Original Article

The Investigation of Methoprim in High Concentration Dose on Rats

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Abstract

Trimethoprim is a dihydropyrimidine antibiotic, antiparasitic, and antiparasitic agent Rash and pruritus are the side effects of trimethoprim that are most frequently reported. The digestive and haematopoietic systems were also engaged in other negative consequences that were described(5). After taking 1000 mg or more of trimethoprim, symptoms of acute overdose can include nausea, vomiting, headaches, dizziness, disorientation, mental depression, and bone marrow depression. 20 Healthy mature rats were used in this experiment. Their age was in the range between 3-5 months , and their weight was around 180±15g. They were divided randomly into four groups and five rats were kept in each cage. A significant increase in the AST enzyme level in the treated groups with 50,100 and 150 mg\kg b.wt. Respectively after 60 days of treatment compared to the control group. Also showed a significant increase in the ALT enzyme in the treated group with 50,100 and 150 mg\kg b.wt. after the passage of 60 days compared to the enzyme level for the control group in Table 1 revealed that there was significant (P≤0.05) increased in the ALP enzyme level in the treated groups with 100 and 150 mg\kg b.wt .(155.34 ±3.16and 188.31 ±4.91)IU\L respectively (80.33 ±2.03)IU\L compared with control group (85.43 ±3.32)IU\L after 60 days , The significant(P≤0.01). increase in blood urea level was found in the treated group with 100 and 150 mg\kg of b. wt.(36.38 ±1.51 and43.42 ±0.35) mg/dl after 60 days compared with the control and treated groups with 50 mg\kg of b. wt.(25.24 ±0.28and26.60 ±0.03) mg/dl.In the table NO.1, the results were showed significant(P≤0.01). increase in Creatinine concentration in the treated group with 150 mg\kg of b. wt.(1.34 ±0.09) mg/dl days compared with the other three groups The results of the determination of malondialdehyde showed a significant (P≤0.05) increased in its concentration of MDA in the treated group with50, 100and150 mg\kg b.wt. (2.90 ±0.34, 4.34 ±0.23and5.22 ±1.24) nmol\lrespectively for a period of 60 days compared with the control (1.19 ±0.28). The results showed a significant (P≤0.01) increased in the level of catalase enzyme in the group treated with concentration 150 mg\kg b.wt.(5.22 ±1.2 4) IU\L duration of 60 days compared with the control group(2.12±1.07) IU\L. while three was no significant difference in the level of catalase in treated group with 50 and 100 mg\kg b.wt.(3.21 ±0.42 and 3.09 ±0.35) compared with control group. On the other hand, the results showed a significant increase in the level of the SOD enzyme in the two treated groups 100 and 150 mg\kg b.wt (4.03 ±0.93 and 4.43 ±0.62) IU\L for a period of 60 days compared to the control group(1.23 ±0.09)IU\L .The results did not show a significant difference in the level of SOD enzyme in the treated group with 50 mg\kg (2.89 ±0.26) IU\L as compared with control group (1.23 ±0.09) IU\L .

Keywords: Artemisia vulgaris, Cisplatin, Hepatoprotective, hepatotoxicity, herbs.

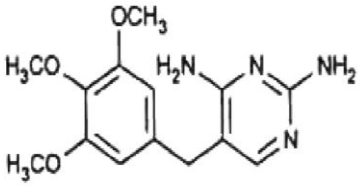
1. Introduction

Trimethoprim is a dihydropyrimidine antibiotic, antiparasitic, and antiparasitic agent. It belongs to a prototype class of non-sulfonamide medicines that inhibit bacteria and protozoa from making dihydrofolate reductase. Despite being developed as an antimalarial drug, it is currently predominantly utilized as an antibacterial drug, particularly when combined with sulfamethoxazole. Dihydrofolate reductase from bacteria and parasites is specifically inhibited by trimethoprim. Patients with normal nutrition who take trimethoprim at the recommended therapeutic levels rarely encounter toxicities. However, those who consume insufficient amounts of folate or who receive prolonged medication treatment may develop megaloblastic anemia(1).

Human were initially treated with trimethoprim (TMP) in 1962 [2], and it was approved for clinical usage in 1968 when combined with sulfonamides. In Finland, TMP was first used as a prophylactic treatment for urinary tract infections in 1972. It was then made available as a treatment for individuals with acute urinary tract infections in 1979. TMP is less likely to have negative effects than sulfonamides. Particularly among AIDS patients, rashes and other hypersensitivity responses have been recorded. Drug-induced aseptic meningitis is one of the most significant causes of other, more uncommon adverse effects [3]. patients with Sjögren's syndrome are more likely to experience allergic reactions to these antimicrobial agents as well as TMP-induced nonallergic systemic reactions (such as CNS irritation) than patients with osteoarthrosis [4]; patients with other rheumatic disorders and HIV-infected patients may also experience more of these reactions.

Trimethoprim may interfere with the metabolism of folic acid, and animal studies have demonstrated that giving trimethoprim at very high dosages during organ development may result in birth abnormalities resembling those caused by folic acid antagonists. It may be necessary to take folic acid supplements if trimethoprim is administered during pregnancy.

Rash and pruritus are the side effects of trimethoprim that are most frequently reported. The digestive and haematopoietic systems were also engaged in other negative consequences that were described. (5) - After taking 1000 mg or more of trimethoprim, symptoms of acute overdose can include nausea, vomiting, headaches, dizziness, disorientation, mental depression, and bone marrow depression. (6)Trimethoprim inhibits bacterial dihydrofolate reductase (DHFR), an enzyme involved in the folate metabolic pathway that converts dihydrofolate to tetrahydrofolate, in a reversible manner. The effect could be, depending on the experimental setup(7). The chemical structure of trimethoprim (8)



In the event that a patient is thought to have trimethoprim/sulfamethazine toxicity, a treatment plan includes the administration of activated charcoal, gastric lavage, and supportive intravenous (IV) and oral fluids. Hemodialysis and alkalizing the patient's urine are the most severe therapy options(9).

2. MATERIALS AND METHODS

2.1 Animal and Grouping

Twenty Healthy mature rats were used in this experiment. Their age was in the range between 3-5 months, and their weight was around 180±15g. They were divided randomly into four groups and five rats were kept in each cage. Water and diet were freely available for the rats and kept under suitable environmental conditions of 20-25°C.

The rats were divided as follow:

G1: This healthy group was considered (negative control).

G2: This group was given concentration 50 mg\kg of methoprim

G3: This group was given concentration 100 mg\kg of methoprim

G4: This group was given concentration 150 mg\kg of methoprim

Three treated groups were administrated orally by gavage tube for 60 days.

Blood collection

The rats being anesthetized with chloroform, then the blood samples were taken from all groups. A 5 ml syringe and a 23 mm gauge needle were used to make a cardiac puncture, which allowed for the direct collection of about 5 ml of blood from the heart. Sera were collected in sterile tubes and centrifuged for 15 minutes at 3000 rpm after blood in sterile plastic test tubes without anticoagulant was collected, then it kept in deep freeze at -18˚C for study biochemical tests .included ALT (Rndox\British),AST (Rndox\British),ALP(Biolabo\Franc), B.Urea and Creatinine were measured by enzymatic and colorimetric methods (Spinreact\Spain),, MDA,. (Fluka, Switzerland), SOD(Abcam, UK), and CAT(Bioassay System, USA).

Table 1: Comparison of several liver enzyme groups (ALT,AST,ALP) and Kidney functions (Blood urea Creatinine and)

Group	Mean± SE				
	AST U\L	ALT U\L	ALP U\L	B. Urea mg\dl	Creatinine mg\dl
Control	42.12 ±2.1 3 c	29.32 ±2.45 b	85.43 ±3.32 c	25.24 ±0.28 c	0.67 ±0.04 b
50mg\kg	55.3 2 ±3.81 b	46.35 ±2.3 0 a	99.37 ±7.14 bc	26.60 ±0.03 c	0.93 ±0.32 ab
100 mg\kg	69.07 ±2.12 a	50.4 4 ±3.3 2 a	155.34 ±3.16 b	36.38 ±1.51 b	1.12 ±0.19 ab
150 mg\kg	78.3 7 ±2.68 a	54.3 2 ±3.25 a	188.31 ±4.91 a	43.42 ±0.35 a	1.34 ±0.09 a
LSD value	11.89 **	13.27 **	27.85 **	2.57 **	0.599 *
P-value	0.0001	0.010	0.0014	0.0001	0.0442
Means having the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).					

In Table No. 1 the results showed a significant increase in the AST enzyme level in the treated groups with50,100 and 150 mg\kg b.wt.(55.3 2 ±3.81, 69.07 ±2.12and 78.3 7 ±2.68) IU/L respectively after 60 days of treatment compared to the control group (42.12 ±2.1 3) IU/L . The results in the above table also showed a significant increase in the ALT enzyme in the treated group with50,100 and 150 mg\kg b.wt. (46.35 ±2.3 0, 50.4 4 ±3.3 2and54.3 2 ±3.25) IU/L after the passage of 60 days compared to the enzyme level for the control group (29.32 ±2.45) IU/L.

The results in Table 1 revealed that there was significant (P≤0.05) increased in the ALP enzyme level in the treated groups with 100 and 150 mg\kg b.wt .(155.34 ±3.16and 188.31 ±4.91)IU\L respectively (80.33 ±2.03)IU\L compared with control group (85.43 ±3.32)IU\L after 60 days ,

The significant(P≤0.01). increase in blood urea level was found in the treated group with 100 and 150 mg\kg of b. wt.(36.38 ±1.51 and43.42 ±0.35) mg/dl after 60 days compared with the control and treated groups with 50 mg\kg of b. wt.(25.24 ±0.28and26.60 ±0.03) mg/dl

In the table NO.1, the results were showed significant(P≤0.01). increase in Creatinine concentration in the treated group with 150 mg\kg of b. wt.(1.34 ±0.09) mg/dl days compared with the other three groups .

Table 2: Comparison between difference groups in MDA and anti-Oxidants enzymes

Group	Mean ± SE		
	MDA	CAT	SOD
Control	1.19 ±0.28 c	2.12 ±1.07 b	1.23 ±0.09 c
50 mg\kg	2.90 ±0.34 b	3.21 ±0.42 ab	2.89 ±0.26 bc
100 mg\kg	4.34 ±0.23 ab	3.09 ±0.35 ab	4.03 ±0.93 ab
150 mg\kg	5.22 ±1.24 a	4.28 ±0.75 a	4.43 ±0.62 a
LSD value	0.975 **	2.576 *	1.40 **
P-value	0.0007	0.049	0.0092
Means having with the different letters in same column differed significantly. * (P≤0.05), ** (P≤0.01).			

The results of the determination of malondialdehyde showed a significant (P≤0.05) increased in its concentration of MDA in the treated group with50, 100and150

mg\kg b.wt. (2.90 ±0.34, 4.34 ±0.23and5.22 ±1.24) nmol\lrespectively for a period of 60 days compared with the control (1.19 ±0.28).

The results showed a significant (P≤0.01) increased in the level of catalase enzyme in the group treated with concentration 150 mg\kg b.wt.(5.22 ±1.2 4) IU\L duration of 60 days compared with the control group(2.12±1.07) IU\L. while three was no significant difference in the level of catalase in treated group with 50 and 100 mg\kg b.wt.(3.21 ±0.42 and 3.09 ±0.35) compared with control group .

On the other hand, the results showed a significant increase in the level of the SOD enzyme in the two treated groups 100 and 150 mg\kg b.wt (4.03 ±0.93 and 4.43 ±0.62) IU\L for a period of 60 days compared to the control group(1.23 ±0.09)IU\L .

The results did not show a significant difference in the level of SOD enzyme in the treated group with 50 mg\kg (2.89 ±0.26) IU\L as compared with control group (1.23 ±0.09) IU\L

The results in table 2 obtained no significant differences in the concentration of .

2.2 Statistical Analysis

To identify the impact of various factors on study parameters, the Statistical Analysis System- SAS (2012) application was employed. In this study, a significant comparison of means was made using the least significant difference (LSD) test (ANOVA).

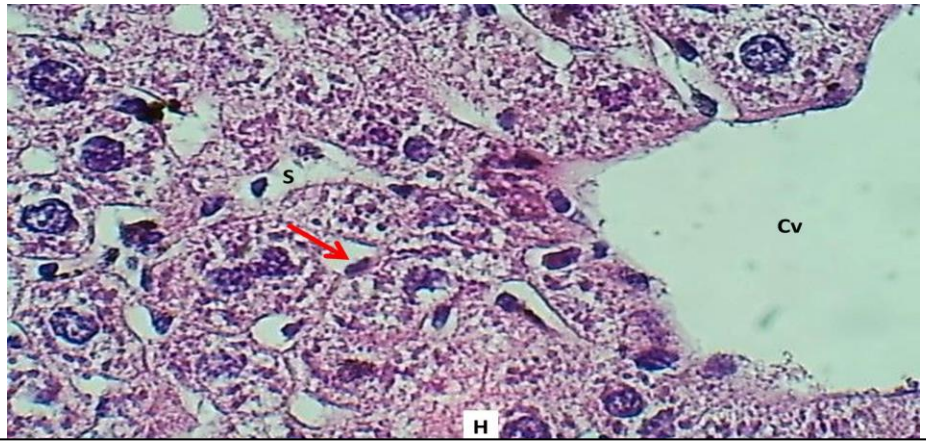


Figure1: Section of liver (control) shows: normal appearance of hepatocytes (H), sinusoid (S) & kupffer cells (arrows). H&E stain. 400x.

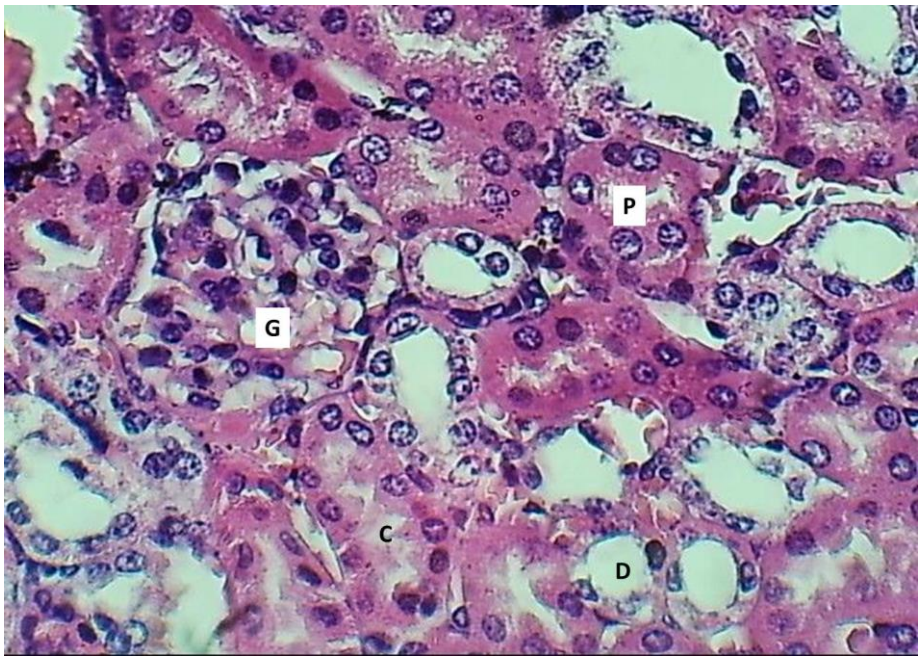
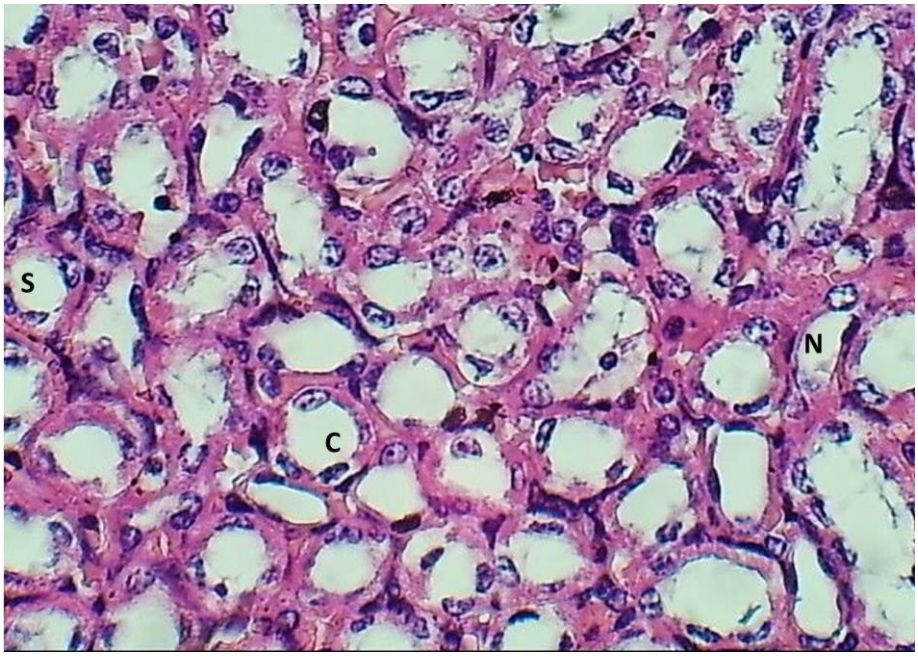


Figure2: Section of renal cortex (control) shows: normal appearance of glomerulus (G), collecting tubules (C,), proximal (P) & distal (D) convoluted tubule. H&E stain.400x.



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fatty degeneration within the cytoplasm of the hepatocyte (Black arrows) &
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Figure3: Section of renal medulla (control) shows: normal appearance of collecting tubules (C,), thick segment (S) & thin segment (N) of loop of henle. H&E stain.400x.

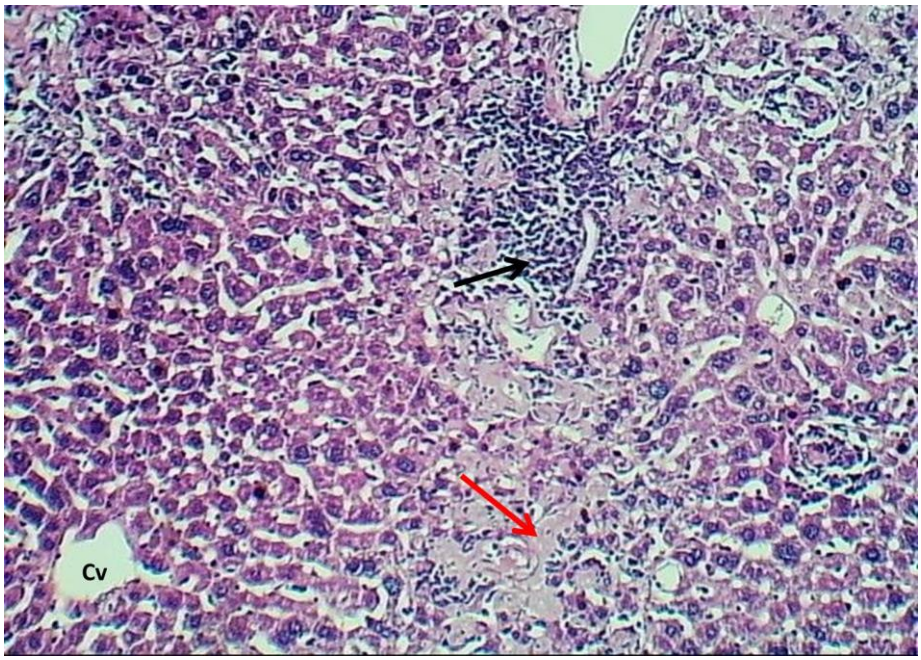

Figure4: Section of liver (50 mg\kg) shows: inflammatory infiltrate (Black arrow). moderate zonal degenerative changes (Red arrow), H&E stain.100x.

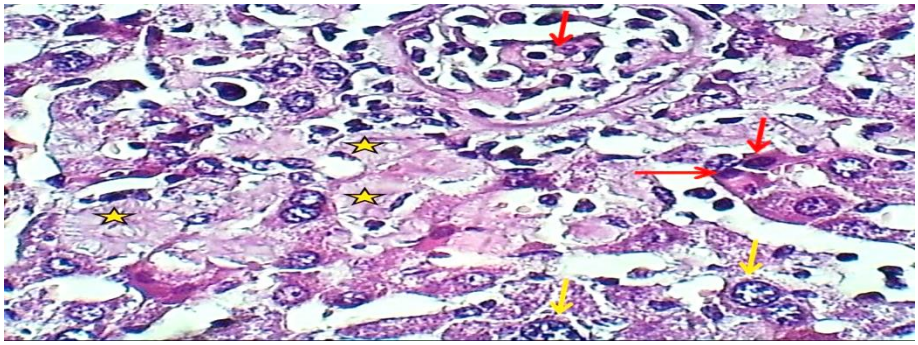

Figure5: Section of liver (100 mg\kg) shows:, apoptosis (Red arrows) and sinusoidal mononuclear leukocytes moderate hyalinization of hepatocytes, nuclear hypertrophy (Yellow arrows) H&E stain.400x.

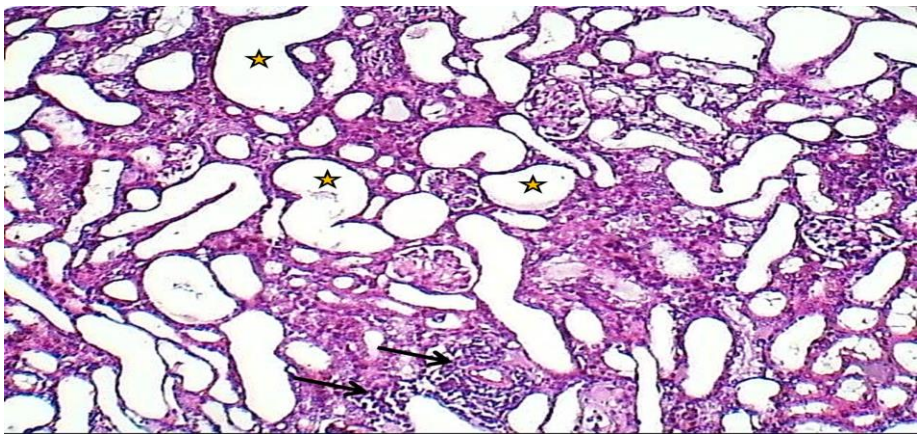

Figure6: Section of renal cortex (100 mg\kg) shows: sever tubulo nephrosis characterized by sever tubular dilation, and focal infiltration of mononuclear leukocytes H&E stain.40x.

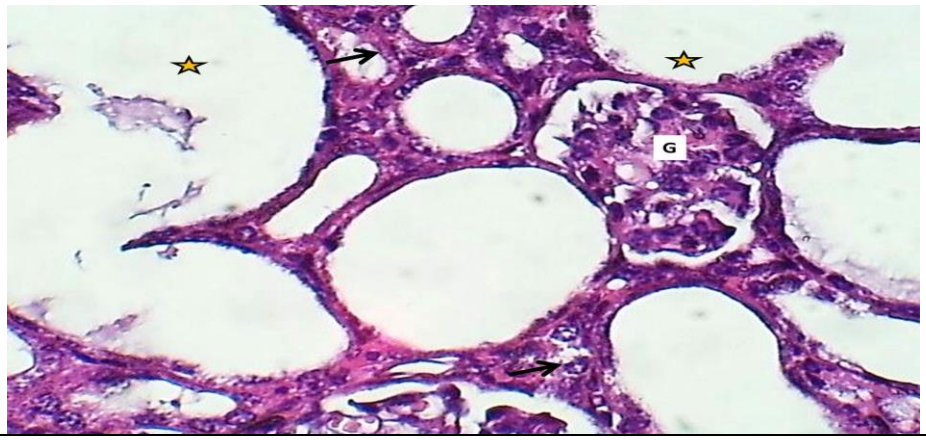

Figure7: Section of renal cortex (150mg\kg) shows: sever tubular dilation and atrophy of remnant tubules (Arrows) and glomeruli (G). H&E stain.100x.

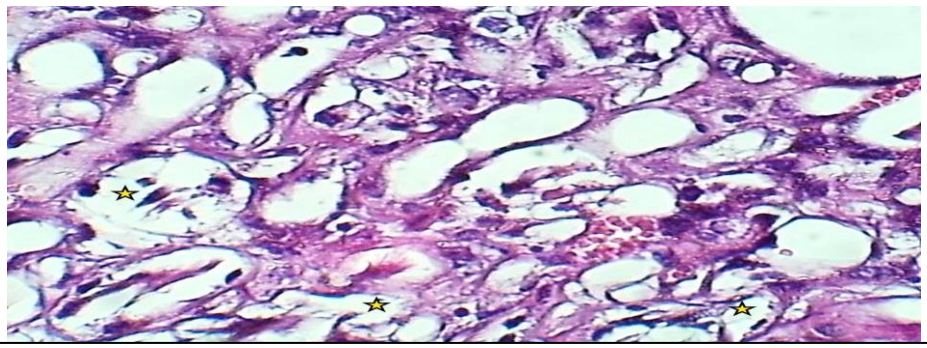

Figure8: Section of renal medulla (100mg\kg) shows: sever vacuolar degeneration of collecting tubules atrophy of remnant tubules and marked desquamation. H&E stain.400x.

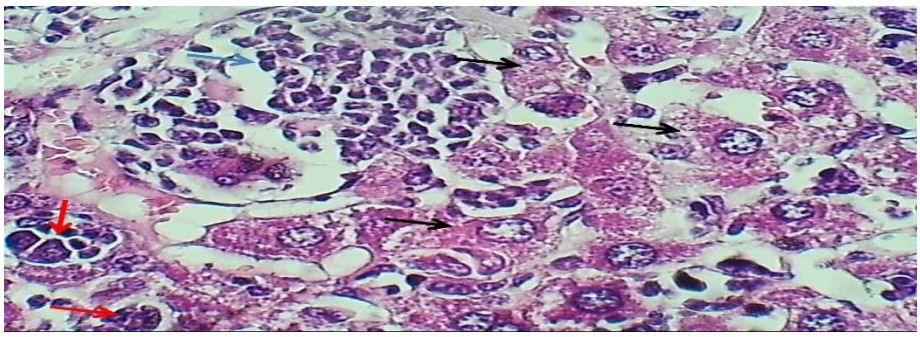

Figure9: Section of liver (150mg\kg) shows: sever degeneration and necrosis of hepatocytes (Black arrows), necrotic tissue occupied with polymorphic nuclear leukocytes (Blue arrows) & giant cells formation (Red arrows). H&E stain.400x.

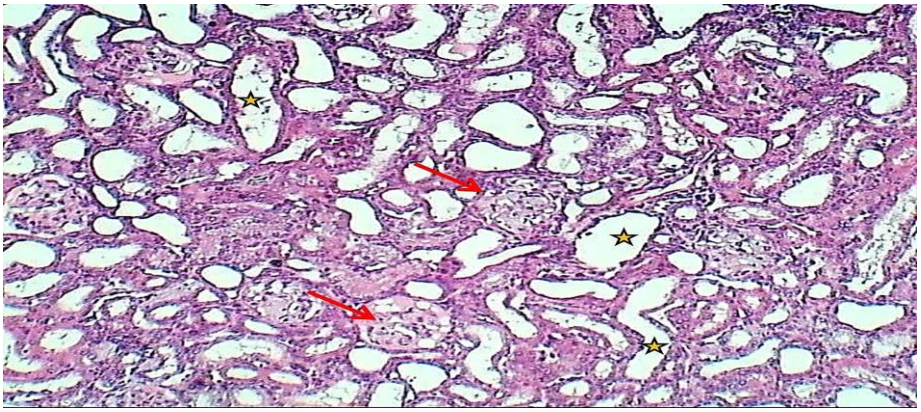

Figure 10: Section of renal cortex (100mg\kg) shows: moderate membranous glomerulonephritis (Arrows) and tubular dilation (asterisks) &E stain.100x.

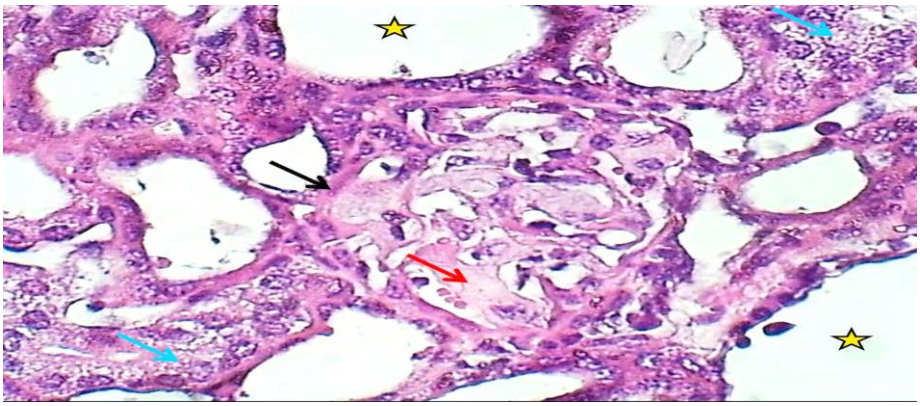

Figure 11: Section of renal cortex (150mg\kg) shows: thickening of glomerular capsule (Black arrow), amyloid deposition (Red arrows) dilation of collecting tubules (Asterisks) & vacuolar degeneration of other tubular parts of nephron (Blue arrows). H&E stain.400x.

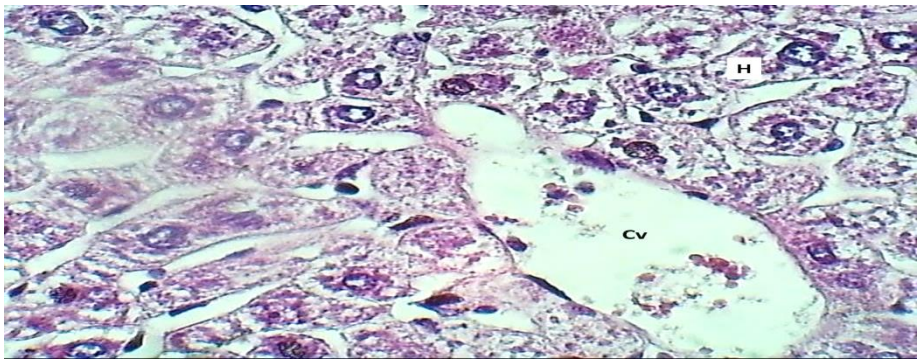

Figure 12: Section of liver (50mg\kg) shows: normal central vein (Cv), mild hypertrophy of hepatocytes (H). H&E stain.400x.

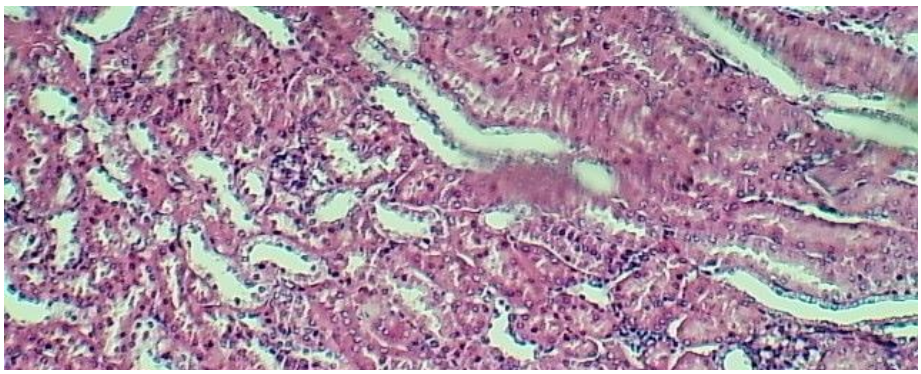

Figure 13: Section of renal cortex (50mg\kg) shows: normal appearance of glomeruli & renal tubule. H&E stain.100x.

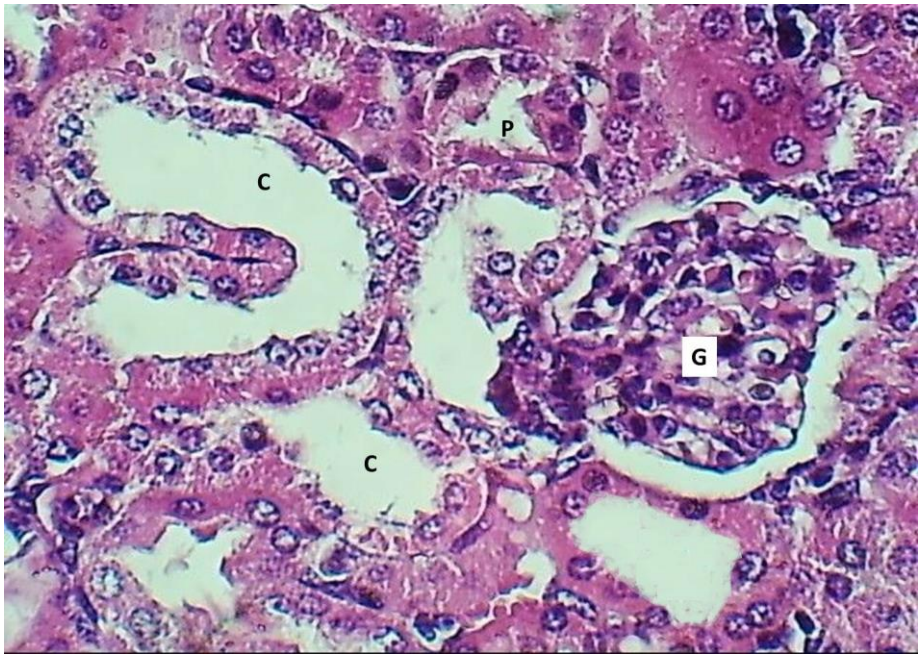

Figure 14: Section of renal cortex (50mg\kg) shows: normal glomerulus (G), collecting tubule (C,) & proximal tubules (P). H&E stain.400x.

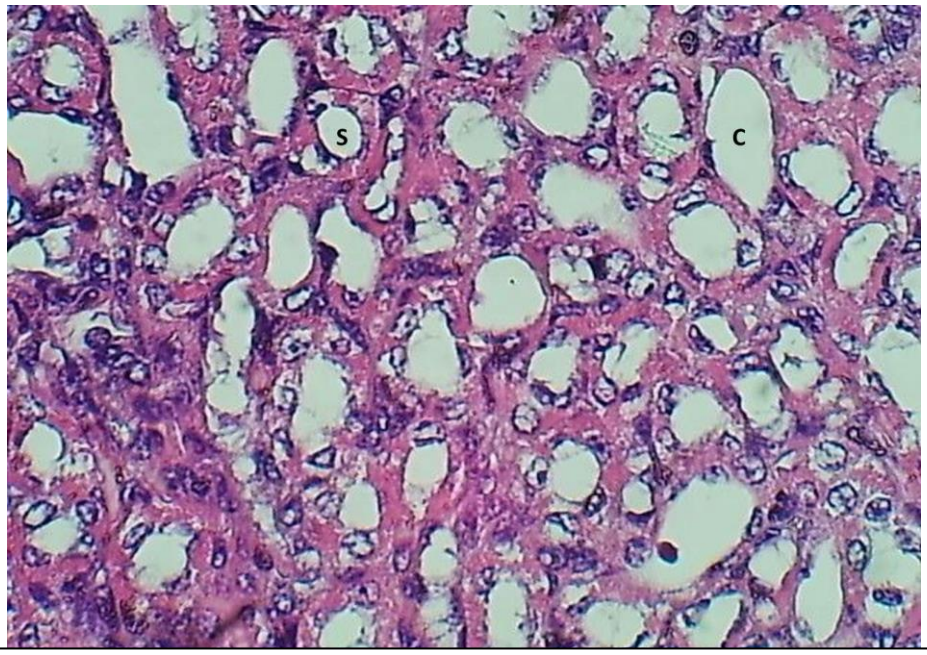

Figure15: Section of renal medulla (50mg\kg) shows: normal collecting tubule (C,) & segment of loop of Henle (S). H&E stain.400x.

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