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Therapeutic effect of methanolic leaves extract of *Solanum incanum* in copper induced cardio-toxicity in Wistar rats

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Abstract
The aim of this investigation was to study the effect of garden eggs on the heart and hematological parameters of Wistar rats induced with copper toxicity. Twenty (20) rats were randomly divided into four groups of five rats each. Group A (negative control) received water only for 15 days, Group B received copper at 200 mg/kg for five days, Group C received copper at 200 mg/kg for five days then treated with garden egg at 250 mg/kg for ten days and Group D received garden egg at 250 mg/kg only for ten days. All the rats were sacrificed on day sixteen and blood was collected through cardiac puncture and analyzed. In a group induced with copper, we observed a decrease in MCV and MCH. However, the result of this study shows that copper caused changes in hematological parameters that lead to anemia, also treatment with garden egg caused a relative increase in hematological parameters which was not significant enough to protect the effect. However, the garden eggs only were able to cause polycythemia.

Keywords: Copper II Oxide, heart, hematology, histopathological, and *Solanum incanum*

Introduction
Copper plays an important role in our metabolism, largely because it allows many critical enzymes to function properly (Jaryum *et al*, 2013) [5]. Copper is essential for maintaining the strength of the skin, blood vessels, and epithelial and connective tissue throughout the body. Cu plays a role in the production of hemoglobin, myelin, and melanin and it also keeps the thyroid gland functioning normally (Bhattacharya *et al*., 2016) [3]. Copper can act as both an antioxidant and a pro-oxidant. Free radicals occur naturally in the body and can damage cell walls, interact with genetic material, and contribute to the development of a number of health problems and diseases. As an antioxidant, Cu scavenges or neutralize free radicals and may reduce or help prevent some of the damage they cause (Mostafa Abd El-Aal, 2012) [6]. When copper acts as a pro-oxidant at times, it promotes free radical damage and may contribute to the development of Alzheimer's disease (Mostafa Abd El-Aal, 2012) [6]. Maintaining the proper dietary balance of Cu, along with other minerals such as zinc and manganese, is important (Uwitonze *et al*., 2020) [13].
Solanum incanum also known as African eggplant, Ethiopian eggplant or scarlet eggplant is a vegetable crop belonging to the family Solanaceae. The genus Solanum includes both the edible and non-edible species. The family is one of the largest and most important families of vegetable grown for their edible fruits (Sifau *et al*., 2014) [10]. They are native to sub-Saharan Africa and are essentially tropical in origin. S. aethiopicum is of high edible quality. The fruits can be eaten fresh without cooking and have a long history of consumption in West Africa (Svobodova & Kuban, 2018) [11]. A basic component of primary health care is nutrition which encourages rural dwellers to grow vegetables within their compounds for easy access. One such plant grown by the rural Nigerian communities and particularly the Igbos of eastern Nigeria is Solanum or garden egg or bitter tomato consumed as an edible vegetable because of its high nutritious value and medicinal properties (Akinola, Pereira, Mabhaudhi, de Bruin, & Rusch, 2020) [1]. The plant contains essential minerals and vitamins as well as other substances such as proteins, riboflavin, thiamine iron, calcium, nicotinemide, carbohydrate, Vitamin C

water, fiber, fat and carotenes which are essential to health (Satheesh & Workneh Fanta, 2020) [8]. The carotene which is present as Provitamin A is converted to Vitamin A in the body which is necessary in the prevention of night blindness and xerophthalmia. The medicinal value of bitter tomato et is due to the presence of certain chemical substances that it contains, such as chaconine which confers on it anticholinesterase activity (Taiwo *et al*., 2021) [12].

Despite its rising popularity as a supplement and/or medicinal agent, little is known regarding the cardio-therapeutic benefits of *Solanum incanum* leaf and seed. As a result, the current study looked at the cardio-therapeutic effects of *Solanum incanum* methanol leaf extract on Wistar rats.

Material and Method

Solanum incanum leaves were obtained from Sokoto, Nigeria. The leaves were crushed into semi powdered form using mortar and pestle and then soaked into 80% methanol and kept at room temperature free of dust for seven days. It was sieved using soft cotton cloths and kept for seven days at room temperature for partial methanol evaporation followed by concentration with a rotary evaporator at 42 ºC and subsequently freeze-dried. The yield of the freeze-dried sample represents the aqueous extract obtained.

Study design

After an acclimatization period of 3 weeks, animals were divided randomly into 4 groups of 5 rats each.

Group 1 served as negative control receiving only feed and water for 15 days.

Group 2 served as positive control receiving copper 200 mg/kg only for 15 days.

Group 3 received copper II oxide 200 mg/kg for 5 days and was later treated with *Solanum incanum* 250 mg/kg for 10 days and.

Group 4 was given *Solanum incanum* only 250 mg/kg for 15 days.

Hematology

The PCV, RBC, Hgb, MCV, total white blood cell counts, and differential white blood cell counts were all determined immediately from the blood samples. Standard protocols were followed for all hematological tests. PCV was evaluated using the microhaematocrit method and Hgb was assessed using the cyanomethaemoglobin method. The hemocytometer method was used to count RBC and WBC utilizing Natt and Herrick's solution as the diluting fluid. The Leishman technique was used to prepare and stain the smears for differential WBC as enumerated by the battlement counting method. Formulas were used to calculate the results of MCV, MCH, and MCH.

Histological Study

The heart was soaked in fixative (10% neutral buffered formalin) for three days before being transferred straight to 70% alcohol, where it was graded to 90%, 99%, and 100% alcohol for eight, twelve, and fifteen hours, respectively. After replacing the alcohol with Xylene and incubating the tissues for 4 hours, they were embedded and inserted into paraffin wax to harden the tissue for easy cutting into thin pieces using the microtome. Tissues were cast into a paraffin block in an 'L' shape to eliminate air bubbles before solidification. The prepared slides were dried in xylene using a dry air oven for 30 minutes before being put on the microscope and observed under oil immersion at 1000 magnification.

Statistical Method cannabis

The data for this study were analyzed using *In vivo* statistical Software (version 4.2). Where statistical differences existed, Behrens Fisher tests were used to separate the mean.

Result

The effect of *Solanum incanum* on copper-induced hematological changes in Wistar rats was presented in Table 1 below. There is a decrease in PCV with a statistical difference ($p<0.05$) between Group B compared to group A, C, and D. There is no statistical difference in Hb ($p>0.05$) among the groups. Statistically difference ($p<0.05$) was observed with decreased RBC in group B compared to A, C, and D. statistically also shows a significant decrease in WBC ($p<0.05$) in group C compared to group B but there is no significant difference ($p>0.05$) between A, C and D. MCHC and MCH also shows a significant increase with a statistical difference ($p<0.05$) between group B compared to group A, C and D. It is statistically significant ($p<0.05$) in group C when compared to group A, B and D. There is decreased in MCV with statistically significant ($p<0.05$). A decrease in monocytes was also observed in group B compared to groups A, C, and D with a statistical difference ($p<0.05$). Lymphocytes, neutrophils, eosinophils, and basophils show no significant changes in all groups.

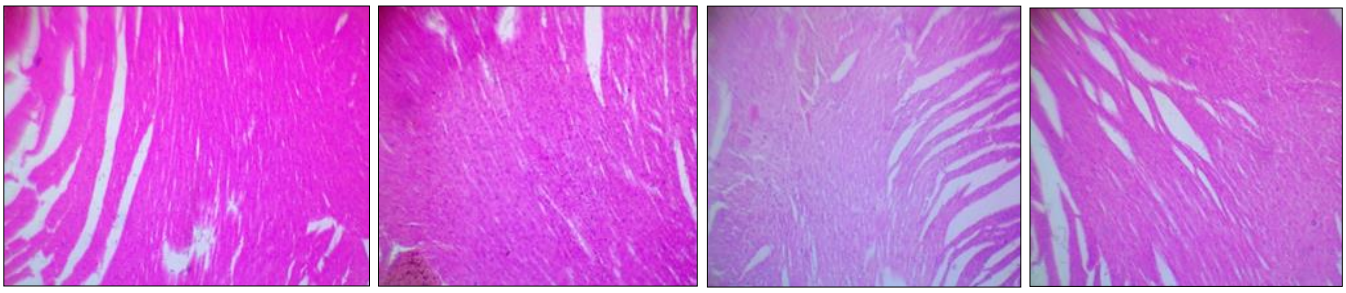
Table 1: Effect of *Solanum incanum* on copper induced hematological changes in Wistar rats. (N=20)

Parameters	Group 1	Group 2	Group 3	Group 4
PCV (%)	37.75±2.43	36.50±2.18	35.00±0.41	39.50±0.96
Hb (g/dl)	13.13±0.33	12.67±0.47	12.56±0.20	12.81±0.27
RBC($x10^6$cells/mm^3)	6.74±0.16	9.88±2.03	5.95±0.32	7.83±1.18
MCV (fl)	$55.83±0.23^a$	$33.55±5.32^b$	$59.20±2.46^a$	$54.08±8.22^a$
MCH (pg)	$19.43±0.14^{ab}$	$12.13±1.81^b$	$21.23±0.79^a$	$17.78±2.58^{ab}$
MCHC (g/dl)	35.03±1.24	34.85±0.77	35.85±0.16	33.95±1.59
WBC $x10^3$ (mm^3)	5.00±0.54	4.78±0.14	5.91±0.50	6.03±0.78
N (%)	$21.00±3.11^b$	$23.25±4.80^{ab}$	$27.75±0.85^{ab}$	$30.00±4.36^a$
L (%)	$77.75±3.68^a$	$74.25±5.19^{ab}$	$70.50±0.65^{ab}$	$67.00±4.74^b$
M (%)	1.25±0.63	2.00±0.71	1.25±0.25	2.75±0.48
E (%)	0.00±0.00	0.50±29	0.50±0.29	0.25±0.25
B (%)	0.00±0.00	0.00±0.00	0.00±00	0.00±00

Key: A (negative control), B (copper only), C (copper then *Solanum incanum*), D (*Solanum incanum* only), PCV (packed cell volume), Hb (hemoglobin), RBC (red blood cells), WBC (white blood cells), MCHC (mean corpuscular hemoglobin), MCH (mean corpuscular hemoglobin), MCV (mean corpuscular volume), L% (lymphocytes), N% (neutrophils), M% (monocytes), E% (eosinophils), B% (basophils). Data is given as means ± standard deviation. ^{abc} means in a row with different superscripts differ significantly ($p<0.05$).

Histopathology

Results of histopathological findings from Wistar rats showed no lesions on the heart in the group of all groups. The heart showed normal myocardium and endocardium with artifact (plate A-D).



A B C D

Plate 1-4: Histopathological assessment of the kidney of Wistar rats: The heart showed normal myocardium and endocardium.

Plate A; heart section of the Wistar rat fed with feed and water only (control group), Plate B; heart section of the Wistar rat exposed to CuO only, Plate C; heart section of the Wistar rat treated to *S. incanum* methanol extract only Plate D; heart section of the Wistar rat treated to *S. incanum* methanol extract and later exposed to CuO.

Discussion

Hematological parameters are essential in disease diagnosis, as they give information about the general condition of the body systems. It can also be used in monitoring or evaluating the efficacy of treatment (Do Nascimento *et al*., 2020) [4]. Therefore, this study investigates those parameters and histological appearance of heart after exposure to copper (ii) oxide and *Solanum incanum* extract.

This study showed decreased in PCV and RBC which is similar to the work of (Atamanalp & Yanik, 2003) [2], who reported decreased values of erythrocytes, hemoglobin and hematocrit after exposure to heavy metal pollution. The decreased of PCV and RBC lead to anemia which occurred as a result of bone marrow suppression by the copper (Pieper, Friedmann, Jones, & Thornton, 2016) [7]. However, treatment with *Solanum incanum* has not prevented the effect.

This study also showed decrease in WBC. The decrease may be as a result of copper effect on suppressing bone marrow activities by altering the maturation of lymphocytes, neutrophils, eosinophils, monocytes and basophils. These may be responsible for reduced immune and or defense mechanism as well as low blood count condition in most consumers (Weng *et al*., 2017) [14]. The level of WBC was not prevented after treating with *Solanum incanum*.

This study showed decreased in MCHC, MCH and MCV which is similar to the work of (Shah, 2006) [9] who reported that, copper induced changes on hemato-biochemical parameters resulted to reduction in MCHC. The decreased of MCHC was due to ability of Copper to cause bone marrow suppression. However, after treating with *Solanum incanum* the effect was not prevented (Weng *et al*., 2017) [14].

Histopathology shows no changes between the control and the treatment groups. This may be due to short time exposure of the rat to the CuO. Use of sub-lethal dose following toxicity study of the CuO to induce degenerative changes in heart also contributed to normal histological appearance.

This study showed decreased in monocytes, this is similar to the work of (Shah, 2006) [9] who reported heavy metal pollution with copper causes decreased in monocytes? However, after treating with *Solanum incanum* the effect was not prevented.

Conclusion and recommendations

The result of this study shows that copper caused changes in hematological parameters that lead to anemia. Treatments with Garden egg prevent the relative increase in hematological parameters which was significant enough to protect the effect. However, the study showed Garden egg causes polycythemia. Further research should be conducted to investigate other means of treating copper toxicity.

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