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Research Article

Structural Destabilization in Renal and Hepatic Cells and Tissues Subjected to $CuSO_4$ Toxicity in Male Mice

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Abstract

This study was conducted to determine the effects of different concentrations of copper sulphate on metabolically active organs-liver and kidneys of male mice on the basis of employing histopathological changes. The observation included careful examination of three batches-defined as normal and treated groups on two concentrations administered by intubation. All animals were acclimatized one week prior to the commencement of the experiment and raised in same conditions in a four weeks long experiment. Upon termination, each specimen was sacrificed and dissected to recover liver and kidney and processed for histopathological examination under microscope. Structural changes of each organ were recognised and categorized organ-wise. The hepatocytes clearly suffered hypertrophy including necrosis; the kupffer cells assumed larger size with disarrangement of hepatic cords. The kidney too affected severely. Most histopathological changes were restricted to Bowman's capsules: suffered haemorrhage, congestion and lymphocytic aggregation observed in the glomerulus. Besides; necrotic changes and apoptosis of epithelial lining of the proximal and distal convoluted tubules and in collecting tubules were prominently observed. The study confirmed that $CuSO_4$ accumulation in organs targeted, inflicts toxic pathological damages, serious enough to impair related physiological processes.

Keywords: Copper Sulphate; Histopathology; Hepato-Renal Toxicity; Heavy Metals, Mice

Introduction

Copper Sulphate ($CuSO_4.5H_2O$) has a ubiquitous presence and so known for wide applications. It is used chiefly in agricultural practice, pesticide control, leather industry and as precipitator in heavy metal poisoning; besides, treating gastric and topical exposure to phosphorous [1,2]. The Cu is an integral component of many enzymes and proteins implicated in many metabolic processes [3]. There is more to it: the copper is required for infant growth, defence mechanism, bone strength, maturation of erythrocytes and leukocytes, and also in cholesterol and glucose metabolism and ion transport [4]. It is no wonder then, the excess of Cu or the deficiency it can alter the normal development in organisms as well [5]. Even as acute copper poisoning is less frequent and so restricted to inadvertent accidental intakes; nevertheless, its toxicity if in small intensity but on long-term consumption is quite serious [6]. Its intake cause health problem and indicative of damage to vital organs like liver and kidney-and each of these are rigorously involved in metabolic processes and excretion of harmful element [7]. Studies, taken earlier on copper toxicity found its affects on erythrocytes, liver and kidneys [2-9]. Besides, these $CuSO_4$ results in caustic burns of esophagus, or causing superficial or deep ulcers in stomach and small intestine [10-13]. The serious effects of copper-on liver, kidney and other organs continued to be of concern because of contradictory results -was the conclusion in the literature.

A great body of literature reported the copper poisoning in diverse organisms between 2002-2015 [14,15] however, reporting on applied aspects such as on fertilization success [16,17] the viability of spermatozoa [18] also appeared in between. Thus, impact

of Cu in male fertility [19] effect on, epididymal fluid [20] and evaluation of epididymal sperm quality-all in male rats are another notable extension, of these [21].

It is not Cu alone, researches are on dealing with the toxicity of heavy metals on albino rats quite recently [22]. But focus is on the assessment of groundwater quality [23] physicochemical properties 24] and genotoxic evaluation [25]. Prior to this studies relating to evaluation of tissue deposition long-term exposure to heavy metal were observed [26-28]). Research using Zn and Pb in male rats are still the research subject [29]. An update on the role of aluminium toxicity is further an important addition but in relation to $CuSO_4$ the studies are scanty even now and those exist are ambiguous [30].

Consider, for example: the accumulation of copper and its effect on liver is inconclusive so far. The liver damage is not always caused by the Cu, even at very high concentration rather it is attributed to its specific binding to proteins in the liver depending on various factor such as species, age, genetics and duration [31]. Under normal condition, much of the Cu in the liver occurs in the cytosol but with increasing accumulation the metal forces its way into nucleus and lysosomes escalating into Cu-induced autophagy, but the study taken long before [32]. The confirmation was not reinstated by contemporary work or by recent citations.

Essentially, the copper is important; copper ions are found in cells and tissues bound to proteins or to some other organic compounds; infact, to most organs of the human body. After the poisonous effects of copper talked, the therapeutic use of the same are also known: given and so astringent and antiseptic properties have recently been highlighted in a recent study [33] but, the high concentration of copper in liver brain, heart, stomach and intestine are quite evident since long [34] and also found in blood and fluids [6].

But we must be more concerned about Cu poisoning in kidney and liver. The effect of Cu accumulation in kidneys was recognized when genetic disorder of Cu metabolism in Long-Evans Cinnamon (LEC) rats-a model for Wilson's disease or a macular mice-a model for Menke's disease were identified [35,36]. Subsequently, it was described that excess of Cu is responsible for the renal injury in LEC rat kidneys [37]. The different localizations of the Cu-MT indicated previously; prompted to investigate the renal Cu metabolism [38]. In this series, [39] did use copper as kusta, but not as $CuSO_4$ solution for renal parameters. Copper metal in all the initial studies, even if scanty, strongly reiterate the need to study infrastructural changes once again the organs such as liver and kidney ascribed to high metabolic rate. These two organs are studied in the recent experiment to resolve the issue.

Materials and Methods

Experimental Animals

The study, an experimental intervention, properly randomized and controlled, was carried on male mice. To begin with, all the animals were kept in the animal house and subsequently, experimental work was processed in the zoology labs utilizing 20 mice in a four-week long study. All the mice were between 6-8 weeks of age: weighing 80-100 gms. These were separated in different cages maintained under controlled conditions of temperature (20-25˚C), humidity (65 ± 5%) and the light-dark cycle (12 hrs. each). The mice received a prescribed dose each day; and cage cards were used to indicate the groups of experimental mice. The procedure for maintenance, tissue collection and treatment was of standard ones [39,40]. The protocol received the formal approval from the ethical committee of the University.

Copper Sulphate Preparation

A blue copper sulphate crystal, use for $CuSO_4$ solution, prepared in two doses: 2% concentration-2 mg of copper sulphate dissolved in 1 liter of Distilled Water (DW) and 4% concentration prepared by dissolving 4 mg of $CuSO_4$ in 1 liter of DW.

Experimental Schedule

All mice inspected prior to the commencement of the experiment. The sex difference was chosen in favor of male rats. This was done to eliminate any difference to male and female hormonal differences. Properly acclimatized mice were divided into three lots. They received nutritionally standard diet and water on every alternate day. Each mice were further assigned an identification number on its back.

The gavage method was used for administrating the $CuSO_4$ for a defined period of times. For this purpose, syringe with a ball-tipped gastric-feeding needle was used. The controlled lot received deionized water whereas, the remaining two has $CuSO_4$ exposure as, altogether grouped into three.

Group A: Acted as a controlled group, included 6 healthy rats, received normal diet and tap water for the duration of experiments.

Group B: Included 6 rats, infused by 2% $CuSO_4$ solution @ of 0.15 mg/kg body weight.

Group C: Included 6 rats, intubated with 4% $CuSO_4$ solution @ of 0.30 mg/kg body weight.

As termination of exposure time neared the animals were sacrificed, dissected and excised for liver and kidney and their biopsies used in processed histopathological preparations.

Histopathological Procedure

The histopathological technique followed the [41] method. The biopsies of liver and kidney were fixed in 10% buffered saline and dehydrated in ascending grades of ethanol and subsequently cleared in xylene. It was followed by infiltration and impregnation procedure in soft paraffin at 50°C and immediately embedded in paraffin and paraffin blocks were prepared; these were cut further to obtain 5 µm thick sections using rotator microtome; mounted glass slides covered by albumin glycerin. Multiple histological stains using hematoxylin and eosin was utilized to explore histological structure and apoptotic changes. These stains ensure better visibility and resolution. The slides were examined under the microscope and followed the standardized method described by [42]. Histopathological changes in hepatic and renal tissues were seen at different magnifications and representative structural changes were photomicrographed.

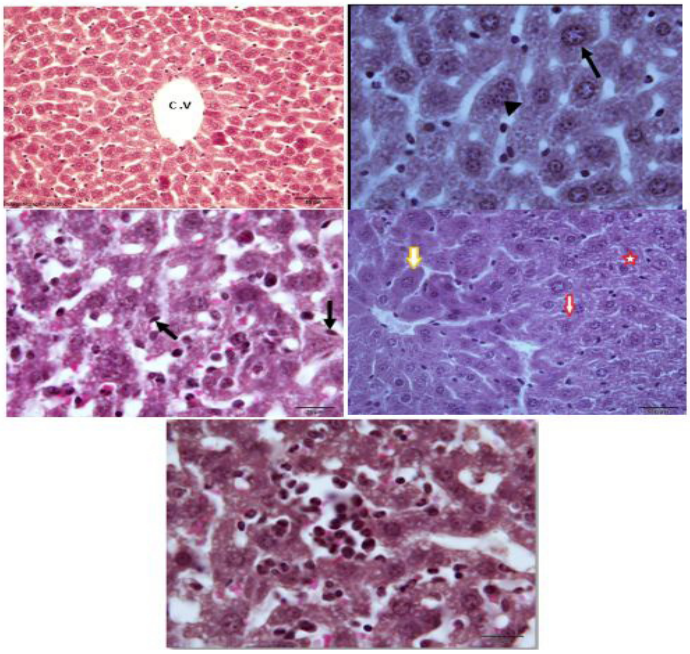
Results

Observation on Hepatic Tissue

The architectural changes of the liver cells have been identified and summed up in Figures 1(a -e). The general outline of the control hepatic tissue showed the typical organization: a thin fibrous connective tissue capsule covering the whole organ at the hilum, where the portal vein and hepatic artery enter the liver at the level of right and left hepatic ducts along with the lymphatic exit- are clearly seen; and the hepatic lobules appeared as polygonal units with a clear plate of epithelial cells and hepatocytes radiating from central vein (1- a).

The representative histopathological changes in hepatic tissue in 2% $CuSO_4$ solution

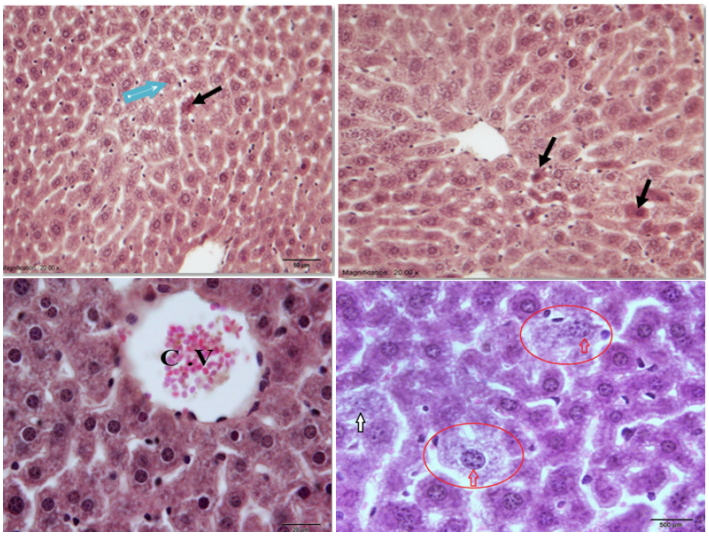
(Figure 1) - b shows typically multinucleated hepatocytes; some having as much as three nuclei; other cells showed karyomegaly with large nuclei. The other changes include: the tendency of increasing vacuolization of the hepatocytes and, kupffer cells appearing as larger size with darkly stained prominent nuclei (1-c). Besides, some lymphocytic infiltration within the sinusoids between the vacuolated hepatocytes was observed additionally (1-d). As the exposure time was increased, the normal appearance of the hepatic lobules gradually lost and the number of affected hepatocytes increased. The hepatic cord became non-distinct from each other, while some hepatocytes showed cytoplasmic vacuoles along with shrinkage and separation from each other: beside, clear pyknotic nuclei could be further seen (1-e).



Figures 1(a-e): Hepatic cell photomicrographs showing structural changes in 2% $CuSO_4$ treated mice. (a), the normal hepatocytes, with a prominent central vein (CV) surrounded by cords of hexagonal nucleated hepatocytes; (b), showing multinucleated hepatocytes (arrow head) some with karyomegaly (arrow); (c), tendency of increasing vacuolization in hepatocytes and Kupffer cells having large and darkly stained nuclei (arrows); (d), showing aggregation of lymphocytes between hepatic cords; (e), large area of affected cells (star) due to degenerated lobules and non-distinct laminae, also some cells with cytoplasmic vacuoles, shrinkage and separation (small arrow) and the pyknotic nuclei ,as against somewhat normal looking cells elsewhere (broad arrow).

Changes in hepatic tissues treated with 4% $CuSO_4$

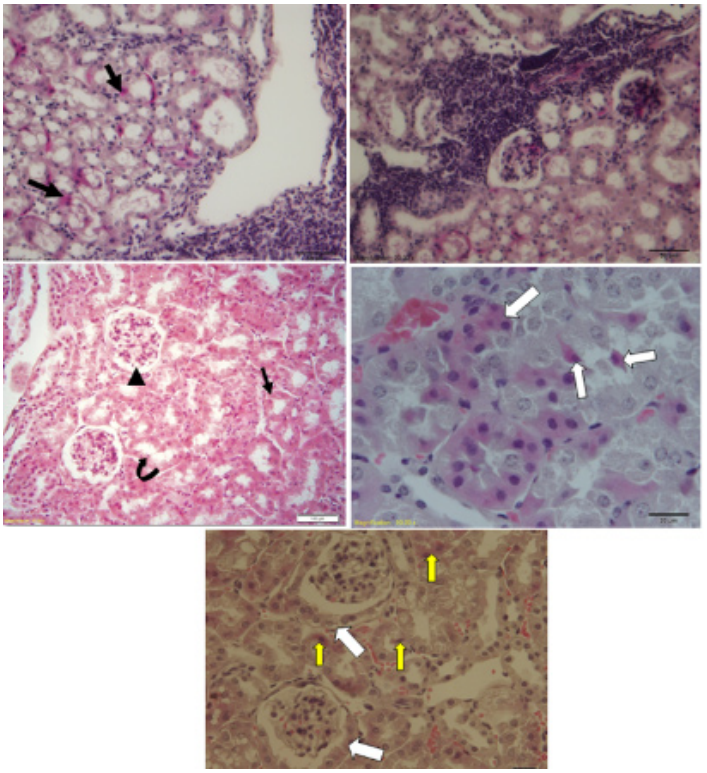
With the more acute treatment of hepatic cells in 4% $CuSO_4$; the cells, in addition to the vacuolization of the cytoplasm, shrinkage and pyknotic nuclei and necrosis, made their appearance along with irregular cell boundary (2-a, b). Another change was noticed in the central vein was the appearance of blood cells in the lumen (2-c). The infrastructural loss of hepatocytes became much more serious with the increase of dosage and duration (2-d); so that the part of the liver suffers gradual loss of the hepatocytes due to of nuclear death and apoptosis. As a result, the nuclei demonstrated condensation of chromatin substance, followed by fragmentation of chromatin with a part of the cytoplasm around and undergoing rupture of nuclear envelope. In case of necrosis, the loss of these acidophilic cytoplasmic materials was accompanied by swelling of the cells.



Figures 2(a-d): Hepatic cell photomicrographs denoting structural changes in 4% $CuSO_4$ treated mice. (a), showing vacuolation of cytoplasm, few cells with necrosis-a sign of degenerative change (small arrow), irregular cell boundary and pyknotic nuclei (broad arrow); (b), necrotic hepatocytes beginning to lose nuclei (arrows); (c), central vein (cv) with blood cells in lumen. (d**),** cells with fragmented chromatin and swelling of cells (circles and arrows). (H & E: a, b x40, c x100, d x1000).

Observation in Renal Tissue

Morphologically, the kidney has a convex lateral surface with concave medial border, the hilum where nerves enter; and further the ureter exits from this point and the blood and lymph vessels make their entry and exit. It is covered by thin fibrous capsule. Broadly, the kidney can be demarcated into an outer cortex and an inner medulla. Each kidney is comprised of numerous functional units called nephrons. The histopathological section of controlled kidney showed cortex with renal capsules and proximal and distal convoluted tubules. These characteristics have been illustrated in 3 - a, other changes are outlined in Figures 3 (b,c).



Figures 3(a-e): Renal cell photomicrographs denoting structural changes in 2% $CuSO_4$ treated mice. (a), an untreated cell showing cortex with renal corpuscle (arrow head), proximal (arrow) and distal convoluted tubules (curved arrow); (b), cortex with lymphocytic infiltration around renal corpuscles and tubules; (c), cortex containing proximal convoluted tubules with necrotic changes of the cell lining (arrows); (d), proximal tubules with degenerated cells in different stages of cell death showing deeply stained cytoplasm and nuclei (arrows); (e), degenerative changes in cells of proximal tubule (small arrows), with the parietal layer of Bowman's capsule partially replaced by cuboidal cells (broad arrows).

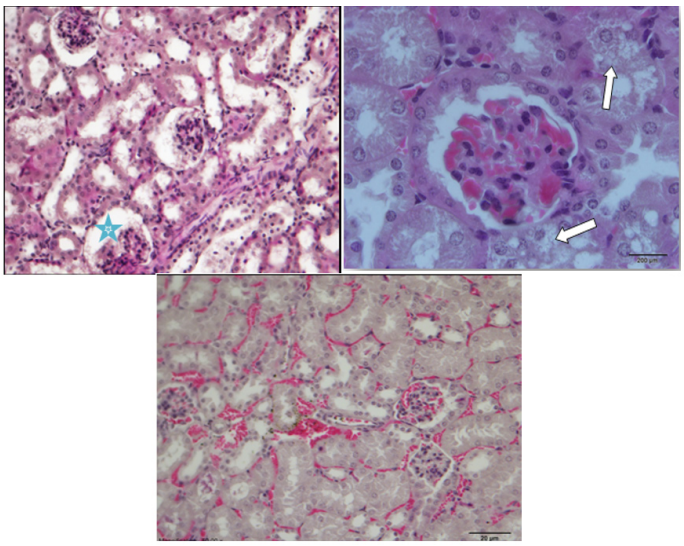
(H & E: a-c X40; (d,e) x1000).

The representative histopathological changes in renal tissue in 2% $CuSO_4$ solution

The kidney showed some lymphocytic infiltration around the renal corpuscles, and some glomeruli suffered degeneration of various grades (3-b). In addition, some cells appeared showing the degenerative changes along lining of the proximal and distal convoluted tubules. These changes were more of the nature of necrosis (3-c). Other figures in plate 3 from (d-e) depicted an increase in the number of degenerated cells lining the proximal convoluted tubules and showed necrosis with darkly stained cytoplasm and equally deeply stained pyknotic nuclei.

Changes in renal tissues treated with 4% $CuSO_4$

At higher dose of $CuSO_4$, serious degenerative changes set and went on becoming complicated with the duration of treatment. For example, in affected tubules, sporadic degenerated cells appeared in dark acidophilic cytoplasm. The proximal and distal convoluted tubules got the cells at different stage of degeneration and noted with pyknotic nuclei. The renal corpuscles showed distorted glomeruli and degenerative cell lining. The Bowman's space becoming wide between the glomerulus and parietal layer - most of these changes have been depicted in Figure (4-a). Further, a change frequented around the kidney tubules and renal corpuscles showed clear sign of congestion of the blood capillaries (4-b). Two other serious signs, as the exposure continued for longer period of time were manifested in the cortex, where the renal corpuscles became crescent shaped as a result of undergoing congestion within, and secondly, an appearance of vacuolated cytoplasm in the cells of proximal tubules (4-c).



Figures 4(a-c): Renal cell photomicrographs denoting structural changes in 4% $CuSO_4$ treated mice. (a), distorted glomeruli with degenerated cells, Bowman's space appearing wide (star); (b), cortex with crescent-shaped renal corpuscles with congestion, proximal tubules showing vacuolated cytoplasm (arrows); (c), cortex showing congestion of the blood vessels around renal corpuscles and proximal and distal tubules (arrows) (H & E: a, b x40; c x1000).

Discussion

The severity of the lesions is reiterated depending upon the dose and duration of treatment. The present results are in agreement with the trend seen earlier [43]. The liver undergoes degenerations with lobular necrosis, a constant feature of all examined sections. The focal point is infiltration of mononuclear cells with apoptosis and severe hemorrhage and proliferated kupffer cells. These cells have phagocytic nature localized in the liver blood sinusoids; and so the sinusoids were seen as markedly large with darkly stained nuclei, more so in the high doses and also towards the end of the experiment.

The effect is in agreement with earlier studies taken on $CuSO_4$, but in rats and not in mice as of present case [44].

Vacuolization in the liver is another phenomenon to many studies and in diverse organisms [45]. Such a change usually frequented near and around the central vein accompanied by pyknosis of nuclei of the hepatocytes. Present study further revealed that in higher doses, the hepatic cells show the sign of karyomegaly-a condition linked to hepatocytes increases. It is predominantly a nuclear change seen commonly, including mice.

The histopathological examination of the kidney is also manifested in many degenerative changes and necrosis of the epithelial lining especially in proximal convoluted tubules and lymphatic infiltration of the chronic inflammatory cells around arteries and near the renal corpuscles also seen. The inflammation can spread to the interstitial tissue, mainly in higher doses and duration. This is also not unique to mice. Such chronic tubulointerstitial nephritis are also reported in human when exposed to $CuSO_4$ [46]. Another marked change is the increase in the Bowman's space and presence of more necrotic cells, a typical sight in the present case associated with the lining of the kidney tubules and extent of vacuolization in the collecting tubules -both are not unique, although variation in the degree of changes are common [47].

These, and other structural changes accompanied with these two metabolically active organs further reveals that, the treatment with respect to heavy metals are equally sensitive manifesting respective changes. Many metals are obtained with, necrosis, blood congestion, infiltration of lymphocytes and degeneration of cells in case of liver causing serious damage to glomeruli [44].

Some noteworthy studies on $CuSO_4$ using nanocopper is quite recently poring in [48]. This line of research indicated an extreme toxicity of nano-Cu in microorganisms, raising the prospects of bactericidal approach [49].

A contemporary study, on $CuSO_4$ nanoparticles although associated with cytotoxicity and apoptosis in human cancer cell line involving mitochondrial pathway due to ROS is equally noteworthy [50]. Nonetheless, potential of $CuSO_4$ nanoparticles in biomedical field is evidently getting credit in *in vitro* and *in vivo* models [51].

It is more expected now that future studies, not only focus on common damages-cytotoxicity, organ toxicity and genotoxicity - should also explore nano prospects-and eco-friendly approaches using synthesis of nano heavy metal not only exploit the *in vivo* toxicity of Copper Nanoparticles (CUNPs) but also reveal functional, morphological changes in organs of mice in an elaborate

way. This will strengthened in our future strategies. There is an urgent need of focusing on molecular mechanism of CUNP-mediated toxicity both in normal and cancer cells of different origins. In conclusion we can say that present update on $CuSO_4$ toxicity, explicitly demonstrated rise in structural abnormalities of metabolically active organs and cells of renal and hepatic tissues, the outcome is serious enough to carry investigation towards immediate consideration in medicine. Going by the reports and researches in $CuSO_4$ as also with other heavy metals, evaluating the protective effects also absolutely essential.

References

1. Barceloux DG (1999) Copper. J Toxicol Clin Toxicol 37: 217-230.
2. Gupta PS, Bhargava SP, Sharma M (1962) Acute copper sulphate poisoning with special reference to its management with corticosteroid therapy. J Assoc Physicians India 10: 287-292.
3. Georgeopoulos PG, Roy A, Yonone LM (2001) Environmental copper: its dynamics and human exposure issues. J Toxicol Environ Health 4: 341-394.
4. Uauy R, Olivares M, Gonales M (1998) Essentiality of copper in humans. Am J Clin Nutr 67: 9529-9599.
5. Shepard TH, Lemire R (2004) Catalog of teratogenic agents eleven edition, The John Hopkins University Press Baltimore Maryland.
6. Underwood EJ (1971) Copper in Trace Elements in Humans and Animals 4th edition. Academic Press, New York.
7. Bendeddouche B, Zellagui R, Bendeddouche E (2014) Levels of selected heavy metals in fresh meat from cattle, sheep, chicken and camel produced in Algeria. Annu Res Biol 4: 1260.
8. Singh M, Singh G (1968) Biochemical changes in Blood in cases of acute copper sulphate poisoning. J Indian Med Assoc 50: 549-554.
9. Mital VP, Wahal PK, Bansal OP (1966) A study of erythrocyte glutathione in acute copper sulphate poisoning. Indian J Pathol Bacteriol 9: 155-62.
10. Chugh KS, Sharma BK, Singhal PC (1977) Acute renal failure following copper sulphate intoxication. Postgrad Med J 53: 18-23.
11. Deodhar LP, Deshpande CK (1968) Acute copper sulphate poisoning. J Postgrad Med 14: 38-41.
12. Papodayanakis N, Katsilambros N, Patsourakos B (1969) Acute copper sulfate poisoning with jaundice. J Ir Med Assoc. 62: 99-100.
13. James LP, Stowe CD, Argao E (1999) Gastric injury following copper sulphate ingestion. Pediatr Emerg Care 15: 429-431.
14. Bidwell C, Livesey C (2002) Copper poisoning: an emerging disease in dairy cattle. State Vet J 12 : 16-19.
15. Gharred T, Ezzine IK, Naija A, Bouali RR, Jebali J, et al. (2015) Assessment of toxic interactions between deltamethrin and copper on the fertility and developmental events in the Mediaterranean sea urchin *Paracentrotus lividus*. Environ Monit Assess 187: 193.
16. Victor S, Richmond RH (2005) Effect of copper on fertilization success in the reef coral *Acropora surculosa*. Mar pollut Bull 50: 1448-1451.
17. Hedouin L, Gates RD (2013) Assessing fertilization success of the coral *Montipora capitata* under copper exposure: does the night of spawning matter? Mar pollut Bull 66: 221-224.
18. Knazica Z, Tvrda E, Bardos L, Lukac N (2012) Dose- and time- dependent effect of copper ions on the viability of bull spermatozoa in different media. J Environ Sci Health a Tox Hazard Subst Environ Eng 47: 1294-1300.
19. Aydemir B, Kiziler AR, Anuran I, Alici B, Ozkara H, Akyolcu MC, et al. (2006) Impact of Cu and Fe concentration on oxidative change in male fertility. Biol Trace Elem Res 112: 193-203.
20. Gaur M, Pruthi V, Prasad R, Pereira BM (2000) Inductively coupled plasma emission spectroscopic and flame photometric analysis of goat epididymal fluid. Asian J Androl 2: 288-292.
21. Sakhaee E, Emadi L, Abhenas J, Kheirandish R, Azari O, et al. (2012) Evaluation of epididymal sperm quality following experimentally induced copper poisoning in male rats. Andrologia 44: 110-116.
22. Tathagat W, Prithviraj B, Basavar B (2017) Acute Toxicity of Heavy Metals from Bheema River Water and Effects on Albino Rat. Trds in Biosci 10: 2246-2252.
23. Adekunle IM, Adetunj MT, Gbadebo AM, Banjoko OB (2007) Assessment of groundwater quality in a typical rural settlement in South West Nigeria. Int J Envoron Res Public Health 4: 307-318.
24. Akubugwo EI, UDe VC, Uhgbu FO, Ugnogu OC (2012) Phsiochemical properties and heavy metal content of selected water sources in Ishiagu Ebonyi State-Nigeria. J Bio Environ Sci 2: 21-27.
25. Danadevi K, Rozati R, Banu BS, Grover P (2004) Genotoxic evaluation of welders occupationally exposed to chromium and nickel using the Comet and micronucleus assay 191: 35-41.
26. Dieter MP, Jameson CW, Tucker AN, Luster MI, French JE, et al. (1988) Evaluation of tissue disposition, myelopietic, and immunologic responses in mice after long-term exposure to nickel sulfate in drinking water. J Toxicol Environ Health 24: 356-372.
27. Foulkes EC (1990) Biological effects of heavy metal in carcinogenesis, Vol. II CRC Press, Florida USA.
28. Ayensu WK, Tchounwou PB (2006) Microarray analysis of mercury-induced changes in gene expression in human liver carcinoma (HepG2) cells: importance in immune esponses. Int J Environ Res Public Health 3: 141-173.
29. Nooshin G, Parvis F, Mostafa M, Faranak S (2016) Zinc and lead poisoning effects on hepatic tissue in male rats. Int Conf Res Sci and Eng.
30. Gajendra M, Sharique AA (2015) An update on the role of medical plants in amelioration of aluminum toxicity. Biosci Biotech Res Comm 8: 175-188.
31. Bremner I (1987) Involvement of metallothionein in the hepatic metabolism of copper. J Nutr 117: 19-29.
32. Goldfischer S, Bernstein J (1969) Lipofuscin (aging) pigment granules of the newborn human liver. J Cell Biol 42: 253-261.
33. Akomolafe RO, Olaoluwa Olukiran S, Eseigbe Imafidon C, Ayodeji Ayannuga O, Oyedele Oyekunle JA, et al. (2016) Effects of chronic copper sulphate administration on feeding pattern and markers of renal and liver functions of wistar rats. Christ Eseigbe Imafidon 26: 1.

34. Shorrocks V (1984) Use of Copper as a Micronutrient for Crops, INCRA Planning Study, Part I, International Copper Research Association, New York.

35. Li Y, Togashi Y, Sato S, Emoto T, Kang JH, et al. (1991) Spontaneous hepatic copper accumulation in Long-Evans Cinnamon rats with hereditary hepatitis: a model of Wilson's disease. J Clin Invest 87: 1858-1861.

36. Danks DM, Campbell PE, Walker-Smith J, Stevens BJ, Gillespie JM, et al. (1972) Menkes' kinky-hair syndrome. Lancet 1: 1100-1102.

37. Tochimaru HY, Akutsu Y, Nagata Y, Taketoshi S, Matsumoto, et al. (1991) Acute tubular necrosis in LEC rats with hereditary hepatic failure-A new model of hepatorenal syndrome. In: M. Mori M. Yoshida, N. Takeichi, N. Taniguchi. Tokyo, (ed.) The LEC Rat: A New Model for Hepatitis and Liver Cancer, Springer-Verlag 103-113.

38. Ebaid R, Elhussainy E, El-Shourbagy S, Ali S, Abomohra AEF et al (2017) Protective effect of *Arthrospira platensis* against liver injury induced by copper nanoparticles. Oriental Pharmacy and Experimental Medicine 17: 203-210.

39. Kareem H, Naseem N, Nagi AH, Haq RU (2015) Deranged levels of Renal Parameters after using heavy metal kushta of copper in diet of albino rats. Pakistan J Med Health Sci 9: 807-810.

40. Liu JY, Yang X, Sun XD, Zhuang CC, Xu FB, et al. (2016) Suppressive Effects of Copper Sulfate Accumulation on the Spermatogenesis of Rats. Biol Trace Elem Res 174: 356-361.

41. Ahmed TME, Saadiya RS, Entesar AS, Rehab AR (2015) Histopathological and histochemical assessment of the protective effects of zinc on ethanol-induced acute hepatotoxicity in adult albino rats. Cytol Histol 6: 321.

42. Bancroft JD, Gamble M (2008) Theory and practice of histological techniques, 60 ed, Churchill living stone, Elsevier 126: 171-601.

43. Al - Naimi RA, Al - Tayar NH, Abdul-Hadi D, (2010) Toxic pathological study due to effect of different doses of copper sulfate ingested in rat. A Anbar J Vet Sci 3.

44. Hamak HM, Thali KM (2012) Ultra-structural Alterations Induced by Doses of Some Heavy Metal in the Kidney and Liver of Rat. J Tikrit Uni Agri Sci 3: 12-22.

45. Abu – Zinadah OA, Hussein HK (2011) The potential of stem cell to lost tissue mass on liver and kidney of rat after copper toxicity. Insig Stem Cell Res 1: 1-5.

46. Nurullah G, Cuma Y, Ozgur K, Nahida EG, Mehmet, et al. (2006) A series of patients in the emergency department diagnosed with copper poisoning. Recog Eq Treat 209: 243-248.

47. Tarasub N, Tarasub C, Ayutthaya WD (2011) Protective role of curcumin on cadmium - induced nephrotoxicity in rats. J of Envir Chem and Ecotox 3: 17 -24.

48. Ghassan MS, Amer TT, Marwa DJ (2018) Biogenic synthesis of copper oxide nanoparticles using Olea European leaf extract and evaluation of their toxicity activities: An *in vivo* and *in vitro* study. Biotechnol Prog 34: 218-230.

49. Camacho-Flores BA, Martinez-Alvarez O, Arenas-Arrocena MC, Garcia-Contreras R, Argueta-Figueroa L, et al. (2015) Copper: synthesis techniques in nanoscale and powerful application as an antimicrobial agent. J Nanometer: 1-10.

50. Shafagh M, Rahmani F, Delirezh N (2015) CuO nanoparticles induce cytotoxicity and apoptosis in human K562 cancer cell line via mitochondrial pathway, through reactive oxygen species and P53. Iranian J Basic Med Sci 5: 1004-1021.

51. Vinardell MP, Mitjans M (2015) Antitumor activities of metal oxide nanoparticles. Nanomaterials 5: 1004-1021.