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Effects of Copper and Zinc Supplementation on Haematological, Renal and Liver Function in Healthy Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Copper and zinc are essential elements that aid in various physiological and biochemical functions. Nevertheless, exposure to these heavy metals could also be detrimental to some vital organs such as the liver and kidney of the body. Increased usage of agrochemicals in crop production has the potential of increasing bioaccumulation of trace elements in both humans and animals as they feed on these plants. The present study, therefore, investigated the effects of copper and Zinc supplementation on haematological, renal, and liver functions in healthy wistar rats.

A total of twenty-five wistar rats of five weeks old (weighing 185g-250g) were recruited for the study. Animals were grouped into five namely Control, Zinc low, Zinc high, Copper low and Copper high as groups 1, 2, 3, 4, and 5 respectively and administered feeds containing low and high

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doses of Copper and Zinc two weeks post-acclimatization. Laboratory investigations on haematological, renal, and liver markers were assessed after three weeks of feeding.
Both copper and Zinc doses significantly (P <0.05) elevated the counts for Red Blood Cell (RBC), Hemoglobin (Hb), Hematocrit (HCT), White Blood Cells (WBCs), and Platelets with no significant effect on their weight gain compared to the control group of rats. Zinc doses elevated the levels of Alanine transaminase (ALT), with a low dosage having a significant effect on AST and ALP. The effect of copper and Zinc doses significantly (P <0.05) reduced creatinine levels, with no significant effect on urea concentrations.
The elevation of ALT and Platelets as a result of Copper and Zinc exposure suggests their deteriorating effect on the liver and other organs of the body. Long-term exposure to these trace elements can lead to a lot of pathologies.

Keywords: Copper; zinc; haematological; renal; liver function.

1. INTRODUCTION

Copper and Zinc play functional roles in diverse physiological and biochemical activities in the body [1,2]. Their effects on the improvement of enzymatic activities, protein synthesis, and stabilization of DNA and RNA have made them ideal for consumption [3,4,5]. Nonetheless, extreme exposure could be detrimental to vital organs of the human body [6,5]. Long-term exposure to these metals has been associated with the slow progression of physical, muscular, and neurological degenerative processes that mimic diseases like Parkinson's disease and Alzheimer's disease [7,8].

Anthropogenic activities such as mining, smelting, and the use of agrochemicals in farming have immensely contributed to the release of these metals into the environment [9,10]. Although chemical fertilizers, pesticides, and weedicides increase plant yield, their application has been associated with the accumulation of trace elements such as Copper and Zinc in the leaves, stems, and roots of crops [11,12]. Consumption of leafy vegetables may therefore expose humans to these heavy metals [13,14].

Available reports suggest increased human exposure to heavy metals, a result of rapid growth and applications in agriculture, industrial, domestic, and technology [15]. While there have been studies on heavy metal exposure, these have largely been limited to assessing their levels and concentrations in environmental matrices (soil, water, and vegetables) [1,16,17,18]. The few available reports however evaluated the concentration of heavy metals in the animal rearing system and the administration of Copper Sulphate on markers of renal functions and feeding patterns of Wistar rats [19,20].

Recent studies point to increasing concentrations of heavy metals in both medicinal plants and herbal distillates, leading to the production of reactive oxygen species upon human consumption and exposure [21,22,23]. Based on these reports, we suspect that exposure to heavy metals (Copper and Zinc) may result in pathophysiological effects in humans. Therefore, we qualitatively, investigated the effects of Copper and Zinc supplementation on haematological, renal, and liver function in healthy Wistar rats.

2. MATERIALS AND METHODS

2.1 Study Design

Twenty-five (25) male and female Wistar rats (185g – 250g) aged five weeks old were randomly selected for the experiment. These rats were obtained from the Pharmacological farm of Kwame Nkrumah University of Science and Technology (KNUST), Ghana.

2.2 Study Site

Animal housing and treatment were carried out at the laboratory of the Department of Science Education, University of Education, Winneba, Asante Mampong Campus. Hematological, Renal, and Liver tests were done at EDEP Laboratory service, Santasi –Kumasi, Ghana.

2.3 Maintenance of Animals

Rats were housed in a one-tier hutch during the study. They were kept in animal rooms at the

laboratory where room temperature was maintained at 26±3◦C under a 12-hour light-dark cycle.

The rats were thoroughly examined and any abnormalities and/or ailments were recorded before the research began. Two hundred and twenty-five grams (225 g) of concentrate feed was weighed with a Camry dial spring scale (ISO9001 APPROVED) every morning and given to the animals as their daily feed. Feeding and water troughs were cleaned every morning before feeding.

2.4 Preparation of Feed

After two weeks of acclimatization, experimental rats were purely subjected to concentrate feed. The feed was formulated and prepared at the University of Education, Winneba, Mampong campus (UEW-M) laboratory. Ingredients were made up of, wheat brand (5 kg/30 kg); maize (15 kg/30 kg); concentrate (10 kg/30 kg); Copper Sulphate and Zinc tablet. They were weighed according to their required quantities with the Camry dial spring scale (ISO9001 APPROVED). The measured quantities were thoroughly mixed by spade, bagged, and then stored in the animals' feed room.

2.5 Preparations and Treatments

A total of 25 experimental rats were used to assess the effect of Copper and Zinc on the haematological, renal, and liver markers. Animals were divided into five (5) groups and housed under controlled environmental conditions. Copper sulphate ($CuSO_4$) and Zinc tablet used for the study were obtained from UEW-M laboratory and Laugh Pharmacy (Mampong-Ashanti) respectively.

In the preparation of the wheat brand maize concentrate with $CuSO_4$ treatment, an amount of 3.929 g and 0.982 g of $CuSO_4$ powder was weighed for the high and low concentrations respectively using an electronic scale. It was then dissolved in 200ml distilled water and was uniformly mixed with 5 kg of the feed concentrate. Also, for the zinc treatment, 200 g and 50 g of zinc tablets were dissolved in 200 ml distilled water for the high and low doses respectively. It was uniformly mixed with 5 kg of the feed concentrate. The mixture was bagged and stored in the animals' feed room. Each group was given an equal amount of feed throughout the experimental period. Weekly measurements of weight were recorded. The Five treatments used for the study were**;**

Group 1 (control) fed on wheat brown maize concentrate and water.
Group 2 (Zinc low) fed on wheat brand maize concentrate with low zinc ppm
Group 3 (Zinc High) fed on wheat brand maize concentrate with high zinc ppm
Group 4 (Copper High) fed on wheat brand maize concentrate with high $CuSO_4$ ppm
Group 5 (Copper low) fed on wheat brand maize concentrate with low $CuSO_4$ ppm.

2.6 Haematological and Biochemical Parameters

Experimental rats were sacrificed and blood was dispensed into EDTA tubes and then analyzed for haematological markers such as red blood cells (RBCs), haemoglobin (Hb), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) using an automated haematology analyzer (MSLAB 12, China). Another portion of blood was collected in empty vacutainer tubes and centrifuged at 13000 rpm for 5 mins. The serum was then retrieved and biochemical parameters such as AST, ALT, ALP, TB, TP, creatinine, and urea concentrations in serum were analyzed using commercially available kits and a standard BS-120 Mindray Chemistry Analyzer.

2.7 Statistical Analysis

GraphPad Prism Version 5.0 for Windows (GraphPad Software, San Diego, CA, USA) was used for data analysis. Data were presented as mean ± SEM online graphs and tables. For comparing the control and treatment groups, independent samples t-tests for the significance of differences were used. To compare the biological effects of the treatment, an analysis of variance (ANOVA) was used. Newman Keuls Posthoc analysis was also used to identify the source of the variation. p values of less than 0.05 were considered statistically significant.

3. RESULTS

3.1 Effect of Copper and Zinc Doses on the Weight of Wistar Rats

Treated groups showed a steady weight gain after two weeks of acclimatization, although it was not statistically significant ($P < 0.05$) from the control group (Fig. 1).

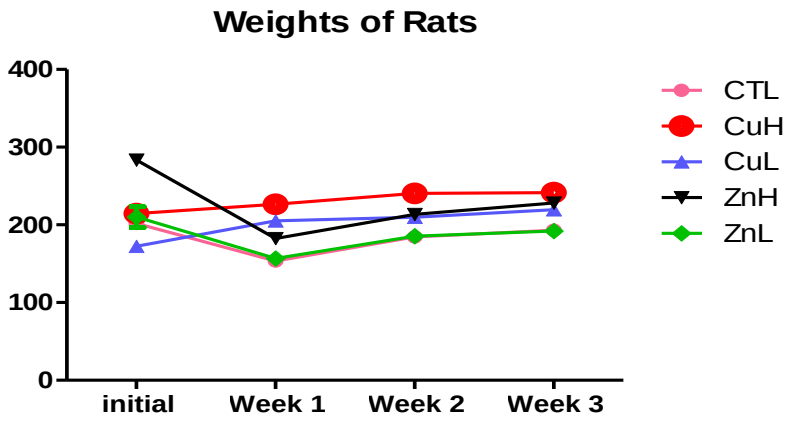

Fig. 1. Effect of Cu High, Cu Low, Zn High, and Zn Low on weight as compared with the weight of the CTL group

3.2 Effect of Copper and Zinc Doses on Haematological Parameters

The effects of copper and zinc dosages on haematological parameters were investigated and compared with similar parameters of normal control rats. Following the feed administration with variously defined dosages, haematological parameters were significantly increased (P <0.05) compared to the control group.

3.3 Effect of Copper and Zinc Doses on Liver Enzymes

Zinc low doses significantly (P <0.05) elevated the levels of the liver enzymes (AST, ALT, and ALP) whereas Zinc high doses only showed a significant (P <0.001), increase in ALT compared to the control. Comparing the different doses of copper, though not significant, the elevation of the biochemical markers was higher in Cu low than in Cu high as compared to the control group (Table 2).

3.4 Effect of Copper and Zinc Doses on Renal Function

Doses of all treatment groups showed a significant (P <0.05) reduction of creatinine levels compared to the control. Zinc high doses showed an increase in urea levels, however, urea levels in copper doses (low and high) and zinc low doses were statistically not significant (Fig. 2).

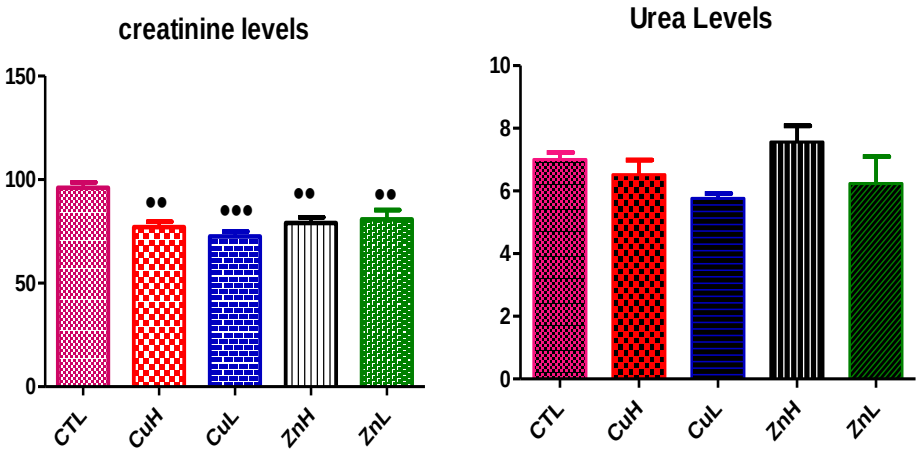

Fig. 2. Effect of CuH, CuL, ZnH, and ZnL on creatinine and urea levels; Each column represents the Mean±SEM. (n= 5). •p <0.05, •p <0.001 compared to control group (One way ANOVA Followed by Newman-Keuls test)

Table 1. Effect of Cu High, Cu Low, Zn High, and Zn Low on levels of haematological markers as compared with the control

	RBC (10^6/µL)	**Hb (g/DL)**	**HCT (**%)	**MCV(fL)**	**MCH(pq)**	**MCHC(g/dL)**	**MPV(fL)**	**PLATELETS (10^3/** µL)	**WBC (103/dL)**
CTL	5.6± 0.1	10.6±0.1	35.00±1.0	59.1±0.7	19.32±0.3	31.78±0.3	9.06±0.4	26.18±2.9	2.36±0.2
CuH	8.3±0.2•••	13.98±0.3•••	51.46±1.3•••	62.96±1.2	17.12±0.3••	27.2±0.5•••	9.8±0.2	897.6±111.3•••	8.82±0.2•••
CuL	8.1±0.2•••	14.04±0.3•••	50.52±1.5•••	62.4±1.6	17.34±0.3••	27.9±0.2•••	9.42±0.3	779.2±67.2•••	5.69±0.7••
ZnH	9.0± 0.5•••	15.22±0.6•••	56.3±2.2•••	60.78±0.8	16.88±0.4•••	27.64±0.4•••	9.20±0.2	805.3±53.6•••	13.25±1.1•••
ZnL	7.4±0.2•••	13.06±0.2•••	44.82±1.6•••	60.64±1.1	17.70±0.4••	29.2±0.9••	8.70±0.3	767.8±45.9•••	11.71±0.6•••

Each column represents the Mean±SEM. (n= 5). •p <0.05, ••p <0.001, •••p <0.0001 compared to control group (One way ANOVA Followed by Newman-Keuls test)

Table 2. Effect of Cu High, Cu Low, Zn High, Zn Low on levels of enzymes and proteins associated with the liver (ALT, AST, ALP, GLB, ALB, TP)

	ALT (U/l)	**AST (**U/l)	**ALP (**U/l)	**GLOBULIN (**g/l)	**ALBUMIN**(g/l)	**TOTAL PROTEIN** (g/l)
CTL	100.5± 1.99	172.4±25.7	235.9±20.3	50.2±1.8	28.4±1.7	5.5±0.1
CuH	79.6±4.56	195.0±21.7	339.5±72.9	56.24±1.3	28.8±1.4	5.8±0.3
CuL	102.6±7.6	235.8±9.4	388.5±52.4	48.74±1.2	29.2±0.4	6.2±0.2
ZnH	157.6±26.9••	227.1±12.6	299.1±55.8	57.94±6.6	28.12±0.6	4.4±0.6
ZnL	147.3±12.3•	259.8±25.1•	783.2±201•	63.8±6.2	27.36±1.8	5.6±0.7

Each column represents the Mean±SEM. (n= 5). •p <0.05, ••p <0.001 compared to control group (One way ANOVA Followed by Newman-Keuls test)

4. DISCUSSION

The present study investigated the effects of Copper and Zinc supplementation on haematological, renal, and liver markers in healthy Wistar rats. The effects of these trace elements administered as feeds significantly (P <0.05) elevated the haematological markers of the treated rats compared to the control. This agrees with [24] who reported significant (P <0.05) changes in haematological parameters, especially white blood cells (WBC) following the administration of copper oxide and/or zinc oxide nano-particles on rats. Also, a study by [25] showed a significant (P <0.05) increase in red blood cells (RBCs), haemoglobin (Hb), and hematocrit (HCT) following zinc supplements.

The significant elevation of the haematological parameters such as RBC, Hb, WBC, and platelets counts, as shown in Table 1 following the supplementation of these trace elements may be a result of bone marrow deficiency leading to left shift production of these markers [26]. Also, high platelets count suggests reactive thrombocythemia as a result of bone marrow suppression. This is in line with studies that suggest that persistent hyperglycemia predisposes individuals to thrombogenesis with platelet aggregation as a result of acute zinc supplements thereby prolonging bleeding time [27,28].

The high elevation of RBC and Hb following Copper sulphate exposure suggest vascular oxidative stress which can lead to impaired oxygen delivery hence the increment in its count for homeostatic balance [24]. This is supported by findings from Lucas and Rifkinds [29] who reported a pronounced increase in RBC and Hb as a result of vascular oxidative stress following copper bound amyloid-β peptide (CuAβ) exposure.

The observed significant difference between treatment and control groups in some of the enzymes associated with liver function (ALT) following zinc doses (high and low) administration suggests organ damage, especially in the liver. Low levels of ALT are generally considered a good marker of liver health; however, low levels of ALT occur as a result of certain underlying medical conditions such as deficiency in Vitamin B6 or chronic kidney disease. Also, high levels of ALT are a good indicator of damage caused to liver cells such as liver inflammation and cirrhosis [30].

Serum alanine transaminases (ALT) are not only measured to determine possible liver damage but also useful in monitoring the general health status [31, 32, 33]. This is an enzyme that is mainly found in the cytosol of the hepatocytes and plays a role in gluconeogenesis [34]. ALT is released as a result of liver injury from the injured liver cells into the serum, which causes the levels to increase significantly in the serum [35, 36]. Also, only low doses of zinc treatment significantly (p <0.05) affected most of the liver enzymes (ALT, AST, ALP) indicating the essential role of zinc and hence it's right proportional supply to the body). Zinc deficiency may induce oxidative stress, compromise the function of oxidative-sensitive transcription factors that can affect cell function, proliferation and survival. Again, it can also result in cell and tissue damage by modulating specific signal cascades with resulting damage to enzymes, mitochondria and ribosomal structures. Hepatic damage following Zinc deficiency sides with other studies that report on zinc deficiency as a predisposing factor for acute and chronic liver disease [37, 38].

Copper doses, both high and low did not have any significant effect on the enzymes associated with liver function. This can be correlated to the significant reduction of creatinine levels following copper treatment. The observable insignificant effect of copper doses on renal function may be due to the body's homeostatic mechanism of regulating excess copper via bile secretion by the liver [39, 40].

Urea is a principal Nitrogenous waste product of metabolism as a result of protein deamination [41], its physiological role in the detoxification of ammonia and conservation of water in the kidney has made it ideal as a biomarker for renal function [42]. Even though statistically, no significant differences were observed in the treatment groups compared to control regarding blood urea levels, its slight increment following high doses of zinc treatment cannot be overlooked. The insignificant differences observed may be because blood urea levels are only affected following an extreme reduction of the Glomerular filtration rate [43].

Albumin and direct bilirubin levels were found to be very low as compared to their normal ranges. Bilirubin acts as an antioxidant that protects tissues throughout the body from damage by substances that can cause the breakdown of

cells. Given this, lower levels of bilirubin indicate a possibility of certain body parts being vulnerable to damage. Several medical conditions are associated with lower levels of bilirubin in the body. These conditions include ulcerative colitis, brain lesions and diabetic retinopathy [44, 33, 24, 45, 25]. On the other hand, lower levels of albumin are associated with inflammation, shock and the inability of the body to absorb and digest protein.

5. CONCLUSION

Results from the study reviewed that, Copper and Zinc at both high and low doses caused high elevation of haematological markers. This affirms the essential role of Cu and Zn in blood formation and immunity in the body. However, low levels of Zn yielded low liver and renal markers, this suggest that long-term exposure to Zinc can have a deteriorating physiological and pathological effects on the liver and other organs of the body.

6. RECOMMENDATIONS

Based on the findings of this current study, it is recommended that;

- Further studies should be carried out to ascertain the histopathological effects of copper and zinc on the liver and kidney.
- Whenever Cu and Zn treatments are administered orally to rats, there may be a high propensity of the gut of the rats being affected, it is therefore recommended that further investigation be made to study the effects of Cu and Zn on the gut of rats as well as possible microbiota present in the gut.
- The Food and Agriculture Organization (FAO) and the Ministry of Food and Agriculture (MOFA) must ensure strict compliance to permissible levels of trace elements in pesticides used in crop production.
- Farmers must be educated on the dangers of breaching the permissible levels of trace elements on-farm produce as well as in the supplementation of animal diets.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

All animal experiments, procedures, and techniques used in this study were conducted in compliance with the National Institute of Health Guidelines for Care and Use of Laboratory Animals, with ethical approval from the Institutional Review Board**,** University of Cape Coast, Ghana (ethical clearance number: UCC IRB/CHAS/2017).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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