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Neurotoxic and Genotoxic Potentials of Short Term Copper Exposure in Adult Male Albino Rats (Biochemical, Histopathological, Immunohistochemical and Genotoxic Study)

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Abstract Objective: Although copper is an essential micronutrient involved in a variety of biological processes indispensable for sustaining life yet it can be toxic when administered in excess. Material and Methods: Thirty six adult male albino rats were equally divided into three groups; the first group was used as a negative control, the second group received normal saline intraperitoneal (positive control group) and the third group received copper chloride 7mg/kg twice weekly intraperitoneal for 8 weeks (treated group). At the end of the experimental period, blood was withdrawn for measuring serum malondialdhyde (MDA) and catalase (CAT), then the rats were sacrificed and the brain was examined by light microscope for detecting histopathological changes. Brain sections were examined immunohistochemically for the detection of the apoptotic BaX protein and finally, the bone marrow was examined for detection of chromosomal aberrations. Results: In Cu chloride treated group (group III), there was a significant elevation in serum malondialdehyde & a significant decrease in serum catalase. Histopathological changes were in the form of increased cellularity of astrocytes, swelling of astrocytes that showed dense eosinophilic cytoplasm, with pyknotic nuclei and multiple apoptotic bodies. Also, there were degenerated neurons with deep eosinophilc cytoplasm using light microscope and BaX showed strong immunoreactions in the brain. Bone marrow showed significant increase in all types of chromosomal aberrations after 8 weeks of Cu chloride treatment. Conclusion: From the previous results, it can be concluded that Cu chloride exposure can induce oxidative stress changes in the form of a significant increase in MDA and significant decrease in CAT enzyme as well as histopathological changes in the brain and genotoxicity in adult male albino rats after short term exposure.

Keywords I copper, brain, genotoxic, BaX, Rats.

Introduction

Copper (Cu) is an essential trace element in living organisms, functioning as a cofactor for many enzymes (Fraga, 2005). However, excessive amounts of Cu are potentially hazardous to human health because Cu can participate in the Fenton reaction, (the Fenton reaction, Cu+1 transform the weak oxidant hydrogen peroxide into hydroxyl radical) (Gutteridge and Halliwell, 2000) producing oxidative stress and subsequent damage to bio molecules (Kozlowski et al., 2009).

Cu-derived substances are used in a wide range of industries, in the production of agrochemicals, and in Cu-containing intrauterine devices (Cu-IUDs) (Arnal et al., 2011).

It has been shown that chronic exposure to Cu ions by farmers handling agrochemicals and women using Cu-IUDs leads to elevated levels of Cu in the plasma (Arnal et al., 2010). Cu is administered parenterally to individuals unable to ingest food by oral or parenteral routes and dialysis patients receiving dialysis via Cu tubing, and in cholestatic infants maintained for long periods on intravenous total parenteral nutrition (Frem et al., 2010).

The widespread potential for human exposure to copper occurs via consumption of food and water

contaminated with copper and also by inhalation of industrial or cosmetics contained dust or fumes with it (Mitra et al., 2012).

Various studies have demonstrated that patients with neurodegenerative diseases had elevated Cu concentrations in their plasma, suggesting a direct or indirect involvement of Cu overload in the progression and/or the etiology of neurologic diseases. Ingestion of excessive Cu through drinking water and vitamin supplements is at least partly responsible for the development of Alzheimer's disease in developed countries, which to date is reaching epidemic proportions (Arnal et al., 2014).

Copper is an essential metal for all living organisms and is a component of many metalloproteins such as antioxidant enzyme Cu–Zn superoxide dismutase (Cu, Zn, -SOD) and cytochrome oxidase . However, copper also plays a role in the pathogenesis of neurodegenerative disease such as Alzheimer's disease (AD) (Lu et al., 2006).

Copper ions themselves, in the presence of reducing agents such as ascorbate or glutathione, causes various kinds of damage to DNA, and also it helps other agents to damage DNA and chromatin, via production of reactive oxygen species (ROS), Hence, it is accused of direct relation to mutagenicity and genotoxicity (Mitra et al., 2013).

The oxidative damage causes disruption of lipid bi-layers, alterations in protein function, and perhaps also altered gene expression. This potential consequence is programmed as cell death, or apoptosis (Linder, 2012).

A pro-apoptotic molecule (Bax) becomes an integral membrane protein and cross-linkable as a homodimer following a death stimulus and blocks the anti-apoptotic effect of Bcl-2 (Xu et al., 2012).

The Bcl-2 gene has been found to inhibit apoptosis. Bax can also dimerise with itself, and appears to promote apoptosis when overproduced. The balance of Bax and Bcl-2 in a cell is one of the critical factors determining whether the cell will undergo apoptosis .Bax mRNA has been found to be up-regulated and Bcl-2 mRNA down-regulated in mouse brain neurons undergoing apoptosis. Various studies showed increased levels of Bax protein in the rat and gerbil hippocampus following cerebral ischemia (MacGibbon et al., 1997).

Genotoxicity is a general term for any type of DNA or chromosome damage or alteration. It includes not only gene mutations, chromosome breaks and rearrangements, but also interaction with or damage to DNA, interference with the DNA replication or repair processes **(**Zeiger and Hill, 2010**).**

The present work aimed to evaluate the potential neurotoxic and genotoxic effects following intraperitoneal injection of copper chloride (Cu cl_2) for 8 weeks in adult male albino rats.

Materials and Methods

Materials

a) Chemicals

1- Copper: copper (II) chloride dihydrate crystals (Cu cl_2) purchased from Biodiagnostic co. 29 El- Tahrer Street- Dokki- Giza, Eygpt.

2- Normal saline: (0.9%Nacl solution) from a local pharmacy.

b) Animals

Ethical consideration of the study:

-All ethically approved conditions used for animal housing & handling were considered. -The animals were acclimatized to experimental conditions prior to the start of dosing for a period of 14 days to ascertain their physical well-being and to exclude any diseased animals (Semler, 1992)**.** -Promotion of high slandered of care & animal well-being at all times. Painless procedures were performed with appropriate sedation to avoid distress & pain.

A total of 36 adult male albino rats, weighing 180-200 gm, were kept on copper-free rat chow and water *ad libitum* at the Breeding Animal House of the Faculty of Medicine, Zagazig University. Acclimatization period lasted for 2 weeks. The animals were housed in filter-top plastic cages at a temperature (23 ± 1°C), humidity (55 ± 5%) and artificially illuminated (12:12 hours light: dark cycle) in a room free from any source of chemical contamination. All rats received human care in compliance with the guidelines of the medical research ethics committee (Institute of Laboratory Animal Resources et al., 1996).

c) Experimental Design:

The rats were divided into 3 numerically equal groups, 12 rats for each group. Six rats subjected to oxidative stress markers measurements & neurotoxic studies and the remaining 6 rats for chromosomal (genotoxic) studies. They were divided as follows:

Group I (negative control group): Kept without treatment for 8 weeks to measure the basic parameters to be compared with other groups.

Group II (positive control group): Each rat in this group received normal saline (vehicle for copper chloride) twice weekly intraperitoneally for 8 weeks.

Group III (copper chloride group): Each rat of this group received copper chloride (7mg/kg) representing 1/10 of Ld 50 according to (Paget and Branes,1964) which is equal to 1mg/kg in mice twice weekly intraperitoneally for 8 weeks according to Mitra et al. (2012) .

After the end of 8 weeks, 6 rats from each group were anesthetized by ether inhalation for drawing blood samples from the retro–orbital plexuses of veins for performing the biochemical studies malondialdehyde (MDA) and catalase (CAT). Then the anesthetized rats were sacrificed & specimens from the brain were taken. The remaining 6 rats in each group were subjected to chromosomal studies.

Methods

1- Biochemical Study

Venous blood samples were collected from the retro-orbital plexus of veins of the animals by capillary glass

tubes after under light ether anaesthesia. The collected blood was used as follows: Blood samples (about 3 mL) were collected in clean test tubes without anticoagulant then the sera were separated by centrifugation of blood 3000 r.p.m for 10 minutes. The supernatant sera were pipetted off using fine tipped automatic pipettes and stored in deep freeze at –20 C until used for estimating serum MDA (Ohkawa et al., 1979) and CAT (Aebi et al., 1984**)**.

2- Histopathological examination

a) Hematoxylin & Eosin stain

After 8 weeks, 6 rats from each group were anaesthetized with ether inhalation then sacrificed. The brain was immediately dissected out and grossly inspected to assess any abnormalities. Tissues from brain were obtained as 5-mm thick sections, fixed with 10 % neutral l formalin for more than 12 hours ,dehydrated, embedded in paraffin, sectioned at5 μm, mounted

b) on glass slides, and stained with hematoxylin and eosin (H&E) stain (Wilson and Gamble, 2002)

Immunohistochemical procedure according to (Bancroft and Gamble, 2002):

Paraffin sections 3-5 um were deparaffinized in the oven at 56 ˚C for 30 minutes, and inserted in xylene for 30 minutes. Tissues were rehydrated in descending grades of alcohol 95%, 85%, and then 75% for 5 minutes each. Slides were rinsed with distilled water for 5 minutes. Antigen retrieval was performed by boiling in sodium citrate buffer (0.001M, pH 6) for 15 minutes in microwave. Endogenous peroxidase activity was blocked by incubation with hydrogen peroxide for 10 minutes, then rinsed with distilled water.

Primary antibodies (2-3 drops of a Bax Ab-4 mouse monoclonal antibody) were applied on each slide to cover the specimen overnight at 4˚C. After washing 3 times with phosphate buffer saline (PBS), the sections were incubated with biotinylated secondary antibodies for 30 min. This was followed by incubation with streptavidin-biotin-peroxidase complex. After 3 rinses with PBS, the slides were incubated with diaminobenzidine for 15 min. The slides were then rinsed with H2O and counerstained with hematoxylin for 3 minutes. This was followed by washing in cold running water, then washing in distilled water. Sections were dehydrated in ascending grades of alcohol and cleared with xylene, then coverslipped and examined.

3- Chromosomal study

After 8 weeks, 6 rats from each group were anaesthetized with ether before sacrifice. The bone marrow was processed using some modifications in the method described by (Rabello-Gay and Ahmed, 1980). 1-Colchicine (BDH Chemicals Ltd Poole, England) was prepared as 0.5% solution and injected intramuscularly in the rats at a dose of 0.25 ml/100 gm body weight. 2-Two hours after colchicine injection, the rats were sacrificed by decapitation and both femora were dissected and cut at both ends. 3-Four milliliters of freshly prepared 0.075 M potassium chloride pre-warmed at 37ºC were injected from one end of the femur while collecting the cell suspension at the other end in a centrifuge tube. 4-The cell suspensions were incubated at 37ºC for one hour then, centrifuged for five minutes at 1000 rpm and the supernatant was discarded. 5-The cells were re-suspended in 2 ml of freshly prepared fixative (3:1, methanol:glacial acetic acid) and the tubes were stoppered and left for at least 30 minutes up to 24 hours at 4ºC. 6-The cell suspension was centrifuged for 3 minutes at 1000 rpm and the supernatant was discarded. The cells were washed by 4 mL of the fixative, centrifuged and the supernatant was discarded for 2-3 times. 7-Finally, 1 ml of the fixative was added to each cell precipitate to obtain a condensed cell suspension. 8-The cell suspensions were dropped and spread on clean glass slides previously put in cold 70% ethanol. 9-The spreads were allowed to air dry and stained in diluted Giemsa stain (1:10, Giemsa stock solution: phosphate buffer pH 6, 8). 10-The glass slides were examined using the oil immersion objective lens of an ordinary light microscope. One hundred metaphase cells were examined in each rat. All metaphases were examined for structural chromosomal abnormalities such as chromatid gaps-break, fragments, deletions, dicentric chromosomes, ring chromosomes, stretched chromatin material and clumping. They were examined also for numerical abnormalities, hypoploidy and hyperploidy.

4- Statistical analysis

For statistical analysis, SPSS version 13.0 for windows programme was used. Data were represented as means ± SD. The differences were compared for statistical significance by ANOVA. Descriptive data were compared by chi-square test. Difference was considered significant at $p<0.05$.

Results

1- Biochemical results: oxidative stress markers (Table-1)

Serum Malondialdehyde (MDA)

Copper chloride treatment (group III) induced a highly significant increase in the serum level of MDA ($p<0.001$) compared to the control groups (group I & II) after 8 weeks as shown in (Table-1).

Serum catalase enzyme (CAT)

The results revealed a highly significant decrease in the serum level of CAT ($p<0.001$) in Cu chloride treated group (group III) as compared to the control groups (group I & II) after 8 weeks as shown in (Table-1).

2- Histopathological result:

Gross inspection of the brain revealed normal appearance in treated and control groups.

Examination of sections from the brain tissue of control groups (group I & II) showed normal neuronal structure formed of nerve cells with pale nuclei (Figure-1). While the brain sections of treated copper chloride group (group III) revealed significant pathological changes in the form of increased cellularity of astrocytes in the cerebral cortex sections, swelling of astrocytes that showed dense eosinophilic cytoplasm, with pyknotic nuclei and multiple apoptotic

bodies. Also, there were degenerated neurones with deep eosinophilc cytoplasm (Figures- 2, 3&4).

3- Immunohistochemical results:

The brains of the both control groups (groups I & II) showed no abnormal BaX protein expression *(Figure-5)* while in the Cu chloride treated group (group III), they showed increased BaX protein expression which appeared as brownish cytoplasmic staining (Figure-6).

4- Chromosomal (Genotoxic) study (Table-2):

The bone marrow cells of the control groups (groups I & II) showed normal metaphase spread having 42 chromosomes (figure-7). The anomalies detected in these groups were too few to show meaningful statistical analysis and there was no statistically significant difference (Table-2).

The Copper chloride treated group (group III), for 8 weeks revealed chromosomal aberrations in their bone marrow cells. These changes were in the form of terminal chromatid deletions (Figure-8), chromosomal fragments, chromosomal gaps (Figure-9), ring chromosomes, dicentric chromosomes (Figure-10), clumping of the chromosomes, hyperploidy (Figure-11), hypoploidy (Figure-12), and stretching of the chromatid materials (Figure-13). Comparing the Cu chloride treated group (group III) with control groups (group I & II) by the end of 8th week, revealed a significant increase in terminal chromatid deletions & hypoploidy and a highly significant increase in the other types of chromosomal aberrations produced in rats bone marrow (Table-2).

Table (1): ANOVA (F-test) statistical analysis of serum malodialdehyde (MDA) & catalase (CAT) in the treated Cu chloride group & both control groups after 8 weeks

	Cu chloride (group III)	+ ve control (group II)	- ve control (group I)	F-test	P-values
MDA (nmol/L)	38.0 ± 6.9	2.2 ± 0.7	2.8 ± 2.1	144.032	0.000**
CAT (U/L)	289.6 ± 21.4	2344.3 ± 203.2	2939.0 ± 114.1	636.342	0.000**

Number of sacrificed rats was 6 rats P value of > 0.05 = Non-Significant. P value of < 0.01= Highly Significant (**)

Table (2): Chi square (X2-test) statistical analysis of chromosomal aberrations produced in the bone marrow cells of the adult male albino rats after 8 weeks in the treated Cu chloride & both control groups .

Parameters	Cu chloride (group III)	+ve control (group II)	-ve control (group I)	X^2 test	P-values
Deletions	9	2	3	6.44	0.040*
Fragments	18	3	2	22.7	0.000**
Rings	20	2	1	32.2	0.000**
Gaps	25	4	3	32.4	0.000**
Dicentric fragments	12	1	2	15.6	0.000**
Hyperploidy	26	4	3	34.5	0.000**
Hypoploidy	11	3	4	6.74	0.034*
Clumping	24	7	9	14.9	0.001**
Stretching	31	10	12	18.5	0.000**

Number of sacrificed rats for each group was 6 rats.
P value of > 0.05 = Non-Significant . P value of < 0.05 = Significant (*).P value of < 0.01= Highly Significant (**).

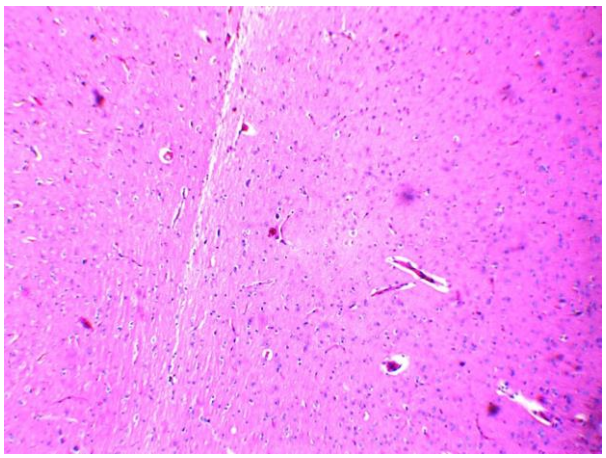

Figure-1: A photomicrograph of a transverse section in the brain obtained from an adult male albino rat of negative & positive control group (group I& II) showing unremarkable changes in neuronal structure (H&E x 200).

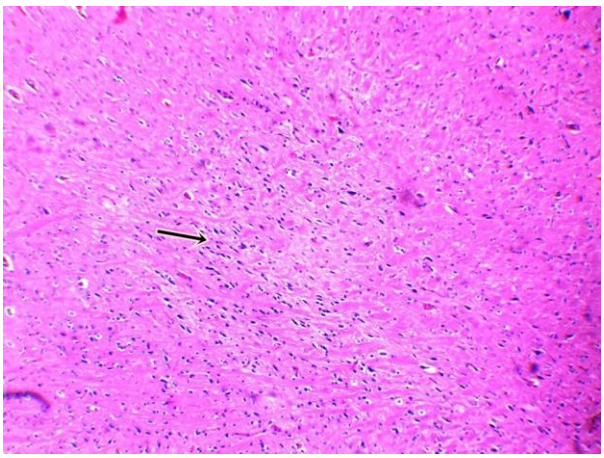

Figure-2: A photomicrograph of a transverse section in the brain obtained from an adult male albino rat of the treated group (group III) showing: Increased cellularity of astrocytes (arrow) of neuronal structure (H&E x 200).

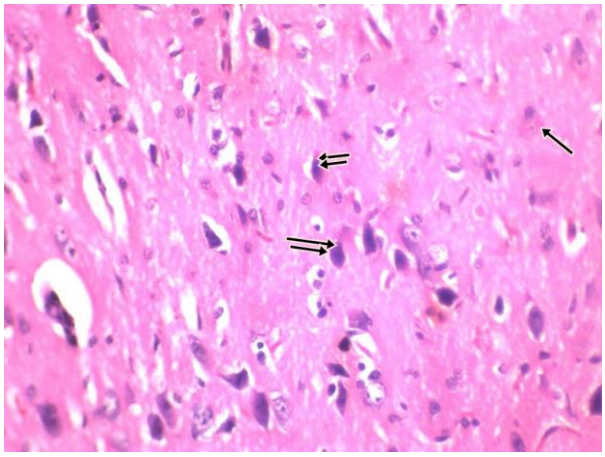

Figure-3: A photomicrograph of a transverse section in the brain obtained from an adult male albino rat of the treated group (group III) showing: degenerated neurones with deep eosinophilc cytoplasm (arrow) and astrocytes with pyknotic nuclei (double arrows) (H&Ex 400).

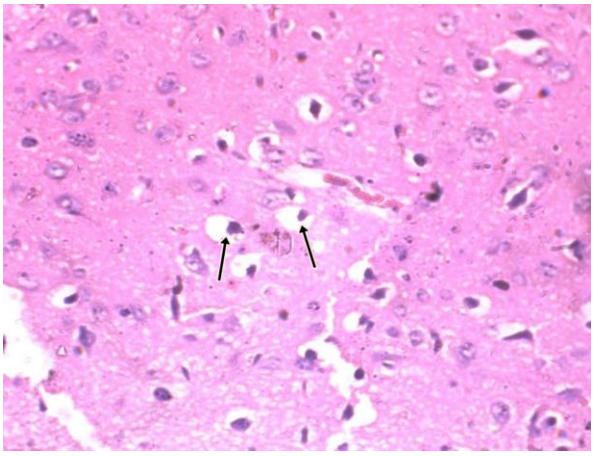

Figure-4: A photomicrograph of a transverse section in the brain obtained from an adult male albino rat of the treated group (group III) showing: Astrocytes with multiple apoptotic bodies (arrow) (H&Ex 400).

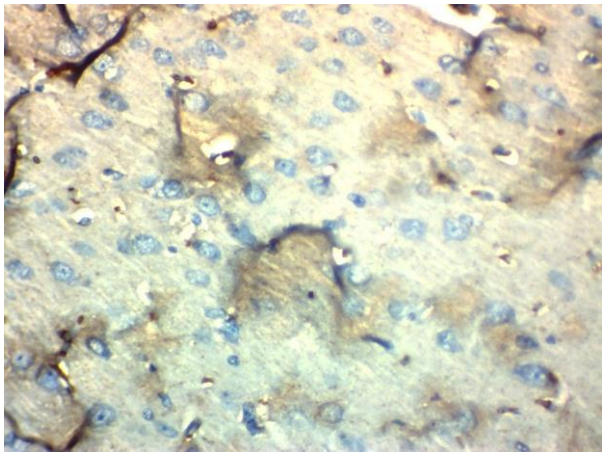

Figure- 5: A photomicrograph of a transverse section of the brain obtained from adult male albino rat immunohistologically stained for Bax. Negative & positive control groups (group I & II) showed no remarkable immunostaining of Bax (immunostain x400).

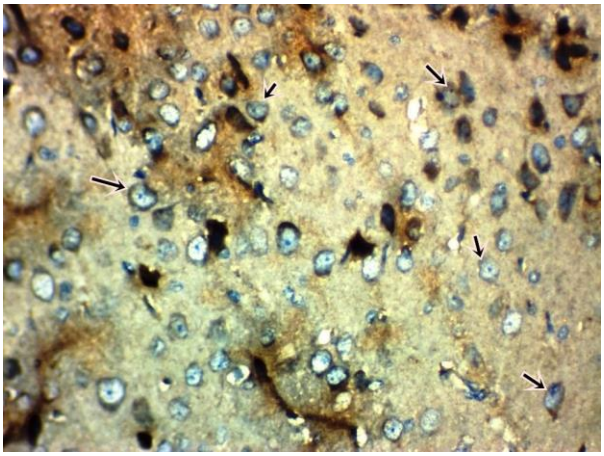

Figure- 6: A photomicrograph of a transverse section in the brain obtained from adult male albino rat immunohistologically stained for Bax of the treated group (group III) showing: Astocytes with cytoplasmic immunostaining of Bax (immunostain x 400).

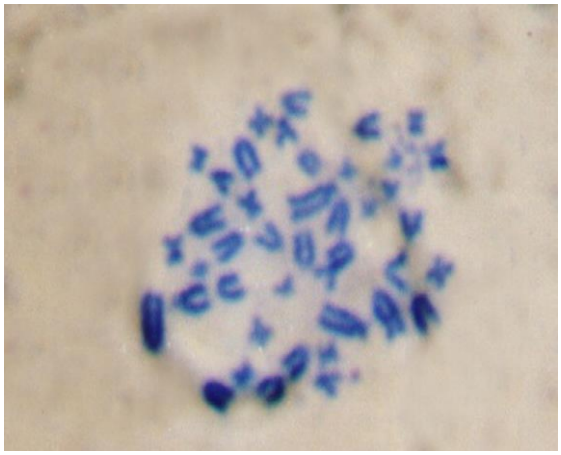

Figure-7: A photomicrograph of a metaphase spread prepared from the bone marrow cells of an adult male albino rat of negative & positive control group (group I&II) showing no remarkable chromosomal changes (Geimsa stain x 1000).

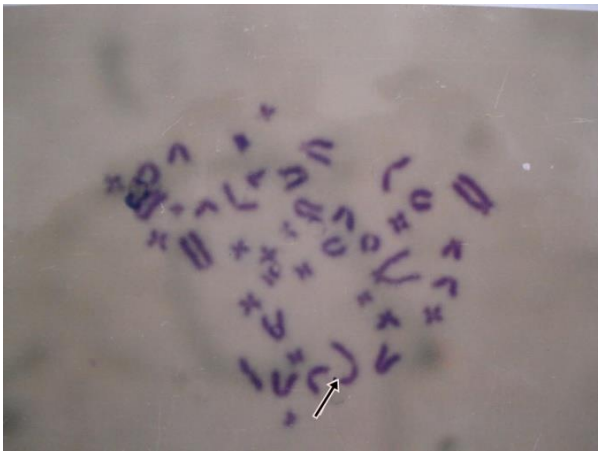

Figure-8: A photomicrograph of a metaphase spread prepared from the bone marrow cells of an adult male albino rat of Cu chloride treated group (group III) showing terminal chromatid deletion (arrow) (Geimsa stain x 1000).

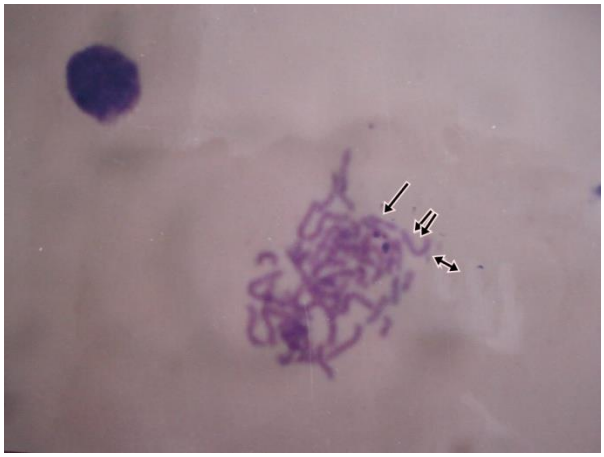

Figure-9: A photomicrograph of a metaphase spread prepared from the bone marrow cells of an adult male albino rat of Cu chloride treated group (group III) showing terminal chromatid deletion (double arrow), gap (arrow) and chromosomal fragment (double arrow heads) (Geimsa stain x 1000).

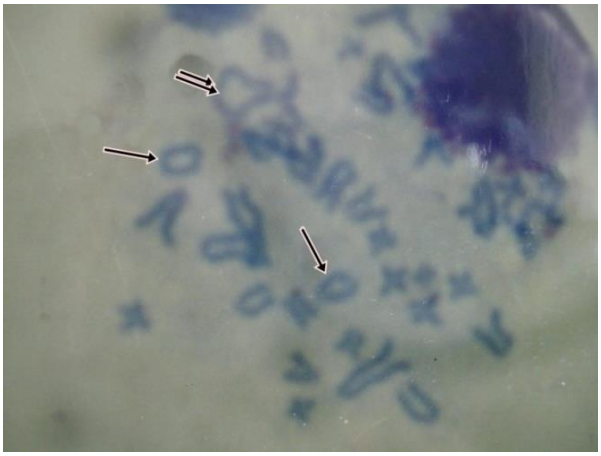

Figure-10: A photomicrograph of a metaphase spread prepared from the bone marrow cells of an adult male albino rat of Cu chloride treated group (group III) showing 2 chromosomal rings (arrow) and dicentric chromosome (double arrow) (Geimsa stain x 1000).

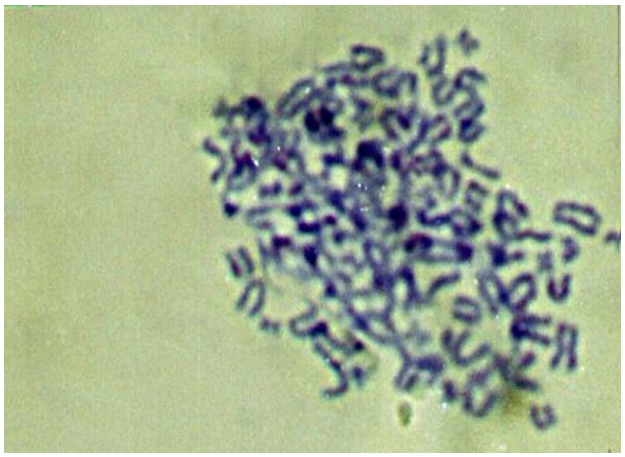

Figure-11: A photomicrograph of a metaphase spread prepared from the bone marrow cells of an adult male albino rat of Cu chloride treated group (group III) showing clumping of the chromosomes and hyperploidy (Geimsa stain x 1000).

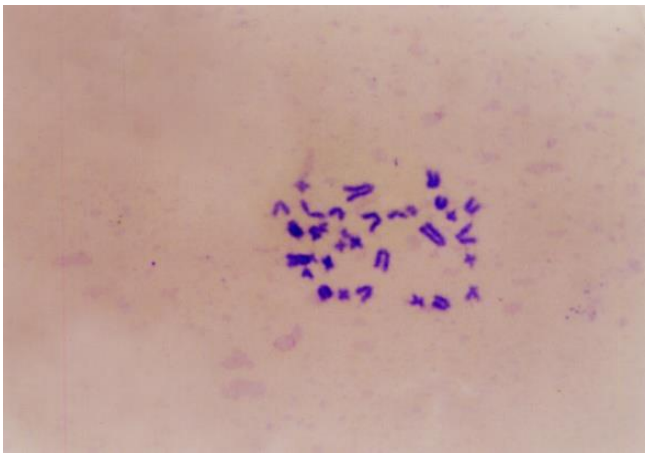

Figure-12: A photomicrograph of a metaphase spread prepared from the bone marrow cells of an adult male albino rat of Cu chloride treated group (group III) showing hypoploidy (Geimsa stain x 1000).

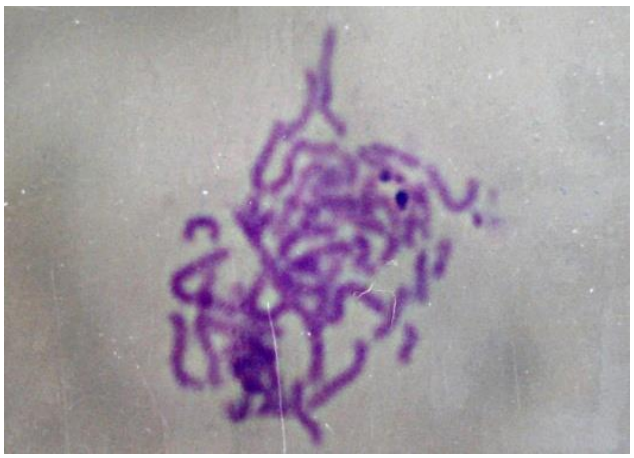

Figure-13: A photomicrograph of a metaphase spread prepared from the bone marrow cells of an adult male albino rat of Cu chloride treated group (group III) showing stretched chromatid materials (Geimsa stain x 1000).

Discussion

In the present study, intraperitoneal injection of Cu chloride for 8 weeks (short term exposure) resulted in a significant increase in serum malondialdehyde with a significant decrease in serum catalase enzyme.

These results are in accordance with Pan et al. (2008) who stated that MDA is widely accepted as a sensitive biomarker of lipid peroxidation (one of the main manifestations of oxidative damage). It is considered as a useful measure of oxidative stress status. Similarly, Arnal et al. (2012) observed that copper caused a significant increase in MDA. It was attributed to reactive oxygen species (ROS) production more specifically hydrogen peroxide causing lipid peroxidation .

In consistence with these results Roy et al. (2009), found that Copper induced oxidation of membrane lipids through generation of ROS in hepatocytes caused a significant increase in thio-barbituric acid reactive substances (TBARS) level.

Furthermore, on a clinical basis Ozturk et al. (2013) showed that MDA level was significantly higher in patients with recurrent aphthous stomatitis (RAS) where higher serum Cu levels were found to be positively correlated with MDA as Cu was released during inflammatory tissue damage.

The obtained decrease in serum CAT in the present study is consistent with the work of Ozturk et al. (2013) which showed that CAT levels were significantly lower in patients with RAS with higher serum Cu levels. Also Pena et al. (2011) found that treatment of sunflower seedling with Cucl2 resulted in decrease of CAT

activity which occurred due to oxidation of the protein structure.

The obtained elevation in serum MDA and decrease in serum CAT due to Cu chloride injection recorded in the present study can be explained by Valko et al. (2004) who stated that copper, at high concentrations, can induce production of (ROS) that are dangerous as they may attack biological macromolecules (DNA, lipids and proteins) inducing oxidation and causing membrane damage, enzyme inactivation and DNA damage.

The results of the present study showed that short term exposure to Cu chloride induced significant pathological changes in the brain as an increase in the number of astrocytes in the cerebral cortex section, swelling of astrocytes with dense eosinophilic cytoplasm, pyknotic nuclei and multiple apoptotic bodies. Also, there were degenerated neurons with deep eosinophilc cytoplasm.

The histopathological findings in the present study coincided with these of Pal et al. (2013) who founded that the effects of intraperitoneally injected copper lactate resulted in swelling and increase in the number of astrocytes with degenerated neurons showing pyknotic nuclei and dense eosinophilic cytoplasm.

Also, astrocytes swelling (Alzheimer type II) and increase in number of astrocytes in the brain of Cu-intoxicated rats were documented by Gouider-Khouja (2009) which may be probably because of the neuroprotective mechanism against direct or indirect Cu induced damage (Tiffany-Castiglioni et al., 2011).

Degenerated neurons observed in this study were in agreement with (Rivera-Mancia et al. (2010) who reported that Cu intoxication-induced neuronal degeneration in the brain of Cu-intoxicated animals.

These histopathological findings were explained by Pietrangelo et al. (1992) who stated that the mechanisms for Cu transport into the brain are not fully understood, it has been shown that Cu is loosely bound to albumin in serum and there is a possibility that Cu ions may be released from the albumin-bound moiety at brain barriers and these free Cu ions are then transported into the brain. Another explanation that brain lesions could be attributed to the toxic effect of CuSO4. It can cause cell injury by damage to lysosomal membrane leading to release of Cu in the cytoplasm of the cells.

Choi and Zheng (2009) suggested that the dopaminergic system may be affected leading to increase of the dopamine level in the brain. In chronic Cu toxicity 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 (Monnot et al., 2011).

The immunohistochemical findings in the present study showed that brain sections of Cu chloride treated group (group III) showed increased BaX protein expression which appeared as brownish cytoplasmic staining that coincided with Zhai et al. (2000) who stated that Bax is upregulated by Cu. Moreover, it increased the production of ROS in a dose-dependent manner. So, Cu might induce apoptosis in BA/F3beta cells via up-regulation of Bax and ROS and subsequent inactivation of NF kappaB.

Also, in agreement with this result Kawakami et al. (2008) found that Bax protein in the cell lysate from PC12 cells treated with copper showed a significant increase compared with that from PC12 cells without copper.

These findings can be explained by Er et al. (2006) who thought that the expression of Bax was activated by addition of copper, and that Bax activated cytochrome **c** release. In response to DNA damage, p53 is activated and capable of regulating gene expression Heimbrook and Oliff, (1998). Also, Schuler and Green (2001) stated that Cu induces the activation of Bax and suppression of Bcl-2 .

Copper induced apoptosis was also explained by Kawakami et al. (2008) who suggested two pathways. At first, an increase of Bax induces the release of cytochrome c from the mitochondria into the cytoplasm owing to binding to apoptotic activating caspase 9 leading to the activation of caspases 3. In the other pathway an increase of Bax and reactive oxygen

species activates the release of apoptosis-inducing factor (AIF) from the mitochondria. The AIF induces apoptosis via a caspase-independent pathway.

The results of the present study revealed genotoxicity (chromosomal aberrations) in Cu chloride treated group (group III) which were in the form of terminal chromatid deletions, chromosomal fragments, chromosomal gaps, ring chromosomes, dicentric chromosomes, hyperploidy, clumping of the chromosomes, hypoploidy, and stretching of the chromatid materials.

Chromosomal aberration and genotoxic potentials of copper observed in this study were consistent with the findings of Prá et al. (2008), who stated that Copper induced the highest mutagenicity as evaluated by comet assay and micro nucleus test.

The genotoxicity of copper compounds has been reported mainly in vitro. For instance, positive results were reported for the Syrian hamster embryo cell transformation/viral enhancement assay (Heidelberger et al., 1983). Also, genotoxicity in vivo has been shown for freshwater planarian, using the comet assay (Guecheva et al., 2001).

These findings can be explained by Linder (2001) who stated that the accumulation of large amounts of copper in cells and organs can be destructive. Destruction is thought to be due to ROS whose formation is catalyzed by free copper ions (or certain complexes) that might occur when the ability of the cells to store excess copper in benign form has been exceeded and hence resulting in DNA damage.

Conclusion

From the previous results it can be concluded that, Cu chloride can induce oxidative stress changes represented by a significant increase in MDA and a significant decrease in CAT enzyme. Histopathological changes in the brain and genotoxicity in adult male albino rats on short term exposure. On the light of the results of the present study, the following guidelines are recommended**:**

Improvement of health education programs to increase public awareness about health impact of copper.

Periodic measurement of serum MDA levels and other oxidative stress biomarkers in populations who are at risk of exposure to copper for a more reliable risk health assessment (or screening determinations) because they are easily detected in clinical labs.

Further studies are needed to evaluate mechanisms of other possible Cu chloride induced toxicities and the reversibility of such changes.

Further studies are needed to evaluate the protective effects of vitamin E and vitamin C against Cu chloride induced toxicities.

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الملخص العربى

السمية العصبية و الجينية المحتمله نتيجة التعرض للنحاس لفترة زمنية قصيرة المدى على ذكور الجرذان البيضاء البالغة

(دراسة كيميائية حيوية و هستوباثولوجية و هستوكيميائية مناعية و جينية)

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المقدمه : يعتبر النحاس من العناصر الهامة التي تدخل في العديد من العمليات الحيوية و التي لا غنى عنها لاستمرار الحياة و لكن من الممكن ان يكون ساما اذا استخدم بكثرة.

المواد و الطرق المستخدمة : ستة و ثلاثون جرذا أبيض من الذكور البيضاء البالغة تم تقسيمهم إلى ثلاث مجموعات متساوية كالأتي : المجموعة الأولى (مجموعة ضابطة سالبة) و المجموعة الثانية (مجموعة ضابطة موجبة) تم اعطاء الجرذان محلول الملح و المجموعة الثالثة تم اعطاء الجرذان في هذه المجموعة كلوريد النحاس الثنائي ٧ مجم / كجم مذاب في محلول الملح عن طريق الحقن البروتيني مرتين أسبوعيا لمدة ٨ أسابيع وفي نهاية الدراسة تم سحب عينات الدم لقياس المالونديالدهيد و الكاتاليز . ثم تم ذبح الجرذان و استخراج المخ لعمل دراسة هيستوباثولوجية وتم عمل دراسة هيستوكيمائية مناعية لقطاعات من المخ للكشف عن بروتين باكس المسبب لموت الخلايا المبرمج. و تم فحص نخاع عظم الفخذ للجرذان بعد استخراجه للكشف عن التشوهات الكروموسومية.

النتائج: في المجموعة الثالثة (المجوعة المعالجة بكلوريد النحاس الثنائي) لوحظ زيادة ذات دلالة احصائية في مستوى المالونديالدهيد وإنخفاض الكاتاليز في مصل الدم, تغيرات باثولوجية مثل زيادة عدد الخلايا النجمية ذات السيتوبلازم الايوسينى الكثيف وانتفاخها , مع وجود انوية ضامرة و ظهور العديد من الأجسام المعبرة عن موت الخلية المبرمج و انكماش وتفتت فى الخلايا العصبيه مع وجود السيتوبلازم الايوسينى الكثيف . بينما أظهر بروتين الباكس تفاعلا مناعيا قويا بالمخ. كما لوحظ زيادة ذات دلالة احصائية في أنواع التشوهات الكروموسومية في نخاع العظام. و قد حدثت هذه التغيرات بعد مرور ٨ أسابيع من الحقن البروتيني بكلوريد النحاس الثنائي.

الأستنتاج: من النتائج السابقة, يمكن استنتاج ان التعرض لكلوريد النحاس الثنائي يؤدي الى اجهاد تأكسدي ممثلا في ارتفاع مستوى المالونديالدهيد وإنخفاض الكاتاليز . و تغيرات هستوباثولوجية في المخ و ظهور السمية الجينية في ذكور الجرذان البيضاء البالغة عند التعرض للنحاس لفترة زمنية قصيرة المدى.

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