Heamatobiochemical and histopathological studies on the effects of vitamin C on experimentally- exposed albino rat to chronic copper toxicity.

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Abstract

The present study aimed to investigate the main effects as well as the interaction effect of supplemental Cu and Vit. C on Heamatobiochemical and histopathological on albino rat. Thirty female Albino rats weighing about 80-100g were used, randomly divided into three equal groups, group (1) considered as a control group. Group (2) received copper sulphate 1g/kg diet, group (3) received copper sulphate 1g/kg diet and Vit. C 1g/1 Litter drinking water. All groups were daily treated for five month. The hematological parameters after 2 months characterized by erythrocytosis, thrombocytosis and leuckocytopenia in copper sulphate treated groups but after five months erythrocytosis with microcytosis and hypochromacia, leuckocytopenia and thrombocytopenia in copper sulphate treated groups with amelioration in Vit. C treated group. Serum biochemical analysis after 2 and 5 months. The AST were significantly increased in the Cu group and Cu + Vit. C group as compared with control group. Cholesterol was significantly decreased in the Cu group and Cu +Vit. C group as compared with control group. Total bilirubin and indirect billirubin was significantly increased in Cu group as a compared with control group and Cu + Vit. C group.

Histopathological changes of spleen of Cu intoxicated rats showed focal necrosis, presence of hemosiderin. Liver showed degeneration and necrosis at parenchymal tissues. The kidney showed damage in the glomerulus and renal tubule. Concomitant administration of Vit. C to Cu-intoxicated rats resulted in mild pathological changes compared to their counterparts exposed to Cu alone. Therefore, the study concluded that the use of vitamin C as a companion to copper sulphate, which is used in some poultry and fish farms as an anti - fungal or as growth promoter that, Vit. C has a protective effect for the effects of toxic incident of copper after the second month and also has a good effect in the case of chronic poisoning for a period of nearly five months. Therefore, our findings suggest a profound ameliorative effect of Vit. C on $CuSO_4$ induced adverse alterations of rats.

Key words:

Copper toxicity, Vit. C, Histopathology, Hematology, Biochemistry.

Introduction

Chronic copper toxicosis is likely to be of greater concern than the acute syndrome and is characterized by a gradual hepatic accumulation of copper that results eventually in liver damage, and in extreme cases in death **(Bremner, 1998; Bjorn *et al.*, 2003)**.The main brunt of $CuSO_4$ is brone in the order by eryth-

rocytes, liver and then kidneys leading to methemoglobinemia, hepatotoxicity and renal failure **(Blundell *et al.,* 2003; Galhardi *et al.,* 2004).**

Vit. C is an important dietary antioxidant, it significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen species that can cause oxidative damage to macromolecules such as lipids, DNA and proteins which are implicated in chronic diseases including cardiovascular disease, stroke, cancer, neurodegenerative diseases and cataractogenesis **(Halliwell and Gutteridge, 1999; Mecll and Balz, 1999).** But copper and vit. C are known to be a prooxidant couple in vitro **(Clopton and Saltman, 1997; Buettner and Jurkiewicz, 1996).** Thus, the aims of the current study to investigate the effects of Vit. C if exacerbate or ameliorate the effect of chronic toxicity of copper through Heamatobiochemical and histopathological investigations.

Materials and methods

1. Chemicals.

Cupric sulfate pentahydrate ($CuSO_4$-$5H_2O$) and Vit. C (ascorbic acid) 20% were obtained from the Elgmhoria co.

2. Animals.

Thirty, clinically healthy, female Albino rats weighing about 80-100g were used throughout this study. Animals were housed in clean metal cages, fed on standard pellet diet and water adlibtium. They were quarantined for 1week, prior to the experiments to acclimatize them.

3. Experimental Design.

Rats were randomly divided into three equal groups (10 rats each) according weight as follows:

Group (1) considered as a control group.

Group (2) received copper sulfate 1g/kg diet (**Elsaid *et al.,* 2005**).

Group (3) received copper sulfate 1g/kg diet and Vit. C 1g/1 Litter drinking water. Groups were daily treated for a five month. Five animals of each group were randomly selected for blood sampling and decapitated for pathological examination after 2 and 5 months from the start of experiment.

4. Hematological Examination.

Blood samples were taken from the retro-orbital venous plexus of rats. The two blood samples were collected one on EDTA for hematological analysis and other for separate serum for biochemical analysis. RBC according to **Feldman *et al.,* (2000).** Hematocrit (Hct), according to **Jain, (1993),** Hb according to **Van Kampan & Zijlistra, (1961),** The values of Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), and Mean Corpuscular Hemoglobin concentration (MCH-C) were calculated by standard formula according to **Dacie and Lewis, (1977).** The prepared fixed thin blood films were stained with Diff & Quick stain. The film then examined for morphological changes in RBCs and WBCs according to **Thrall *et al.,* (2004).** The percentage values of differential leukocytic count were then calculated according to **Jain, (1993).**

5. Serum Biochemical Studies.

Serum total protein, albumin, aspertate aminotransferase (AST), alanine aminotransferase (ALT), Serum alkaline phosphatase, (Alk. P). Serum Total Billirubin according to **Doumas *et al.,* (1981).**, Cholesterol as mentioned by **Fossati and Medici, (1987).** Creatinine according to **Husdant and Rapaport, (1958)** and urea were analyzed according to **Patton and Crouch, (1977),** using commercial kits (Diamond Diagnostics Company, Egypt). Lipid peroxidation in liver tissue (LPO) was measured by the method of **Buege and Aust, (1978).** Copper in liver tissue was measured according to the method described by **Mehmet and Akdeniz, (2004).**

6. Pathological examination.

Tissue samples were collected twice, the first after the second months of experiment and the second after fifth months.

Postmortem examination was done and samples from kidneys, liver and spleen were taken. These samples were fixed in formalin 10% for at least 24-72hrs, then washed with water, dehydrated, cleared, and embedded in paraffin wax. Then, the paraffin embedded tissues were blocked and underwent microtomay. Sections

of 5-7 microns were stained with Heamatoxylin and Eosin, according to **Bancrofts *et al.*, (1996).**

7. Statistical analysis.

Data were computer analyzed using SPSS version 16.0 for windows (Statistical Package for the Social Sciences Inc, Chicago, Illinois). One way analysis of variance (One-way ANOVA) was used to determine differences among means of investigated groups. The differences were considered to be statistically significant at $P<0.05$. Data were analyzed by computer using SPSS version 16.0 for windows. (Statistical Package for the Social Sciences Inc, Chicago, Illinois).

Results

Hematological and biochemical Examination.

The hematological parameters after 2 months in this study are presented in (Table 1). RBCs count, hemoglobin and PCV significantly increased in the Cu group, and Cu + Vit. C group as compared with control. No significant differences between Cu group and Cu + Vit. C group were observed. While, WBCs count significantly ($p < 0.05$) decreased in Cu group and Cu + Vit. C group as compared with control. In addition, WBCs count in Cu group showed significantly decrease as compared with Cu+Vit. C group. While platelets count increased in Cu group and Cu + Vit. C group as compared with control. In addition, platelets in Cu group showed significantly increase as compared with Cu + Vit. C group.

Serum biochemical analysis after 2 months is presented in (Table 2.) The AST were significantly increased in the Cu group and Cu + Vit. C group as compared with control. Total protein significantly decreases in Cu + Vit. C group as a compared with control and Cu group, while cholesterol and creatinine were significantly decreased in the Cu group and Cu + Vit. C group as compared with control. LPO was significantly decreased in Cu group as compared with control group and Cu + Vit. C group.

After 5 months in this study, the hematological parameter are presented in (Table 3). RBCs count showed significantly increased in the Cu group and Cu +Vit. C group as compared with control and without significantly differences between Cu group and Cu + Vit. C group. Hemoglobin, MCV, MCH and MCHC significantly decreased in the Cu group as a compared with control and Cu + Vit. C group except MCHC parameter no significantly differences between Cu group and Cu + Vit. C. While WBCs count, platelets count significantly decreased in Cu group and Cu + Vit. C group as compared with control. But WBCs showed significantly increased in Cu group as compared with Cu + Vit. C. Differential leukocytic count result showed no significant difference between groups.

Serum biochemical analysis after 5 months is presented in (Table 4.) AST was significantly increased in the Cu group and Cu +Vit. C group as compared with control group. But ALT, ALP were significantly decreased in the Cu group and Cu +Vit. C group as compared with control group. Total protein significantly increase in Cu group and Cu +Vit. C group as a compared with control group but Cu group significantly decrease than Cu + Vit C group, while cholesterol and creatinine were significantly decreased in the Cu group and Cu +Vit. C group as compared with control group but Cu group significantly increased than Cu + Vit. C group, urea no significant differences between Cu group and control, but significantly decreased in Cu + Vit. C group as a compared with control group and Cu group. Total bilirubin and indirect bilirubin was significantly increased in Cu group as a compared with control group and Cu + Vit. C group. LPO was significantly decreased in Cu group as compared with Cu +Vit. C group. Copper level in liver tissue of Cu group was highly significantly increased as compared with control group and Cu + Vit. C group.

Table (1). Hematological parameters (mean values ± S.D) of 5 rats in each group after two months from start of experiment.

	Control group	CU Group	CU + VIT C Group
RBCs (10^6/µl)	6.05 ±0.287	7.5±0.332^{A}	7.05±0.183^{A}
HB (mg/dl)	12.8±0.255	14.5±0.474^{A}	13.86±0.607^{A}
PCV %	37.98±1.702	44.74±1.126^{A}	43.9±1.418^{A}
MCV (fl)	62.8±1.812	60.04±1.198	62.27±1.465
MCH (pg)	21.1±0.752	19.4±0.611	19.6±0.594
MCHC %	33.18±1.997	31.852±0.995^{A}	32.27±0.290^{A}
WBCs (10^3/µl)	9.1±0.940	5.4±0.437^{A}	6.6±0.4 64^{A}
Platelets (10^3/µl)	1284±100.2	2089±318.7^{Aa}	1682±222.7^{A}

A $p<0.05$ (cu) (cu+vit) group compared with control.
a $p< 0.05$ (cu) group compared with (cu+vit) group
The mean difference is significant at 0.05 level.

Table (2). Biochemical parameters (mean values ± S.D) of 5 rats in each group after two months from start of experiment.

	Control group	(CU) Group	(CU+VIT C) Group
S.AST u/l	153±16.8	257.2±61^{A}	241.2±39.4^{A}
S.ALT u/l	62.4±2.4	61.6±12.7	58±4.2
S.Alp u/l	509.8±47.6	473.6±101	572±169
Total bilirubin mg/dl	0.2±0.06	o.2±0.06	0.2±0.05
Conjugated b.	0.1±0.01	0.1±0.02	0.1±0.01
Uncongugated b.	0.1±0.01	0.1±0.01	0.1±0.01
Cholesterol mg/dl	122.8±1.9	90.6±7.8^{A}	87±13.6^{A}
Total protein g/dl	7.7±0.15	7.8 ±0.3^{a}	7.3±0.15^{aA}
Albumin	4.2±0.17	4.2±0.19	4±0.12
Globulin	3.5±0.06	3.6±0.5	3.4±0.06
A/G ratio	1.2±0.1	1.2±0.3	1.2±0.06
Urea mg/dl	55.4±1.1	59.6±4.6	57.5±5
Creatinine mg/dl	1.12±0.08	0.72±0.15^{A}	0.87±0.07^{A}
Lipidperoxidase in liver tissue mmol/mg protein	0.254±0.012	0.181±0.005^{Aa}	0.207±0.01^{A}

A $p< 0.05$ (cu) (cu+vit) group compared with control.
a $p< 0.05$ (cu) group compared with (cu+vit) group
The mean differences is significant at 0.05 level.

Table (3). Hematological parameters (mean values ± S.D) of 5 rats in each group after five months from start of experiment.

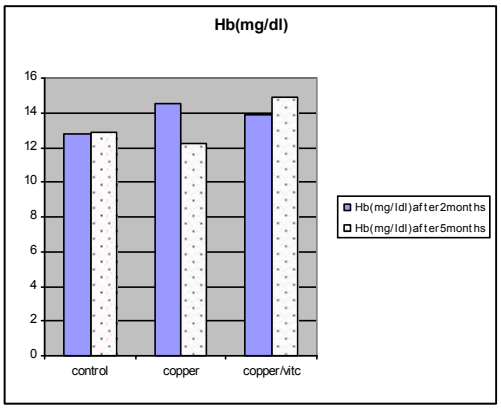
	Control group	CU Group	CU+ VIT. C Group
RBCs (10^6/µl)	6.05±0.3	$7±0.5^{A}$	$7.3±0.19^{A}$
HB (mg/dl)	12.86±0.32	$12.23±0.19^{Aa}$	$14.86±0.69^{A}$
PCV %	38.88±1.6	$39.31±0.68^{a}$	$48.28±2.06^{A}$
MCV (fl)	64±0.47	$56±3.8^{Aa}$	66±2.8
MCH (pg)	21.3±0.8	$17.5±1.2^{A}$	$21±1.2^{a}$
MCHC %	33±1.3	$31±0.4^{A}$	$30.8±0.9^{A}$
WBCs (10^3/µl)	9.6±0.7	$6±0.4^{A}$	$5.8±0.5^{A}$
Platelets (10^3/µl)	1275±101	$1183±100^{a}$	$542±16.9^{A}$

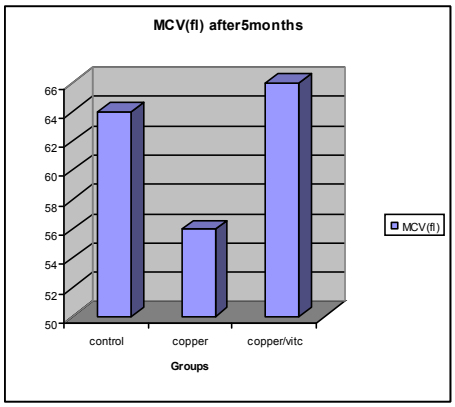
A p< 0.05 (cu) (cu+vit) group compared with control.
a p< 0.05 (cu) group compared with (cu+vit) group
The mean differences is significant at 0.05 level.

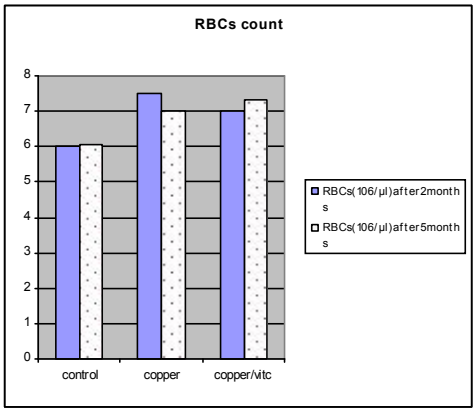
Table (4): Biochemical parameters (mean values ± SD) of 5 rats in each group after five months from start of experiment.

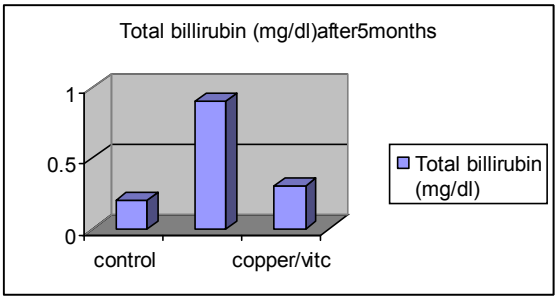
	Control group	(CU) Group	(CU+VIT C) Group
S.AST u/l	124±13.6	$153.4±7.2^{A}$	$156.4±15.5^{A}$
S.ALT u/l	40.4±11.5	$29.3±1.9^{A}$	$30±1.6^{A}$
S.Alp u/l	370.4±125	$166.2±16^{A}$	$125.2±7.3^{A}$
Total bilirubin mg/dl	0.2±0.06	$0.9±0.4^{Aa}$	0.3±0.06
Conjugated b.	0.15±0.05	0.13±0.01	0.13±0.01
Uncongugated b.	0.12±0.02	$0.82±0.6^{Aa}$	0.23±0.05
Cholesterol	121.4±2.3	$1o7.2±10.2^{Aa}$	$94.2±3.6^{A}$
Total protein g/dl	8.6±0.7	$9.6±0.2^{Aa}$	$10.4±0.6^{A}$
Albumin	4.5±0.25	$5.8±0.13^{A}$	$5.24±0.3^{A}$
Globulin	4.14±0.5	$4.5±0.3^{a}$	$5.2±0.6^{A}$
A/G ratio	1.2±0.3	1.1±0.1	1.01±0.1
Urea mg/dl	64.2±4.3	$60.1±1.6^{a}$	$50.8±6.9^{A}$
Creatinine mg/dl	1.2±0.2	$1±0.06^{Aa}$	$0.8±0.07^{A}$
Lipid peroxidase in liver tissue mmol/mg protein	0.258±0.004	$0.261±0.003^{a}$	$0.227±0.006^{A}$
Copper in liver tissue µg/L	6.8±0.26	$164.5±4.9^{Aa}$	$24.07±1.4^{A}$

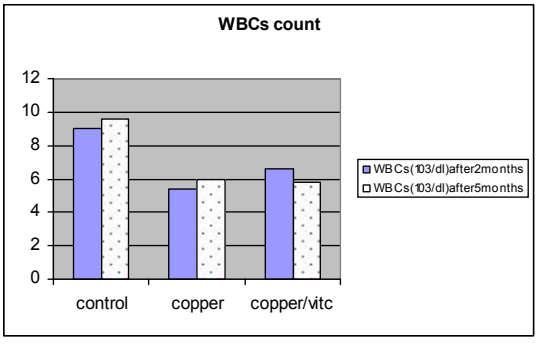
A p< 0.05 (cu) (cu+vit) group compared with control.
a p< 0.05 (cu) group compared with (cu+vit) group
The mean differences is significant at 0.05 level.

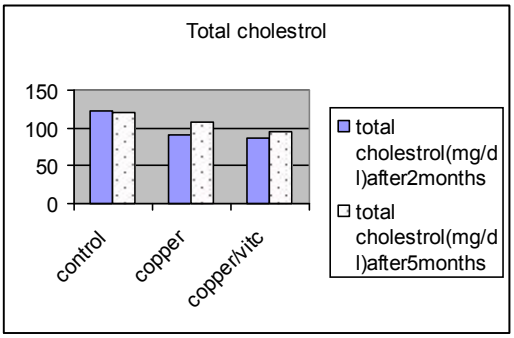

Hb(mg/dl)
16
14
12
10
8
6
4
2
0
control
copper
copper/vitc
Hb(mg/ldl)after2months
Hb(mg/ldl)after5months

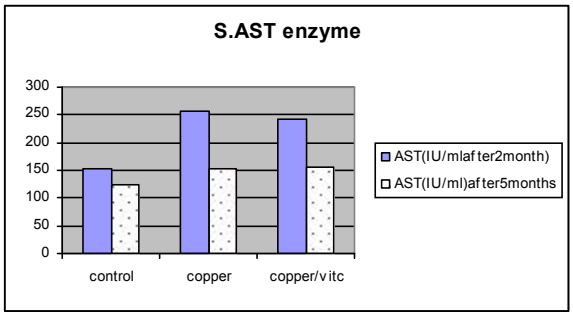

MCV(fl) after5months
66
64
62
60
58
56
54
52
50
control
copper
copper/vitc
Groups
MCV(fl)


RBCs count
8
7
6
5
4
3
2
1
0
control
copper
copper/vitc
RBCs(106/µl)after2months
RBCs(106/µl)after5months


Total billirubin (mg/dl)after5months
1
0.5
0
control
copper/vitc
Total billirubin (mg/dl)


WBCs count
12
10
8
6
4
2
0
control
copper
copper/vitc
WBCs(103/dl)after2months
WBCs(103/dl)after5months


Total cholestrol
150
100
50
0
control
copper
copper/vitc
total cholestrol(mg/dl)after2months
total cholestrol(mg/dl)after5months


S.AST enzyme
300
250
200
150
100
50
0
control
copper
copper/vitc
AST(IU/mlafter2month)
AST(IU/ml)after5months

Pathological examination.

1. Clinical symptom and Post-mortum examination.

Clinically, no mortality was detected throughout the period of the experiment.

● Control group: There were no significant macroscopic findings in the control untreated rats used in the experiment.

● Group treated with Cu: the kidney appear enlarged with brown discoloration, the spleen were swollen and congested with slightly congested liver consistently.

● Group treated with Cu +VitC: Mild gross changes were observed.

2. Histopathological examination.

● Control group: There were no significant microscopic findings in the control untreated group along experiment period.

The histopathologic examination of parenchymatous organs in different treated groups revealed that :-

Spleen:

The histopathologic examination of spleen exposed to Cu after 2 months showed depletion of lymphocytes of white pulps (Fig. 1 a). Focal areas of necrosis showed pyknotic, karyolitic nuclei (fig.1b).

Congestion and dilatation of blood vessels which studded by large numbers of erythrocytes with destructive wall (Fig. 1c). Proliferation of mononuclear cells infiltration at pervascular region were observed (Fig. 1c).

The histopathologic examination of Spleen Cu treated group after 5months showed depletions in lymphoid follicles with presence of hemosiderin pigment. (Fig.1d).

Spleen exposed to Cu &Vit. C after 2 months showed some improvement in white and red bulb element (Fig.2a).

Spleen of Cu &Vit. C treated group after 5months showed ameliorated in the histopathological alternation as a result of cu toxicity (Fig.2b).

Kidney:

The main microscopic changes of kidney after 2 months showed degeneration and necrosis of the epithelial lining cells of tubules. The most alterations were cloudy swelling, occlusion of tubular lumen (Fig. 3a&b), and some sections showed renal tubular fibroses (fig.3a).The glomeruli were shrunked or atrophied and absence of the Bowman's space (fig.3a&b). Focal cellular necrosis was observed, tubular necrosis showed pyknotic and karyolitic nuclei (fig.3c).

The histopathologic examination of kidney exposed to Cu after 5 months showed degeneration and vacuolation of the renal tubules (Fig. 3d) , necrosis of the renal tubules and glomeruli (Fig. 3e).The renal tubules showed different, necrobiotic changes (Fig3d), dilatation and degeneration of Bowman's capsule, increase filterate space (Fig3e).

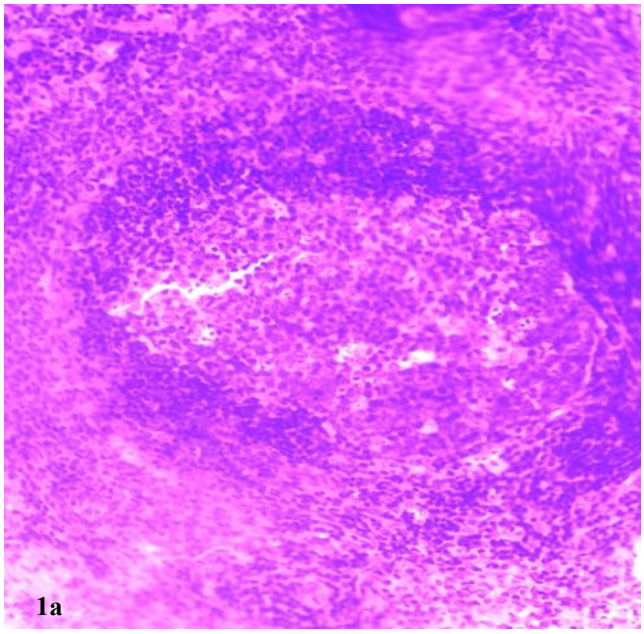
Kidney of animals treated with copper sulfate and vitamin Vit C after 2 months appeared more or less as normal (Fig. 4a). The administration of Vit. C conjugated with cu , Vit. C showed ameliorated of kidney pathological lesions after 5 months (Fig. 4b &c).

Liver:

The histopathologic examination of liver exposed to Cu after 2 months showed diffuse cytoplasmic degeneration with infiltration of few mononuclear cells (Fig. 5a&e). Some hepatic cells showed degeneration in which the cells swollen with irregular vacuoles in the cytoplasm (Fig. 5a). Other hepatic cells showed coagulative necrosis with pyknotic and karyolitic nuclei (Fig. 5b). Central veins, hepatic sinusoids were congested dilated and filled with erythrocytes (Fig, 5c, e). kupffer cells were proliferated, apoptosis was seen in many sections (Fig.5c,e). The hepatocytes showed disorganization and disorientation of hepatic plates (fig. 5d). Blood vessel showed exhibited engorged and congested vasculature (Fig.1d) with degenerative changes in the hepatocytes around blood vessels (Fig.5e).

The histopathologic examination of liver After 5 months Copper sulphate treated group showed degeneration ,cell membrane degeneration of hepatocytes dense cytoplasmic granules, pycnotic nuclei, atrophy of nucleus of hepatocytes, nuclear pyknosis, (Fig.5f) and Periportal degeneration (Fig.5f).

Liver of animals treated with copper sulfate and Vit.C after 2 months showed more or less as normal with small area of swelling hepatocytes (Fig.6a, b&c). Concomitant administration of Vit. C to Cu-intoxicated rats and Vit.C after 5 months resulted in mild pathological changes compared to their counterparts exposed to cu alone (Fig.6d).



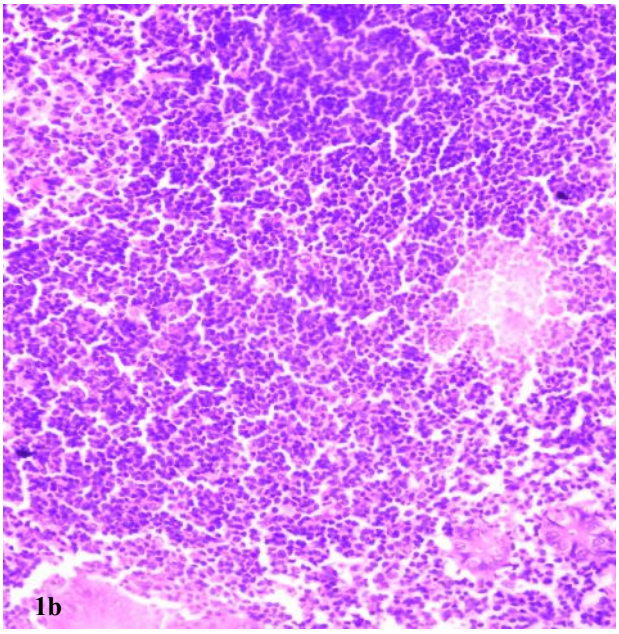
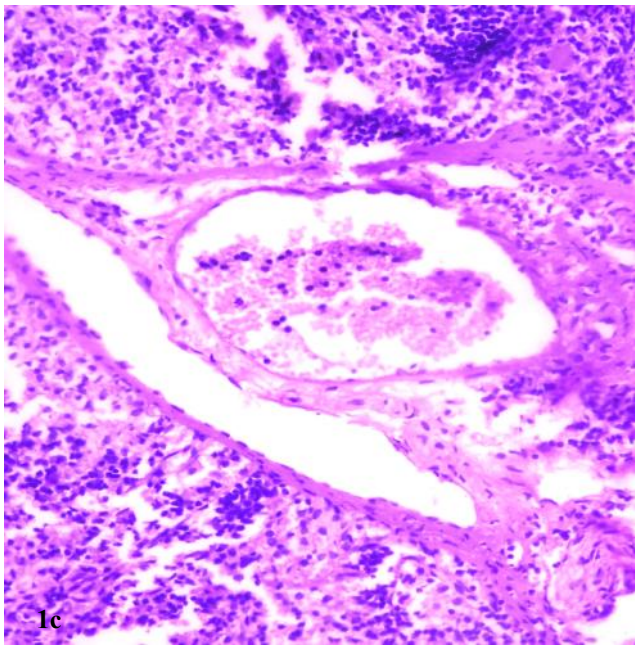


Fig. 1a: spleen from rat received Cu for 2months showing depletion of lymphocytes of white pulps. H&E X100.
Fig. 1b: spleen from rat received Cu for 2months showing focal necrosis, increase lymphocytic infiltration H&E X200.



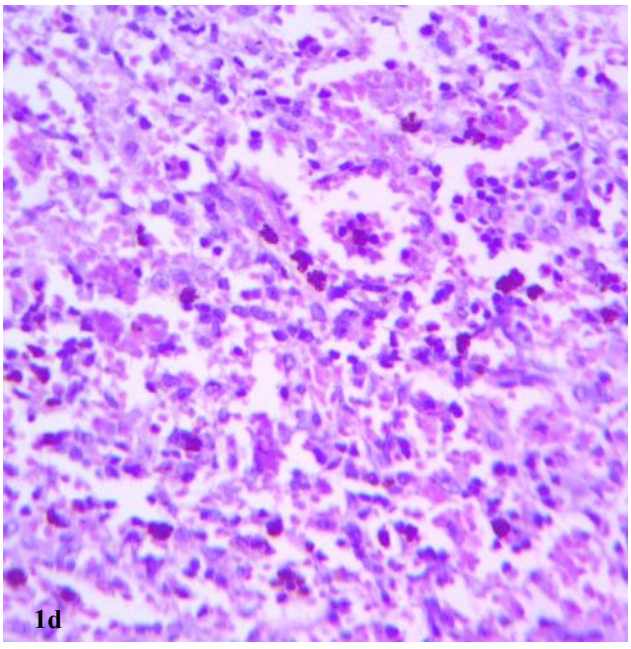


Fig. 1c: spleen from rat received Cu for 2 months showing Congestion and dilatation of blood vessels, mononuclear cells infiltration at pervascular region. H&E X200.
Fig. 1d: Spleen of cu treated group for 5 months showing depletions in lymphoid follicles, hemosiderin pigment. H&E X250.

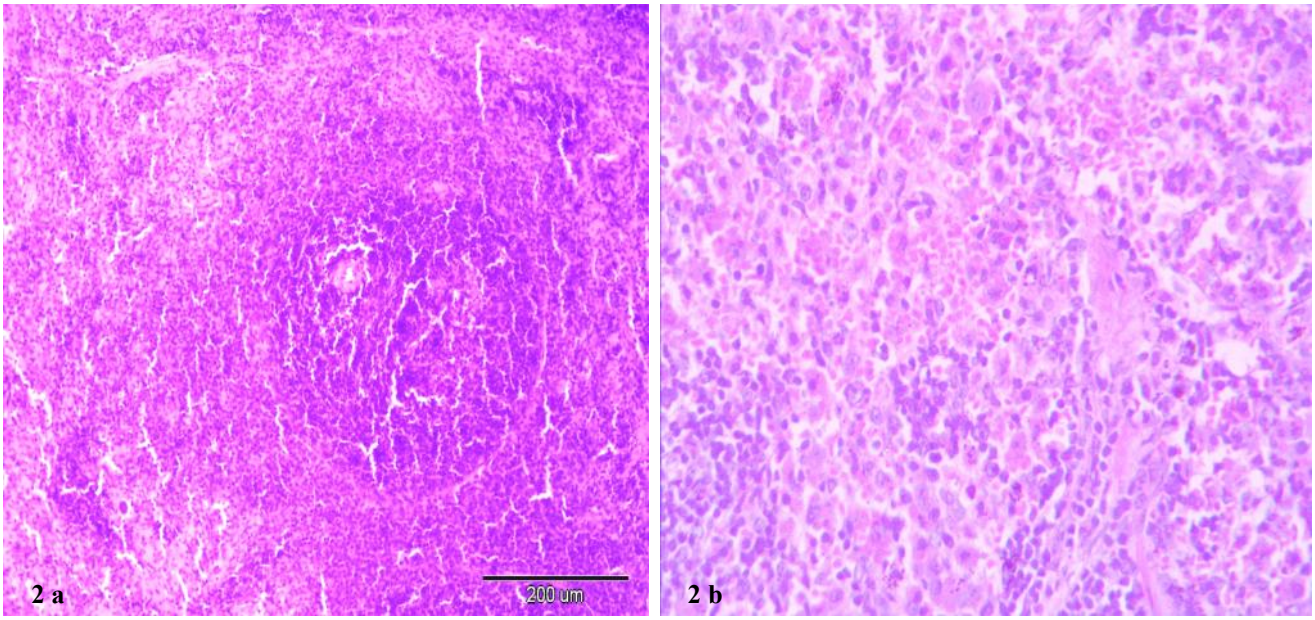


Fig. 2a: Spleen from rat received Cu &Vit. C for 2 months showing improvement in white and red bulb element. H&E X100**.**
Fig. 2b: Spleen from rat received Cu &Vit. C for 5 months showing ameliorated in the histopathological alternation as a result of cu toxicity. H&E X250

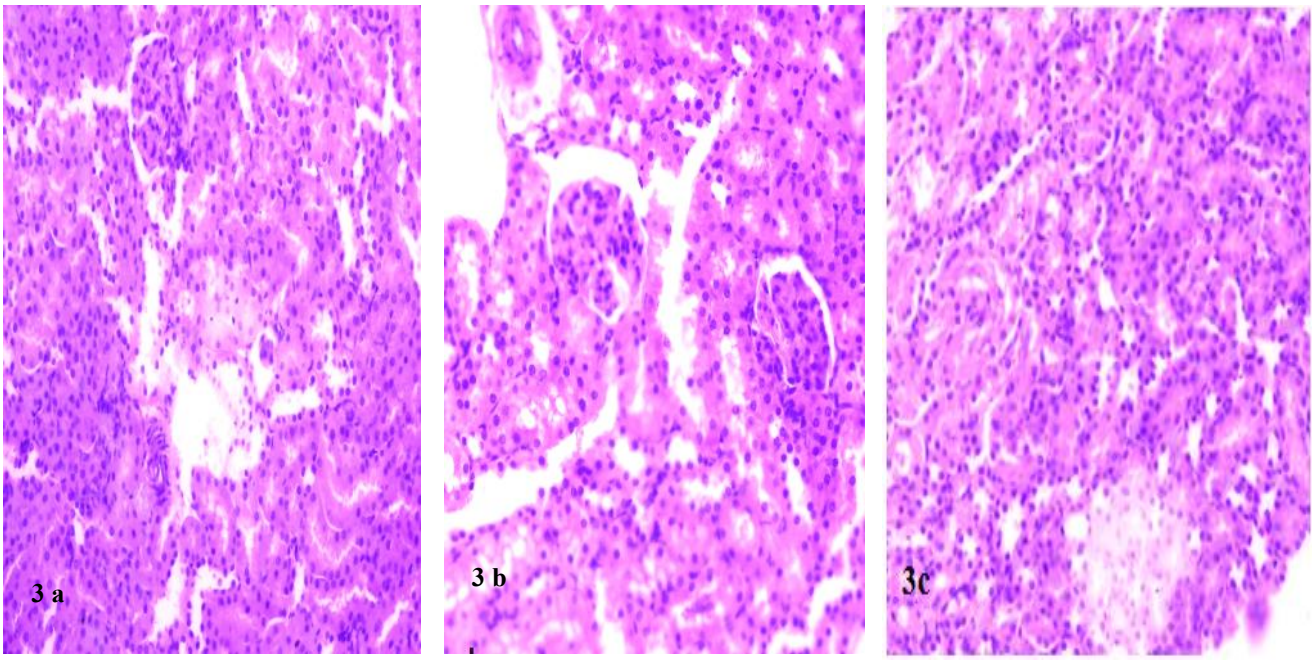
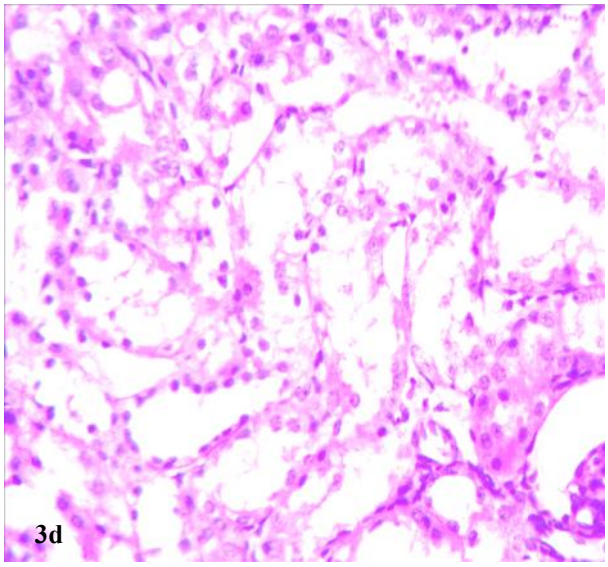


Fig. 3a: Kidney from rat received Cu for 2 months showing cloudy swelling, occlusion of tubular lumen, renal tubular fibroses. H&E X200.
Fig. 3b: Kidney from rat received Cu for 2 months showing shrunked or atrophied glomeruli and absence of the Bowman's space. H&E X200.
Fig. 3c: Kidney from rat received Cu for 2 months showing focal necrosis pyknotic, karyolitic nuclei. H&E X200.



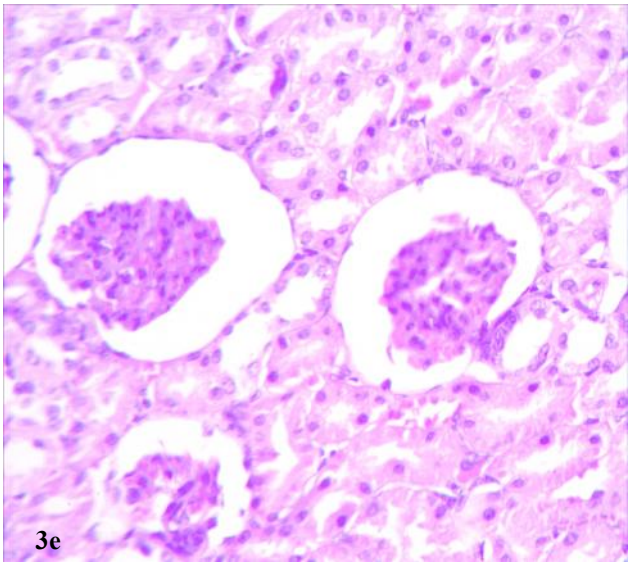
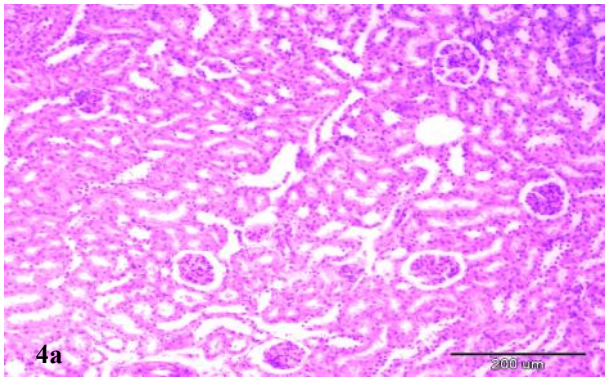
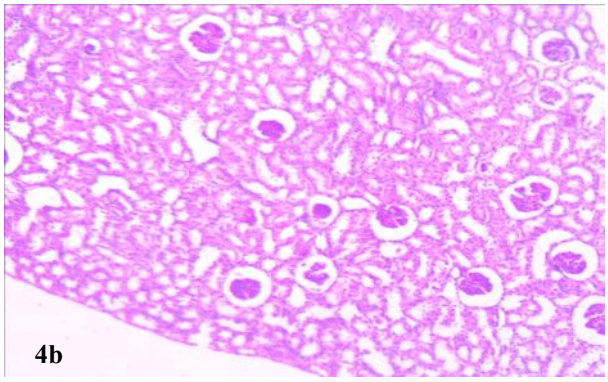


Fig. 3d: Kidney from rat received Cu for 5 months showing degeneration and vacuolation of the renal tubules, necro-biotic changes. H&E X400.
Fig. 3e: Kidney from rat received Cu for 5 months showing degeneration of Bowman's capsule, increase filterate space. H&E X400.





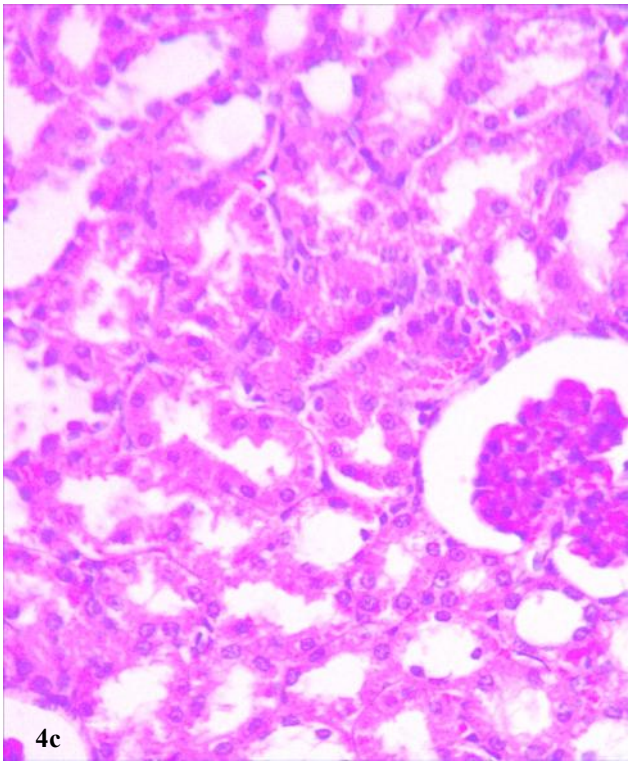
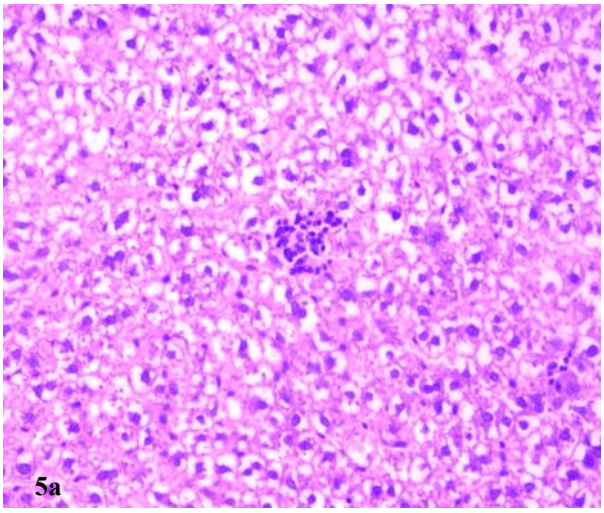
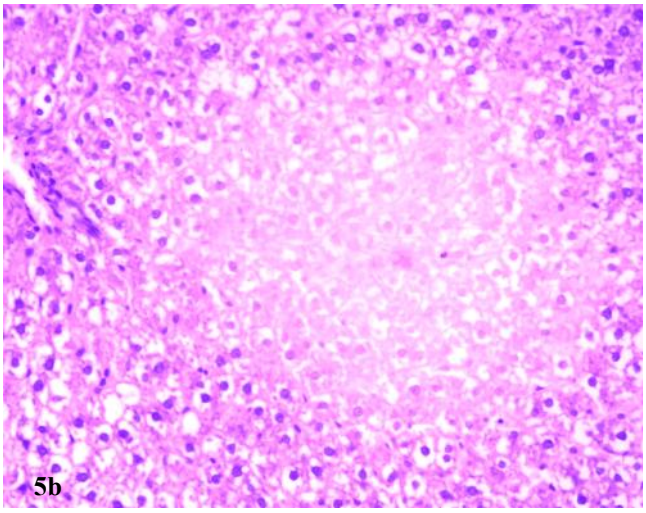
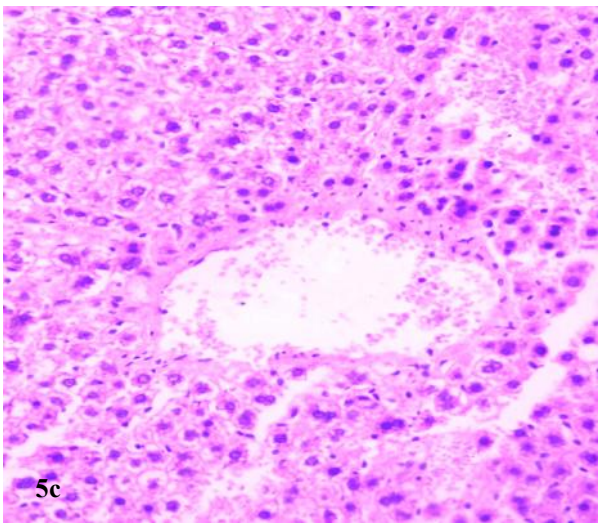


Fig. 4a: Kidney from rat received Cu &Vit. C for 2 months showing more or less as normal as a result of cu toxicity. H&E X100.
Fig. 4b: Kidney from rat received Cu &Vit. C for 5 months showing mild cytoplasmic vacuolation. H&E X100.
Fig. 4c: Kidney from rat received Cu &Vit. C for 5 months showing ameliorated in the histopathological alternation of kidney as a result of cu toxicity. H&E X400.







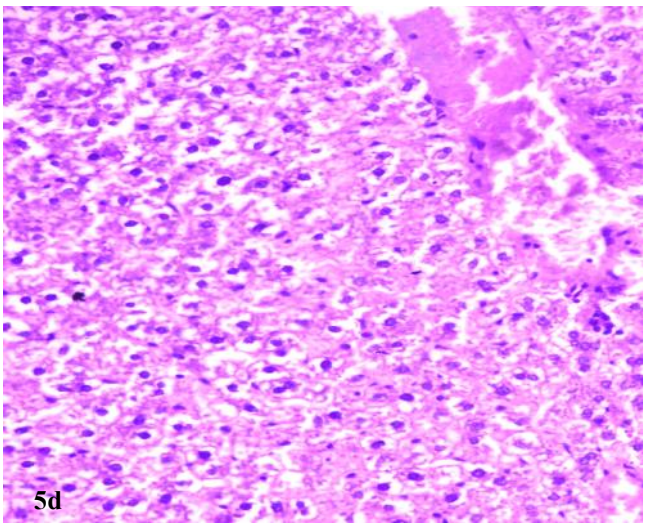
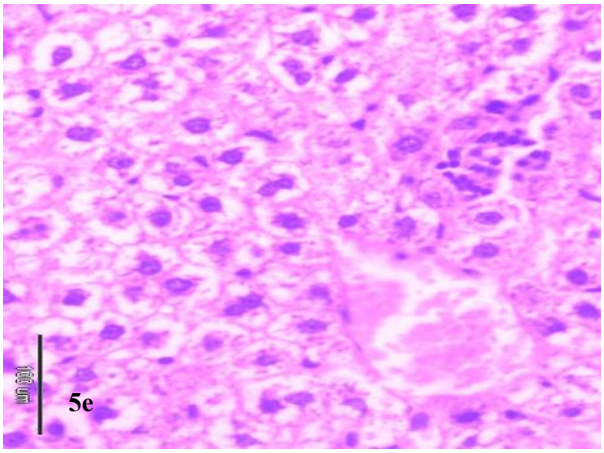


Fig. 5a&1b: Liver from rat received Cu for 2 months showing focal infiltration of mononuclear inflammatory cells together with vacuolar degeneration, coagulative necrosis with pyknotic and karyolitic nuclei. H&E. X 200.
Fig. 5c&d: Liver from rat received Cu for 2 months showing intercellular haemorrhage, some necrotic cells, cell membrane degeneration of hepatocytes, degeneration around the central vein, dilated blood vessel. H&E. X 200.



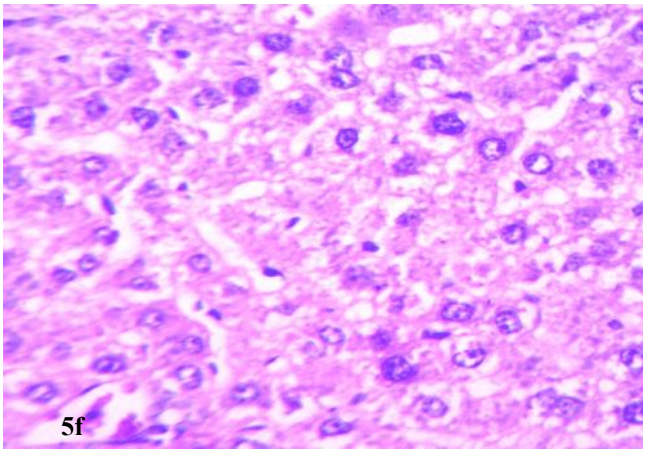


Fig. 5e: Liver from rat received Cu for 2 months showing focal infiltration of leucocytes, dilated central veins together with activation of kuffer cells .H&E X 400.
Fig. 5f: Liver from rat received Cu treated for 5 months showing degeneration, atrophy of nucleus of hepatocytes, Periportal degeneration. H&E X 400.

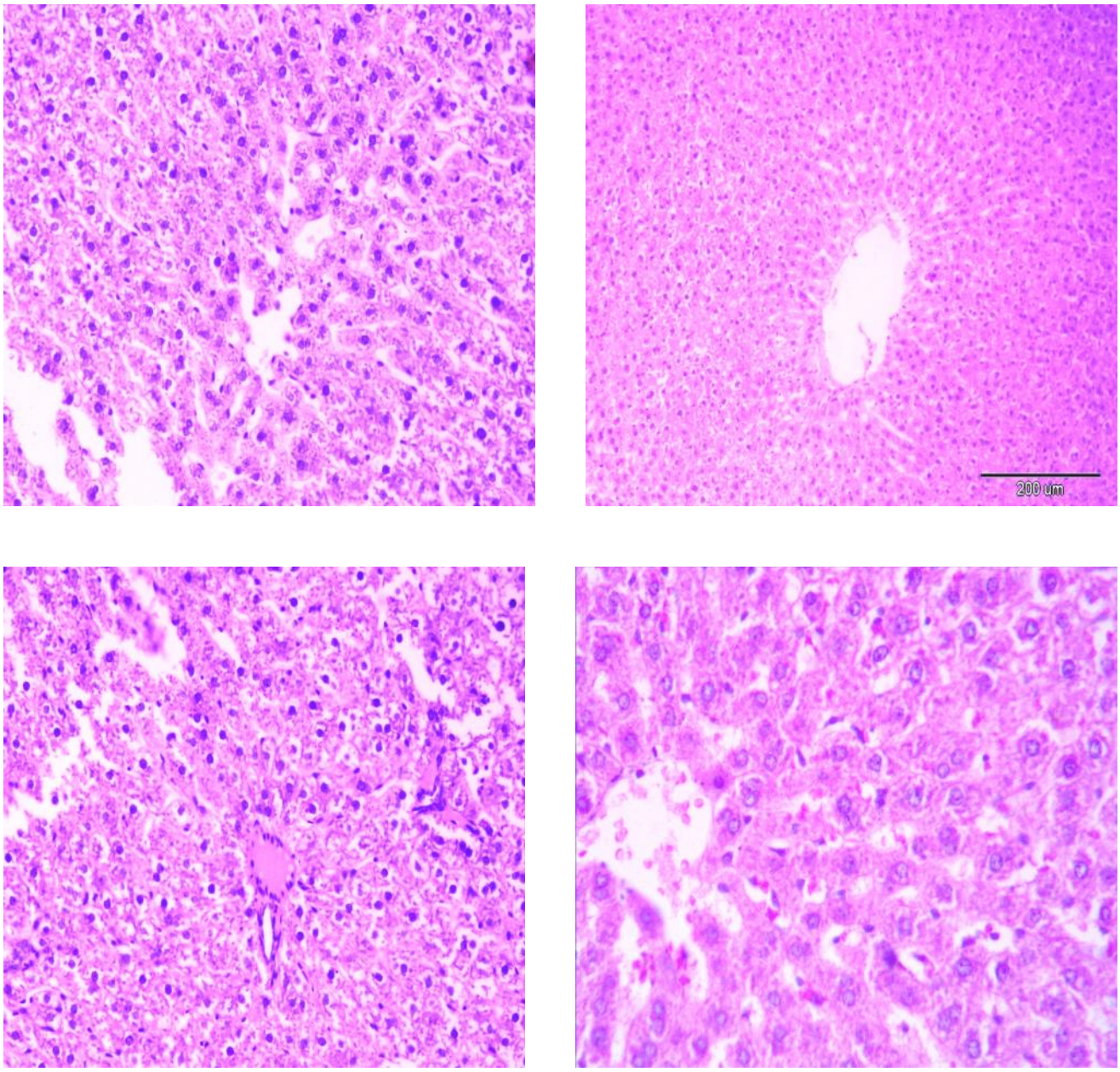


Fig. 6a: Liver from rat received Cu &Vit. C for 2months showing dilated central vein .H&E X100.
Fig. 6b&c: Liver from rat received Cu &Vit.C for 2 months showing mild cytoplasmic vacuolation, pycnotic nuclei, dilated sinusoids. H&E X200.
Fig. 6d: Liver from rat received Cu& Vit. C for 5 months showing congestion, dilatation of central veins and sinusoids, activation of kuffer cells. H&E X400.

Discussion

In present study, copper was mixed in the feed of albino rat, showed various post mortum finding, the kidney appear enlarged brown in color, swollen, congested and dark brown spleen surface and slightly congested Liver. **Shahzad *et al.,* (2012)** showed the liver was pale to yellowish and fragile. The liver colour varied from pale yellow to orange, while that of the kidneys from brown to black **(Abd Al Majeed, 2010).** Similar result obtained by **(Iqbal, 2012), w**ho reported kidney was pale, hypertrophic, hemorrhagic and deposition of urates was present on the kidney.

The evaluation of hematological indices showed that hemoglobin, PCV and RBCs count increased sharply in Cu group and Cu + Vit. C group as compared with control group, suggesting the erythrocytosis. These finding are in agreement with those described by **Bassuny, (1991); Ahmed *et al., (*1997)**. We can explain that by Copper ions can oxidize hem iron to form methaemoglobin. This blood loses its oxygen carrying capacity **(Saravu *et al.,* 2007)**. The resulted methemoglobin related anemic hypoxia could induce erythrocytosis through increase of erythropiotin **(Stockham & Scott, 2002).** But after five months of experiment our result indicated to hypochromic with microcytosis meaning low in iron that was clear in cu treated group, this is in agreement with **(Prince *et al.,* 1979)** said that Liver iron levels were decreased approximately 50% by the addition of 250 *ppm* copper to the diet. But our finding indicated to ameliorative effect of Vit. C and this is consistent with **(Milne & Omaye, 1980)** who said the iron and heme parameters increased as the vitamin dose increased. These data are consistent with the hypothesis that interaction between vitamin C, copper and iron influence normal heme formation through the oxidation/reduction of iron and/or by regulating iron absorption and availability at the gut level. Histopathological findings confirmed the toxic effects of copper consistent with biochemical determination. In cupper group, the spleen was swollen and congested, spleen showed depletion of lymphocytes of white pulps. Focal areas of necrosis, Congestion and dilatation of blood vessels. **Al Naimi *et al.,* (2010)** reported the pathological changes of spleen as hyperplasia of lymphoid tissues. Depletion of the lymphoid tissues appeared clearly at the end of the 5month that could be related to the effect of the long duration of cu toxicity causing reduction in the immune system. Those results were in agreement with **(Hebert *et al.,* 1993)**. Spleen from treated animals indicated a marked depletion of iron stores in both male and female rats, while hematologic and clinical chemistry alterations in rats **(Heberet, 1993**).

The increase in indirect bilirubin after 5months in cu treated group in our study indicated to heamolysis which was explained by **Abu-Zinadah *et al.,* (2012)** considered copper as a modulator factor of haemolysis which increases osmotic fragility of red cell. However all the values of the white blood cells fall below the normal range reported by **Adu and Egbunike, (2010)** which could be as result of defense nature of the white blood cells in fighting the inclusion of copper.

In present study, histopathologic examination of spleen Cu treated group after 5months showed presence of hemosiderin. The congestion was severe in high dosage group with deposition of large amount of hemosderin, these results could be related to the excessive hemolysis accompanied Cu toxicosis as stated by **IPCS, (1999)**. Hemosiderosis of spleen are considered dominant features of histological picture in diagnosis of various forms of chronic Cu intoxication **(Donald, 2005)**.

The present data clearly demonstrate that oral administration of copper for long time did not significantly alter urea and creatinine indicates little or no adverse effect on kidney function. Similar observations have been reported by **Adu *et al.,* (2010)**. This may be explained by **Fuentealba *et al.,* (I989)** who said that temporary tubular nephrosis is succeeded by recovery and adaptation of regenerated cells whereby excess copper is excreted by exocyto-

sis in the form of Cu-MT and also an α-2 urinary protein-copper complex.

In histopathological examination, Copper induced the most alterations, cloudy swelling, the glomeruli were shrunken or atrophied and absence of the Bowman's space,focal cellular necrosis was observed, tubular necrosis showed pyknotic and karyolitic nuclei. Similarly **(Heo, 1997)** showed albino rats subjected to a chronic toxicity reveled copper causes lumen closure of renal tubules as a result of epithelial edema and causes structural damage to the epithelial cells of renal tubules and parenchymal cells in the kidney**.** These changes are more severe in fifth month of experiment group and that might be attributed to the releasing of Cu which may caused necrosis of epithelial lining of the tubules. This result was in agreement with **(Dashy, 1989). Elgarwi, *et al.,* (1998)** related the damage of the proximal convoluted tubules from direct effect of the Cu. The obtained results showed that the damage of the epithelial lining tubules was constant in all examined sections and that agreemend with **(Hocher *et al.,* 1992 and Nurullah *et al.,* 2006)**. **Wangsongsak *et al.,* (2007)** found prominent tubular and glomerular damage in the kidney. Also **Koponen *et al.,* (2001)** stated that histopathological examination revealed abnormal cellular changes in the renal corpuscle, dilation of glomerular capillaries, mesangial edema, an adhesion between visceral and parietal layers of Bowman`s capsule. **Al-Bairutya *et al.,* (2013)** demonstrated damage to the epithelium of some renal tubules and increased Bowman's space in the kidney. **Shahzad *et al.,* (2012)** revealed mild cytoplasmic vacuolation and condensation pyknosis or disappearance of the nucleus in the cells of the kidney were the salient changes observed. **Abd Al Majeed, (2010)** showed sever damage to glomerulus and glomeruli are swollen and dwindle in the lumen of Bowman's corpuscle being signs of glomerulonephritis.

The histopathologic examination in copper group revealed hepatocellular degeneration, coagulative necrosis with pyknotic and karyolitic nuclei. The hepatocytes showed disorganization and disorientation of hepatic plates. Those results were in agreement with **(Kamunde *et al.,* 2002)** Copper induced changes included centrilobular necrosis and perilobular sclerosis**.** The extent of necrosis vary with the dose of the drug and the length of treatment and this agreed with **(Hojo *et al.,* 2000)** They recorded that necrosis of liver lobules was a constant feature of Cu poisoning cases. Similarly, **(Kasai *et al.,* 1990)** reached the same that the extent of lesion observed in the examined organs were affected most by the length of the action and the dose of Cu. **Al-Naimi *et al.,* (2010)** revered the most significant changes in the liver due to Cu toxicosis was the centri-lobular coagulative necrosis which began centrally and progress peripherally in the lobule. parenchymal cell necrosis and swelling of the Kupffer cells **(Ishmael *et al.,* 1971, 1972; Abd Al Majeed 2010 and Al-Bairutya *et al.,* 2013)**. Some hepatocytes showed signs of dying by apoptosis **(Lewis *et al.,* 1997). Suchismita *et al.,* (2013)** reported liver of exposed to sublethal doses of Cu showed several reactions including fatty changes in hepatocytes, liver cord disarrangement, nuclear pyknosis and extensive degeneration of cytoplasm, which are characteristic abnormalities and well supported by many workers **(McCarthy and Shugart, 1990; Pourahamad and O'Brien, 2000)** found that chronic metal accumulation in the liver causes hepatocyte lysis, cirrhosis. Studies have shown that long-time ingestion of water containing Cu greater than the maximum contaminant level can lead to liver damage **(Varanka *et al.,* 2001; Zietz *et al.,* 2003). Maiorka, *et al.,* (1998)** stated that rats showed increased extent of liver necrosis with associated inflammatory response and changes in clinical chemistry parameters which were indicative of hepatocellular damage and cholestasis. The clear decrease in the cholesterol level due to copper administration is consistent with the findings of **Zaghloul, (2001)** who revealed this effect to reduce cholesterol synthesis or high degradation or excretion rate.

Severe liver damage in chronic copper poisoning could lead to increase in AST activity as suggested by **(Mozaffari *et al.*, 2009).** In a clinical serum examination, AST and ALT activities in serum represent biomarkers for liver functions. **(Ronald and Koretz, 1992)** Alterations of AST and ALT activities are liver-specific and have been used as a tool to study varying cell viability and changes in cell membrane permeability **(Galhardi *et al.*, 2004).** The present study demonstrates that in the copper-loaded rat there was a significantly increased activity of serum AST ($P < 0.05$). Based on these observations, alterations in serum AST activities (Table 3 and 4) reflect adverse effects of copper intake on the hepatic function indicating copper-related injury to the liver.

No significant effect was also observed in the serum globulin content and these observations concurred with the findings of **(Kerr and McGrain, 1991)** who reported no significant influence on serum globulin in sheep with high level of copper poisoning during grazing. Increase in copper in liver tissue in copper treated group in our study is consistent with observations of **(Turnlund *et al.*, 2004).** In contrast, copper supplementation studies have shown that indexes of copper status do not tend to be influenced by additional dietary copper. For example copper status did not change in healthy infants supplemented with copper **(Salmenpera *et al.*, 1989)**. Vit. C administration in our study leads to lower the level of copper in liver tissue in (Cu + Vit. C) treated group as compared with copper treated group this is in agreement with the observation of **(Van den berg & Beynen, 1992).** It has been known that copper storage begin in the centrilobular hepatocytes, where most of copper is sequestered in hepatic lysosomes **(Kelly, 1993 and Schilsky & Sternlieb, 1993)** lysosomal membranes lose integrity as copper accumulates and copper lysosomal hydrolyses are released, irreversibly injuring the cell **(Kelly, 1993)** hepatocellular necrosis and apoptosis occur. The accelerated loss of hepatocytes leads to acute massive copper release causing hemolytic and accumulation of hemoglobin cast in the renal tubules **(Kelly, 1993 and Schilsky & Sternlieb, 1993)**. These changes probably occurred due to the accumulation of Cu in the mitochondria and lysosomes causing progressive hepatocyte organelle damage and cellular degeneration and necrosis. This result agreed with **(NTP, 1993).** Vit. C is the most important hydrophilic Free radical scavenger in extracellular fluids, trapping radicals in the aqueous phase and protecting biomembranes from peroxidative damages **(Abd Al Majeed, 2010).**

Increase in lipid peroxidase along with hepatic elevation of copper was in agreement with **(Zhang *et al.*, 2000).** Very high levels of excess dietary Cu are needed to induce significant liver injury in rats. Increased MDA content was not detected in rats with morphologic evidence of liver damage, suggesting that lipid peroxidation may not play a major role in this model of copper toxicity **(Aburto *et al.*, 2001).** Cu + Vit. C group showed less pathological effect or normal spleen, liver and kidney tissues. However, Cu + Vit. C group were showed mailed affect or showed similar to those of the control group. Similar findings were also reported by **(Thophon *et al.*, 2003; Takashima and Hibya, 1995).** Other finding were also reported by **(McCal and Balz, 1999)** that antioxidants nutrients, as ascorbic acid are considered to give protection against damage induced by different toxicants and reduce the activity of free radical – induced reaction. **Abd Al Majeed (2010)** found the Cu + Vit. group showed less pathological effect or normal liver and kidney tissues.

Conclusion

- The present data indicate that the adverse effects of copper administration are dose-and duration dependant.
- Vit. C with ameliorative effect on chronic copper toxicosis by decreasing level of hemolysis and improving iron advantage.
- The administration of Vit. C in copper intoxicated mice caused a pronounced pro-

tective effect to liver and kidney damage, In addition, the treatment with Vit. C after the administration of Cu reduced these lesions caused by Cu.

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دراسات هيماتوبيوكيميائية وباثولوجية علي تاثير فيتامين سي على الفئران البيضاء عند تعرضها لسمية النحاس المزمن

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الملخص العربى

أجريت الدراسة الحالية لدراسة الآثار السلبية لسمية النحاس المزمن في إناث الفئران واستخدام فيتامين سي في التخفيف من حدة هذه الاثار. ثلاث مجموعات من إناث الفئران تحتوى 10 لكل منهما، المجموعة الاولي كانت ضابطة ، تلقت المجموعتين الاخرتين 1 جرام من كبريتات النحاس / 1كجم عليقة. اما المجموعة االثالثة فقد اعطيت بالإضافة إلى كبريتات النحاس 1 جرام من فيتامين سي / 1لتر من المياه يوميا لمدة 5 شهور. وأظهرت المجموعة التي تلقت كبريتات النحاس زيادة الكريات الحمراء، زيادة الصفائح الدموية ،انخفاض في عدد كريات الدم البيضاء ، زيادة في انزيمات الكبد AST مع زيادة في الصبغة الصفراء ، زيادة في مستوي النحاس بالكبد مع زيادة في انزيم ليبيد اوكسيديز انزيم بالمقارنة بالمحموعة الضابطة ، اظهرت المجموعة المعاملة بفيتامين سي تحسين التأثير السلبي كبريتات النحاس بارتفاع الهيموجلوبين وكرات الدم البيضاء ، وانخفاض AST بالإضافة إلى أن انخفاض مستوى الصفراء ومستوي النحاس بالمقارنة مع المجوعة الضابطة.

وأظهرت التغيرات المرضية في نسيج الكبد والكلية والطحال منها ترشح الخلايا الالتهابية في نسيج الكبد ,تنكس و تنخر الخلايا الكبدية. وظهر فيالكلية تلف حاد في الكبيبة والأنابيب مع احتقان في الاوعية الدموية مع ترسب للهيموسيدرين للطحال في مجموعة النحاسعند مقارنتها بمجموعة النحاس والفيتامين.

ولذلك فقد خلصت الدراسة الى ان استخدام فيتامين سي مصاحبا لكبريتات النحاس الذي يستعمل في بعض مزارع الدواجن والاسماك كمضاد للفطريات او محفز للنمو ان فيتامين سى له تاثير وقائي لاثار السمية الحادثة من النحاس بعد الشهر الثانى وايضا له تاثير جيد في حال التسمم المزمن لفترة قاربت من الخمسة اشهر .ولذلك يوصى باستخدام فيتامين سى مصاحبا لكبريتات النحاس.