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Evaluation of protective effect of methanolic leaves extract of *Solanum incanum* in copper induced nephro-toxicity in wistar rats

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Abstract
The study investigated the protective effect of methanolic leaf extracts of *Solanum incanum* in copper-induced nephrotoxicity. Animals were divided into four groups: A Control (maintained on food and water only), B treated with 300 mg/kg *S. incanum*, C treated with 200 mg/kg CuO, and D treated with 300 mg/kg and then 200 mg/kg CuO. Serum electrolytes (sodium, potassium, chloride, bicarbonate) and kidney functional tests (urea, creatinine, and erythropoietin) were evaluated using enzyme-linked immunosorbent assay (ELISA). Prepared slides from the kidney were dehydrated using a dry air oven in xylene for 30 minutes and later mounted on the microscope and viewed using oil immersion ×1000 magnification. The result of electrolytes assays showed a significant decrease ($P<0.05$) with sodium, chloride, and bicarbonate concentration between the control groups 137.00 ± 4, 100.00 ± 3.46, 9.33 ± 3.18 compared with the group exposed to CuO only 93152.00 ± 1.00, 110.00 ± 2.04, 21.33 ± 0.88 (mmoL/L). The kidney function test shows no significant difference between the control group and the group treated with *S. incanum* methanol extract and then CuO. For the histopathological study, there were mild renal tubular degeneration and tubular dilation blue dot in groups exposed to CuO only. Because of the presence of a significant protective effect of electrolytes and as well kidney function, *S. incanum* is a potential source of remedy against CuO poisoning.

Keywords: Cupper II oxide, electrolytes, histopathological, kidney function and *Solanum incanum*

Introduction
CuO is found in a variety of cells and organs, with the highest concentrations in the liver and brain (Cholewiska *et al*., 2018) [5]. Cu is most commonly found in biological systems in the cupric form (Cu++), however, Cu-containing enzymes can contain a variety of different bonded cations, often in combination within a single protein (Su *et al*., 1982) [25]. For example, the Cu enzyme lysyl oxidase is essential for cross-linking collagen and elastin, both of which are crucial for connective tissue growth. Ceruloplasmin, also known as ferroxidase I, is a Cu protein that facilitates the transport of hemoglobin from the interstitial lumen and storage sites to erythropoiesis sites.

CuO is needed for the production and maintenance of myelin, a protective coating that covers neurons, as well as the synthesis of melanin pigment in skin, hair, and eyes. CuO is also a component of cytochrome c oxidase, which catalyzes the reduction of oxygen to water, an important step in cellular respiration, and copper, zinc-superoxide dismutase (Cu, Zn-SOD), which scavenges the free radical superoxide. Non-specific Cu^{++} binding to thiol enzymes may alter the catalytic activity of cytochrome P450 monooxygenase, and Cu^{++} may oxidize and bind to certain amino acid residues of the P450 monooxygenase but not to its heme group (Park and Lee, 2021) [20]. CuO is required for the creation of melanin pigment in the epidermis, fur, and optic, as well as the formation of myelin, a defending covering that surrounds nerve cell. CuO is also found in cytochrome c oxidase, it speeds up the rate of O_2 conversion to H_2O, a crucial step in aerobic oxygen transport, and CuO, Zn-superoxide dismutase, that scavenges superoxide free radicals. Random Cu binding to thiol enzymes may affect Cyt-P450 monooxygenase enzymes exertion, and Cu may be metabolized and attached to particular

amino acid remainders of the Cyt-P450 monooxygenase but not to its heme group (Park and Lee, 2021) [20]. Cu is also element of dopamine-beta-hydroxylase, a crucial protein in the catecholamine biosynthesis process. It isn't unanticipated, also, that Cu-enzyme mutation lead to the pathogenesis of liver, brain, and other ailments (Gaetke *et al*., 2014) [10].

A large variety of medicinal plants have been tested for biological activity in order to justify their use in traditional medicine (TM) (Vujanović *et al*., 2019) [26]. Among these, *Solanum incanum* L. is a re-known traditional herbal used to treat a variety of ailments. In tropical Africa, especially Ethiopia, the plants are used in traditional medicine to cure problems such as sore throat, stomach ache, malaria, common cold, hypertension, diabetes, headache, painful menstruation, liver discomfort, and pain caused by onchocerciasis, pneumonia, and rheumatism (Mukungu *et al*., 2016) [17]. Since the turn of the century, there has been a surge of interest in the study of medicinal plants and their historic uses in many regions of the world. Bringing such research together is critical in order to give ethnomedicinal and pharmacological information on those plants (Adotey *et al*., 2012) [2].

Despite the growing popularity of *Solanum incanum* leaves as an additive and or protective agents, little is known about its nephron-protective effects. As a result, this research evaluated the nephron-protective effect of methanol leaves extract of *Solanum incanum* on Wistar rats.

Material and Method

Identification of Plant

Solanum incanum leaf was obtained from Gwaski village, Sakwa ward, Hawul LGA, Borno State, Nigeria. S. Sanusi from the University of Maiduguri's Department of Biological Science in Borno State, Nigeria, identified it, and a voucher number (DCPT 014) was assigned. The leaf was cleaned and exposed to air for 14 days at ambient temperature.

Extraction of Plant

About 200 g of the grounded leave was soaked in 80% methanol (1000 mL) in beaker (sgma-Aldrich, USA) and allowed to stand for 3days. Diluted leave sample was shaken daily to obtained high yield of the bioactive compounds. Filtration of the sample was carried out with clean muslin cloth and then concentrated to semi-solid form with rotary evaporator (IKA RV 10, USA) at 42 ºC. The concentrated semi-solid form was then transferred into sample bottles and stored at 4 ºC (Hassan *et al*., 2020) [11].

Dilution of plant sample

A 100 g of *S. incanum* leaves extract was dissolved in 1 L of 100 percent DMSO (100 g/L) to make the stock solution. Diluting the stock solution to 10 mg/mL with distilled water was used to make the sub-stocks solution. Working solution was generated from sub-stock solution. In all concentrations of extract, DMSO (vehicle) was kept at 0.1%. (Hassan *et al.*, 2020) [11].

Toxicity study of the extract

The extract's oral toxicity effects were carried out in compliance with the Organization for Economic Cooperation and Development's (OECD) 423(7) recommendations (Alli *et al.*, 2011) [3]. The Institutional Animal Care and Use Committee (IACUC) at Usmanu Danfodiyo University's Faculty of Veterinary Medicine in Sokoto, Nigeria, accepted the protocol for these studies under the number UDUS/IACUC/AUP-R005/2020. Rats were given 1000, 500, and 250 mg/kg of crude extract for two days in order to test its acute toxicity (Clemente *et al*., 2019) [8]. The LC_{50} was calculated using Probit analysis. The crude extract was given at doses of 500, 250, and 125 mg/kg for 14 days in a chronic toxicity study (Adekola *et al*., 2020) [1].

Induction of Nephrotoxicity using Copper (ii) oxide

Nephrotoxicity in rats was induced by administering Copper (ii) oxide at 200 mg/kg/rat cupper trioxide (200 mg/kg bw) orally for 5 days (Patlolla and Tchounwou, 2005) [21].

Protective effects of the extract on Copper (ii) oxide-induced Nephrotoxicity

Nephro-protective effects of the extract on Copper (ii) oxide - induced nephrotoxicity were carried out in accordance with the OECD guidelines. Animals were divided into three groups of 5 rats each. The groups were treated as follows:

A. Normal control, received only food and water.
B. In addition to food and water, the rats were administered 300 mg/kg bw of *S. incanum* methanol extract.
C. Rats received 200 mg/kg bw of Copper (ii) oxide in addition to food and water.
D. Rats were given 300 mg/kg bw of *S. incanum* methanol extract for 10 days followed by administration of 200 mg/kg bw of Copper (ii) oxide for 5 days in addition to food and water.

Animals were anesthetized with chloroform vapor, and blood was taken through heart puncture with a 5 ml syringe and needle and put into EDTA-free bottles 48 hours after the previous treatment. Each rat's brain was extracted after the head was dissected with a dissecting kit. The dissected brains were dipped into a beaker containing normal saline to remove excess blood before being placed in a clean sample container containing 10% neutral buffered formalin. (Parasuraman *et al*., 2010) [19].

Biochemical Tests

Total proteins (Kashyap *et al*., 2020) [13], Sodium ion (Na^+) (McCabe *et al*., 1988) [16], Potassium ion (K^+) (Chuang *et al*., 1992) [7], Chloride ion (Cl^-) (Selman *et al*., 2012) [24], Bio carbonate ion (HCO_3^-) (Orlinska and Newton, 1995) [18], Urea (Christgau *et al*., 1998) [6], Creatinine (Christgau *et al*., 1998) [6] and Erythropoietin (Juul *et al.*, 1998) [12], were determined from serum using enzyme-linked immunosorbent assay (ELISA) assay kits from Thermo Fisher Scientific, USA as described in the manufacturer's guide.

Histological Study

The kidney 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 a '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 (Sabdyusheva *et al*., 2020) [23].

Statistical Method

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.

Results

Toxicity study

The chronic toxicity studies revealed the LC_{50} of 676.10 mg/Kg body weight for the extract and 387.4 mg/Kg body weight for the Copper (ii) oxide.

Result of protective effects of Garden egg on cupper induced electrolyte changes

Protective effects of Garden egg on cupper induced electrolyte changes shows 137.00 ± 4.93 and 152.00 ± 1.00 (mmoL/L) with significant difference (P<0.05) between the control group and group exposed to cupper only on sodium ion concentration. There was no significant difference between the control group and groups treated with crude extract, crude extract and copper (II) oxide (CuO). There was no observed significant difference on potassium (K^+) concentration between the control and the remaining treated groups. Significant difference (P<0.05) was also recorded between the control group 100.00 ± 3.46 and group exposed to cupper only 110.00 ± 2.04 on chloride ion (Cl^-) concentration. There was no significant difference between the control group and groups treated with crude extract, crude extract and copper (II) oxide (CuO). Statistical analysis also shows significant difference (P<0.05) between the control group 9.33 ± 3.18 and group exposed to cupper only 21.33 ± 0.88 on bicarbonate ion (HCO_3-) concentration. Statistical difference was also not observed the control group and groups treated with crude extract, crude extract and copper (II) oxide (CuO) Table 1.

Table 1: Protective effects of Garden egg extract on copper induced electrolytes changes.

Parameters (MMOL/L)	A (C)	B (CuO)	C (Ge)	D (Ge + CuO)
NA^+ (Mmol/L)	137.00 ± 4.93^a	152.00 ± 1.00^b	146.67 ± 0.67^a	138.50 ± 1.85^{ac}
K^+ (Mmol/L)	6.03 ± 0.61^a	5.68 ± 0.30^a	6.34 ± 0.20$^{a\ b}$	5.47 ± 0.15^a
Cl^- (Mmol/L)	100.00 ± 3.46^a	110.00 ± 1.00^{bcd}	104.00 ± 1.00^{ac}	100.00 ± 2.04^a
HCO_3^- (Mmol/L)	9.33 ± 3.18^a	21.33 ± 0.88^b	13.67 ± 1.86^a	8.50 ± 1.19^{ac}

Keys: Group A (General control), Group B (Copper only), Group C (Garden Egg only), and Group D (Garden egg induced with copper), the values with different superscript showed statistical significance difference Superscript ^{A} = non significance, Superscript ^{B}= significance.

Result of protective effects of Garden egg on cupper induced Nephro toxicity

Protective effects of Garden egg on cupper induced nephro toxicity show no significant difference on urea, creatinine and erythropoietin concentration between the control and the remaining treated groups Table 2.

Table 2: Protective effects of Garden egg on cupper induced Nephro toxicity

Parameters	A (GC)	B (CU)	C (GE)	D (GCo)
Urea (mg/dl)	6.60 ± 0.87^a	6.55 ± 0.42^a	6.73 ± 0.55^a	6.50 ± 0.32^a
Creatinine	0.90 ± 0.15^a	1.05 ± 0.18^a	1.00 ± 0.10^a	0.83 ± 0.15^a
Erythropoietin	29.32 ± 11.02^a	38.13 ± 3.49^a	29.31 ± 9.74^a	25.52 ± 5.86^a

Keys: Group A (General control), Group B (Copper only), Group C (Garden Egg only), and Group D (Garden egg induced with copper), the values with different superscript showed statistical significance difference Superscript ^{A} = non significance, Superscript ^{B}= significance

Histopathology

Results of histopathological finding from Wistar rats showed lesions such as infiltration, mild renal tubular degeneration and renal tubular dilation of the kidney in group exposed to CuO only (plate B). Control (plate A) and remaining treated groups (plate C and D) show normal histological appearance.

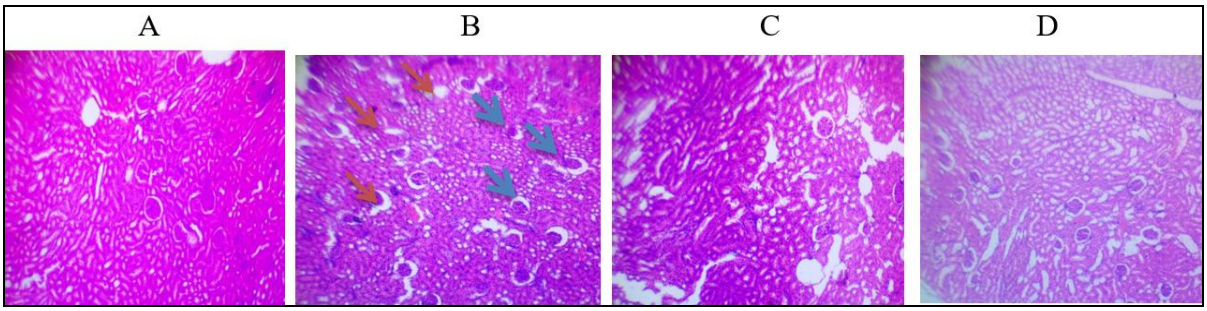

Plate 1-4: Histopathological assessment of the kidney of Wistar rats: The kidney showed mild renal tubular degeneration (red arrows) and tubular dilation blue dot (black arrows).

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

Discussion

The aim of this study was to determine the protective effect of garden egg (*Solanum incanum*) on CuO induced nepro-toxicity in Wistar albino rats.

Effect of CuO on animals' electrolyte balance was not available online. Groups that were exposed to CuO only showed significant decrease in total sodium, chloride and bicarbonate (p<0.05) compared to the control and other treated groups. This may be due to hepatocellular necrosis and acute tubular necrosisas as reported by Lotfi *et al.*, (2018) [15]. There was no observed significant difference on urea, creatinine and erythropoietin between the control and the treated groups. Infiltration, mild renal tubular degeneration and renal tubular dilation of the kidney in group exposed to CuO only in this study is similar to the finding reported by (Cuzzocrea *et al.*, 2003) [9].

Cu is harmful to a number of cells, including kidney cells, in its ionic state (Xiang and Liu, 2021) [28]. Excessive intracellular Cu buildup stimulates the creation of reactive

oxygen species (ROS), which catalyze the interaction between the superoxide anion and hydrogen peroxide, resulting in the generation of the hydroxyl radical (Pierson *et al*., 2018) [22]. Cu can also bind directly to free cysteine thiols (CYS), causing oxidation and crosslinking between proteins, inactivating enzymes or weakening structural proteins (Lorincz, 2018) [14]. Cu-induced ROS trigger apoptotic/necrotic processes as well as other diseases such as cancer, nephrosis, and renal failure (Bhattacharya *et al*., 2016) [4].

Conclusions

This study showed that the *S. incanum* methanol extract has a significant protective effect on kidney function. Histopathologic results revealed the protective effect of this extract on CuO induces nephron toxicity. Because of the presence of significant unknown bioactive compounds alongside its protective effects, this plant could be a potential source of lead compounds for the development of drugs that can be used in the management of kidney diseases.

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