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

A I Ja'afaru, I M Hassan, N Abdulazeez, I I Ogido, SU Ambursa
Department of Veterinary Physiology and Biochemistry, Usmanu Danfodiyo University Sokoto, Nigeria

Abstract
The study investigated the therapeutic of methanolic leaves extract *Solanum incanum* in cupper induced nephro toxicity. 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) and kidney functional test (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 significant decrease ($P<0.05$) with sodium, potassium chloride, and bicarbonate concentration between the control groups 138.00 ± 1.85, 6.03 ± 1.05, 101.25 ± 2.71 compared with the group exposed to CuO only 137.00 ± 4.93, 5.68 ± 0.30, 100.00 ± 3.46 (mmoL/L). The kidney function test show no significantly difference between the control group and group exposed to CuO and then treated with *S. incanum* methanol extract. For histopathological study, there were mild renal tubular degeneration and tubular dilation blue dot in groups exposed to CuO only. Because of the significant therapeutic effect on electrolyte 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

Copper (Cu) 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. Cu is required for the formation of melanin pigment in skin, hair, and eyes, as well as the formation and maintenance of myelin, a protective covering that surrounds neurons. Cu is also found in cytochrome c oxidase, which catalyzes the reduction of oxygen to water, a key step in cellular respiration, as well as copper, zinc-superoxide dismutase (Cu, Zn-SOD), which scavenges the free radical superoxide. Non-specific Cu++ binding to thiol enzymes may affect cytochrome P450 monooxygenase catalytic activity, and Cu++ may oxidize and bind to particular amino acid residues of the P450 monooxygenase but not to its heme group (Park and Lee, 2021) [20]. Cu is also found in dopamine-beta-hydroxylase, an important enzyme in the production of catecholamines. It's hardly surprising, then, that aberrant Cu-enzyme/protein interactions lead to hepatic, neurological, and other diseases (Gaetke *et al*., 2014) [10]. To justify their use in traditional medicine (TM), a wide range of medicinal plants have been evaluated for biological activity (Vujanovi *et al*., 2019) [26]. *Solanum incanum* L., for example, is a well-known medicinal herb that has long been used to cure a number of ailments. The plants are used in traditional medicine in tropical Africa, particularly Ethiopia, to treat sore throats, stomachaches, malaria, the common cold, hypertantion, diabetes, headaches, painful menstruation, liver discomfort, and pain caused by onchocerciasis, pneumonia, and rheumatism (Mukungu *et al*., 2016) [17]. There has been a rise of interest in the study of medicinal plants and their historical applications in various parts of the world since the turn of the century. It is vital to bring such studies together in order to provide ethinonomedicinal and pharmacological knowledge on such plants (Adotey *et al*., 2012) [2]. Despite its rising popularity as a supplement and/or medicinal agent, little is known regarding the nephron-therapeutic benefits of *Solanum incanum* leaf and seed. As a result, the current study looked at the nephroprotective effects of *Solanum incanum* methanol leaf extract on Wistar rats.

Materials and Method

Plants Collection and Identification

Gwaski village, Sakwa ward, Hawul LGA, Borno State, Nigeria is the village where *Solanum incanum* leaf. It was identified by S. Sanusi of the Department of Biological Science at the University of Maiduguri in Borno State, Nigeria, and a voucher number (DCPT 014) was issued. The leaf was washed and air-dried at room temperature (26°C) for two weeks.

Plant Extraction

Solanum incanum leaves were washed and crushed to a semi-powdery texture (40–60 mesh). The powder was allowed to dry for two weeks at room temperature (26 1°C). In flat bottom flasks, 200 g of leaves were steeped in 1000 mL of 80% methanol for 3 days (Sigma-Aldrich, USA). To obtain a high yield of extract, the semi-powdered leaves in methanol were shaken daily for three days at 251°C. The extract was then filtered through a fresh white clean muslin cloth before being concentrated to semisolid form at 42°C using an IKA® RV 10 rotary evaporator. The semi-solid crude extract was then weighed and placed in sample vials, which were kept at 4 °C until they were needed.

Yield (percentage) = [weight of extract (g)/weight of plant] (Hassan *et al*., 2020) [11].

Plants Sample Dilution and Dose Preparation

The stock solution was made by dissolving 100 g of S. incanum leaves extract in 1 L of 100 percent DMSO (100 g/L). The sub-stocks solution was made by diluting the stock solution to 10 mg/mL with distilled water. Working solution was prepared from sub-stock solution by twofold serial dilution with distilled water at concentrations of interest (1 mg/mL). DMSO (vehicle) was preserved at 0.1 percent in all extract concentrations (Hassan *et al*., 2020) [11].

Toxicity study of the extract

The 423(7) guidelines of the Organization for Economic Cooperation and Development (OECD) were followed while testing the extract's oral toxicity effects (Alli *et al*., 2011) [3]. The protocol for these investigations was approved by the Institutional Animal Care and Use Committee (IACUC) at Usmanu Danfodiyo University's Faculty of Veterinary Medicine in Sokoto, Nigeria, under the number UDUS/IACUC/AUP-R005/2020. To assess the acute toxicity of crude extract, rats were administered 1000, 500, and 250 mg/kg for two days (Clemente *et al*., 2019) [8]. Probit analysis was used to compute the LC_{50}. In a 14-day chronic toxicity trial, the crude extract was administered at dosages of 500, 250, and 125 mg/kg (Adekola *et al*., 2020) [1].

Induction of Nephrotoxicity using Copper (ii) oxide

Copper (ii) oxide at 200 mg/kg/rat cupper trioxide (200 mg/kg BW) was given orally to rats for 5 days to produce nephrotoxicity (Patlolla and Tchounwou, 2005) [21].

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

The extract's nephron-therapeutic properties on Copper (ii) oxide-induced nephrotoxicity were tested in accordance with OECD criteria. The rats were separated into three groups, each with five rats. The following was done to the groups:

a. Food and water were the only things given to the normal control group.
b. The rats were given 300 mg/kg bw of *S. incanum* methanol extract in addition to food and water.
c. In addition to food and water, rats were given 200 mg/kg bw of Copper (ii) oxide.
d. In addition to food and water, rats were given 200 mg/kg bw of Copper (ii) oxide for 5 days followed by 300 mg/kg bw of *S. incanum* methanol extract for 10 days.

Animals were sedated with chloroform vapor, and blood was drawn through heart puncture with a 5 ml syringe and needle 48 hours after the last treatment and placed in EDTA-free bottles. After dissecting each rat's skull with a dissecting kit, the brain was retrieved. Before being put in a clean sample container containing 10% neutral buffered formalin, the dissected brains were dipped in a beaker containing normal saline to remove excess blood. (Parasuraman *et al*., 2010) [19].

Biochemical Tests

Proteins content (Kashyap *et al*., 2020) [13] The concentrations of 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 ($HCO3^-$) (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 measured in serum using enzyme-linked immunosorbent assay (ELISA) kits from Thermo Fisher Scientific, USA, according to the manufacturer's instructions.

Histological Study

The kidney was fixed in 10% neutral buffered formalin for three days before being transferred to 70% alcohol and graded to 90%, 99.9%, and 100% alcohol for eight, twelve, and fifteen hours, respectively. The tissues were imbedded and placed into paraffin wax to harden the tissue for easy cutting into tiny pieces using the microtome after replacing the alcohol with Xylene and incubated for 4 hours. To remove air bubbles before solidification, tissues were cast into an L-shaped paraffin block. The prepared slides were placed on the microscope and viewed under oil immersion at 1000 magnification after 30 minutes of drying in xylene using a dry air oven (Sabdyusheva *et al*., 2020) [23].

Results

Toxicity study

The extract's LC_{50} was 676.10 mg/Kg body weight, whereas Copper (ii) oxide's was 387.4 mg/Kg body weight, according to the chronic toxicity experiments.

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

The Therapeutic effects of Garden eggs on cupper-induced electrolyte alterations are 137.00 ± 4.93 and 138.00 ± 1.85 (mmoL/L) respectively, with a significant difference (P<0.05) in sodium ion concentration between the control group and the group exposed to cupper exclusively. The difference between the control group and the groups treated with crude extract, copper (II) oxide and crude extract was not different (CuO). Between the control 6.03 ± 1.05 and the group exposed to CuO only, there was statistical difference (P<0.05) in potassium (K^{+}) levels. On chloride ion (Cl^{-}) concentration, a significant difference (P<0.05) was found between the control group 101.25 ± 2.71 and the group exposed to cupper alone 100.00 ± 3.46. The difference between the control group and the groups treated with crude extract, copper (II) oxide and crude extract was not significant (CuO). On bicarbonate ion ($HCO3^{-)}$) concentration, statistical analysis showed a significant difference (P0.05) between the control group 9.33 ± 3.18 and the group groups treated with crude extract, copper (II) oxide and crude extract 21.33 0.88. There was no statistical difference between the control group and the 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	A(GC)	B(CO)	C(GE)	D(CG)
Na^{+} (mmol/l)	138.00 ± 1.85^{b}	137.00 ± 4.93^{b}	146.67 ± 0.67^{a}	147.25 ± 1.03^{a}
K^{+} (mmol/l)	6.03 ± 1.05^{b}	5.68 ± 0.30^{b}	6.37 ± 0.35^{a}	6.55 ± 0.49^{a}
Cl^{-} (mmol/l)	101.25 ± 2.71^{b}	100.00 ± 3.46^{b}	104.00 ± 1.73^{a}	104.75 ± 1.31^{a}
HCO_3^{-}(g/dl)	9.33 ± 3.18^{c}	8.50 ± 1.19^{c}	13.67 ± 1.86^{b}	14.25 ± 1.65^{a}

KEYS: A = General control, B = Copper only, C = Garden egg only, D = Copper then treated with garden egg, Superscript **a** = Increase, Superscript b = Decrease, $Na^{+\,=}$ Sodium, K= Potassium, Cl^{-} = Chloride, HCo_3^{-} = Bicarbonate.

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

Table 2 shows that the protective effects of the Garden egg on copper-induced nephrotoxicity reveal no significant differences in urea, creatinine, or erythropoietin concentrations between the control and the other treatment groups.

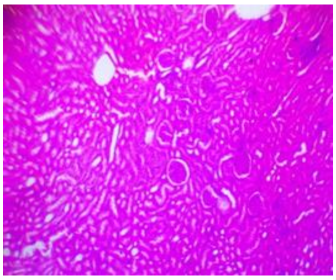
Table 2: Therapeutic effects of Garden egg on cupper induced Nephro toxicity

Parameters	A(GC)	B(CO)	C(GE)	D(CG)
Urea (mg/dl)	6.60 ± 0.87	6.55 ± 0.42	6.73 ± 0.55	6.53 ± 0.54
Creat. (mg/dl)	0.90 ± 0.15	1.05 ± 0.19^{A}	1.00 ± 0.10^{A}	0.95 ± 0.20^{A}
Erythr. (mg/dl)	29.32 ± 11.02	38.13 ± 3.49^{A}	29.31 ± 9.74^{B}	32.73 ± 2.88^{B}

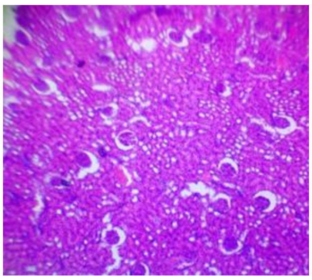
KEYS: A = General control, B = Copper only, C = Garden egg only, D = Copper then treated with garden egg, Superscript **A** = Increase, Superscript B = Decrease.

Histopathology

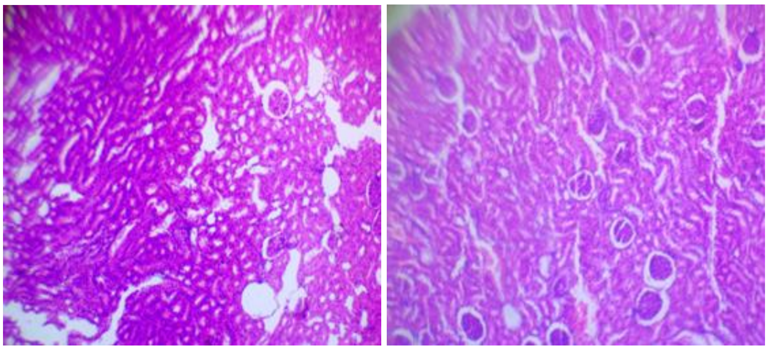
In the group exposed to CuO alone, histological findings revealed diseases such as infiltration, moderate renal tubular degeneration, and renal tubular dilatation of the kidney (plate B). The remaining treatment groups (plate C and D) and the control (plate A) had normal histological appearances.



A



B



C D

Plate 1-4: Histopathological evaluation of Wistar rats' kidneys: Mild renal tubular degeneration (red arrows) and tubular dilatation (blue dot) were seen in the kidney (black arrows).
Plate A: Wistar rat kidney section fed only feed and water (control group), Plate B: Wistar rat kidney section exposed to CuO only, Plate C: Wistar rat kidney section treated only with G. egg methanol extract, Plate D: Wistar rat kidney section exposed to CuO and later treated with G. egg methanol extract.

Discussion

The goal of this study was to see if Garden egg (*Solanum incanum*) might cure Wistar albino rats from CuO-induced nephrotoxicity.

The effect of CuO on the electrolyte balance of animals could not be found on the internet. CuO-exposed groups had a substantial reduction in total salt, chloride, and bicarbonate ($p< 0.05$) when compared to control and other treatment groups. According to Lotfi *et al*., (2018) [15] this might be attributed to hepatocellular necrosis and acute tubular necrosis. Between the control and treatment groups, no significant differences in urea, creatinine, or erythropoietin were found. This study's findings of infiltration, moderate renal tubular degeneration, and renal tubular dilatation in the CuO-only group are comparable to those reported by (Cuzzocrea *et al*., 2003) [9].

Cu, in its ionic state, is toxic to a variety of cells, including kidney cells (Xiang and Liu, 2021) [28]. Excessive intracellular Cu accumulation promotes the formation of reactive oxygen species (ROS), which catalyze the reaction of the superoxide anion with hydrogen peroxide, culminating in the development of the hydroxyl radical (Pierson *et al*., 2018). Cu can also bind directly to free cysteine thiols (Cys), resulting in protein oxidation and crosslinking, inactivating enzymes or weakening structural proteins (Lorincz, 2018) [14]. Cu-induced ROS drive apoptotic/necrotic processes, as well as cancer, nephrosis, and renal failure, among other disorders (Bhattacharya *et al*., 2016) [4].

Conclusions

The methanol extract of S. incanum was shown to have a considerable therapeutic impact on kidney function in this investigation. The curative effect of this extract on CuO-induced nephron toxicity was demonstrated by histopathologic results. This plant might be a potential source of lead compounds for the development of medications that can be used to treat kidney illnesses due to the presence of considerable undiscovered bioactive chemicals as well as its therapeutic benefits.

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