Toxicopathological study due to effect of different doses of Copper Sulfate ingested in rat

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Abstract

The object of this study were to evaluate the effect of different doses with regard toxicopathological changes in rat ingested CuSO4.5H2O. The study was done on (66) female rats of approximately the same age and body weight. The first experiment was done on (30) rats, the animals were divided into (5) groups, each group composed of (6) animals ingested toxic doses of CuSO4.5H2O for studying the symptoms of acute toxicosis, time of appearance and disappearance, and for determination of LD50 by using probit method. The result of the experiment showed that the value of LD50 was 809 mg/kg.bw. The acute toxic signs appeared after short time and become more severe with increasing rate of the death according to the dose of the drug. Second experiment was done on (36) rats divided into (3) groups each groups contain (12) animals as in the followings, First group: Administered orally with distilled water\ day for (3) months and considered as a control group. Second group: Administered orally (8) mg /kg.b.w\ day of CuSO4.5H2O for 3 months and considered as Treated 1 group (T1). Third group: Administered orally (40) mg /kg.b.w\ day of CuSO4.5H2O for 3 months and considered as Treated Two group (T2). The clinical signs were checked continously. The main signs were anorexia, decrease in body weight, the mucous membranes were pale-yellow, with rough skin, ruffled hair and alopecia especially in the abdominal regoin, hemaglobinurea and dyspnea. The severity of signs increased with high dose and long duration. Toxicopathological changes due to CuSO4.5H2O showed the followings. The gross lesions of liver characterized by hepatomegaly or atrophy with pale-yellow color. Kidney darkish brown descolaration (gunmetal) colored with presence of small whitish nodules in high dose group, splenomegaly due to congestion or atrophy and fibrosis. The brain showed congestion and edema, with thickening of the stomach wall and catarhal enteritis. The microscopic examination showed that the severity of the lesions depend upon the dose and duration of treatment. The liver undergo fatty degenerations with centri-lobular necrosis which was a constant feature of all examined sections, with focal infiltration of mononuclear cells, with apoptosis, severe hemorrhage with formation of hyperplastic nodules and deposition of hemosiderin pigment in hepatocytes, proliferated kupffer cells and periportal fibrosis. The lesions of the kidney were characterized by degeneration and necrosis of the epithelial lining of the proximal convoluted tubules with deposition of hemosiderin pigment and dilation of cortical and medullary renal tubules with formation of hyaline cast , cortical tubular basophilia and interstitial infiltration of mononuclear cells and fibrosis of medullary region especially in high dose. The brain sections showed perineuronal and perivascular edema with focal gliosis and demyelination with focal aggregation of microglial cells around the blood vessels with hemorrhage especially in high dose. The heart lesions were represented by fatty degeneration and myocarditis and epicarditis with infiltration of mononuclear cells and areas of necrosis and hemorrhages with fibrosis in high dosage group. Spleen showed severe depletion of white pulp lymphoid tissue with extramedullary...
hemopoiesis characterized by the presence of large numbers of megakaryocytes, severe congestion and deposition of hemosiderin pigment and fibrosis especially in high dosage group, the non-glandular stomach showed hyperplasia and hyperkeratosis. Bone sections showed suppression of hemoietic tissue with signs of osteoporosis. The study have been concluded that CuSO4.5H2O poisoning in rat causes toxicopathological changes internal abdominal organs with precancerous lesions.

دراسة التغيرات المرضية السمية حول تأثير جرعات فموية مختلفة من كبريات النحاس في الجرذان

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الخلاصة

هدف الدراسة إلى بحث تأثير الجرعات المختلفة لمحلول كبريات النحاس المائية وذلك من خلال دراسة التغيرات المرضية. استخدمت في الدراسة (66) من إناث الجرذان (ذا تأوزاً و أعمار متقاربة) وقد تضمنت الدراسة ما يلي:

التجربة الأولى: أجريت التجربة على (30) من إناث الجرذان حيث قسمت إلى (5) مجاميع احتجت كل مجموعة على (6) من الإناث أعطيت جرع مماثلة من كبريات النحاس المائية وذلك لعرض دراسة الأعراض السمية الحادة ومشدتها وقت ظهورها واختفاءها وعمر وقود تحليل الجرعة المميتة النصفية (LD50) وحسب طريقة Probit وقعت بها حيث قدرت ب809 ملغم/كم من وزن الجسم والأعراض السمية تم ظهورها بأوقات أقل وبشدة أكبر مع زيادة نسبة الموت بزيادة الجرعة في مجاميع التجربة.

التجربة الثانية: أجريت التجربة على (36) من إناث الجرذان حيث قسمت إلى (3) مجاميع احتجت على أعداد متساوية من الحيوانات وكما يأتي: المجموعة الأولى: أعطيت ماء مقطور يوميا عن طريق التجربة الفموي واعثرت مجموعة سيطرة ولمدة (3) أشهر. المجموعة الثانية: أعطيت (8) ملغم / كغم من وزن الجسم محلول كبريات النحاس المائية يوميا عن طريق التجربة الفموي ولمدة (3) أشهر واعتبرت مجموعة معالجة أولى. المجموعة الثالثة: أعطيت (40) ملغم / كغم من وزن الجسم محلول كبريات النحاس المائية يوميا عن طريق التجربة الفموي ولمدة (3) أشهر واعتبرت مجموعة معالجة ثانية.

تم ملاحظة الاعلامات السريرية بصورة مستمرة ووجد أن هناك فجأة للإسهال مع انخفاض في وزن الجسم وهذا يتناسب عكسيا مع زيادة الجرعة والوقت مع شحم وأصابع الأرضية خجولة الجلد وتجمع الشعر مع وقود الحافة خصوصا في منطقة البطن بالبلية الهيموجلوبينية وتطبيق السفت. أما علامات السرطان ولم تظهر علامات سريرية متعدة فقد كانت شائعة ومرة الجلد. وبعد أجزاء الصفة التشريحية تبين أن كل الأعضاء الداخلية كانت مستهدفة خصوصا الأعضاء الحيوية وقد أظهر الفحص العصبي ضخامة الكبد أو صغر حجمه مع شحم- اصابع اللون وثنو الكلي باللون الغامق. مع وجود عقيدات صغيرة بيضاء اللون في الحيوانات المجرعة بالجرعة العالية. وكثير حجم الطحال واحترق أو صغر حجمه وتمثله مع احترق الدماغ وامتصال بالسوائل الخزبية.
Introduction

Copper (Cu) is a mineral found in trace amount in all tissues in the body. It has been known, mined, and used by humans for more than 7000 years (1). Cu is required for normal iron metabolism, elastin and collagen synthesis, melanin production and integrity of the central nervous system, numerous metalloprotein enzymes and non-enzymes metalloprotein in animal that require Cu to be biologically active, recently it has been shown that Cu is one of the key trace minerals required for an effective immune response (2). The total body content of Cu is 150 mg (3). Approximately 30% absorbed from gastrointestinal (gI) tract (4). The kinetics of Cu during over dose differs from that during the normal. In normal poisoning, albumin rather than ceruloplasmin, binds the excess Cu. liver is the major site of deposition of Cu following large ingestion (5). There are a number of complicating factors involved in the metabolism of Cu. The dietary ratio of Cu to molybdenum (Mo) is extremely important. Mo and sulfur act to neutralize the toxic effect of Cu (6). In humans acute Cu poisoning has occurred through the contamination of beverages by storage in Cu containing containers as well as from contaminated water supplies (7). In animals acute responses to Cu vary with species and Cu compound (8). While ingestion or cutaneous absorption of adequate amount of Cu salt is able to produce acute gastroenteric poisoning. The usual way of poisoning in domestic animals is a chronic disorder that terminates principltu in acute symptoms when Cu stored in the liver reaches a critical level (9). Copper sulfate (CuSO₄) is a powerful oxidizing agent and irritant to mucous membranes leading to ulceration of stomach and small intestine. A dose response effect following ingestion is difficult to define but approximately 10 grams may be fatal in an adult (10). The main brunt of CuSO₄ is brone in the order by erythrocytes, liver and then kidneys leading to methemoglobinemia, hepatotoxicity and renal failure. Elevated levels of liver enzymes are seen in severe cases of poisoning both in humans and animals (11). Because of the importance of Cu mineral for both humans and animals, this study was designed to
evaluate the effect of different doses of this element on different organs such as liver, kidney, stomach, lymphoid tissue, brain and hemopoietic tissue in 3 months period study in female rats.

Materials and methods

Experimental design:

- First experiment- Acute toxicity study: Determination of median lethal dose (LD50) by probit method (12). Thirty female rats were divided into five groups, each group consists of six animals, dosed orally by using stainless steel stomach tube with the following lethal doses of CuSO4.5H2O according to the animal body weight in each group, Group 1: 750 mg/kg bw conc. 7.5% Group 2: 800 mg/kg bw conc. 8% Group 3: 850 mg/kg bw conc. 8.5% Group 4: 900 mg/kg bw conc. 9% Group 5: 950 mg/kg bw conc. 9.5%. The animals were watched for 24 hr. for development of toxicity symptoms and lethality. The following parameters had been studied: Recording the toxic symptoms with their time of appearance and disappearance in each group. by probit method (12) Recording animal death time and mortality percent of each dose group. Converting the mortality percent for each lethal dose into probit number according to probit converting table (12). Drawing the log dose-probit response curve from which LD50 was determined according to probit method (13).

- Secondary experimental- chronic toxicity study: Thirty female rats were used to determine chronic toxicity. The animals were divided equally into three groups. Control group (1) Ten rats ingested orally with distilled water for three months via stomach tube considered as control group. First treated group (2) Ten rats daily ingested for three months via stomach tube with selected dose of CuSO4.5H2O at a dose 8mg/Kg, bw conc 0.8%. The selected dose was less than the reported NOEAL dose in rats (8). Second treated group (3) Ten rats daily ingested for three months via stomach tube with selected dose of CuSO4.5H2O at a dose 40mg/Kg, bw conc. 0.4%The selected dose was same as reported LOEAL dose in rats (8).

- Histopathology: At the end of each month three animals from each group were sacrificed by injection of high dose ketamin hydrochlorid and post mortem were done for all animals. The macroscopic appearance were recorded to detect any abnormal gross changes in internal organs; the tissues were kept in 10% formaldehyde solution, for fixation, then processed routinely by using the histokinette. Tissue specimens were embedded in paraffin blocks, and sectioned by microtome with hematoxylin and eosin stain, some sections also stain for specific consideration necessary, then examined under light microscope (14).

Results

- Acute toxicity signs: The acute toxicity signs of different high single doses of CuSO4.5H2O were characterized by bloody diarrhea, bloody vomitus or green blue vomitus (Fig.1) tachycardia, hemoglobinurea, rapid respiration, asphyxia, petechial hemorrhage in ear, paralysis to be convulsion and exophthalmia (Fig.2). It was noticed that more acute toxicity signs were developed according to increasing dosing groups with more severity and short duration period delayed when disappeared. More dead animals were recorded in high doses group.

- Chronic toxicity signs: The chronic toxicity signs are characterized by loss in body weight, depression and slow activity, paleness of mucous membranes of eyes and pads extremities, jaundice, hemoglobinurea, dyspnea, rough dried skin with alopecia especially in the abdominal region.

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

Treated groups: After one month of treatment:


Treated group (2): Liver: Enlarged and its borders were swollen, friable, and yellow in color (Fig.3). kidney: Enlarged in size and more darker in color than G (T1). Spleen: More congested than the previous group, and enlarged in size. Heart: Flabby with the and severely congested. Other internal organs showed the same macroscopic lesions as in the previous group.

- After two months of Treatment:

Treated group (1): Liver: Slightly congested. In two animals enlarged, pale with accentuation of lobular markings. Spleen: Enlarged and congested. In one animal it was small in size with granular surface. Heart: Flabby with presence of pale spots on the epicardium. Other internal organs showed the same macroscopic lesions as in the previous month.

Treated group (2): Liver: Pale in color with hemorrhagic spots. kidney: Deep brown in color and enlarged. Spleen: swollen and dark brown. Other internal organs showed the same macroscopic lesions as in the previous sections.

- After three months of Treatment:

Treated group (1): Liver: Showed large hemorrhagic areas. Kidney: Enlarged and dark brown. The urinary bladder enlarged with bloody-urine content. Spleen: Small in size with granular surface and slightly brownish in color. Stomach: Distended with gases, with same gross lesions as in the previous month.

Treated group (2): Liver: Enlarged and friable with large hemorrhagic areas. Kidney: Deep brown in color with the presence of small whitish nodule appeared on the surface of the capsule (Fig.4). Spleen: Enlarged, severely congested in one animal, other animals showed small size spleen and firm consistency. Other internal organs showed the same macroscopic lesions as in the previous month.

- Microscopic Findings:

Control group: There were no significant microscopic findings in the control untreated group along the period of experiment (3 months).

Treated groups (after one month of treatment):-

Treated group (1): Liver: Enlargement of hepatocytes with large prominent nuclei containing more than nucleoli, many cells were binucleated (Mitosis).with minimal diffuse vacuolation with infiltration of few mononuclear cells in periportal areas, kupffer cells were proliferated, apoptosis was seen in many sections with deposition of hemosiderin pigment. The central veins and sinusoids were dilated and congested with dilation of bile ducts (Fig. 5). Kidney: The lesions were restricted to the cortex, the main microscopic changes were degeneration and necrosis of the epithelial lining cells of the proximal and distal convoluted tubules, these cell containing large amount of hemosiderin pigment, with formation of epithelial cast in the lumen of the tubules, with congestion in the intertubular blood vessels and glomerular tufts, Medulla showed slight congestion (Fig.6). Spleen: Congestion of blood sinuses with deposition of hemosiderin pigment, and infiltration of neutrophils. The white pulps were hyperplastic proliferation of lymphocytes and macrophages in the periarterial sheath region (T-cell region) and remainder areas of white pulp (B-cells region) with reticuloendothelial cell hyperplasia.
Brain: Perineuronal and perivascular edema, focal gliosis due to proliferation of microglial cells, perivascular cuffing and congestion of blood vessels of the meninges (Fig. 7). Stomach: Non-glandular region: Papillary proliferation of epithelial lining with slight hyperkeratosis and infiltration of large numbers of eosinophils in the mucosa and submucosa. Heart: Edema between muscle fibers with fatty degeneration and congestion of blood vessels. Skin: Thickening of the epidermis due to hyperplasia, with hyperkeratosis and extensive sclerosis of dermal region due to fibrosis, many sections showed cystic dilation of hair follicles with eosinophilia and infiltration of mononuclear cells within the dermis. Bone marrow: Extensive erythropoiesis with the presence of large numbers of megakaryocytes (Fig. 8).

**Treated group (2):** Liver: The same lesions as seen in the previous group in addition to that there is marked fatty degeneration with infiltration of inflammatory cell in periportal areas. Significant lesion characterized by the presence of hyperplastic nodules in 2 animals these nodules contained large number of hepatocytes that undergo fatty degeneration and caused pressure atrophy to the adjacent liver parenchyma (Fig. 9). Kidney: The same lesion as in the previous group, the degeneration and necrosis are more severe than G(T1) with infiltration of mononuclear cells within the interstitial tissue of the cortex and presence of hyaline cast within the medullary tubules (Fig.10). Spleen: The same as in the previous group many sections showed congestion with deposition of hemosiderin pigment. Other organs showed the same lesions as seen in treated G (T1).

**Treated groups (After 2 month of treatment):**

**Treated group (1):** Liver: The same lesions as seen in the previous sections, with focal infiltrations of large numbers of mononuclear cells forming nodular aggregation between hepatocytes (Fig.11). Kidney: The degeneration and necrosis are more severe as seen as in the previous month, with cystic dilatation of medullary tubules many of them contain hyaline cast. Brain: more pronounced edema of the nerve cell and blood vessels than the previous month. Heart: Inflammation of epicardium with infiltration of neutrophils and lymphocytes forming nodular aggregation with congestion of blood vessels. Infiltration of large numbers of chronic inflammatory cells between muscles fibers causing pressure atrophy to muscle fibers. Other organs showed the same lesions as seen in the previous month.

**Treated group (2):** Liver: The same as in the previous sections, the liver parenchyma showed centrilobular necrosis which extended peripherally with increase in amount of fibrous connective tissues in perportal areas and around central veins (Fig.12). Spleen: The congestion was more severe than treated G(T1) with macrophages laden with hemosiderin pigment, thickening of the capsule and trabeculae with areas of fibrosis. Heart: Massive hemorrhage causing pressure atrophy and necrosis of muscles fibers. Brain: Demyelination of white matter with large areas of hemorrhages. Kidney: In addition to the previous lesions there is a nodular infiltration of inflammatory cells in the cortical areas forming pressure atrophy to the adjacent tissue with cortical tubular basophilia. Heart: Large areas of hemorrhages with atrophy of muscles fibers. Other organs sections showed the same lesions as seen in the previous month.

**Treated groups (after 3 months of treatment):**

**Treated group (1):** Liver: Presence of patchy areas of necrosis blood oozing to the necrotic areas with presence of serum protein between hepatocytes. Spleen: Severe depletion of white pulp lymphoid tissue and extramedullary hemopoiesis, with presence of megakaryocytes, many areas showed fibrosis (Fig.13). Kidneys: The same as in the previous month with infiltration of mononuclear cells in the interstitial tissue and in periglomerular region with dilation of glomerular urinary space. Bone marrow: showed
degeneration of bone marrow cells, with decrease in total number of cells and increase sinus numbers (Fig.14). Heart: Marked fibrosis of myocardium causing pressure atrophy of muscle fibers (Fig.15).

**Treated group (2):** Liver: Increase in numbers of inflammatory cells forming focal aggregation, the necrotic areas are larger than treated G (T1) with blood oozing to the necrotic areas and periportal fibrosis (Fig.16). Kidney: Severe dilation of cortical and medullary renal tubules. The cortical areas showed focal aggregation of mononuclear cells in the interstitial tissue, severe congestion of blood vessel of cortex and medulla with perivascular cuffing, slight fibrosis of medulla near the pelvic region, the perirenal adipose tissues showed the same aggregation of cells (Fig.17). Spleen: Severe congestion with deposition of large amount of hemosiderin pigment which appeared greenish –blue using iron stain (Fig.18). Brain: The same lesions as in the previous section with aggregation of microglial cells around congested blood vessels forming slight elevation above the surface of the cerebrum, with focal vacuolations (Fig.19). Stomach: Hyperkeratosis and hyperplasia were more marked than treated G (T1) and previous months (Fig.20).
Fig. (5) liver of rat treated with (8mg/kg.bw/day for 1 month). Showed minimal vacuolation ( ) and apoptosis of hepatocytes and kupffer cell proliferation ( ). H&E X40.

Fig. (6) kidney of rat treated with (8mg/kg.bw/day for 1 month). Showed degeneration and necrosis of epithelial cell lining renal tubules with hemosiderin pigment ( ). H&E X40.

Fig. (7) Brain of rat treated with (8mg/kg.bw/day for 1 month). Showed focal gliosis ( ) with edema ( ). H&E X10.

Fig. (8) Bone marrow of rat treated with (8mg/kg.bw/day for 1 month). Showed extensive erythropoiesis and high no. of megakaryocytes ( ). H&E X40.

Fig. (9) liver of rat treated with (40mg/kg.bw/day for 1 month). Showed hyperplastic nodule ( ). H&E X10.

Fig. (10) kidney of rat treated with (40mg/kg.bw/day for 1 month). Showed severe dilation of medullary renal tubule with hyaline cast ( ). H&E X10.
Fig. (11) Liver of rat treated with (8mg/kg.bw/day for 2 months). Showed focal nodular aggregation of inflammatory cells. H&E X40.

Fig. (12) Liver of rat treated with (40mg/kg.bw/day for 2 months). Showed centri-lobular necrosis. H&E X40.

Fig. (13) Spleen of rat treated with (8mg/kg.bw/day for 3 months). Showed depletion of white pulp. H&E X40.

Fig. (14) Bone marrow of rat treated with (8mg/kg.bw/day for 3 months). Showed decrease in hemopoietic tissue. H&E X40.

Fig. (15) Myocardium of rat treated with (8mg/kg.bw/day for 3 months). Showed proliferation of F.C.T between muscle fibers. H&E X40.

Fig. (16) Kidney of rat treated with (40mg/kg.bw/day for 3 months). Showed periportal fibrosis, infiltration of inflammatory cells, hyperplasia of bile ductless. H&E X40.
Fig. (17) kidney of rat treated with (40mg/kg.bw/day for 3 months). Showed fibrosis in medullary region. H&E X10.

Fig. (18) Spleen of rat treated with (40mg/kg.bw/day for 3 months). Showed hemosiderin pigment stained greenish blue. Perls prussian blue X10.

Fig. (19) Brain of rat treated with (40mg/kg.bw/day for 3 months). Showed marked vacoulation of white matter. H&E X40.

Fig. (20) Stomach of rat treated with (40mg/kg.bw/day for 3 months). Showed papillary proliferation of epithelial lining with marked hyperkeratosis. H&E X40.

**Discussion**

**Clinical signs:**
- **Acute toxicity:** The results showed that the acute toxicity signs were significantly related to CuSO4.5H2O concentration, the highest dose the more effects of the drug which leads finally to death.
- **Chronic toxicity:** The main clinical signs seen along the period of experiment were depression and slow activity, paleness of mucous membranes of eyes and pads of extremities, hemoglobinurea, dyspnea, leathery and dried skin with ruffled fur and alopecia especially of abdominal region.

Singh and Singh (15) explained that jaundice in CuSO₄ poisoning is partly hepatic in origin in addition to hemolysis which appeared on the second or third day following ingestion. The level of bilirubin was directly proportional to the severity of the poisoning (16). This was in agreement with our results which showed significant increase in bilirubin level in the groups with high toxic group. Hemoglobinurea resulted from hemolysis of the blood and the overloading of the kidney with hemoglobin. Kidney: Hemolysis resulted in the urine being discolored with the hemoglobin degradation products and this explain the dark –brown coloration in the urine of the treated groups, this agreed with (17) who stated that intravascular hemolysis lead to
hemoglobinurea and plays a major role in the pathogenesis of renal failure. Chronic Cu toxicity in cattle caused depression, anorexia, icterus and hemoglobinurea (18). Other features seen in all treated groups especially of high dose, were the loss of elasticity of the skin due to hyperkeratosis which lead to the stiffness and hardness of the skin and piloeretion with ruffled fur (8). The skin of the treated groups showed alopecia (hair loss) and this may be attributed to the cytotoxic effects of CuSO₄ on the cells of hair follicles causing cystic dilation and reduction in their numbers (Telagen) (19).

- **Pathological findings:**
- **Gross lesions:** The lesions found in this study were classic for chronic Cu toxicity as previously described (20).
- **Histopathological lesions:**
  - Liver: The liver showed diffuse minimal vacoulations in low dosage group while the vacoulations were more marked in higher dosage group. These vacoulations appeared because the fatty degeneration which occurred due to excessive release of free fatty acids from adipose tissue due to starvation, resulting in delivery of increased amounts of these acids to the liver which may not be able to utilize the increased fatty acids, also it occurred due to failure of protein synthesis, thus reducing the protein component available for lipoprotein particles which are the means of excretion of lipid from the liver. These disturbances occurred due to toxic effects of CuSO₄ which was responsible for accumulation of fat in liver. These results were in agreement with the results of (21) which showed that increasing of triglyceride in the serum of the blood results in non-alcoholic fatty liver disease. The most significant changes in the liver due to CuSO₄ toxicosis was the centri-lobular coagulative necrosis which began centrally and progress peripherally in the lobule. The extent of necrosis vary with the dose of the drug and the length of treatment, it become more extensive at the end of the experiment and it appeared as a constant feature of all examind sections and this agreed with (22). They recorded that necrosis of liver lobules was a constant feature of CuSO₄ poisoning cases. 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 (23) who reported that in chronic Cu toxicity, Cu accumulated in the lysosomes of hepatocytes until their storage capacity is exceeded. The lysosomes then released the Cu to the cytoplasm where it causes necrosis and inflammation. Similar to our results were described by (24,25), they stated that rats in the dosed feed studies had a related dose in the increased extent of liver necrosis with associated inflammatory response and changes in clinical chemistry parameters which were indicative of hepatocellular damage and cholestasis. Similarly (26) reached the same conclusions that the extent of lesion observed in the examined organs of sheep were affected most by the length of the action and the dose of Cu, and extensive focal necrosis of hepatic tissue had been found in biopsies taken during the hemolytic crisis. Centrilobular necrosis may result from hypoxia in the perivenular regions, with increase in hepatic oxygen demand without an appropriate increased in hepatic blood flow.

Other important change occurred due to higher dose of CuSO₄ was the formation of hyperplasic nodules. This change was due to the fact if Cu concentration is almost 1000 mg/g dry weight the liver maintains its regenerative ability and continuous production of new hepatocytes to take up Cu released by dying cell as stated by (27), or it might be due to mutagenic effect of CuSO₄ since chromosomal aberrations have been produced in isolated rat hepatocytes incubated with CuSO₄(24). These nodules were considered as a preneoplastic nodules which could lead to hepatocellular carcinoma in LEC rats (28). The histopathological examination of the liver showed hepatic degeneration of
different types, some hepatocytes showed signs of dying by apoptosis. Apoptotic cells produce IL-1 which is known as lymphocytic activated factors. Apoptosis was seen during acute hepatitis in LEC rats (28). The apoptosis of hepatocytes and the presence of macrophages in the sinusoidal zone, tubular nephrosis with granular and hyaline casts and hemosiderosis of spleen are considered dominant features of histological picture in diagnosis of various forms of chronic Cu intoxication (29). Other pathological changes seen in the liver were the periportal fibrosis with infiltration of inflammatory cells and hyperplasia of bile ductules which may be due to cholestasis. The fibrosis was more prominent at the high dosage and long duration which may be considered as a healing process.

- Kidney: Histopathological examination of kidney sections showed degenerative changes and necrosis of the epithelial lining of proximal convoluted tubules which including epithelial and hyaline casts. These changes are more severe in highly dosage group and that might be attributed to the intravascular hemolysis and releasing of Cu which may caused necrosis of epithelial lining of the tubules. This result was in agreement with (17). They stated that intravascular hemolysis plays major role in the pathogenesis of renal failure. The hemosiderin pigment released due to hemolysis and direct toxic effect of Cu from lysed red cells contribute to tubular epithelial damage of the kidney. Similarly (30) related the damage of the proximal convoluted tubules either from direct effect of the CuSO₄ or from the effect of the hypotension and/or hemoglobinuria. Tubular nephrosis with granular and hyaline casts were seen in industrial Cu intoxication in sheep (31). Our results showed that the damage of the epithelial lining tubules was constant in all examined sections and that agreed with (31). They mentioned that renal tubular damage is predominant in chronic Cu toxicity.

Other histopathological changes were cortical tubular dilation which is more marked in higher dosage and long duration they undergo cystic dilation. These results may be attributed to the toxic effect of CuSO₄ leading to renal insufficiency resulting in decrease excretion of the drug and that will lead to accumulation of CuSO₄ metabolites within the cortical tubules. The examined sections showed cortical tubular basophilia which refered to regenerative process which followed by degeneration. Similar results seen in sheep receiving a daily drench of CuSO₄ at a rate of 20 mg/kg.bw there was some indication of a regenerative process in the damaged renal tissue based on the slight recovery of certain enzyme activities that are markedly reduced during the hemolytic crisis like glutamate dehydrogenase and succinic tetrazolium reductase. The regenerated epithelium is histologically different from undamaged epithelium (32). Our results showed infiltration of chronic inflammatory cells in the interstitial tissue and fibrosis of medullary region especially in high dose and long duration of treatment and that could be attributed to the damage that affected the renal parenchyma. These results were in agreement with (33). They reported that chronic tubulointerstitial nephritis had developed as a consequence of parenteral CuSO₄ in human.

- Brain: The main lesions of the brain are perineuronal and perivascular edema which is clear in all examined sections. The edema increased fluid increased with the time and especially in higher dosage, with extensive demyelination, focal gliosis and vacuolation of pyramidal cells of white matter. Hemorrhages, congestion and perivascular cuffing seen in sections of higher group. Animals of higher dosage group showed aggregations of microglial cells around the blood vessels forming nodular like structure just beneth the meninges. Brain lesions could be attributed to the toxic effect of CuSO₄. It caused cell injury by damage to lysosomal membrane leading to release of Cu in the cytoplasm of the cells as explained by (34) Similarity
of ataxic signs of lambs to those of the extrapyramidal motor disease such as parkinson`s disease suggests that the dopaminergic system may be affected (35) leading to increase of the dopamine level in the brain of ataxic lambs. In chronic toxicosis in sheep, the histopathological examination revealed gliosis at the junction of the molecular and granular layers of the cerebellum and status spongiosis in the medulla (36).

- Stomach: Results showed hyperplasia and hyperkeratosis of the mucosa of forestomach with marked changes were seen in higher dosage and long duration. The available human and animal data suggest that gastro-intestinal tract is a sensitive target of toxicity (37). Studies in rats showed that a dietary concentration of 34 mg/kg.bw/day in female for 13 weeks were associated with hyperplasia and hyperkeratosis of the squamous epithelium of the limiting ridge of the forestomach (24) and this result agreed with our study.

- Heart: The present results showed degenerative changes characterized by fatty degeneration and necrosis with hemorrhages the amount of necrotic tissues and hemorrhage, are more in the group with the higher dose of CuSO₄ with infiltration of mononuclear cells and fibrosis which were more pronounced at the 2nd month of treatment. These cells also infiltrate the epicardium. These changes were due to toxic effect of CuSO₄ on myocardial cells leading to their necrosis. This result agreed with (38), they established a positive correlation between serum Cu levels and risk of acute myocardial infarctiion. A high tissue Cu levels ,by causing a zinc deficiency predisposes one to hypertension, heart attacks and strokes (39). High Cu levels have been cited a possible risk factor for heart disease (40). Animal studies showed that heart disease occurred in the surviving offspring of pregnant hamsters given intravenous Cu salts on day eight of gestation and rats fed a diet supplemented with Cu exhibited increased systolic blood pressure. (38) reported toxic myocarditis as a cause of death following CuSO₄ poisoning (39).

- Spleen: The pathological Changes showed hyperplasia of lymphoid tissues in both groups during the first two months. Depletion of the lymphoid tissues appeared clearly at the end of the 3rd month, that could be related to the effect of the drug causing reduction in the immune system. Those results were in agreement with (8). They stated that there is a marked depletion of the lymphoid tissues in both male and female rats but not in mice especially at high concentration at 13 weeks of ingestion in a study of subchronic toxicity of CuSO₄. In addition to that the histopathological examination of the spleen showed congestion with deposition of hemosiderin pigment, the congestion was severe in high dosage group with deposition of large amount of hemosiderin pigment which appeared clearly when specific stain were used, these results could be related to the excessive hemolysis accompanied CuSO₄ toxicosis as stated by (41). Our results showed that there is a decrease in erythropoiesis which explained the presence of large numbers of megakaryocytes in the red pulp region that extramedullary hemopoiesis takes place in the spleen as a secondary mechanism (42).

- Bone marrow: Bone marrow undergo hyperplasia of hemopoietic tissues with presence of large numbers of megakaryocytes at the 1st and 2nd month, while at the 3rd month there is severe suppression of hemopoietic tissue and increased in numbers of sinuses with thin trabeculae especially in higher dosage group. The hyperplasia of hemopoietic tissue was due to hemolytic anemia and it is a usual physiological response in order to increase the production of blood cells by the bone marrow with immature forms which appear in the circulation suggesting that compensatory erythropoiesis has been suppressed. In other words, the bone marrow
of toxicated groups far from being depressed, is in fact producing red cells at a considerable faster rate than normal and this is of a great value in helping the animal maintaining its hematological indices to meet the continuous blood loss. Continuous stimulation and toxic effect of the drug resulting in exhaustion and suppression of hemopoietic tissue which is very clear in sections of later stages of treatment. Evidence of osteoporosis could be related as stated by (19) to manganese deficiency which is considered one of the principal causes of loss of calcium from the bone.

References


