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Effects of Aqueous Extract of Solanum Gilo Fruit on Mercuric Chloride-Induced Kidney Toxicity in Adult Wistar Rats (Rattus Norvegicus)

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ABSTRACT

The kidney is one of the target organs of mercury toxicity. Mercuric chloride produces oxidative damage by enhancing peroxidation of membrane lipid. Studies have shown that *Solanum gilo* has nutritional and medicinal features such as high level of antioxidant compounds. The aim of this study was to investigate the effects of aqueous extract of *Solanum gilo* fruits on mercuric chloride- induced kidney toxicity. Thirty adult Wistar rats were purchased from the animal holding of the Department of Anatomy, University of Benin, Benin city, Edo State, Nigeria. The rats were allowed to acclimatize for two weeks. They were allowed free access to food and water throughout the duration of the experiment. They were randomly divided into six groups of five rats each. Animals in group A served as control animals. Those in group B were administered orally with 5 mg/kg body weight of mercuric chloride only; those in group C were administered 200 mg/kg body weight of aqueous extract *Solanum gilo* fruits only; those in group D were administered 400 mg/kg body weight of aqueous extract of *Solanum gilo* fruits only; those in group E were administered 5 mg/kg body weight of mercuric chloride and 200 mg/kg body weight of aqueous extract *Solanum gilo* fruits while those in group F were administered 5 mg/kg body weight mercuric chloride and 400 mg/kg body weight of aqueous extract of *Solanum gilo* fruits. The experiment lasted for 28 days. The experimental rats were sacrificed under chloroform anaesthesia, kidneys were harvested and immediately subjected to antioxidant or histological analyses. The results showed that there was a significant decrease ($P<0.05$) in kidney superoxide dismutase and significant increase ($P<0.05$) in kidney malondyaldehyde of rats treated with mercuric chloride when compared with control groups. *Solanum gilo* was able to reverse this condition when co-administered with mercuric chloride. The result also showed that *Solanum gilo* protects the kidney against mercuric chloride-induced histological changes. In conclusion *Solanum gilo* has a protective effect on mercuric chloride-induced kidney toxicity.

Keywords: mercuric chloride, kidney toxicity, *Solanum gilo, Wistar rats*

INTRODUCTION

The potential hazards of heavy metals to human is of immense importance as man continues to be exposed through anthropogenic events that lead to bioaccumulation across food chain.[1] One of such heavy metals is mercury.

Mercuric chloride ($HgCl_2$) is a white powder, soluble in water. It could be found in some homoepathic medicine, lightening creams, and some batteries.[2]It is also present in the atmosphere associated with other particles.[3] Mercuric chloride is used in the manufacture of calomel, chemical reagents, metallurgy, tanning, as a catalyst for vinyl chloride, as an intensifier in photography as well as in electroplating. It is also used as an inorganic reagent.[4]

Mercuric chloride can be absorbed through any route, ranging from ingestion, inhalation and dermal absorption. Thus, mercuric intoxication can result from exposure through any of these means,[5] and may be highly toxic and corrosive once absorbed into the blood stream. From nutritional point of view, humans could be exposed to mercury intoxication by consumption of freshwater fish and sea food.[6,7]

On absorption into the blood stream, mercuric chloride readily combines with plasma proteins or enters the red blood cells. Mercuric chloride excretion is largely through urine; stool, although significant amounts are shed through sweat, tears, breast milk and saliva.[8] However, mercuric chloride has been regarded as one of the most toxic forms of mercury as a result of the ease with which it forms organomecury complexes with plasma proteins,[9]resulting in alteration in the

structure and function in many organs such as kidney, liver, gastrointestinal tract.[10]

Mercuric chloride exposure has been reported to affect many organs of the body including kidney, liver and gastrointestinal tracts.[1,11] Symptoms may include oliguria, anuria, weak pulse, seizures, psychic disturbance, circulating collapse, chest pain and dyspnea.[12] Much of the body burden of mercuric chloride intoxication resides in the proximal convoluted renal tubule,[13] where it exists bonded in metallothionein.[14] Studies had recorded autoimmune kidney damage in adult rats exposed to mercuric chloride.[15] Others had reported acute renal failure attributed to tubular and glomerular pathology in adult rats exposed to mercuric chloride, experienced.[16]

The toxic effects of mercuric chloride on the kidney is due to its interaction with sulfur-containing plasma proteins[17,18] and its ability to induce oxidative stress.[19-21] Its ability to induce oxidative damage had been linked to its high affinity for cellular cysteine thiols.[22]

Studies had predicted that antioxidants play an important part in counteracting the adverse effects of metabolic free radicals.[23] Treatments with antioxidants and radical scavengers such as vitamin C, vitamin E and herbal antioxidants were found to decrease oxidative stress induced mercuric chloride.[24] Thus, plant-based remedies had continued to provide protections against mercuric chloride-induced toxicity with fewer side effects.

Eggplant (*Solanum gilo)* is one of the prevalent tropical plants widely used in most part of the world; Asia, Africa, Sub-tropics, and cultivated in warm temperate regions; Mediterranean and South America[25] for food and medicinal purposes.[26] The plant genus *Solanum*, belonging to the family solananceae, also known as nightshade. There are over 1000 species worldwide of which 25 species are known in Nigeria including those domesticated and wild ones with their leaves, fruits or both used as vegetables and in traditional medicine.[27]

The use of this eggplant in indigenous medicine range from weight reduction to treatment of several ailments including constipation, weight reduction, obesity, diabetes, rheumatic disease, swollen joint pains, gastro-esophageal reflux disease, dyspepsia.[28] It has proven benefit to patient suffering from anemia, is used to induce lactation in freshly delivered women.[29] It reduces intraocular pressure (glaucoma) and convergence insufficiency.[26] In addition, it prevents heart diseases and blood pressure.[30,31] These pharmacological properties have been attributed to the presence of certain chemical substances in the plant such as crude fibre, phenols, ascorbic acid, alkaloid, steroidal alkaloid, glycoalkaloid, anthocyanin.[32]

The aim of this study was to investigate the effects of aqueous extract of *Solanum gilo* fruits on mercuric chloride-induced kidney toxicity in adult Wistar rats.

MATERIALS AND METHOD

Purchase of *Solanum gilo* and Preparation of Extract

Fresh *Solanum gilo* fruits were purchased from New Benin market, Benin-City, Edo State, Nigeria. The fruits were identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Benin City, Edo State, Nigeria. They were pulverized using pestle and mortar after which they were macerated with distilled water for 24 hours, under constant shaking and stirring. The sample was then filtered to separate the residue from the filtrate. The filtrate was then concentrated with crucible water bath to form a semi-solid paste which was subsequently preserved in refrigerator at 4°C for further use.

Qualitative Phytochemical Screening of *Solanum Gilo*

The aqueous extract of *Solanum Gilo* subjected to phytochemical analyses for evidence of the presence of bioactive compounds following standard procedures.[33-35] The plant extract was screened for the presence of carbohydrates, saponin, flavonoids, tannins and alkaloids

Experimental Design

Thirty adult Wistar rats weighing between 180 g and 200 g were used for this experiment. The animals were obtained and bred from the Animal Holdings of the Department of Anatomy, University of Benin, Benin City, Edo State, Nigeria. The animals were allowed to acclimatize for 2 weeks and were all allowed free access to feed and water.

The animals were randomly divided into six groups; A, B, C, D, E and F, of five rats each and were treated as follows: Group Aanimals served as control. They were neither administered mercuric chloride nor aqueous extract of *Solanum gilo* fruits, but were administered 1 ml of normal saline using the same method of administration as other group; group B animals were treated with oral administration of 5 mg/kg body weight mercuric chloride only, daily for 28 days; group C animals were treated with oral administration of 200 mg/kg body weight of only aqueous extract of *Solanum gilo* fruits daily for 28 days; group D animals were treated with oral administration of 400 mg/kg body weight of only aqueous extract of *Solanum gilo* fruits daily for 28 days; group E animals were treated with oral administration of 5 mg/kg mercuric chloride while 200 mg/kg body weight of aqueous extract of *Solanum gilo* fruits was administered orally, one hour later, daily for 28 days; Group F animals were treated with oral administration of 5 mg/kg of mercuric chloride while 400 mg/kg body weight of aqueous extract of *Solanum gilo* fruits was administered an hour later, daily for 28 days.

Method of Sacrifice and Tissue Collection
At the end of the experimental period, a midline incision was made through the ventral abdominal wall of the rats under slight anesthesia using chloroform. The kidneys were harvested and fixed immediately in 10 % formal saline for 24 hours.

Histological Technique
Paraffin Tissue Processing: Following the fixation of the kidney tissue in 10% formal saline, the tissues were dehydrated in ascending grades of alcohol, cleared in xylene, infiltrated in molten paraffin wax in an oven, embedded with embedding mold in molten paraffin wax and sectioned using a rotary microtome (thickness of 5μ) prior to routine haematoxylin and eosin staining.

Haematoxylin and Eosin Staining Method: Good tissue sections which came out as ribbons were placed in 20% alcohol for spreading of the tissue, which was then floated in a water bath at temperature of 30^{o}C. The sectioned tissues were picked with slides and allowed to dry.

The tissues were placed in Xylene to remove excess paraffin wax, rehydrated by passing them through descending grades of alcohol and water, for about 2 minutes each. The tissues were stained in Haematoxylin for about 10-15 minutes and rinsed in water. Excess stains were removed by washing under tap water for 2-3 minutes, followed by differentiation of tissues in 1% acid alcohol for a minute. The tissues were then blued in running tap water, counter-stained with 1% Eosin for 3-5 minutes, rinsed in water and dehydrated rapidly by passing through ascending grades of alcohol, cleared in Xylene and finally mounted in DPX (Distrene Plasticizer and Xylene) covered with a cover slip, for photomicroscopic studies.

Photomicrography: The tissue sections were examined under Leica DM750 research microscope with a digital camera (Leica ICC50) attached. Digital photomicrographs of the tissue sections were taken at various magnifications (X100 and X400).

Antioxidant Enzyme Estimation
Preparation of Sample: Known weights of different samples of the kidneys from the experimental animals were dissected out, homogenized in a chilled mortar and pestle with a pinch of acid-washed sand and a total of 5ml normal saline (0.95%) added sequentially during the homogenization process. The homogenates were centrifuged at 3500rpm for 5 minutes. The clear supernatants were collected using a micropipette and transferred into an empty specimen container and refrigerated at 4^{o}C till needed for the assays.

The Superoxide (SOD) activities in these tissues were determined by the method of Misra and Fridovich[36] while malondialdehyde (MDA) levels were estimated by the method of Beuge and Aust using Thiobarbituric Acid (TBA).[37]

Statistical Analysis
The data generated were analyzed using descriptive and inferential statistics. All the values were presented as mean $\pm$ Standard Error of Means (S.E.M). All statistical analyses were carried out using Statistical Package for Social Sciences (SPSS, version 16, Chicago, Illinois). The significance of difference in the means of all parameters was determined using One Way Analysis of Variance (ANOVA; 95% confidence interval). *Post hoc test* was carried out for all groups and compared with control.

RESULTS
Phytochemical Constituents of *Solanum gilo*
Phytochemical analysis of aqueous extract of *Solanum gilo* fruit showed the presence of carbohydrates, saponin, tannin, flavonoid and alkaloids (table 1).

Antioxidant Enzyme Studies
The antioxidant studies showed that while superoxide dismutase was significantly lower ($P<0.05$) in group B when compared with Groups A, C, D, E and F, there was no statistically significant difference ($P>0.05$) when groups A, C, D, E and F were compared with each other (table 2). More so, while malondyaldehyde was significantly higher ($P<0.05$) in group B when compared with Groups A, C, D, E and F, there was no statistically significant difference ($P>0.05$) when groups A, C, D, E and F were compared with each other (table 2).

Histological Findings
The photomicrograph of the control group (group A) showed normal features of the kidney such as glomeruli, renal tubules and interstitial spaces (plate 1, A). In the rats treated with 5 mg/kg body weight of mercuric chloride only (group B), there were mild tubular casts, interstitial inflammation and focal tubular necrosis (plate 1, B). The groups treated with both 200 mg/kg body weight and 400 mg/kg body weight of aqueous extract of *solanum gilo* only (groups C and D) presented normal glomeruli and tubules (plate 1, C and plate 1, D). The same normal histology of the kidney was noted in the groups treated daily with oral administration of 5 mg/kg body weight along with 200 mg/kg body weight and 400 mg/kg body weight of aqueous extract of *Solanum gilo* fruit only, for 28 days respectively (plate 1, E and plate 1, F).

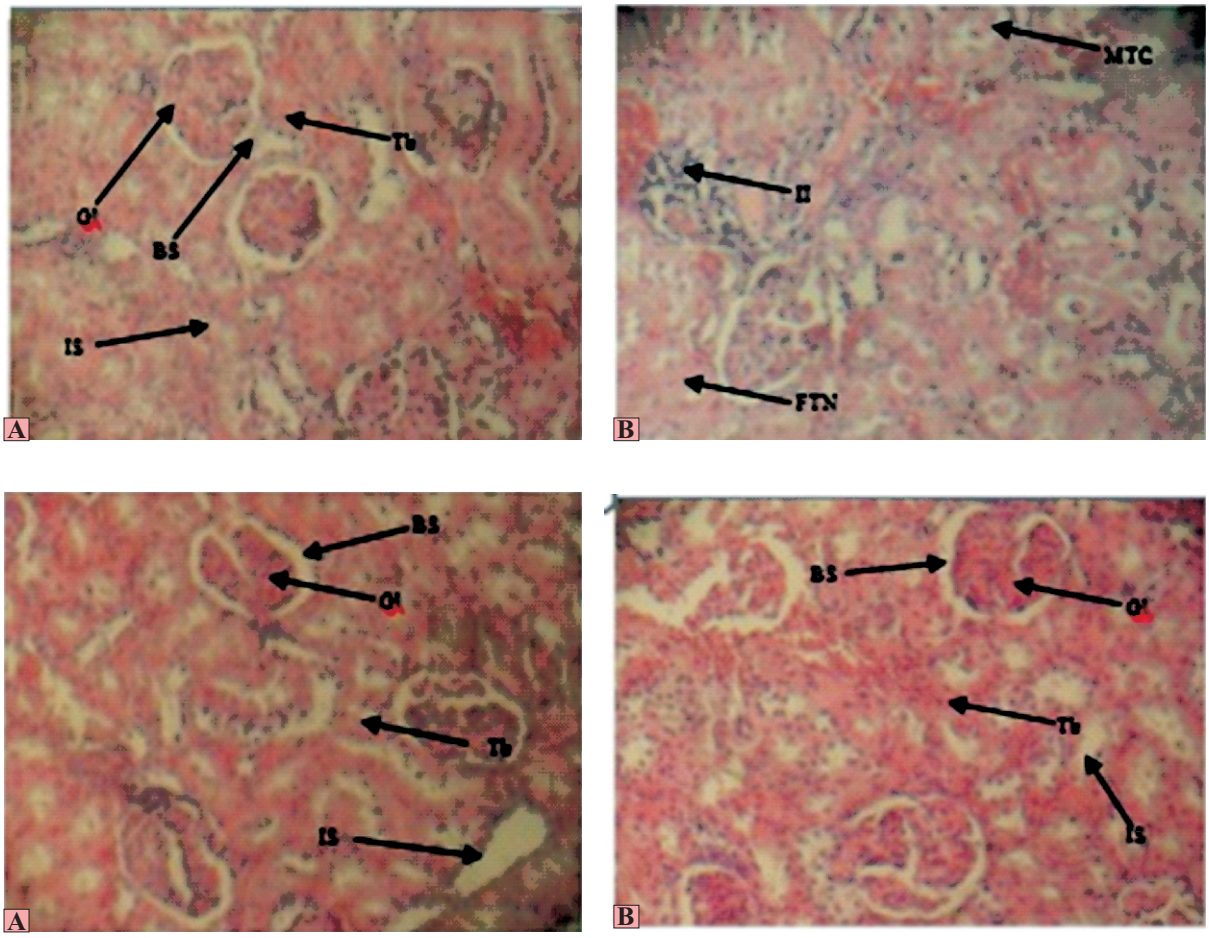
Table 1: Phytochemical Constituents of Aqueous Extract of *Solanum gilo fruits*

Constituents	Present/absent
Carbohydrate	present
Saponin	present
Tannin	present
Flavonoid	present
Alkaloid	present

Table 2: Effects of Aqueous Extract of *Solanum Gilo Fruits* on Mercuric Chloride induced Oxidative Damage in Kidneys of Adult Wistar Rats

Parameters	Group A	Group B	Group C	Group D	Group E	Group F
Number	5	5	5	5	5	5
Superoxide Dismutase	16.78±0.08	12.18±0.48*	17.10±0.10	17.28±0.05	16.88±0.10	17.01±0.09
Malondyaldehyde	16.75±0.7	63.52±1.84*	17.33±1.90	16.07±0.63	16.46±1.20	17.81±1.58

Photomicrograph of H&E Sections of the Kidney



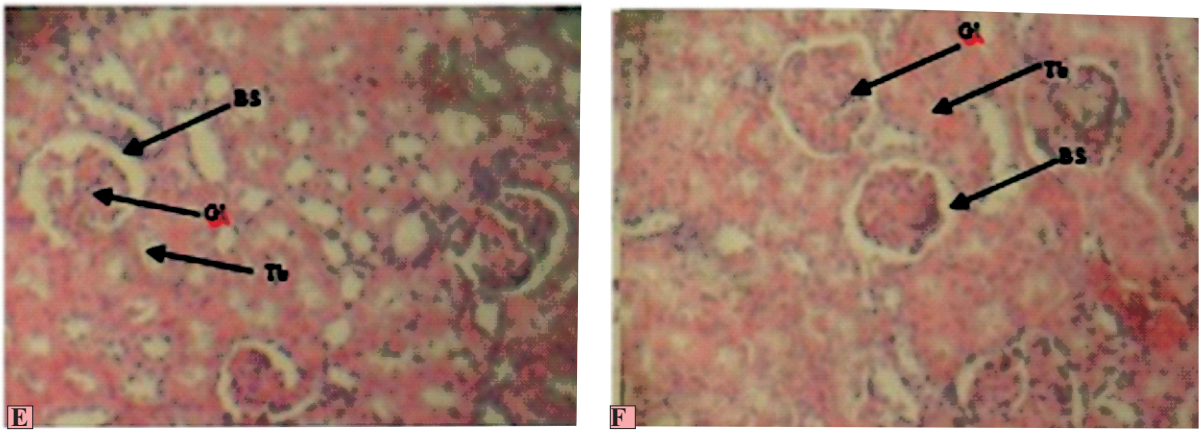


PLATE 1: Photomicrographs of the kidneys of the experimental animals. 'A' shows the Group A (Control group) showing normal glomeruli **'Gl'**, Bowman's spaces **'BS',** tubules **'Tb'** and interstitial space **'IS'**. 'B' shows Group B (treated daily with oral administration of 5 mg/kg body weight $HgCl_2$ only for 28 days) showing mild tubular casts 'MTC', focal tubular necrosis 'FTN' and interstitial inflammation 'II'. 'C' and 'D' show Groups **C and D** (treated daily with oral administration of 200 mg/kg body weight and 400 mg/kg body weight of aqueous extract of *Solanum gilo* fruit only, for 28 days respectively) showing normal histology of the kidney similar to the control group with glomerulus **'Gl' and** tubules **'Tb'**. 'E' and 'F' show Groups E and F(treated daily with oral administration of 5 mg/kg body weight along with 200 mg/kg body weight and 400 mg/kg body weight of aqueous extract of *Solanum gilo* fruit only, for 28 days respectively) showing normal histology of the kidney similar to the control group with glomerulus **'Gl' and** tubules **'Tb'**.

DISCUSSION

Millions of people in developing nations including Nigerians, have resorted to the use of plants sources to treat or manage their ailments; this could be due to the high cost of orthodox health care or as a result of global shift towards the use of natural sources rather than synthetic drugs.[38] This study showed that *solanum gilo* fruit contains phytochemicals which include carbohydrate, saponin, flavonoid and alkaloids, which were revealed from the phytochemical screening.

One of the mechanisms of actions in mercury induces toxicity is oxidative stress,[39-41] resulting in the excessive release of reactive oxygen species and increased lipid peroxidation in the cells.[20] Free radicals and intermediate products of peroxidation have the ability to destroy the integrity and altering the function of biomembranes, with many consequent pathological conditions.[42] Enzymes that inhibit formation of free-radicals, such as superoxide dismutase (SOD), play a significant part role in defending the cell membranes against oxidative stress.[43]

This study showed a significant decrease in kidney Superoxide Dismutase in rats treated with mercuric chloride. This is in agreement with an earlier study by Gutierrez *et al*.[44] The resultant increase in Superoxide Dismutase (SOD) levels in the kidneys of rats treated with mercuric chloride and various doses of aqueous extract of *Solanum gilo* fruit could be as a result of the possible antioxidant properties of *Solanum gilo* fruits.

Malondialdehyde is one of the major oxidation products of peroxidized polyunsaturated fatty acids. Increased malondialdehyde content is an important indicator of lipid peroxidation.[45]This study showed significant increase in malondialdehyde levels in the kidney of rats treated with mercuric chloride when compared with control. This result agrees with previous studies that mercuric chlorideincrease MDA level in tissues.[46,47] It was observed from this study that there was a significant decrease in MDA levels in all the groups treated with aqueous extract of *Solanum gilo* fruit following mercuric chloride intoxication. The antioxidant effect of *solanum gilo* on the kidney might be due to its content such a flavonoids and alkaloids.[32]

In conclusion, the aqueous extract of *solanum gilo* has a protective effect on the mercuric chloride induced kidney toxicity in adult Wistar rats by acting as antioxidant.

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