST, ALP ($P<0.001$) as compared to normal group (20) also our results in agreement with (21) who reported an increase in LDH and AST levels after CCT fruits dose 0.25 g/kg/day to sheep for 42 day. This was attributed to the increase in glucokinase, glucose -6- phosphate, phosphate and phosphofructokinase values and a decrease in hexokinase value reported (22) that discuss the ingestion of *Citrullus Colocynthis* seed stimulate the activities of LDH and AST (23,24). The Results indicated that the *Citrullus Colocynthis* in treated groups showed a significant elevating tendency in the serum ALT and AST. These results confirmed the finding of (25). The amino transferases (enzymes) involved in this reaction AST and ALT are present in hepatocytes and leak into blood with liver cell damage.

- **Toxic Signs (Clinical Signs):** Toxic signs were also noticed in dose treated group: including: depression, increase respiration, changes in stools, decrease weight in T2 and T_3 comparison with the control group.

Table (2) Effect of alcoholic extract of CCT on the Clinical Signs

Clinical signs	C	T_1	T_2	T_3
Depression	-	-	+	+
Decrease weight	-	+	+	+
Increase respiration	-	-	-	+
Stool change to terry color	-	-	-	+

N = 6
c = control group
T_1 = Treated groups with 30 mg/kg CCT .
T_2 = Treated groups with 60 mg/kg CCT.
T_3 = Treated groups with 120 mg/kg CCT.
+ = presence signs
- = No presence signs

The severity of toxicity was positively proportional with increase in CCT doses, this indicant that toxicity was due to the presence of same toxic components like saponine and alkaloids that induce toxicity according with the dose. It has been postulated that administration of this plant to the female rats for 30 days in different dosages induces dose-dependent decrease in the size of the offspring with no toxicological sign. Animal of T2 and T3 group showed significant decline in mice weight at different experimental period 7, 14, 21, 30 days in comparison with control group and before treatment. While T1 group same pattern of no increase weight as that of control one show in (Table 3).

Table (3) Effect of alcoholic extract of CCT of on mice the body weight (gm)

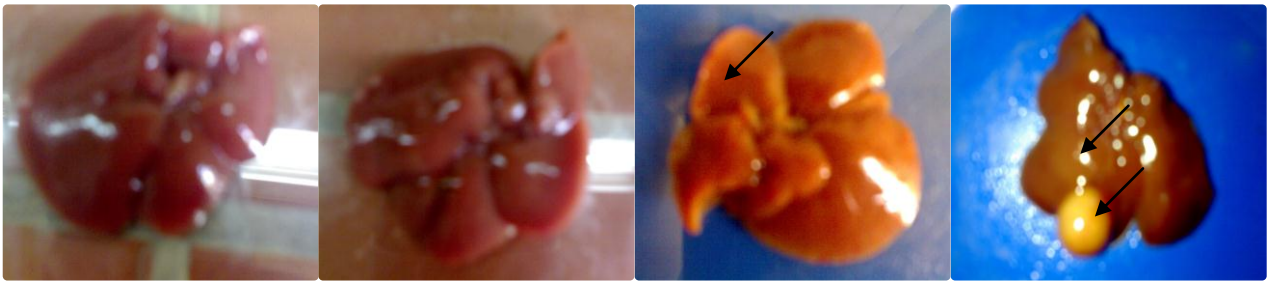
Group	Before treatment	After 7 day treatment	After 14 day treatment	After21day treatment	After30 day treatment
C	22±0.50 Aa	22±0.52 Aa	22±0.55 Aa	22.3±0.55 Aa	22.5±0.30 Aa
T_1	24±0.85 Aa	24±0.96 Aa	23±0.31 Aa	22.7±0.39 Aa	22±0.45 Ab
T_2	22±0.32 Aa	21.7±0.13 Aa	20.8±0.21 Aa	19.9±0.29 Bb	18.7±0.35 Bc
T_3	24 ±0.75 Aa	22.5±0.40 Ab	20.4±0.82 Ac	19±0.22 Bc	17±0.52 Cd

n = 6
c = control group
T_1 = Treated groups with 30 mg/kg CCT.
T_2 = Treated groups with 60 mg/kg CCT.
T_3 = Treated groups with 120 mg/kg CCT.
Capital letter refers significant ($p<0.01$) Between groups, small letter refer significant ($p<0.01$) within group.

This effect of CCT may be attributed to the bitter and irritant effect of the plant on GIT that cause stress and purgative effect as reported by (26). Who use Powder of ripped CCT fruit pulp has been used a purgative acting directly on the gastrointestinal tract affect demonstrated.

- **Gross Lesion:**

Liver of T_2 and T_3 group showed yellowish spots on the surface increase the with increased in dose of extract while T1 show no change on the surface of liver and appear nearly control group as that show clear glistening surface. As shown in Fig. (1).



C **T1** **T2** **T3**

Fig. (1) Effect of alcoholic extract of CCT on mice liver

n = 6
c = control group
T_1 = Treated groups with 30 mg/kg CCT.
T_2 = Treated groups with 60 mg/kg CCT.
T_3 = Treated groups with 120 mg/kg CCT
↑= presence yellowish spots on the liver of treated in T_2 and T_3 CCT in mice.

Various components of extract might be responsible for mice as reported by (25) saponine extracted form CCT induce hepto renal damage including necrosis of liver cell and renal tissue it seem that the toxicity observed in this study might be due to the damage to the liver (27).

- **Kidney:** All treated group show no change on the surface of kidney that appear nearly as the control group. This results may be because the doses of alcoholic extract of CCT were not enough to cause gross lesion effect on kidney while the histopathological Study revealed necrotic change with in infiltrations of inflammatory cell and presence multiple hyaline casts in kidney section in accordance with extract doses used.
- **Histopathological Study:**
- **Liver:** The effect of extract CCT appear with dose which was given that more sever lesion noted with high dose 120 mg/kg which characterized by highly infiltrations of mnc (mononuclear cell in the liver parenchyma show focal aggregation of mnc in the liver (Fig.1) in addition of the presence of cuffing of the mnc (lymphocyte) surrounding the blood vessels (Fig. 2) and also in portal areas (Fig. 3). Wide areas of necrosis were noted especially with high dose infiltrated with inflammatory cells (Fig. 4, 5). Less areas of necrosis were noted in the dose (60 mg/kg) that necrotic cell appear with different stage (Fig.5, 6) in addition for degenerative cell were seen. Slight lesion were noted at the dose (30 mg/kg) were seen in (Fig. 7). No changes were seen in the control were seen in (Fig. 8) our result concurrence with (28). They found presence of degeneration and necroses of the hepatic cells in addition for the inflammation and these gross lesion and histological changes noticed in liver indicated the toxic effect of CCT extract and mention the increase (AST, ALT) proportionally with its doses in mice.

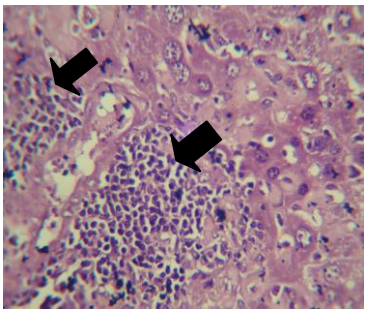


Fig. (1) Histopathological section of liver in mouse treated with T3 shows mnc aggregation in liver parenchyma and near blood vessels (⬆). (H&E×100)

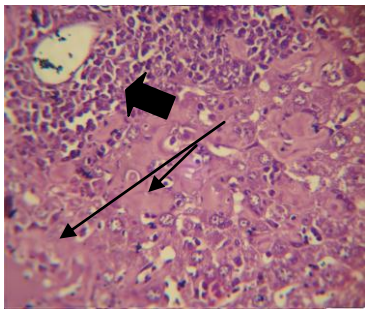


Fig. (2) Histopathological section of liver in mouse treated with T3 shows cuffing of mnc (lymphocyte) surrounding central vein (⬆) and presence of necrotic hepatocyte (↑) (H&E×100)

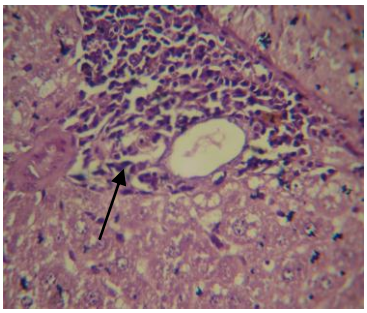


Fig. (3) Histopathological section of liver in mouse treated with T3 shows cuffing of mnc (lymphocyte) surrounding blood vessels (↑) (H&E×100)

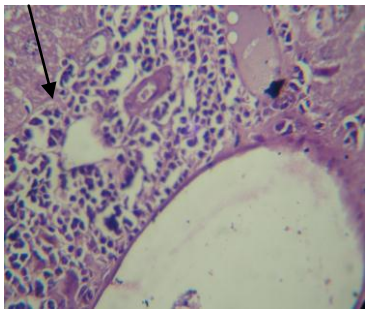


Fig. (4) Histopathological section of liver in mouse treated with T3 shows infiltrations of inflammatory cells (↑) in portal area (H&E×100)

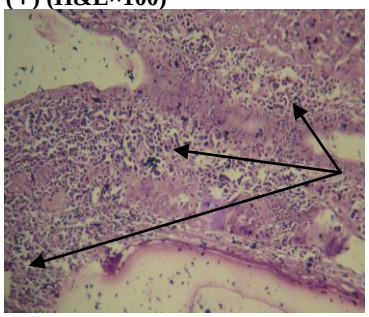


Fig. (5) Histopathological section of liver in mouse treated with T3 shows wide area of necrosis infiltrated with inflammatory cells (↑) (H&E×100)

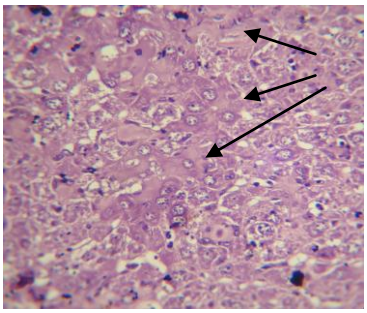


Fig. (6) Histopathological section of liver in mouse treated with T2 shows necrotic heptocyte with different stage (↑) (H&E×100)

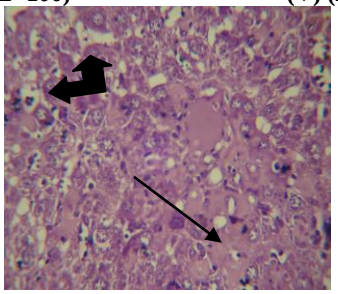


Fig. (7) Histopathological section of liver in mouse treated with T1 shows. Slight changes degenerative cell (vacoulation (↘) & bollonic heptocyte(↑)) (H&E×100)

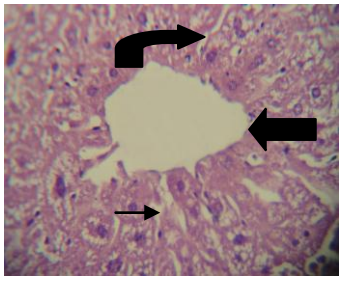


Fig. (8) Histopathological section of liver in control mouse shows lobe of liver involve central vein (⬆) with hepatic cord (↑) & sinusoid (↱) (H&E×100)

- **Kidney:** Microscopic lesions give the same changes that severity depended upon the dose. high dose (120mg/kg) gives more sever lesions appearance that shows wide areas of necrotic tissue infiltrated with inflammatory cells. Periglomerular and peritubular and around blood vessel (Fig.9-10), renal casts in the tubules also noted in (Fig.11-12-13), cystic dilation of renal tubules in addition for necrosis and degeneration in epithelial lining the tubules. This changes appear less sever at dose (60mg/kg) were seen in (Fig. 14) while disappear in the dose (30mg/kg) no changes appear just congestion was shown in (Fig. 15). No changes observed in control in (Fig. 16). This result agree with (29) that record presence of necrosis in tubular and glomerular mononuclear cellular infiltration and renal castes and calcified mass in the collecting tubules in rat treated with amended diet containing 50-75% CCT powdered seeds these change credit with toxic effect of CCT.

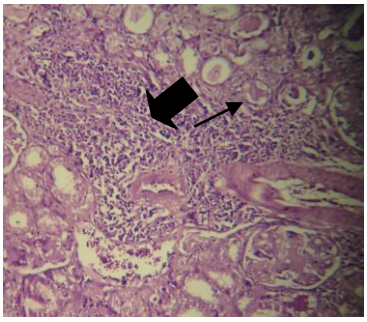


Fig. (9) Histopathological section of kidney in mouse treated with T3 shows wide areas of necrotic tissue infiltrated with inflammatory cells (⬆) in addition presence of hyaline cast (↑) (H&E×100)

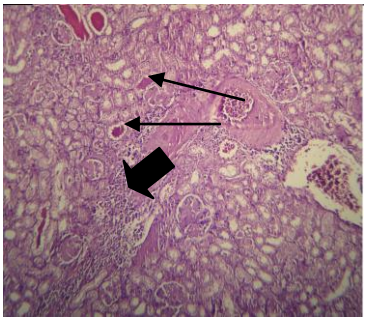


Fig. (10) Histopathological section of kidney in mouse treated with T3 shows wide areas of necrotic tissue infiltrated with inflammatory cells (⬆) in addition presence of hyaline cast (↑) (H&E×100)

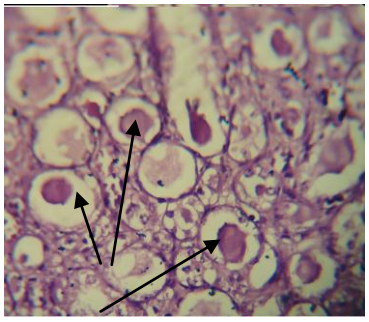


Fig. (11) Histopathological section of kidney in mouse treated with T3 shows multiple hyaline casts (↑) (H&E×100)

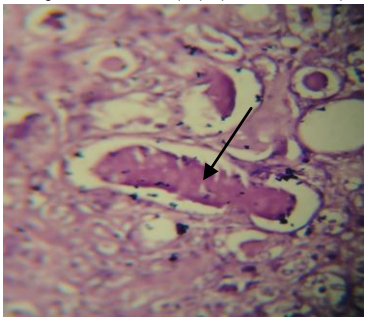


Fig. (12) Histopathological section of kidney in mouse treated with T3 shows multiple hyline cast (↑) (H&E×100)

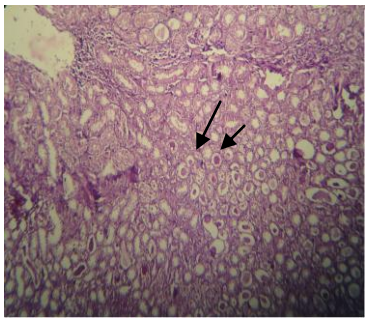


Fig. (13) Histopathological section of kidney in mouse treated with T3 shows multiple hyaline cast (↑) (H&E×100)

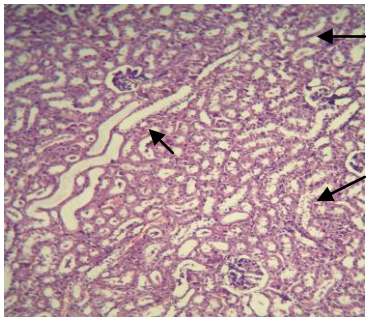


Fig. (14) Histopathological section of kidney in mouse treated with T2 shows cystic dilation of renal tubules (↑) (H&E×100)

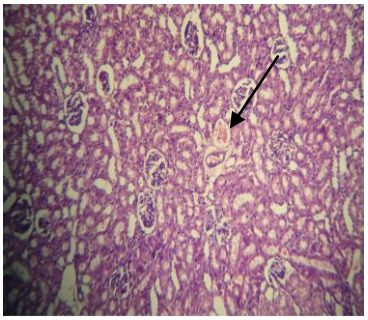


Fig. (15) Histopathological section of kidney in mouse treated with T1 shows congestion of blood vessels (↑) (H&E×100)

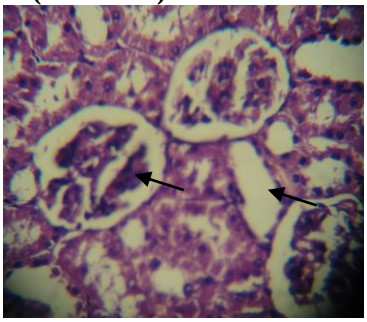


Fig. (8) Histopathological section of kidney in mouse shows treated with D.W (control) no changes observed (↑) (H&E×100)

Herbal hepto and renal toxicity has been recognized for many year but new agent are contently being identified (30,31) CCT extract was found to have hepototoxic effect and renal effect at high dose hepototoxic effect and renal effect be proved by the result of study through the enzyme of liver AST, ALT (21,28) in addition for toxopathological change which presence of high dose that give the wide necrosis in liver and kidney with infiltration of renal castes this agreed with result (27,28, 29). The severity of toxicity was positively proportional with increase in CCT doses, this indicant that toxicity was due to the presence of same toxic components like saponine and alkaloids that induce toxicity according with the dose.

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