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RESEARCH ARTICLE

Hepatotoxicity and nephrotoxicity of colchicine prolonged use in the rats.

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Abstract

Colchicine is used as a treatment for gout, arthritis, and familial Mediterranean fever. It has a narrow therapeutic index and its overdose is associated with a high mortality rate. Assessment of hepatotoxicity and nephrotoxicity of colchicine prolonged use was evaluated by studying biochemical and histopathological abnormalities. Sixty adult albino rats were divided into three equal groups (each = 20). First group (control) received distilled water daily; while second and third group received 2 and 3 mg / kg/day of colchicine dissolved in distilled water, respectively, by intraperitoneal route for three months. Results: Prolonged use of colchicine induced hepatotoxicity and nephrotoxicity. Hepatotoxicity was manifested by the statistically significant increase of liver enzymes such as ALT, AST, ALP and histopathological changes in the form of hepatocytes necrosis, dilatation of central vein, and degeneration in the form of pyknosis of hepatocytes nuclei, fibrosis and cytoplasmic vacuolation. Neprotoxicity was manifested by the statistically significant increase of serum urea, creatinine; degenerative changes of renal tubules and hypertrophy of glomeruli. Conclusions: Prolonged use of colchicine induced hepatotoxicity and nephrotoxicity depending on its dose.

Introduction

Colchicine is used as a treatment for gout, arthritis forms, familial Mediterranean fever, Behcet disease and primary biliary cirrhosis. It has a narrow therapeutic index and colchicine overdose is associated with a high mortality rate **(Lainé et al., 2012).** Colchicine induces protection against a variety of hepatotoxic insults and improves survival in a clinical trial for alcoholic cirrhosis **(Maxwell et al., 2002)**.

The British Society for Rheumatology and British Health Professionals in Rheumatology indicated that the efficacy of colchicine in the treatment of gout, but it recommended that the use of colchicine should be by low doses in order to reduce the risk of its adverse effects (**Jordan et al., 2007).** Colchicine poisoning is a serious and fatal event. It consists of three phases, the first phase is characterized by gastro-intestinal symptoms; the second phase represents as multi-organ dysfunction, metabolic derangements and bone marrow suppression. And the third phase is a recovery phase of bone marrow depression and resolution of organ failure **(Finkelstein et al., 2010).** The U.S. Food and Drug Administration (FDA) reported that life-threatening and fatal colchicine-related toxicity can occur

with usual doses of colchicine in patients with certain risk factors, including drug-drug interactions, impaired renal or hepatic function and with age more than 65 years because of susceptibility to cumulative toxicity **Guven et al., (2002).** The aim of the present study was to assess hepatotoxicity and nephrotoxicity of colchicine prolonged use of studying biochemical and histopathological parameters.

Materials and methods

Sixty adult albino healthy rats weighing (250–300 g) were subjected to the study. They were maintained on a standard laboratory conditions. Rats had free access to water and food during the experimental period. Animals were randomly allocated to three equal groups of twenty mice each. First group "control" was orally received distilled water daily for three months. The second and the third group were received 2 and 3 mg/kg/day of colchicine dissolved in distilled water, respectively by intraperitoneal route for three months (**Terkeltaub et al., 2010)**.
Colchicine drug was in the tablet form and obtained from El-Nasr pharmaceutical Chemicals Company (ADWIC) Abu-Zaabal-Egypt. One tablet contains 500 microgram of active ingredient, was dispersed in 5 ml distilled water (freshly prepared). Animals were sacrificed 24 hours after the last dose of colchicine. Blood samples were collected from the hearts of rats in all groups on the last day (the ninety days). Samples were centrifuged at 3000 rounds for 10 minutes to separate the serum. The serum was analyzed for the biochemical analysis:

1- Liver functions tests:

A. Aspartate Aminotransferase "AST"
Assay of AST was performed by mixing the serum to buffered solution of L- aspartic acid and 2-ketoglutarate and then incubated for one hour at 37 ˚C. After incubation, 1 mm of DNPH and 0.4m of NaOH was added **(Chatterjee, 1993)**.

B. Alanine Aminotransferase "ALT"
Assay of ALT was performed by mixing the serum to buffered solution of DL- alanine and 2-ketoglutarate, and then incubated for thirty minutes at 37 ˚C. After incubation, 1 mm of DNPH and 0.4m of NaOH was added **(Daniel and Marshall, 1999)**.

C. Alkaline Phosphatase "ALP"
Assay of ALP was performed by using p- nitrophenol phosphate as substrate, in alkaline buffer with fresh unhemolysed serum for 45 min at 12°C **(Daniel and Marshall, 1999)**.

2- Renal function tests:

A. Serum creatinine
It was determined by Jaffe reaction **(Wilding and kennedy, 1977).**

B. Serum urea
It was determined by Berthelot method **(Alexander and Griffith, 1992).**

Histological studies:

Abdominal viscera were exposed by midline incision. Livers and kidneys of the experimental rats were rapidly excised, sliced and fixed immediately after collection in 10% neutral buffered formalin for at least 24 hours. The organs were then processed in an automated tissue processing machine, embedded in paraffin, and cut into 5 µm sections. Subsequently, the sections were stained with hematoxylin-eosin, Mallory and Periodic acid Schiff (PAS). Tissue sections were investigated using a light microscope to evaluate histopathological changes **(Drury and Wallington, 1980)** and **(Bancroft and Gamble, 2002)**.

Ethical considerations

The most appropriate animal species was chosen for this research. Promotion of a high standard of care and animal well-being at all times was done. Appropriate sample size was calculated by using the fewest number of animals to obtain statistically valid results. Painful procedures were performed under anesthesia to avoid distress and pain. Our standards of animal care and administration met those required by applicable international laws and regulations.

Statistical analysis

Statistical analysis was performed using SPSS version 16. Variability of results was expressed as mean $\pm$ SD. Results of this study were analyzed by using non - parametric test (Mann Whitney U test). The significance of differences between mean values was determined using Chi-Square test and independent sample T-test for comparisons. $P < 0.05$ represents level of significance.

Results

1- Biochemical findings:

A- Liver function tests:-

Table (1) shows mean ±SD values of rats liver function tests (alanine aminotransferase "ALT", aspartate aminotransferase "AST" , alkaline phosphatase "ALP"). Mean ±SD values of ALT were 10.16± 3.1 in the control group which received distilled water, 22.1±2.1 in the second group which received 2 mg/kg/day of colchicine dissolved in distilled water and 45.9±12.1 in the third group which was received 3 mg/kg/day of colchicine dissolved in distilled water. Mean ±SD values of AST were 30.7±2.8 in the control group which received distilled water, 34.13±1.9 in the second group which received 2 mg/kg/day of colchicine dissolved in distilled water and 51.3±8.2 in the third group which was received 3 mg/kg/day of colchicine dissolved in distilled water. Mean ±SD values of ALP were 85.8±5.3 in control group which received distilled water, 89.1±2.3 in the second group which received 2 mg/kg/day of colchicine dissolved in distilled water and 144.4±9.1in the third group which was received 3 mg/kg/day of colchicine dissolved in distilled water.

B- Renal function tests:-

Table (2) shows mean ±SD values of rats renal function tests (serum urea and creatinine). Mean ±SD values of urea were 12±1.1 in the control group which received distilled water, 30.3±2.2 in the second group which received 2 mg/kg/day of colchicine dissolved in distilled water and 45.2 ± 2.4 in the third group which was received 3 mg/kg/day of colchicine dissolved in distilled water. Mean ±SD values of creatinine were 0.32±0.5 in the control group which received distilled water, 0.59±0.1 in the second group which received 2 mg/kg/day of colchicine dissolved in distilled water and 0.97±0.1 in the third group which was received 3 mg/kg/day of colchicine dissolved in distilled water.

2- Histopathological findings

A- Hepatic histopathological findings:-

The Liver section of the control rat showed normal hepatic structure **(Fig.1&2&3)** with normal positive reaction of PAS **(Fig.4).** But liver sections of the second group rats showed fragmentation of hepatocytes nuclei, lighting of the cytoplasm of hepatocytes, decreased widening of central vein and blood sinusoid **(Fig.5&6&7)** with mild positive reaction of PAS **(Fig.8)**. In the third group, liver sections showed marked hepatic disorganization which represented as necrosis of hepatocytes, contracted and fragmented pycknotic nuclei, increased number of cytoplasm vacuoles, degenerated kupffer cells and fibrosis of central vein **(Fig.9&10&11)** with marked decrease in the positivity of PAS reaction **(Fig.12)**.

B- Renal histopathological findings:-

The Kidney section of the control rat showed normal renal structure **(Fig.13&14&15)** with normal positive reaction of PAS **(Fig.16).** But kidney sections of second group rats showed mild hyperaemia of renal vessels, some degenerative changes of tubular epithelium and cystic dilatation **(Fig.17&18&19)** with mild positive reaction of PAS **(Fig.20)**. Kidney sections of third group rats showed severe tubular damage, enlarged vascular glomeruli, tight filling of Bowman's capsule and absence of capsular spaces **(Fig.21&22&23)** with marked decrease in positivity of PAS reaction **(Fig.24)**.

Table (1): Effect of colchicine prolonged use on Mean + SD of Liver function tests in rats.

L.F.T / Group	ALT (IU/L)	AST (IU/L)	ALP (IU/L)
Group 1	10.16± 3.1	30.7±2.8	85.8±5.3
Group 2	22.1±2.1*	34.13±1.9*	89.1±2.3*
Group 3	45.9±12.1**	51.3±8.2**	144.4±9.1**

L.F.T= Liver function tests. **ALT =** alanine aminotransferase. **AST** = aspartate aminotransferase. **ALP** = alkaline phosphatase. **SD** = standard deviation.

Number per group = 20
First group (control) received distilled water daily.
Second group received 2 mg/kg/day of colchicine.
Third group received 3 mg/kg/day of colchicine.
* =p< 0.05 (significant difference in comparison with control group)
** = p < 0.05 (significant difference in comparison with second group)
Statistical analysis was performed by non - parametric test (Mann Whitney U test).

Table (2): Effect of colchicine prolonged use on Mean + SD of renal function tests in rats.

Group / R.F.T	Group 1	Group 2	Group 3
S.Urea (mg/dl)	12±1.1	30.3±2.2*	45.2 ± 2.4**
S.Creatinine (mg/dl)	0.32±0.5	0.59±0.1*	0.97±0.1**

R.F.T= Renal function tests.**SD** = standard deviation. **Number per group** = 20
First group (control) received distilled water daily.
Second group received 2 mg/kg/day of colchicine.
Third group received 3 mg/kg/day of colchicine.
* =p< 0.05 (significant difference in comparison with control group)
** = p < 0.05 (significant difference in comparison with second group)
Statistical analysis was performed by non - parametric test (Mann Whitney U test).

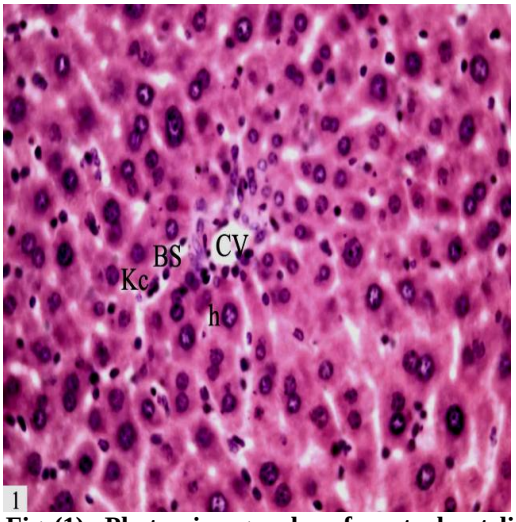

Fig (1): Photomicrography of control rat liver showing normal hexagonal hepatic lobules with portal triads at the vertices and a central vein (CV) in the middle. Hepatocytes (h) are arranged into hepatic cords and separated by adjacent blood sinusoids (BS) containing Kupffer cells (Kc). (H&E X400)

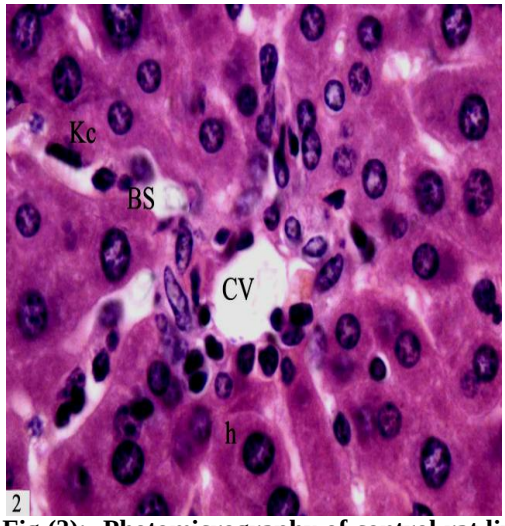

Fig (2): Photomicrography of control rat liver showing normal hepatic lobules, central vein (CV), hepatocytes (h), blood sinusoids (BS) and Kupffer cells (Kc). (H&E X1000)

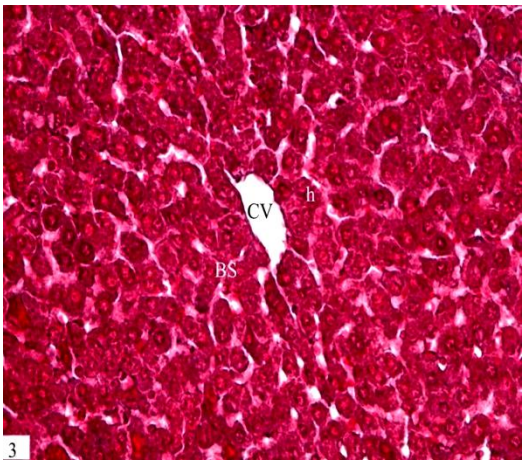

Fig (3): Photomicrography of control rat liver showing normal hepatic structures such as hepatocytes (h), central vein (CV) and blood sinusoids (BS). (Mallory X400)

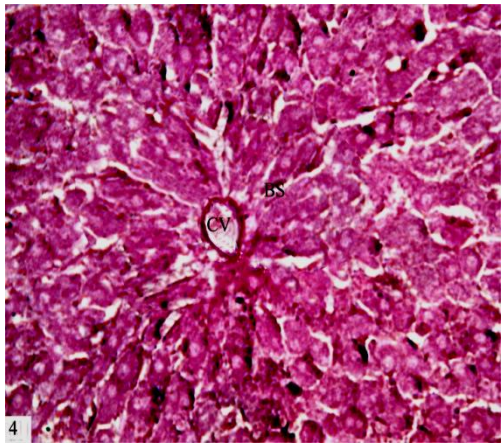

Fig (4): Photomicrography of control rat liver showing normal positive reaction of PAS. (Periodic acid-Schiff's X400)

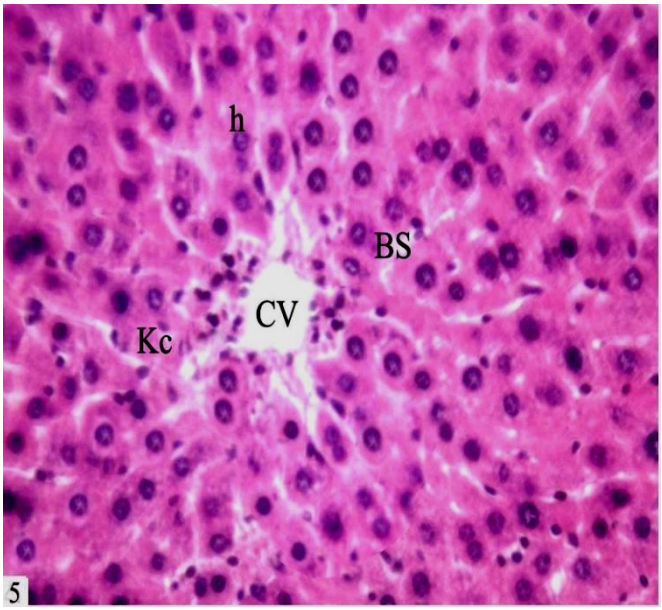

Fig (5): Photomicrography of liver rat in second group showing normal liver organization, decreased widening of central vein (CV) and blood sinusoid (BS), mild fragmentation of hepatocytes nuclei with mild lighting of the cytoplasm of hepatocytes (h) and normal Kupffer cells (Kc). (H&E X400).

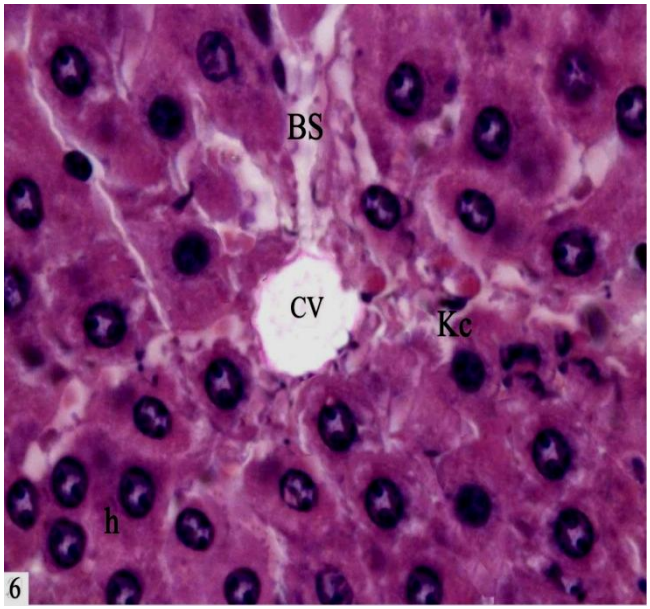

Fig (6): Photomicrography of liver rat in second group showing normal liver organization with decreased widening of central vein (CV) and blood sinusoid (BS) which lined with Kupffer cells (Kc), mild fragmentation of hepatocytes nuclei and lighting of the cytoplasm of hepatocytes (h). (H&E X1000).

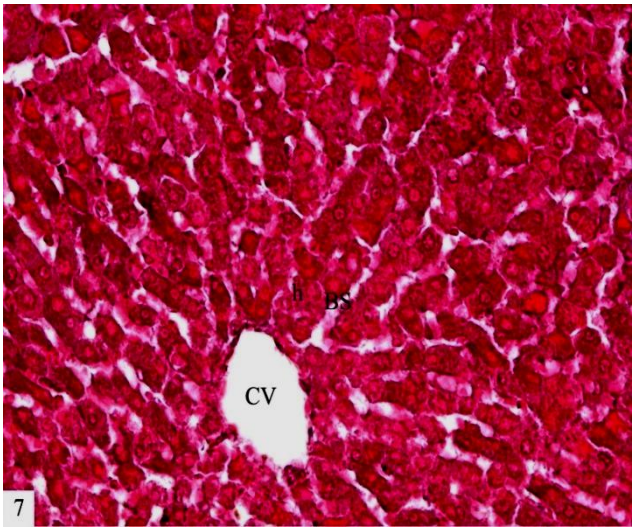

Fig (7): Photomicrography of liver rat in second group showing decreased in portal mononuclear cells infiltration around central vein (CV), bile duct (B), hepatic artery (A) and portal vein (V). (Mallory X400).

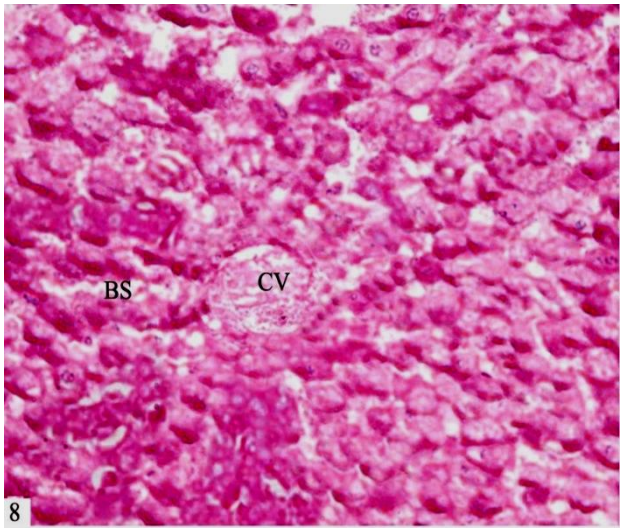

Fig (8): Photomicrography of liver rat in second group showing mild positive reaction of PAS. (Periodic acid-Schiff's X400).

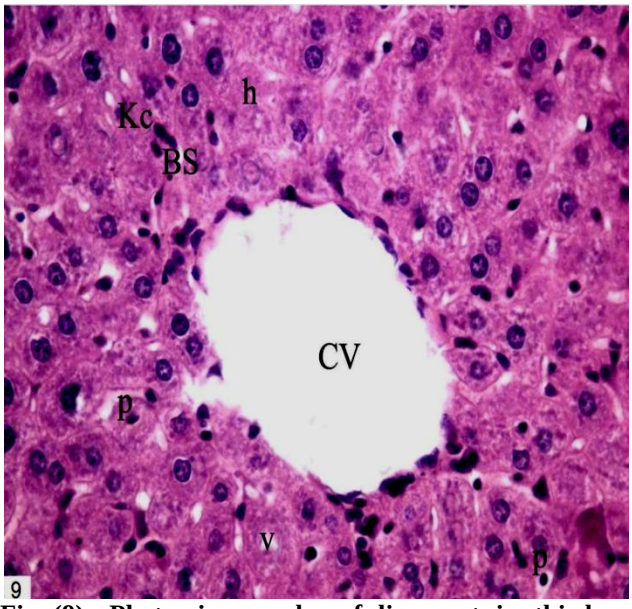

Fig (9): Photomicrography of liver rat in third group showing light, foamy hepatocyte cytoplasm filled with vacuoles (v), necrosis of some hepatocytes (h) and their nuclei are contracted, pycknotic with condensed chromatin. Decrease size of blood sinusoids (BS) and degenerated Kupffer cells (Kc) of sinusoid walls. (H&E X400).

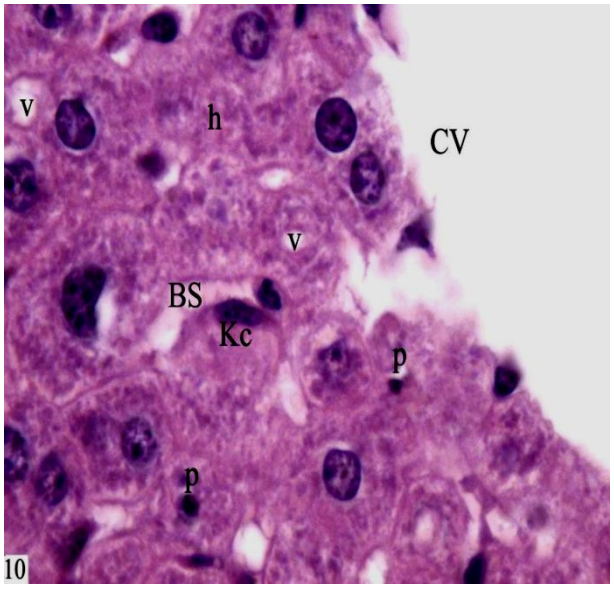

Fig (10): Photomicrography of liver rat in third group showing necrosis of some hepatocytes (h), vacuoles of cytoplasm (v), small, pycknotic nuclei with decrease size of blood sinusoids (BS) and degenerated Kupffer cells (Kc). (H&E X1000).

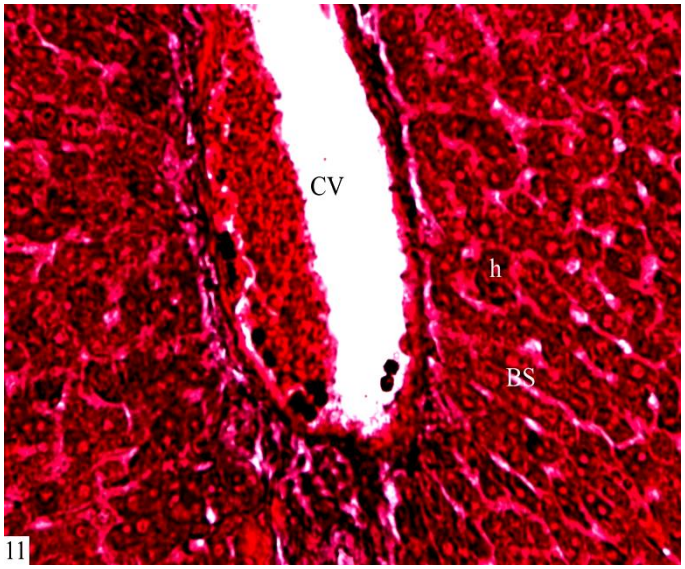

Fig (11): Photomicrography of liver rat in third group showing widening and fibrosis of central vein (CV), decrease size of blood sinusoids (BS) and enlarged hepatocytes (h). (Mallory X400).

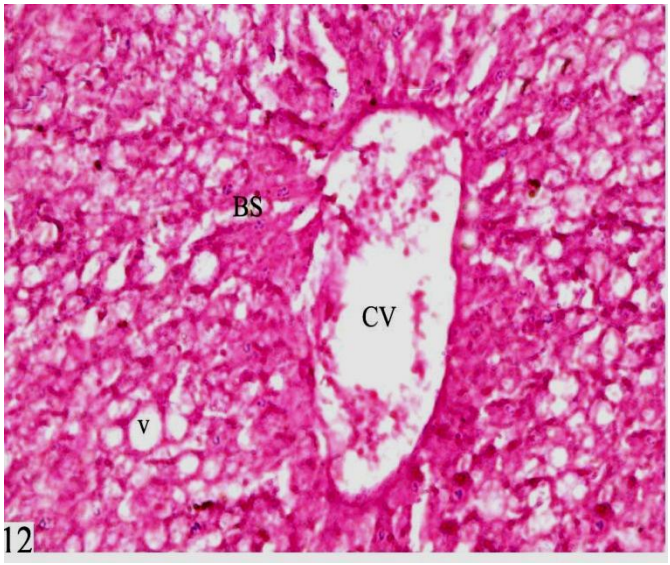

Fig (12): Photomicrography of liver rat in third group showing marked decrease in PAS reaction. (Periodic acid-Schiff's X400).

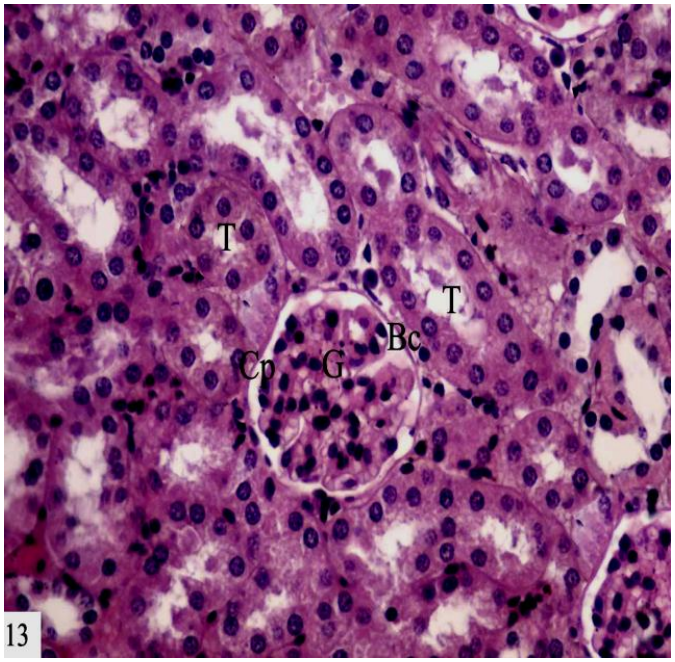

Fig (13): Photomicrography of kidney control rat showing normal glomeruli (G) and flat epithelium lining glomerular capsule (BC) with distinct capsular space (CP), normal proximal and distal convoluted tubules (T). (H&E X400).

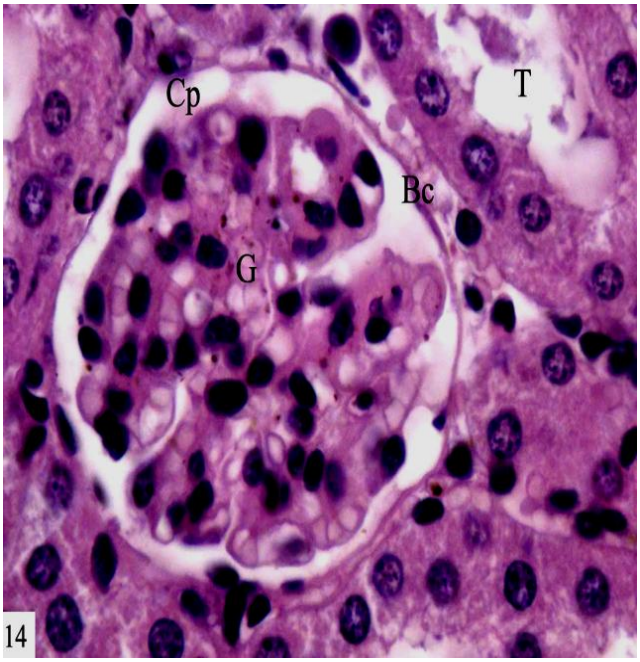

Fig (14): Photomicrography of kidney control rat showing normal glomeruli (G) and flat epithelium lining glomerular capsule (BC) with distinct capsular space (CP), normal proximal and distal convoluted tubules (T). (H&E X1000).

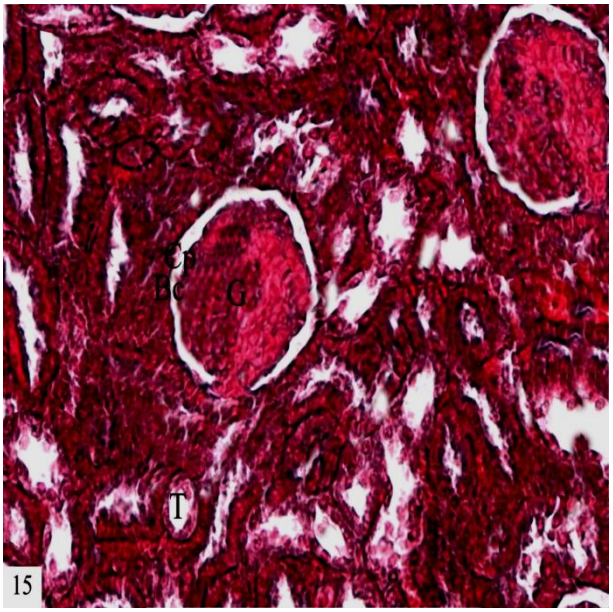

Fig (15): Photomicrography of kidney control rat showing normal glomeruli (G) and flat epithelium lining glomerular capsule (BC) with distinct capsular space (CP) with normal proximal and distal convoluted tubules (T). (Mallory X400).

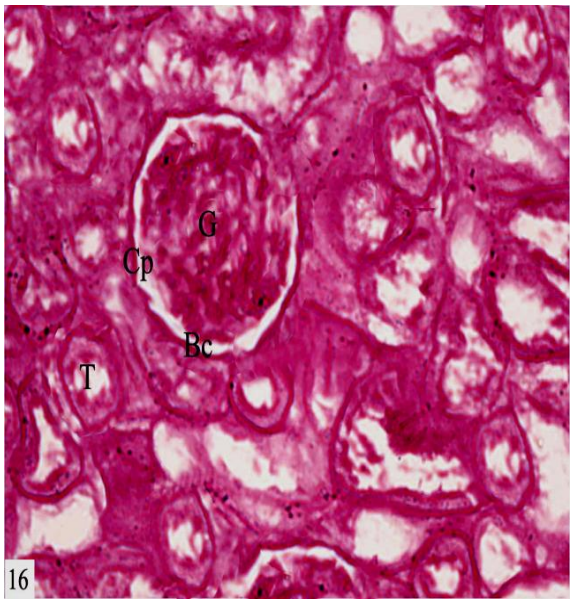

Fig (16): Photomicrography of kidney of control rat showing normal positive reaction of PAS. (Periodic acid-Schiff's X400).

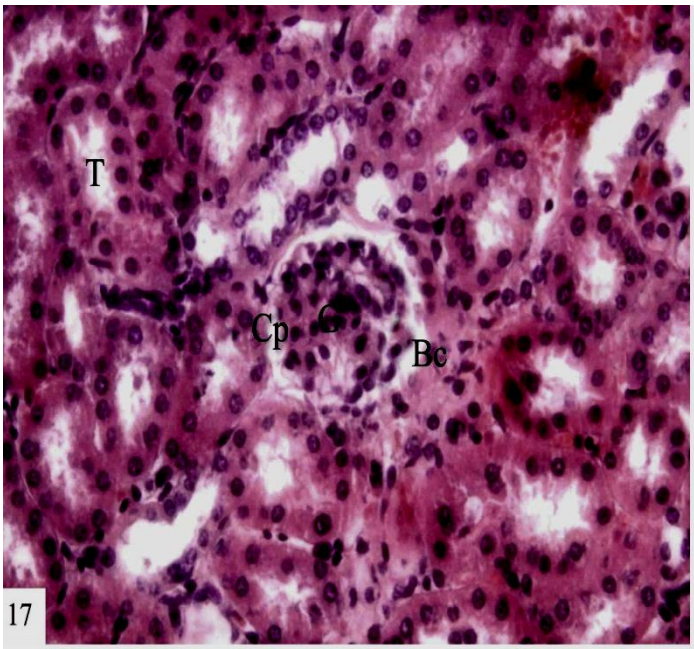

Fig (17): Photomicrography of kidney rat in second group showing decrease of renal glomeruli vasculature (G), normal glomerular capsular space (CP) and mild oedema of tubular epithelium (T).(H&E X400).

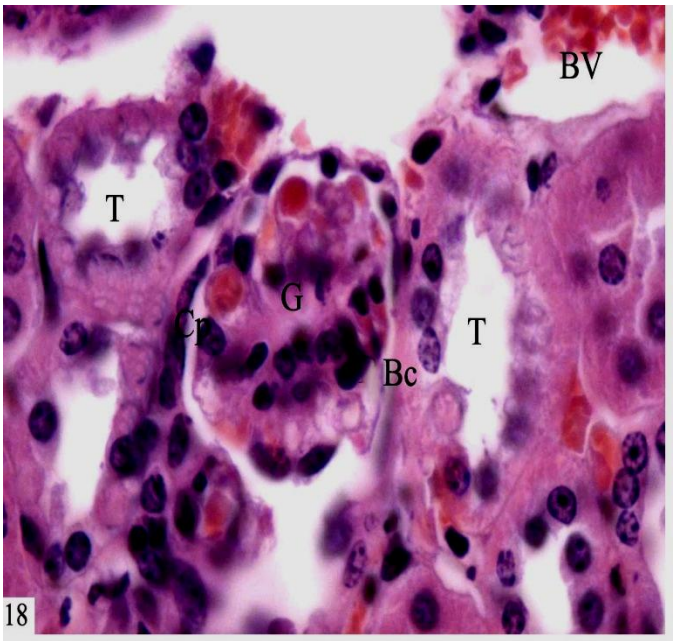

Fig (18): Photomicrography of kidney rat in second group showing decrease of renal glomeruli vasculature (G), normal glomerular capsular space (CP) and mild oedema of tubular epithelium (T). (H&E X1000).

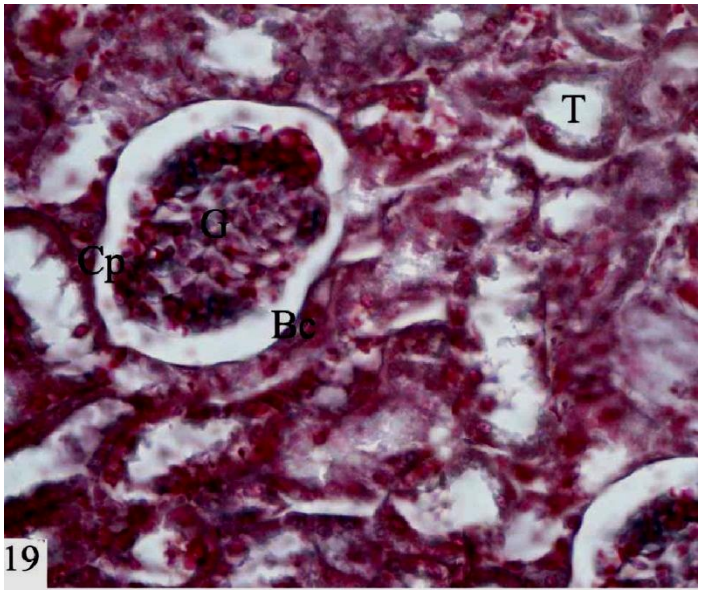

Fig (19): Photomicrography of kidney rat in second group showing mild fibrosis of renal glomeruli (G), normal appearance of glomerular capsular space (CP) and mild oedema of tubular epithelium (T). (Mallory X400).

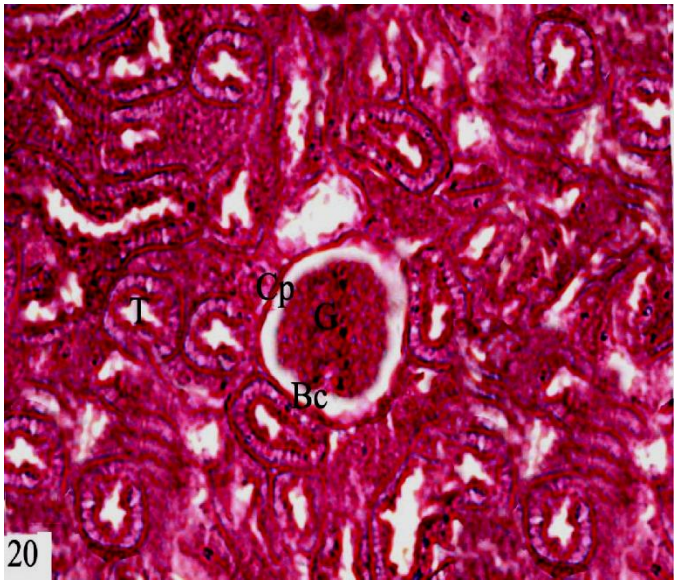

Fig (20): Photomicrography of kidney rat in second group showing positive reaction of PAS. Periodic acid-Schiff's X400).

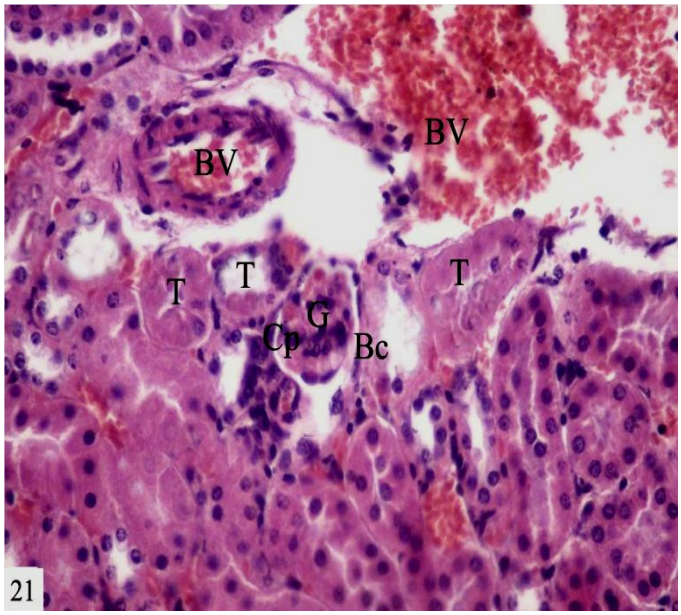

Fig (21): Photomicrography of kidney rat in third group showing severe decrease of vascular glomeruli size (G), tight filling the glomerular capsular space (CP), with degenerated epithelial lining the Bowman's capsule (BC), oedema and degeneration of some tubular epithelium cells (T). (H&E X400).

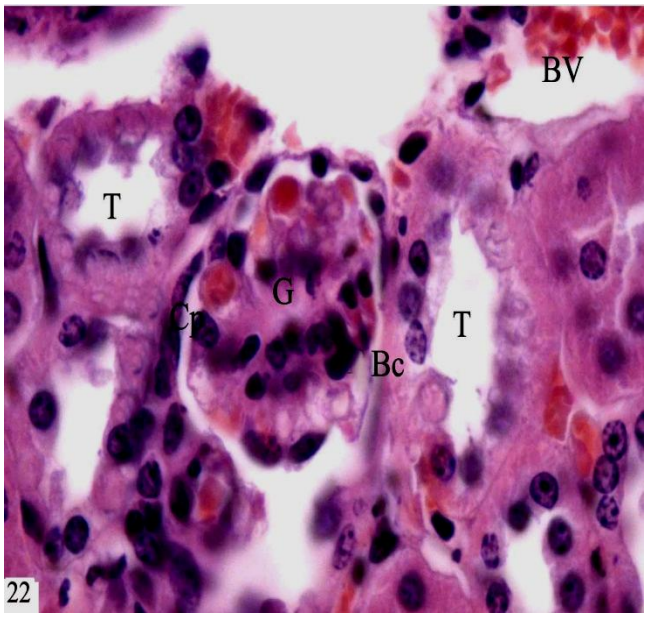

Fig (22): Photomicrography of kidney rat in third group showing severe decrease of vascular glomeruli size (G), tightfilling the glomerular capsular space (CP), with degenerated epithelial lining the Bowman's capsule (BC), oedema and degeneration of some tubular epithelium cells (T). (H&E X1000).

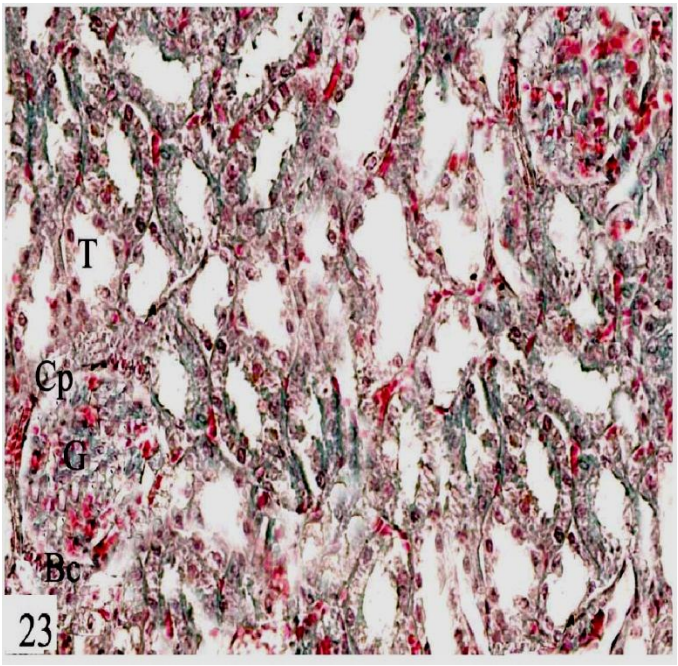

Fig (23):Photomicrography of kidney rat in third group showingfibrosis of vascular glomeruli (G), tight filling of glomerular capsular space (CP), with degenerated epithelial lining Bowman's capsule (BC), oedema and fibrosis of tubular epithelium cells (T).(Mallory X400)

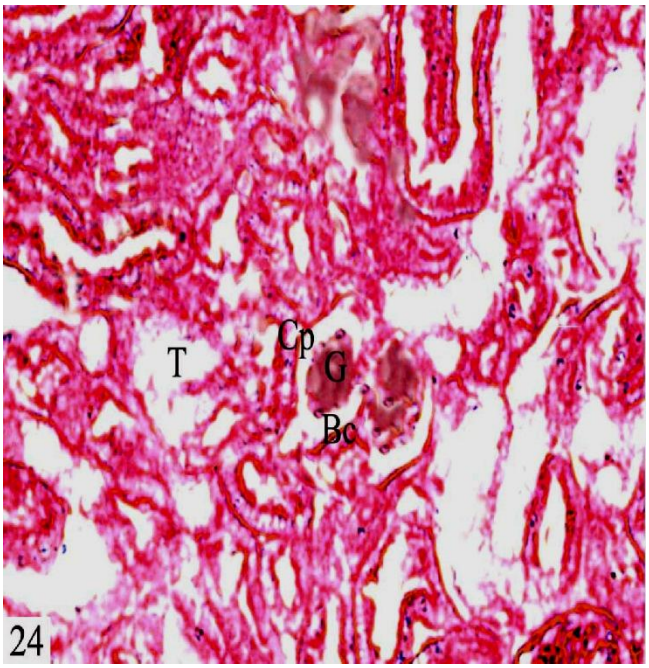

Fig (24): Photomicrography of kidney rat in third group showing marked decrease of positive reaction of PAS. (Periodic acid-Schiff's X400)

Discussion

Colchicine is a safe drug if its use for short periods and under recommended dose. It is treatment for chronic diseases such as gout and familial Mediterranean fever. We need to use it for prolonged period for these diseases. So the present study investigates its toxic effects on main organs which involved in its metabolism and excretion such as liver and kidney by biochemical and histological parameters.

The present study showed a statistical significant increase of liver enzymes such as ALT, AST and ALP in third group which received 3mg/kg/day of colchicine in comparison with the control group and the second group which received 2mg/kg/day. **Sherlock (1997)** referred that the use of liver function tests is associated with high specificity, especially when more than one test is abnormal and these results were in agreement with **Brncic et al., (2001).**

Toxic effect of colchicine in the liver reflects on histopathological changes such as distortion of hepatocytes, dilatation of liver central vein, and degeneration in the form of hepatocytes nuclei pyknosis, fibrosis and cytoplasmic vacuolation. These histopathological changes were consistent with biochemical results depending on the dose of colchicine. AST and ALT were increased as result of hepatic necrosis which induced by prolonged use of colchicine. Serum alkaline phosphatase is represented near the canalicular membrane of the hepatocyte. Accordingly, diseases that predominately affect hepatocyte secretion will be accompanied by elevations of alkaline phosphatase levels. This was consistent with **Marcelo et al., (1997)**

According to **(Nagesh et al., 2011),** Volume of distribution of colchicine is large. It is 2 to 3 L /kg. This is suggests rapid entry of the drug into tissues. Deacetylation of colchicine occurs in the liver with large amounts of the

drug and its metabolites undergoing enterohepatic circulation. As a result of the cytotoxic effect of colchicine on the liver, detoxification processes and other functions of the liver will be affected.

Colchicine induced Oxidative stress which was responsible for hepatocellular injury development and then increase of enzyme activity (ALT and AST). This was consistent with **Ozdemir et al., (2011)** and **Brncic et al., (2001**), who reported that colchicine is intracellular poison because it alters cellular division, intracellular transport, nuclear structure and cytoplasmic motility. It binds with tublin and prevents its polymerization into microtubules. But this was contrasted with **Klintschar et al., (1999)** who refereed that mechanism toxicity is unrelated to binding microtubules.

Results of the current study were contrast with **Mourelle et al.,** (**1988**), who showed that long-term colchicine treatment exerts an anti-inflammatory, anti-fibrotic and immunomodulatory effect in patients with hepatic fibrosis and then it reduces acute liver injury, inhibits collagen secretion and increases collagen degradation, thereby it reduces liver fibrosis.

The present study indicated that there is a statistical significant increase of serum urea and creatinine in third group which received 3mg/kg/day of colchicine in comparison to control group and second group which received 2 mg/g/kg/day of colchicine. This was consistent with **Zemer et al., (1991)**, who confirmed that renal failure develops a result of direct toxic effect of colchicine on the renal tubules.

Our study confirmed that prolonged use of colchicine with high different doses leads to disturbance of renal physiological function which depends on pathological condition of the kidney. According to **Zhang et al., (2004),** Increase of serum creatinine and urea due to functional capacity disturbance of tubular excretion, nuclear and mitochondrial protein function change. These changes results from oxidative stress generating reactive oxygen species induced by colchicine on the kidney depending on its dose.

The current study referred to renal histopathological changes after prolonged use of colchicine high doses. These pathological changes represent as degenerative changes of renal tubules and hypertrophy of glomeruli. These results were consistent with **Wagenaar (2004)**, who indicated that the toxic effect of colchicine depends on dose and duration of exposure and in agreement with **Nabila (2006)**, who refereed that double therapeutic dose of colchicine leads to histological changes in different organs such as spleen, liver and kidney.

Conclusion

Prolonged use of colchicine leads to hepatotoxicity and nephrotoxicity which represents as biochemical and histopatholgical changes in adult albino rats depending on its dose.

Recommendations

The results of this study may be significant to animal but we need further researches in human to investigate our results. We suggest further studies with other parameters of liver and kidney assessment such as serum albumin, serum bilirubin, prothrombin time, creatninine clearance, glomerular filtration rate and determination of oxidative stress parameters to complete this work.

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