Exacerbation of Symptoms of Renal Toxicity by Aqueous Extract of *Cyperus esculentus* in a Gentamicin Nephrotoxicity Model

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**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; Kužma et al., 2021). Gentamicin, a widely used aminoglycoside against infections by gram-negative bacilliary microorganisms can result in acute renal failure in 10-30% of patients once given beyond seven days (Abdel-Raheem et al., 2009). The mechanism of gentamicin-induced nephrotoxicity comprises of oxidative stress due to radical generation, a rise in lipids peroxidation, a decrease in the activity of both endogenous enzymatic and non-enzymatic 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 *Offioraki* 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-picyrhydrazyl (DPPH) and hydroxyl (OH) radicals and Fe⁺⁺ 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 scopalamine-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 nephrototoxic male Wistar rats.
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 ApaydinYıldırım 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 auto-analyzer (Fawcett and Scott, 1960) creatinine (alkaline picrate method in auto-analyzer) (Bartel et al., 1972) and serum electrolytes (i.e. sodium, potassium, chloride, and bicarbonate) 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>
<thead>
<tr>
<th>S/N</th>
<th>SECONDARY METABOLITE</th>
<th>TEST</th>
<th>RESULT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saponins</td>
<td>Frothing</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannins</td>
<td>Gold beaters skin test</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Flavonoids</td>
<td>Shinoda’s</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sodium hydroxide</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ferric chloride</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrate</td>
<td>Molisch</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Fehlings</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Alkaloids</td>
<td>Mayers</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dragendorff</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wagners</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Steroids</td>
<td>Salkowski</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liebermann Burchard</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Cardiac glycosides</td>
<td>Keller-Kelliani</td>
<td>+</td>
</tr>
</tbody>
</table>

\( \pm \) 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 et al., 2020).
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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

Table 2: Effect of aqueous extract of Cyperus esculentus on serum electrolytes of gentamicin intoxicated rats

<table>
<thead>
<tr>
<th>GROUPS</th>
<th>SODIUM ION (mmol/L)</th>
<th>POTASSIUM ION (mmol/L)</th>
<th>CHLORIDE ION (mmol/L)</th>
<th>BICARBONATE ION (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>141.20±0.2</td>
<td>4.3±0.63</td>
<td>96.20±0.73</td>
<td>23.00±0.63</td>
</tr>
<tr>
<td>Group II</td>
<td>140.20±0.49</td>
<td>3.86±0.18</td>
<td>95.80±0.66</td>
<td>21.80±0.73</td>
</tr>
<tr>
<td>Group III</td>
<td>137.60±1.63</td>
<td>4.40±0.24</td>
<td>95.80±0.70</td>
<td>20.00±0.63</td>
</tr>
<tr>
<td>Group IV</td>
<td>133.4±1.33</td>
<td>4.46±0.24</td>
<td>95.30±2.73</td>
<td>18.60±1.86</td>
</tr>
<tr>
<td>Group V</td>
<td>136.00±1.26</td>
<td>4.72±0.18*</td>
<td>95.80±2.41</td>
<td>17.40±1.17*</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (N=5) *= significant at p < 0.05 when compared with Group II. 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 (Kakalji 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-Chalikias et al., 2016) and Ekeanyanwu et al. (2011). Hence, 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-acetylcyesteine (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 esculentus* 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 *kwu nu* 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 unverified 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 gentamicin-induced nephrotoxicity in rats.

**REFERENCES**


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