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RENAL TOXICITY POTENTIATION BY AQUEOUS EXTRACT OF CYPERUS ESCULENTUS IN A GENTAMICIN-INDUCED

[1]Kabiru Suleiman Musa and [2]Hauwa Zainab Umar

[1]Department of Morbid Anatomy and Forensic Medicine, College of Health Sciences, Usmanu Danfodiyo University, Sokoto

[2]Department of Pharmacology and Toxicology, Usmanu Danfodiyo University, Sokoto

Abstract: Oxidative stress plays a central role in the mechanism of gentamicin-induced nephrotoxicity. The antioxidant properties of *Cyperus esculentus* (CE) have been reported. This study aimed to explore its potential in ameliorating symptoms of nephrotoxicity in a gentamicin-induced model. Tubers of CE were extracted with distilled water. Phytochemical analysis was done using standard protocols. Twenty-five male Wistar albino rats were divided into five groups of five animals each and treated for 8 days: Group I received normal saline, Group II (Gentamicin group)-gentamicin 80 mg/kg + distilled water, Group III-V were administered 80 mg/kg gentamicin +100, 200 and 400 mg/kg CE respectively 1 hour after gentamicin treatment. The rats were anesthetized and blood was collected via cardiac puncture for biochemical analysis. The gentamicin group showed significant increase in serum urea and creatinine levels compared to the control group. *Cyperus esculentus* resulted in a further elevation of these parameters in a dose-dependent manner. Furthermore, there was a significant increase in serum potassium and a significant decrease in bicarbonate at 400 mg/kg CE. Histological results further buttressed the biochemical findings. In conclusion, oral administration of *Cyperus esculentus* in gentamicin-induced nephrotoxicity resulted in a further elevation in serum levels of urea, creatinine, and potassium as well as a decrease in serum bicarbonate, and failed to protect against gentamicin-induced nephrotoxicity.

Keywords: *Cyperus esculentus*, Nephrotoxicity, Gentamicin, Tiger nut.

INTRODUCTION

The kidney is a vital organ in health and disease, many environmental contaminants and chemical variables, including drugs may alter its functions (Kataria *et al*., 2015;
Kuźma *et al*., 2021). Gentamicin, a widely used aminoglycoside against infections by gram-negative bacillary microorganisms can result in acute renal failure in 10-30% of patients once given beyond seven days (AbdelRaheem *et al*., 2009). The mechanism of gentamicininduced nephrotoxicity comprises of oxidative stress due to radical generation, a rise in lipids peroxidation, a decrease in the activity of both endogenous enzymatic and nonenzymatic antioxidants, inflammation of the renal tubules, and consequent release of pro-inflammatory cytokines. These result in reduced glomerular filtration rate and renal dysfunction (Balakumar *et al*., 2010; Lee *et al*., 2012). Consequently, several compounds with antioxidant and anti-inflammatory activity have been shown

to prevent or ameliorate gentamicin-induced nephrotoxicity (Adil *et al*., 2016; Ehsani *et al*., 2017; Hajihashemi *et al*., 2020; Atsamo *et al*., 2021).

Cyperus esculentus commonly called tiger nut, chufa, earth almond, or yellow nutsedge belongs to the family *Cyperaceae*. It is native to the warm temperate or subtropical regions of the northern hemisphere and cultivated in China, Spain, and West African countries like Niger, Mali, Senegal, Ghana, Togo, and Northern Nigeria. In Nigeria, *Cyperus esculentus* is locally known as *Aya* among the Hausas, *Imumu* among Yorubas, and *Ofiooraki* Hausa among the Igbos. The nut is either consumed in its fresh state or after being dried, roasted, or made into a beverage known as Kunnu (Oladele and Aina, 2007). Tiger nut has been reported to be rich in energy content (starch, fat, sugars, and protein) (Belewu and Abodunrin, 2008). The mineral content of tiger nuts in a decreasing order comprises potassium (P), phosphorus (P), magnesium (Mg), calcium (Ca), sodium (Na), iron (Fe), zinc (Zn), and copper (Cu) respectively. It was also shown to contain a high level of antioxidant vitamins; vitamin E and C and a little vitamin A content (Suleiman *et al*., 2018), high phenolic content (Ogunlade *et al*., 2015) and a rich source of flavonoid (El-Habashy, 1988). The antioxidant properties of Tiger nut against 1,1-diphenyl-2 picrylhydrazyl (DPPH) and hydroxyl (OH) radicals and Fe^{2+} induced malondialdehyde (MDA) production has been reported (Ademosun and Oboh, 2015; Ogunlade *et al.,* 2015). It has been shown to reduce oxidative stress in the liver and inflammation in atherosclerosis (Achoribo and Ong, 2017). Tiger nut powder has also shown antioxidant, anti-inflammatory, and anti-apoptotic effects to prevent testicular dysfunction in rats (Adelakun *et al*., 2021, Udefa *et al*., 2020). It decreased scopolamine-induced deregulated glutathione (GSH) contents and antioxidant enzymes in the mouse brain (Umukoro *et al*., 2020). The medicinal value of plants lies in the plant phytochemical (bioactive) constituents, which show various physiological effects on the human body (Adebisi *et al*., 2018). This present study determined the phytochemical constituents and explored the potential of aqueous extract of *Cyperus esculentus* in ameliorating renal failure in gentamicin-induced nephrotoxic male Wistar rats.

MATERIALS AND METHODS Chemicals, Reagents, and Drugs

Gentamicin injection (DERM GENTAMYCIN®) was purchased locally and manufactured by Shanxi Zhungbao Shuguang. Chemicals such as Mayer's reagent, Wagner's reagent, Dragendroff reagent, gold beater's skin, Ferric chloride, Molisch's reagent, Fehling solution, sodium hydroxide, hydrochloric acid, sulfuric acid and other chemicals used to were analytical grade and obtained from the research laboratory of Pharmacognosy and Ethnopharmacy, Faculty of Pharmaceutical Sciences, Usmanu Danfodiyo University Sokoto.

Experimental Animals

Thirty (30) albino Wistar rats comprising five (5) females and twenty–five (25) males weighing (180 – 220 g) were purchased from Department of Pharmacology and

Therapeutics, Ahmadu Bello University Zaria, , Nigeria. The animals were kept in a well-ventilated cage at room temperature of 12 hours light and dark cycle and acclimatized for 14 days. They were fed with standard chow and had access to food and water *ad libitum.* The study protocol was approved by the animal research ethics committee of the Department of Pharmacology and Toxicology UDUS (PTAC/Ce/(Ae)/OT/41-22). The care and handling of the animals were according to the established public health guidelines on the use of laboratory animals in research.

Collection and Identification of *Cyperus esculentus* the plant was collected from Yarkuhuji village, Mafara local government, Zamfara state. It was identified at the herbarium unit of the Department of Pharmacognosy and Ethnopharmacy, Usmanu Danfodiyo University Sokoto (UDUS), and authenticated by the taxonomist Mallam Abdul-Azeez Salihu of the Department of Biological Sciences (UDUS). The plant was given a voucher number PCG/UDUS/CYPE/0001 and preserved in the herbarium for future reference

Preparation of Aqueous Extract of *Cyperus esculentus* the tubers were thoroughly screened, washed and airdried. The samples were ground using wooden mortar and pestle until a coarse sample was obtained. Aqueous extract of the sample was prepared by soaking 100 g of dried powdered sample in 200 ml of distilled water for 24 hours. The extract was filtered using Whatman filter paper No. 42 (125 mm) and dried in a water bath at a temperature of 50 °C.

Phytochemical Analysis

The qualitative analysis of the aqueous extract was assessed using the methods described by Trease and Evans (1999) to detect the presence of chemical constituents including alkaloids, tannins, saponins, flavonoids, carbohydrates, cardiac glycosides, and steroids

Acute Oral Toxicity Study

The acute oral toxicity study was done in healthy adult female rats according to OECD guidelines no 425 (OECD, 2008). A limit dose of 2000 mg/kg was used for the study. Five female rats were labeled for identification. An animal was picked at a time, weighed, and dosed with an equivalent volume of extract containing 2000 mg/kg body weight dissolved in distilled water as a vehicle after overnight fasting. Oral administration of the extract was done using a gastric feeding tube.

Each animal was observed after dosing for the first 5 minutes for signs of regurgitation and then kept in a metallic cage. Each was then observed every 15 minutes in the first 4 hours after dosing, then every 30 minutes for 6 hours, and then daily for 48 hours for the short-term outcome according to the specifications of the OECD. The animals were monitored for a total of 14 days for the long-term possible lethal outcome.

Experimental Design

The method of ApaydinYildirim *et al*. (2017) was adopted with slight modification (modified in terms of vehicle used and three extract treated dose levels as against two). Twenty-five male Wistar rats were used for the study. The animals were divided into five groups of five animals each. Each group received treatment as follows: Group I (Normal control) received normal saline (1 ml/kg) ip, Group II (Gentamicin group) received gentamicin 80 mg/kg ip and distilled water (10 ml/kg) by oral gavage, Group III-V received gentamicin 80 mg/kg ip plus a graded dose 100 mg/kg, 200 mg/kg and 400 mg/kg *Cyperus esculentus* extract respectively by oral gavage 1 hour after gentamicin treatment. All treatments were given once daily for 8 days. Afterward, animals were fasted overnight, anesthetized and blood was collected via cardiac puncture for biochemical analysis.

Assessment of Kidney Function Parameters

Blood samples were gently placed in plain bottles to avoid haemolysis of the blood cells. Blood serum was obtained by centrifugation at 4000 rpm for 5 minutes. Assessment of kidney function was done by estimating serum urea (modified Urease-Berthelot colorimetric method in autoanalyzer (Fawcett and Scott, 1960) creatinine (alkaline picrate method in auto-analyzer) (Bartel *et al*., 1972) and serum electrolytes (*i.e* sodium, potassium, chloride, and biocarbonate) using ion selective electrode method (electrolyte analyzer- K-Lite 8 series).

Histopathological Analysis

After blood collection, the kidneys were carefully dissected longitudinally, and gross pathological observation was performed to check for any gross lesions. It was then fixed in 10% buffered formalin solution, dehydrated in an ascending series of alcohol, cleared in xylene and embedded in paraffin wax melting at 60 °C. Serial sections (4-5-µm thick) were mounted on 3- aminopropyltriethsilane- coated slides and dried for 24 hours at 37 °C (Baravalle *et al*., 2006). The sections on the slides were deparaffinized, hydrated, stained with Mayer's hematoxylin and eosin dyes, dried, and mounted on a light microscope (Carl Zeiss Microscope at magnification x100 for histopathological examination.

Statistical Analysis

Data were presented as mean ± standard error of the mean (SEM). The results were analyzed using Graph Pad Prism version 8 software. Multiple comparisons of means amongst groups were made using one-way analysis of variance (ANOVA) and Dunnett's post-test comparison was
used to test for statistically significant differences at $p< 0.05$

RESULTS

Preliminary Phytochemical Screening The preliminary phytochemical screening of the aqueous extract of *Cyperus esculentus* show the presence of alkaloids, cardiac glycosides, sterols, saponins, and carbohydrates as presented in Table 1

Table 1: Qualitative phytochemical screening of *C. esculentus* aqueous extract

S/N	SECONDARY METABOLITE	TEST	RESULT
1	Saponins	Frothing	+
2	Tannins	Gold beaters skin test	-
3	Flavonoids	Shinoda's	-
		Sodium hydroxide	-
		Ferric chloride	-
4	Carbohydrate	Molisch	+
		Fehlings	+
5	Alkaloids	Mayers	+
		Dragendroff	+
		Wagners	+
6	Steroids	Salkowski	+
		Liebermann Burchard	+
7	Cardiac glycosides	Keller-Kelliani	+

+= Detected; - = Not detected

Acute Oral Toxicity

A single oral administration of the extract at 2000 mg/kg caused restlessness and itching of the ear and lower limbs in two out of five animals. No mortality occurred within 24 hours after administration and after fourteen days of observation. Therefore, the LD_{50} of the aqueous extract of *Cyperus esculentus* is greater than the test dose.

Effect of Aqueous Extract of *Cyperus esculentus*on Kidney Function Parameters

The results indicated that administration of gentamicin at a dose of 80 mg/kg/day for 8 consecutive days showed a significant increase in serum urea and serum creatinine compared to the normal animals. Aqueous extracts of *Cyperus esculentus* showed a dose-dependent increase in these parameters compared to the untreated group as shown in Figures 1 and 2. An insignificant decrease in serum electrolytes was observed in the gentamicin group compared to the normal control.

However, in the extract-treated groups, a significant increase in serum potassium and a significant decrease in serum bicarbonate were observed at 400 mg/kg of the extract as compared to the gentamicin group. There was no significant difference in serum sodium and chloride ions across the groups. This is shown in Table 2.

Histological analysis

Histological examination of the kidney sections showed necrosis of the tubular epithelium, presence of hyaline materials in the lumen, and mild inflammatory infiltrates in the interstitium in all the groups that received gentamicin (*i. e* Group II-V) compared to the normal control group, which showed normal glomeruli and normal tubules without hyaline materials in the lumen nor signs of interstitial inflammation. Administration of *Cyperus esculentus* extract did not avert these effects. Further increased tubular secretions were seen with the highest dose of the extract (400 mg/kg). This is shown in Figure 3.

DISCUSSION

In this study, the possible renoprotective effect of *Cyperus esculentus* extract against renal injury induced by gentamicin was investigated against the backdrop of its reported antioxidant and anti-inflammatory properties (Ademosun and Oboh, 2015; Adelakun *et al*., 2021, Udefa

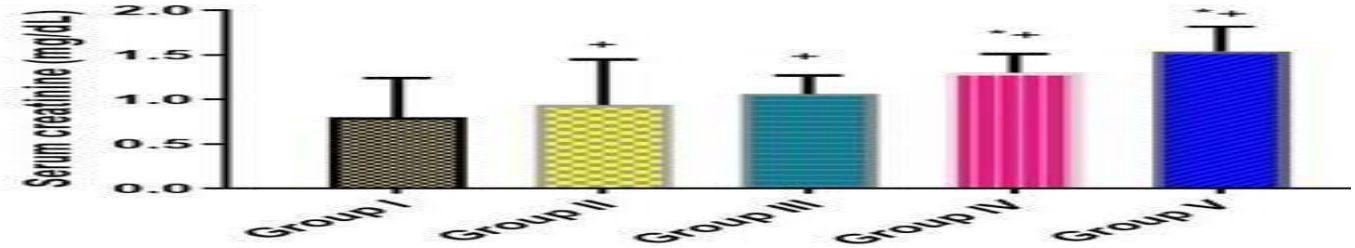

Figure 1: Effect of aqueous extract of *C. esculentus* on serum creatinine

Values are mean ± SEM (N=5) *= significant at p < 0.05 when compared with Group II. += significant at *p* < 0.05 when compared with normal control; Group I = normal control group; Group II = untreated gentamicin group; Group III = 100 mg/kg *C. esculentus* Aq. Extract; Group IV = 200 mg/kg, *C. esculentus* Aq. Extract;
Group V = 400 mg/kg *C. esculentus* Aq. Extract

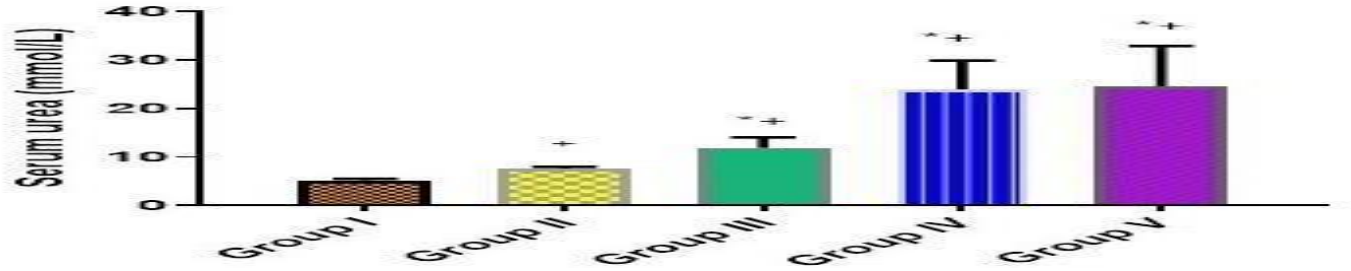

Figure 2: Effect of aqueous extract of *C. esculentus* on serum urea.

Values are mean ± SEM (N=5) *= significant at p < 0.05 when compared with Group II. += significant at p < 0.05 when compared with normal control; Group I = normal control group; Group II = untreated gentamicin group; Group III = 100 mg/kg *C. esculentus* Aq. Extract; Group IV = 200 mg/kg, *C. esculentus* Aq. Extract; Group V = 400 mg/kg *C. esculentus* Aq. Extract
Group V = 400 mg/kg *C. esculentus* Aq. Extract

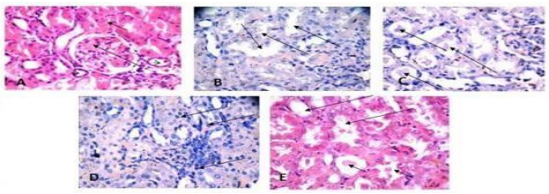

Figure 3: Photomicrograph of kidney sections of gentamicin intoxicated experimental rats administered *C. esculentus* aqueous extact (x 100)

A= Kidney section of normal rats showing normal glomerulus (long arrow), proximal convulated tubules and distal convulated tubules (short arrow); B= kidney section representing groups II-V showing tubular necrosis

evidenced by the loot of tubular epithelium (arrow); C= kidney section (II-V) showing hyaline materials within the tubules (arrow); D= kidney section (groups II-V) showing interstitial inflammatory infiltrate (arrow); E = kidney section (V) showing increased ubular secretions (long arrow) and necrosis (short arrow); Group I = normal control group; Group II = untreated gentamicin group; Group III = 100 mg/kg *C. esculentus* Aq. Extract; Group IV = 200 mg/kg, *C. esculentus* Aq. Extract; Group
V = 400 mg/kg *C. esculentus* aq. Extract

The acute dosing of 2000 mg/kg of the extract in rats did not result in any observed toxic effect. Other studies have shown that the oral lethal dose of *Cyperus esculentus* is above 5000 mg/kg (Oladipipo *et al.,* 2016; Udefa *et al*., 2020). The results of the preliminary phytochemical analyses show the presence of saponins, alkaloids, cardiac glycosides, steroids, and carbohydrates. This is similar to the report of Chukwuma *et al*. (2010). However, Imam *et al*. (2013), Charles *et al*. (2018) and Nwosu *et al*. (2022) reported the presence of tannins and flavonoids in their study. This may be probably due to the difference in the solvent of extraction. Moreover, Felhi *et al*. (2017); Thouri *et al*. (2017); Gonfa *et al*. (2020), and Sharma *et al*. (2021) have shown that extraction solvent influences the phytochemical constituents detected in plant materials. Furthermore, the effect of seasonal changes/ time of collection on phytochemical constituents of plant materials have been previously reported (Gololo *et al*., 2016; Tavhare *et al*., 2016; Adegbaju *et al*., 2020).

Renal function is evaluated by measuring its ability to excrete creatinine and urea. Gentamicin, a known nephrotoxic agent significantly increases serum urea and creatinine indicating altered glomeruli and tubular functions (Nafiu *et al*., 2019). The toxicity of aminoglycosides, including gentamicin, is related to the generation of reactive oxygen species (ROS) in the kidney and a relationship between nephrotoxicity and oxidative stress has been confirmed in many experimental models (Kakalij *et al*., 2014). The extract of *Cyperus esculentus* at 200 and 400 mg/kg led to a further increase in serum urea and creatinine. A significant increase in serum potassium and a decrease in bicarbonates in the group that received 400 mg/kg extract compared to the gentamicin group were also observed. Tiger nut is reportedly rich in mineral content such as potassium, sodium, calcium, magnesium, phosphorus, copper, *etc*, with potassium being the highest (Suleiman *et al*., 2018, Chinedu *et al*., 2019, Aladekoyi *et al*., 2019). Potassium is important in maintaining electrolyte and chemical balance between the tissue cells and the blood (Aladekoyi *et al*., 2019). This may interfere with the extracellular fluid's osmotic balance leading to loss of body water and a subsequent electrolyte imbalance that may be responsible for the observed elevations of some of the kidney parameters in this study. This biochemical result is consistent with the histopathological examination that revealed the presence of hyaline materials within the tubules, interstitial inflammatory infiltrate and increased tubular necrosis in the renal tissue of the extract-treated groups. Therefore, aqueous extracts of *Cyperus esculentus* could not ameliorate the symptoms of renal toxicity in a gentamicin model despite its reported antioxidant property. A review by Dennis and Witting (2017) that examined the protective role of antioxidants in acute kidney injury reported that although some pre-clinical studies have demonstrated that antioxidants ameliorate the symptoms of renal injury and improve kidney function by reducing oxidative damage and/or inflammation, some therapeutic antioxidants have generally failed to show benefit in human acute renal injury. For instance, Selenium though possesses antioxidant properties, its supplementation inhibited renal oxidative damage and inflammation, yet was not reno-protective in an animal model of acute kidney injury (Shanu *et al*., 2013). Similar studies with synthetic polyphenol, tert-butyl-bisphenol, and vitamin E supplementation, despite ameliorating oxidative stress and decreasing biomarkers of inflammation, demonstrated a lack of amelioration of acute kidney injury in animal models (Kim *et al*., 2011). N-acetylcysteine (NAc), a synthetic derivative of cysteine and precursor of Glutathione (GSH) that exhibits reactive oxygen species scavenging

activity has undergone several trials but has proved largely inconclusive in alleviating kidney injury (Chalikias *et al*., 2016; Dennis and Witting, 2017).

Agbabiaka *et al.* (2013) reported significantly higher creatinine values ($p<0.05$) in birds fed diets containing 25%, 50%, 75%, and 100% *Cyperus esculentus* meal and higher urea values in those fed 50%, 75%, and 100% *Cyperus esculentus* meal compared to control. Akpojotor *et al.* (2015) also reported that *Cyperus escule ntu* extract significantly increased the serum biochemical parameters for liver function indicating the plant could likely be hepatotoxic. Dhouha *et al*. (2016) and Ekeanyanwu *et al*. (2010) reported that antinutrients such as tannins, saponins, phylates, oxalates, and cyanogenic glycosides are present in *Cyperus esculentus* nut in considerable quantities and consumption of foods that contain a high quantity of antinutrients over a long period could be harmful to the body. Oxalates for example can remove calcium in the form of calcium oxalate in the blood and this may result in kidney damage (Ekeanyanwu *et al*., 2010). Furthermore, kidney injury has been reported as one of the common adverse effects caused by herbal materials (Bagnis *et al*., 2004).

Nonetheless, the tuber of *Cyperus esculentus* is used in making a refreshing beverage called *kuunu* in Nigeria which is consumed mostly in the Northern region of Nigeria (Belewu and Abodurin, 2008) and there have not been any reported cases of toxicity in humans (Ekeanyanwu *et al*., 2010). A study of the effect of a four-week daily administration of *Cyperus esculentus* on heamatological and biochemical parameters in Wistar rats by Oladipupo *et al*. (2016) did not significantly affect liver and kidney function parameters. This may suggest that *Cyperus esculentus* extract did not ameliorate the underlined nephrotoxicity induced by gentamicin in this study.

CONCLUSION

In conclusion, oral administration of aqueous extract of *Cyperus esculentus* in gentamicin-induced nephrotoxicity resulted in a further elevation in serum levels of urea, creatinine, and potassium as well as a decrease in serum bicarbonate, hence it failed to protect against gentamicininduced nephrotoxicity in rats.

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