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Histological, Immunohistochemical Expression and Biochemical Changes in the Liver and Kidney of Wistar Rat Neonates after Prenatal Exposure to Copper Oxychloride

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Abstract | Copper oxychloride (CO) is a commonly employed antifungal pesticide in the fields of agriculture and public health. The objective of the present research is to explore the influence of maternal acquaintance to CO during pregnancy on renal and hepatic function in neonatal rats. Thirty pregnant rats were categorized into 3 groups; first one is the control group received oral administration of carboxymethyl cellulose (CMC) via gavage, high-dose group (73.5 mg/kg) of CO was given by gavage 1/20 LD_{50} of CO mixed with CMC, and a low-dose group (36.75 mg/kg) of CO was given by gavage 1/40 LD50 of CO mixed with CMC. The administration of the CO began on the 6th day gestation day and continued orally until the 20th day of gestation. Thirty days after birth, blood, and organ samples, including liver and kidney, were taken from both male and female newborns. The blood samples were analyzed using a biochemical assay, while the kidney and liver tissues were investigated for histopathological and immunohistochemical expression. The results indicated there was significant tolerance of female offspring than male as indicated by ALT, urea and creatinine. A notable alteration in the biochemical parameters of blood serum (ALT, AST, albumin, total protein urea, uric acid and creatinine), as well as in the histopathological changes and immunohistochemical expression of tumor necrosis factor (TNF) in liver and caspase-3 in kidney tissues were observed when the pregnant rats exposed to CO especially the high dose that were significantly altered than the low dose CO. These findings suggested that a higher dose treatment of CO to the mother can lead to adverse effects on the liver and kidneys. Nevertheless, the small dose treatment of CO to the mother resulted in less harm to them than the high dose.

Keywords: Copper oxychloride, Histopathology, Hepatotoxicity, Nephrotoxicity, Neonates

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INTRODUCTION

Fungicides have been employed in agriculture for more than a century, and there were no initial indications of reduced effectiveness in real-world conditions (Brauer *et al*., 2019). The regular application of fungicides to control crop diseases is a crucial aspect of current agricultural intensification. It has a significant role in enhancing crop yields, improving quality, and assuring stable output (Pretty, 2018). A wide range of potent pesticides with minimal dosage requirements and strong disease-fighting capabilities are readily available to farmers and producers (Ayaz *et al*., 2023).

Copper oxychloride (CO) is one of fungicides used to control bacterial and fungal diseases in vegetable and fruit crops, stone fruit, citrus, ornamentals and pome fruit. It is considered a common broad-spectrum fungicide used to protect vegetables and fruits against various diseases (Thind, 2014). Despite the advantageous effects of CO, it poses health hazards due to its genotoxicity, developmental toxicity, and hepatic toxicity in experimental animals (Burandt *et al*., 2024).

Copper is the primary constituent of CO is a dense metallic element that can build up in different tissues of all organisms (Sattanathan *et al*., 2019). The continuous use of copper can result in the accumulation of this element in crops, soil, and water (Adrees *et al*., 2015). Because of this, copper can get into people and animals through the food chain which may cause many illnesses like kidney problems, hepatocellular neoplasia, and hemolytic anemia (Pandey, 2013). The estimated daily requirement of copper for adults is 2 mg, as copper intoxication may lead to hemolytic anemia. Conversely, excessive copper accumulation in the liver and other organs has resulted in various diseases in humans, including chronic renal and hepatic dysfunctions (Karim, 2018). Chronic copper poisoning in humans results damage to the kidneys and liver (Ashish *et al*., 2013). The accumulation of excess copper in the liver may cause pathological damage by disrupting mitophagy and apoptosis pathways (Yu *et al*., 2021). Extended indirect exposure to copper is linked to neurodegenerative and cardiovascular diseases, liver disorders, atherosclerosis, and certain studies associate it with nephrotoxicity (Sailer *et al*., 2024). Copper can induce oxidative stress by producing reactive oxygen species (ROS), resulting in DNA damage and the oxidation of thiol-containing molecules such as glutathione. The mechanism of action for CO and Cu involved the induction of oxidative stress, as they elevate the production of free radicals that can accumulate within cells, causing damage to biological macromolecules such as RNA, DNA, and DNA repair proteins. This process adversely affects antioxidant capacity and defense mechanisms while also enhancing lipid peroxidation (Rajizadeh and Pourbabaki, 2024). Furthermore, agrochemicals including pesticides and fertilizers, heavy metals, and conditions of occupational heat stress and dehydration have been identified as risk factors for liver and kidney diseases (Buralli *et al*., 2024).

Copper oxychloride is readily absorbed through the gut, reaches maximal blood concentrations, binds serum proteins, and is eliminated in the urine after being metabolized in the liver (Additives and Feed, 2016). The high tissue distribution and low renal clearance of CO resulted in a high elimination half-life in rats (Elalfy *et al*., 2021).

Prior research on CO has primarily examined the toxic effects on adult animals, but there has been limited exploration into the organ toxicity of CO in male and female neonatal mammals following exposure of their mothers. The exposure of mother during pregnancy represents a crucial developmental phase (Margolis and Gabard-Durnam, 2024) wherein exposure to agrochemical compounds, including CO, can adversely influence fetal development. The fetus is especially susceptible during this critical period due to its restricted ability to metabolize and process these chemicals (Vinnars *et al*., 2023). This may lead to enduring changes at the tissue level that contribute to persistent negative health outcomes in adulthood (Sajdel-Sulkowska, 2023). This study examined the comparison impact of CO on the kidney and liver functions and structure of the male and female neonatal rats after exposing their mothers to CO orally.

MATERIALS AND METHODS

Chemicals

Commercial CO with a linear formula $(CuCl)\bullet[Cu(OH)_2]_3$ has a purity of 95% of copper and 5% is co-suspension inert compounds according to manufacture sheet. The product was acquired from the Central Agricultural Pesticide Laboratory, ARC, Egypt is a light green, finely textured powder that does not flow freely and consists of delicate clusters. The chosen doses were determined based on the observed LD_{50} of CO in pregnant female rats, which was detected to be 1470 mg/kg of the body weight.

Animals

A total of fifty female rats and ten male rats, with an average weight of 150-157 grams, acquired and cared for at the animal house of the Faculty of Veterinary Medicine, Suez Canal University, located in Egypt. Before the experiment, the subjects housed in metallic cages (5 subjects per cage) with wood shavings for a period of 2 weeks to allow for acclimation. The rats were kept in a controlled environment with a temperature of 24°C (±2°C) and 55±5% relative humidity. They were exposed to natural daylight conditions. Rats had unrestricted admission to both water and food.

The experimental producers adhered to the ethical norms for the utilization of animals in laboratory settings at the Faculty of Science, Port Said University, Egypt (PSU. Sci.69). Each female rats were undergone a daily collection of vaginal smears to check the regularity of their estrous cycle (Hamid and Zakaria, 2013). Only female individuals who are in the regular cyclic state and the proestrus stage were selected to be introduced to a single male for mating. The ratio of males to females was 1:3. The initiation of pregnancy was documented when sperm was detected in a vaginal smear. The pregnant females (thirty in number) only were selected.

Design of the Experiment

Thirty pregnant female rats were separated into 3 groups, each containing ten females. The rats were treated from the 6th gestation day (GD6) to the 20th day of gestation (GD20). The pregnant females were categorized into 3 distinct groups: Group I: The control group received oral administration of carboxymethyl cellulose (CMC) via gavage. Group II: The high-concentration group of CO was given by gavage at a dose (1/20 LD_{50}), which is 73.5 mg/kg body weight, mixed with CMC. Group III: Rats were given a low concentration of CO by gavage at a dose of 1/40 LD_{50}, which is 36.75 mg/kg body weight, mixed with CMC. Throughout the testing period, all experimental animals were carefully monitored daily for any indications of poisoning.

Organ Weight and Body Weight of the Male and Female Weaned Babies

The born animals were examined daily for signs of toxicity and mortality rate. After the neonates were weaned and reached an age of 30 days old, they were weighed. Liver and kidney weights were examined.

Blood Sampling and Blood Parameters

Six randomly neonate's male and female rats from each group were administered pentobarbital sodium to induce anesthesia. Neonate samples of blood were got from the retroorbital venous plexus of the eye (Van Herck *et al*., 2001). The obtained blood samples were left to coagulate. Subsequently, the serum was separated by spinning it at a speed of 3000 revolutions per minute for a duration of 20 minutes using a centrifuge. The resulting clear serum, free from any hemolysis, was collected and divided into multiple portions. These portions were then held at a temperature of -20 °C until they were analyzed. Following the method described by Bucolo and David (1973), The concentration of triglycerides in the serum was determined using the CliniChem kits Cat. No 47161, Budapest. The method depends on rapid enzymatic hydrolysis method using microbial protease and lipase that split glycerol which quantitatively measuresed at absorbance 340 nm. Additionally, the total protein concentration was evaluated using the CliniChem kits Cat. No 41951, Budapest (Okutucu *et al*., 2007). The method implied Biüret calorimetric method with a CV %<6. The total protein was estimated at 550 nm absorbance. The levels of liver enzymes, specifically serum aspartate aminotransferase (AST) and alanine transaminase (ALT), were tested using CliniChem kits (Cat. No 46361, Budapest for ALT and Cat. No 46263, Budapest for AST) (Huang *et al*., 2006). The later kits depend on spectrophotometric assay method where the absorbance 340 nm was implemented for ALT and 412 nm for AST. The levels of serum creatinine, albumin, cholesterol, uric acid, and urea were measured using CliniChem kits (Cat. No 41751, 41253, 41411, 46761, and 46661, respectively) from Budapest (Sharma *et al*., 1987; Isra'a, 2010). Simple spectrophotometric methods were implemented to test the later parameters at wave length 510 nm for cholesterol, 630 nm for albumin, 492 nm for creatinine, 294.46 nm for uric acid and 430 nm for urea.

Histopathological Preparation

Kidney and liver samples were taken, weighed, and then preserved in 10% neutral buffer formalin so that they could be studied histologically. Next, the specimens were subjected to dehydration using a sequence of increasing concentrations of alcohol. After that, they were washed and treated with xylene to remove any remaining impurities. Finally, the specimens were coated with paraffin wax for preservation. The paraffin blocks were sliced into sections that were 5 µm thick and placed on glass slides. After that, these sections underwent hematoxylin and eosin staining by the procedure Drury and Wallington described in 1980. The renal and hepatic tissue slices were inspected under a microscope (Olympus, Tokyo, Japan) to observe any histological alterations. A pathologist, who was unaware of the different treatment groups, analyzed the findings. The evaluation of liver and kidney histologic features were divided: hydrobic degeneration, fatty degeneration, inflammation, glomerulus atrophy, glomerulus hypertrophy and necrosis according to Kleiner *et al*. (2014) and Eadon *et al*. (2020). The extent of liver damage was evaluated using a scoring system ranging from 1 to 3 as outlined below: (1) slight; (2) intermediate; (3) intense according to Gad El-Hak *et al*. (2022).

Immunohistochemical Expression

For immunohistochemistry analysis, a representative section of the liver and kidney were chosen for each rat. The chosen portions underwent deparaffinization, rehydration, and were subjected to microwave heating using 0.01 M citrate buffer (pH 6.0) for a duration of 30 minutes. The activity of naturally occurring peroxidase was inhibited by exposing it to a 3% solution of hydrogen peroxide for a duration of 10 minutes. This was then followed by rinsing with a solution

of phosphate buffered saline. The liver sections were held in reserve at 4°C overnight and treated with the primary antibodies: Anti-TNF (rabbit polyclonal IgG, 100 µg/ml, with dilution rate 1:50, catalog number sc-130220; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and the kidney sections were held in reserve at 4°C overnight and treated with the primary antibodies: caspase-3 antibodies, with dilution rate 1:50, (catalog number sc-271759 ; Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The avidin-biotin peroxidase was used to detect the primary antibody. This solution consisted of streptavidin biotin reagent labelled with Dakocytomation and System-horseradish peroxidase, both from Dakocytomation in Glostrop, Denmark. Visualization of the signal was achieved using diaminobenzidine from Dakocytomation and Substrate Chromogen-System from Dako in Glostrup, Denmark. The slides were stained with Harris's hematoxylin, then underwent dehydration, clearing, and mounting. The tissue was initially examined at magnification (×100) to evaluate the overall dispersal of the main antibody. Following that, the tissue specimens were analyzed at a greater level of ×400 magnification power. The assessment of cellular immunostaining was conducted in the liver and kidney tissue.

Statistical Analysis

The data were first tested for the normality and homogeneity of variance using Shapiro-Wilk test for univariate normality. The data was scrutinized using a Two-way analysis of variance (ANOVA) for sex difference and parameters followed by Bonferroni test (IMB-SPSS version 28.0 for Mac OS) to compare the mean values attained in the diverse groups. The data was expressed as the mean ± standard error (SE), and statistical significance was considered as a P-value less than 0.05.

RESULTS AND DISCUSSION

Body Weight and Organ Weight of the Male and Female Weaned Babies

During the experimental duration, no noticeable toxic sign was noticed in the weaned control rats and the CO-intoxicated rats. No animal death was noticed along experimental duration of the study. The final body weights were significantly low (P<0.05) in high and low doses CO groups as matched to control. The high CO group exhibited statistical (P<0.05) reduction body weights as matched with the low dose group. The kidneys and liver weights of CO-treated weaned male and female rats in both high and low dose groups were statistically (P<0.05) decreased compared with those of control rats (Table1). Non- statistical variation was noticed between males and females within the same group.

Biochemical Parameter of the Male and Female Weaned Babies

The high and low dose treatment of mothers with CO produced a significant elevation (P<0.05) of ALT and AST as matched to control with higher values (P<0.05) in high value than the low one. Within the high and low groups there were significant reductions (P<0.05) in females ALT activities than males however, AST revealed non-significant variation between males and females. The low and high dose treatment of mother with CO produced a statistical lessening (P<0.05) of total protein and albumin as matched to control with lower values (P<0.05) in high group than the low one.Within the low group there was a substantial increment (P<0.05) in females' total protein than males however, albumin was statistically (P<0.05) advanced in females than males within high group. The cholesterol, triglyceride, urea, uric acid and creatinine concentrations were statistically higher in low and high Co groups than those of control with the high Co group higher than the low Co group for all of them except for triglyceride whereas there was no statistical variation between low and high groups. Urea levels displayed statistical (P<0.05) lessening in female offspring than males within the low and high Co groups. Creatinine levels showed statistical (P<0.05) decrease in female offspring than males within the high Co groups however within low Co group there was non-statistical variation between male and female offspring (Table 2).

Table 1: Effects of CO on organ weight and body weight of the male and female weaned babies.

Weight/g	Control group		Low dose CO		High dose CO		P value within ttt	P value between ttt
	Male	Female	Male	Female	Male	female		
Final body	53.5 ± 1.08^{a}	49.5 ± 2.69^{a}	32.8 ± 0.30^{bc}	33.6 ± 1.37^{b}	29.1 ± 1.34^{c}	29.0 ± 1.7^{c}	*P*=1.000	P=0.000
Liver	2.61 ± 0.26^{a}	2.60 ± 0.26^{a}	2.33 ± 0.01^{a}	1.57 ± 0.07^{b}	1.30 ± 0.18^{b}	1.04 ± 0.11^{b}	*P*=1.000	P=0.000
Kidney	0.52 ± 1.97^{a}	0.53 ± 2.1^{a}	0.34 ± 5.3^{b}	0.31 ± 2.3^{b}	0.38 ± 3.1^{b}	0.39 ± 4.3^{b}	*P*=1.000	P=0.000

Data was expressed as means ± SEM, n=6 per sex. Data were statically analyzed using Two-way ANOVA followed by Bonferroni test $p \leq 0.05$. Different letters showed data of different row which is statistically significant $p \leq 0.05$. (alanine transaminase (ALT) and aspartate aminotransferase (AST), High dose CO group: maternal rats were given by gavage 73.5 mg/kg body weight copper oxycloride from the 6th day of gestation (GD6) to the 20th day of gestation (GD20). Low dose CO group: maternal rats were given 36.75 mg/kg body weight copper oxycloride from the 6th day of gestation (GD6) to the 20th day of gestation (GD20).

Table 2: Effects of CO on the biochemical parameter of the male and female weaned babies.

	Control group		Low dose CO		High dose CO		P value within ttt	P value between ttt
	Male	Female	Male	Female	Male	Female		
ALT	25.03±0.22^{e}	24.90±0.23^{e}	36.33±0.099^{c}	32.90±0.56^{d}	85.43±0.11^{a}	82.66±0.50^{b}	P=0.000	P=0.000
AST	55.96±0.1^{c}	56.26±0.49^{c}	66.76±0.71^{b}	66.63±0.69^{b}	84.10±0.63^{a}	82.66±0.50^{a}	P=1.000	P=0.000
Total protein	6.30±0.04^{a}	6.21±0.09^{b}	5.83±0.009^{d}	5.89±0.02^{c}	5.22±0.01^{e}	5.19±0.009^{e}	P=0.001	P=0.000
Albumin	4.27±0.007^{a}	4.21±0.01^{a}	4.14±0.03^{ab}	4.06±0.01^{b}	3.44±0.01^{d}	3.83±0.10^{c}	P=0.000	P=0.000
Cholesterol	54.68±0.27^{c}	55.90±0.20^{c}	61.57±0.27^{b}	60.95±0.20^{b}	70.03±0.80^{a}	69.27±0.82^{a}	P=1.000	P=0.000
Triglyceride	4.27±0.007^{a}	4.37±0.007^{a}	1.59±0.33^{b}	1.80±0.22^{b}	1.80±0.22^{b}	1.45±0.01^{b}	P=1.000	P=0.000
Urea	16.81±0.06^{e}	16.77±0.07^{e}	20.45±0.06^{c}	19.55±0.01^{d}	26.87±0.15^{a}	23.91±0.44^{b}	P=0.003	P=0.000
Creatinine	0.43±0.003^{d}	0.43±0.003^{d}	0.57±0.01^{b}	0.51±0.002^{c}	0.74±0.008^{a}	0.71±0.02^{a}	P=1.000	P=0.000
Uric acid	2.22±0.01^{c}	2.23±0.03^{c}	2.33±0.003^{b}	2.32±0.004^{b}	2.43±0.006^{a}	2.44±0.009^{a}	P=1.000	P=0.000

Data were expressed as means ± SEM, n=6 per sex. Data were statically analyzed using Two-way ANOVA followed by Bonferroni test $p \leq 0.05$. Different letters showed data of different rows which is statistically significant $p \leq 0.05$. (alanine transaminase (ALT) and aspartate aminotransferase (AST), High dose CO group: maternal rats were given by gavage 73.5 mg/kg body weight copper oxychloride from the 6th day of gestation (GD6) to the 20th day of gestation (GD20). Low dose CO group: maternal rats were given 36.75 mg/kg body weight copper oxychloride from the 6th day of gestation (GD6) to the 20th day of gestation (GD20).

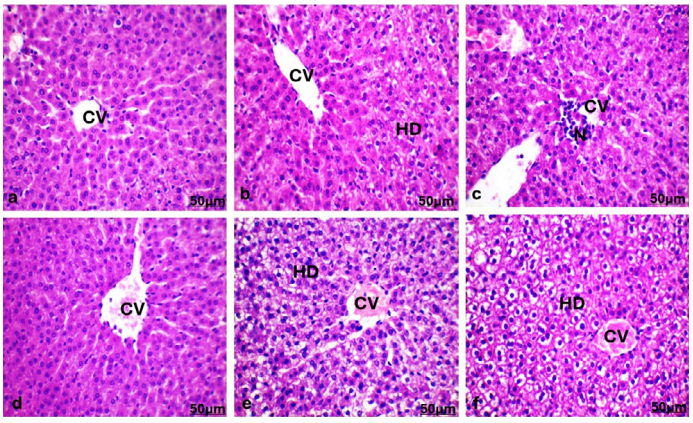

Figure 1: Photomicrograph to the liver of neonates from (a) control male group (d) control female group showed hepatocytes spread in an organized fashion around the lobular central vein (CV). (b and e) low dose male and female neonates where maternal rats were given 36.75 mg/kg body weight copper oxychloride (CO) from the 6th day of gestation (GD6) to the 20th day of gestation (GD20) showed diffuse cellular vacuolization (HD) surrounded the central vein (CV). (c and f) higher dose male and female neonates where maternal rats were given by gavage 73.5 mg/kg body weight CO from the 6th day of gestation (GD6) to the 20th day of gestation (GD20) showed diffuse cellular vacuolization (HD) and focal necrosis (FD) surrounded the central vein (HE, 400X).

Histopathology of the Liver and Kidney

The liver of neonates from the control group showed hepatocytes spread in an organized fashion around the lobular portal area and the central vein (Figure 1a, 2a, 1d and 2d). However, the presence of slight diffuse cellular vacuolization, characterizing hepatic hydopic degeneration, was observed in the liver of neonates from low dose CO (Figure 1b, 1e, 2b and 2d). This clinical picture was expressed in the liver of male and female neonates whose mothers were treated to the lowest dose of the CO (Figure 1b, 2b, 1c and 2c). Diffuse cellular vacuolization, focal necrosis, hypertrophy of the bile duct and portal area were also observed intense in the male group receiving the highest doses of the CO (Figure 1c, 1f, 2c and 2f).

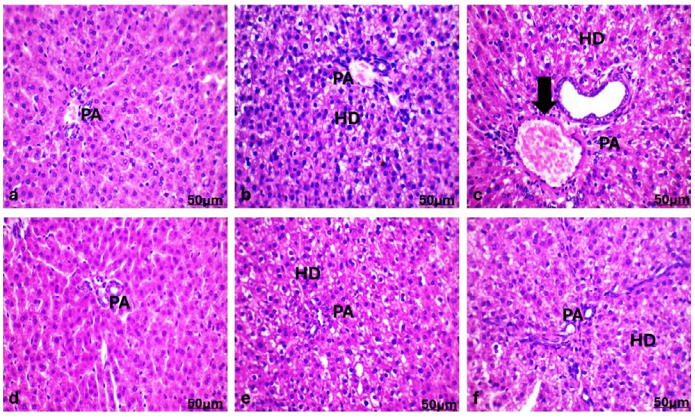

Figure 2: Photomicrograph to the liver of neonates from (a) control male group (d) control female group showed hepatocytes spread in an organized fashion around the portal area (PA). (b and e) low dose male and female neonates where maternal rats were given 36.75 mg/kg body weight copper oxychloride (CO) from the 6th day of gestation (GD6) to the 20th day of gestation (GD20) showed diffuse cellular vacuolization (HD) surrounded the portal area (PA). (c andf) higher dose male and female neonates where maternal rats were given by gavage 73.5 mg/kg body weight CO from the 6th day of gestation (GD6) to the 20th day of gestation (GD20) showed diffuse cellular vacuolization (HD) and focal necrosis (FD) surrounded the congestion portal area and hypertrophy to the bile duct (HE, 400X).

The kidneys of male and female rats from the control group presented well defined cortical and medullar regions. The kidney cortex displayed normal renal corpuscle's structure, collecting ducts and renal tubules (proximal and distal). The renal corpuscle is classically shaped by a clump of blood capillaries and a Bowman's capsule surrounds the glomerulus (Figure 3a and 3d). The absence of histopathological alterations was not observed in the kidneys of neonates from the group of low doses of CO (Figure 3a and 3d). Observations of histopathological changes, including intense atrophy in the glomerulus, were made in the kidneys of both male and female neonates that were exposed to a greater dose of CO (Figure 3c and 3f).

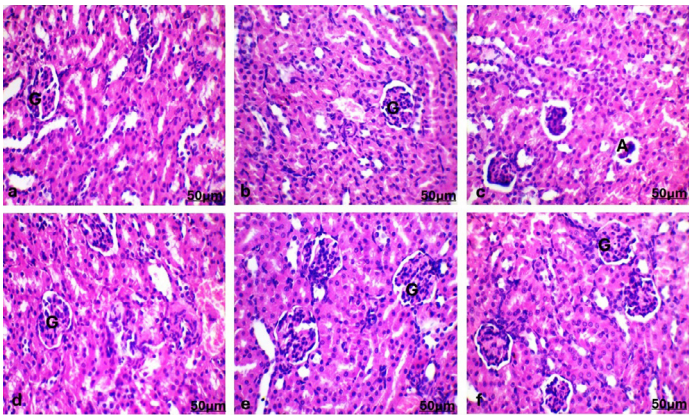

Figure 3: Photomicrograph to the kidney of neonates from (a) control male group (d) control female group showed normal renal structure of renal corpuscles (G), renal tubules (proximal and distal) and collecting ducts. (b and e) low dose male and female neonates where maternal rats were given 36.75 mg/kg body weight copper oxychloride (CO) from the 6th day of gestation (GD6) to the 20th day of gestation (GD20) showed normal renal structure of renal corpuscles (G), renal tubules (proximal and distal) and collecting ducts. (c and f) higher dose male and female neonates where maternal rats were given by gavage 73.5 mg/kg body weight CO from the 6th day of gestation (GD6) to the 20th day of gestation (GD20) showed atrophic shrinkage renal corpuscles (A), renal tubules (proximal and distal) and collecting ducts (HE, 400X).

Immunohistochemical Expression of TNF in the Liver and CASPASE-3 in the Kidney

Statistical analysis ($P \leq 0.05$) revealed an increase in the positive immunohistochemical expression of TNF in the hepatocytes of low and high dose of CO maternally treated rats, as matched to control. Positive immunohistochemical expression staining for TNF was most prominent on hepatocytes in the periportal areas (Figure 4).

Statistical analysis ($P \leq 0.05$) revealed a rise in the positive immunohistochemistry expression of caspase 3 in the kidney of low and high dose of CO maternally treated rats, as matched to control. Positive immunohistochemical expression staining for caspase 3 was most prominent on the renal tubules Figure 5.

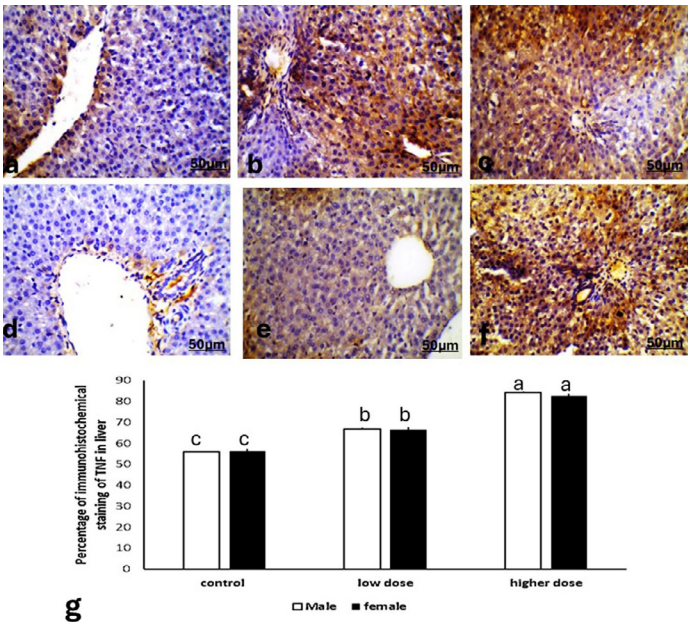

Figure 4: Photomicrograph depicting the immunohistochemical expression of tumor necrosis factor (TNF) in the liver of neonates. (a) control male group (d) control female group. (b and e) low dose male and female neonates where maternal rats were given 36.75 mg/kg body weight copperoxychloride (CO) from the 6th day of gestation (GD6) to the 20th day of gestation (GD20). (c and f) higher dose male and female neonates where maternal rats were given by gavage 73.5 mg/kg body weight CO from the 6th day of gestation (GD6) to the 20th day of gestation (GD20) (400X). (G) Histogram showed the percentage in the immunohistochemical expression staining to the TNF in the liver of neonates. Data were statically analyzed using One-way ANOVA followed by Bonferroni multiple comparisons test $p \leq 0.05$. Different letters showed data of the same bars which is statistically significant $p \leq 0.05$.

This study utilized rats as subjects to investigate the prenatal hepatotoxicity and nephrotoxicity of CO. Male and female neonatal rats have been utilized as test animals to research the hepatotoxicity and nephrotoxicity of CO. This is because rats exhibit similar toxicological symptoms and tissue structure changes to humans.

Treatment of pregnant mothers did not show signs of toxicity in their male and female rats offspring; there were also no deaths of either male or female rats during the 20-day experimental period. The final body weight of the male and female offspring significantly decreased in comparison to control group. The weight loss of treated neonates may be a consequence of reduced milk intake (Furman *et al.*, 2003). Maternal exposure to CO chiefs acute phase drawbacks that induced troubles in the mothers' metabolism with perturbation of nutrients in their milk which supplied to its

neonates (Zhen *et al*., 2022). Absolute weight of liver and kidney showed significant decrease with CO treatment. The present observations are in harmony with the observed final weight decrease.

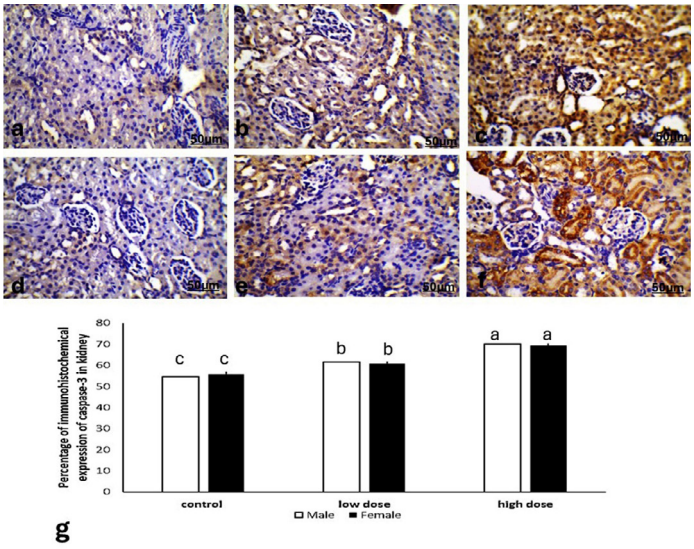

Figure 5: Photomicrograph depicting the immunohistochemical expression of caspase-3 in the kidney of neonates. (a) control male group (d) control female group. (b and e) low dose male and female neonates where maternal rats were given 36.75 mg/kg body weight copper oxychloride (CO) from the 6th day of gestation (GD6) to the 20th day of gestation (GD20). (c and f) higher dose male and female neonates where maternal rats were given by gavage 73.5 mg/kg body weight CO from the 6th day of gestation (GD6) to the 20th day of gestation (GD20) (400X). (G) Histogram showed the percentage in the immunohistochemical expression staining to the caspase-3 in the kidney of neonates. Data were statically analyzed using One-way ANOVA followed by Bonferroni multiple comparisons test $p \leq 0.05$. Different letters showed data of the same bars which is statistically significant $p \leq 0.05$.

Biochemical analysis displayed promotion in both AST and ALT enzyme activities and decreased of albumin and total protein of the offspring maternally treated with low and high dose of CO which may indicate some liver damage (Lala *et al*., 2023). The CO can pass easily the murine placenta to the feti where free radicals and lipid peroxides could be produced and persist a serious DNA damage (Elalfy *et al*., 2019). Furthermore, the liver is a target where CO bio-transformed primarily in it (Solaimalai *et al*., 2004). During the biotransformation process free radicals can be generated which may cause liver damage and inflammation (Gad El-Hak and Mobarak, 2018). The liver of neonates, in the current study, appeared to be exposed to significant inflammation and injury as evidenced by increased the immunohistochemical expression of TNF with the higher and lower dose of CO. The liver of CO-treated group showed hydropic degenerative deviations in the liver cells with hyperplasia of the bile ductulus and focal necrosis near the central vein. Similar finding have been described after treatment with CO (Gad El-Hak and Mobarak, 2018). Focal necrotic cells a s a result of CO toxicity (Elalfy *et al*., 2021) were noticed. Hepatic inflammation is often associated with increased TNF (Tilg *et al*., 2006). The hallmark of the response to inflammation comprises activation and migration of both circulating and resident inflammatory cells and the manufacture of growth factors and cytokines (Chen *et al*., 2018).

In the same way, creatinine, urea and uric acid levels in all treated male and female groups showed a progressive increase compared to control group with mild evidence of the histopathological alteration in the kidney tissue. In the current research, renal tissue of CO treated neonates revealed glomerular atrophy with increased immunohistochemical expression of caspase-3. Consistent with the present results of Elalfy *et al*., (2021) reported damage in the kidney as a response to CO. Moreover, the raised serum echelons of kidney function parameters after CO have been also observed by Abomosallam *et al*. (2020). The rise in the kidney biochemical parameters may indicate the presence of a substantial level of cellular breakdown (Kari *et al*., 1997). Moreover, elevated levels of serum creatinine, urea, and uric acid due to CO exposure serve as reliable indicators of both renal dysfunction and the harmful effects of any treatment on the kidneys of rats (Fontana *et al*., 2015). The increased immunohistochemical expression of caspase-3 suggested possibly that CO induced chemically the apoptotic machinery for glomerulus atrophy (Guvenc *et al*., 2013). This change could be claimed to the oxidative stress induced by CO that promotes apoptosis in direct or indirect way. The direct way is through depletion of antioxidant enzymes and promoting mitochondrial mediated oxidative stress that promotes apoptotic genes expression such as caspase-3 (Sarkar *et al*., 2011; Abdelazeim *et al*., 2020). The indirect way was mediated via activation of ROS mediated death inducing genes like (TNF and Fas) that promoted caspase cascade for apoptosis (Sarkar *et al*., 2011; Furuichi *et al*., 2012).

The results indicated there is significant tolerance of female offspring than male as indicated by different biochemical parameters. The gender-specific difference observed in that study may be due the presence of estrogen hormone in the female as explained by Bagheripuor *et al*. (2015). The liver and the kidney were sexually dimorphic organ, which influenced by gonadal hormones like androgen and estrogen, exhibiting drug toxicity and a drug-dose sex disparity (Farkouh *et al*., 2021).

The reduction in histological changes in the kidneys may be linked to the hepatic detoxification process, as neonates have a quicker metabolism compared to adults. This pro-

cess causes lipophilic chemicals, which can easily cross the placenta, to have a shorter duration of presence in the neonate's body compared to the mother's body (Ku and Smith, 2015). Even though the liver in neonates had more significant changes than the kidney tissue, it appears that the liver's functioning prevented the toxin from spreading to the kidneys (Kieffer *et al*., 2016).

CONCLUSIONS AND RECOMMENDATIONS

Our findings indicate that the maternal consumed CO during pregnancy can produce deleterious changes in hepatic and kidney structure and function; that are somehow pronounced in males than females offsprings. Consequently, it poses a specific risk to both the environment and humans, necessitating future research to examine this risk, particularly through the food chain. Plants and crops using this antifungal should be used with caution especially during pregnancy. It is recommended to use natural antifungal products also the people who are frequently exposed to antifungal products should use antioxidant supplements to ameliorate the toxic effect. A limitation of the present study, is the lack of several doses of CO that could determine the safety margin of such fungicide exposure during pregnancy.

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NOVELTY STATEMENT

This research article provides new insights about the toxic effect of prenatal copper oxychloride exposure on liver and kidney of male and female rat offspring with specifying influence of their sex.

AUTHOR'S CONTRIBUTIONS

Hadeer M. Shosha, **Heba M. A. Abdelrazek and Heba Nageh Gad El-Hak**: Conceptualization.
Heba M. A. Abdelrazek and Hadeer M. Shosha: Methodology.
Sherine Abbas, **Hala M. Ebaid and Hadeer M. Shosha**: Formal analysis.
Hala M. Ebaid, Heba M. A. Abdelrazek, Sherine Abbas and Hadeer M. Shosha: Investigation.
Hadeer M. Shosha, Heba M. A. Abdelrazek and Sherine Abbas: Resources.
Hadeer M. Shosha, and Hala M. Ebaid: Writing- original draft preparation.
Hadeer M. Shosha, Heba M. A. Abdelrazek, **Sherine Abbas and Heba Nageh Gad El-Hak**: Writing-review and Editing.

All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

All authors have no conflict of interest to disclose

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