AMELIORATIVE EFFECTS OF TIGER NUT (Cyperus esculentus) ON ALUMINUM CHLORIDE INDUCED RENAL HISTOPATHOLOGY ON ADULT WISTAR RATS

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ABSTRACT

Tiger nut (Cyperus esculentus) is known in Nigeria as aya in Hausa, Ofio in Yoruba and Aki Hausa in Igbo, is an edible perennial grass-like plant. Tiger nut tubers can be eaten snacked, roasted, fried or baked and liquid (Kunun aya). Tiger nut contain lipid, protein, and carbohydrate (fiber and starch included). Tiger nut can be used to ‘mop up’ and scavenge free-radicals generated by essential metabolic body reactions and environmental pollutants. The major route of aluminium elimination is by the kidneys. Due to its reactivity, aluminum in nature is found only in combination with other elements like chlorine as in aluminum chloride (AlCl3). Al compound are used in a variety of foodstuffs, medications, domestic water supplies, kitchen equipments. herbs and cosmetics. If the kidney cannot excrete Al, it will accumulate in the body. The aim of this work was to evaluate the protective effects of tiger nut against aluminium chloride induced renal histopathology in the kidney of wistar rats. Twenty adult males and females wistar rats weighing 80 – 170g were grouped into 4. Group 1 - Control group, received normal saline for 14 days, Group 2 – received 1250mg of liquid tiger (ethanolic extract) nut extract daily by oral route for 14days. Group 3 – received aqueous aluminium chloride 500mg/kg followed by 2500mg/kg of liquid tiger nut extract daily by oral route for 14days and Group 4 – received aqueous aluminium chloride 500mg/kg followed by 3750mg/kg of liquid tiger nut extract daily by oral route for 14days. After sacrifice and tissue processing, groups 1 and 2 presented normal renal histology while groups 3 and 4 presented normal renal histology with lymphocytes between their distal convoluted tubules around the medulla. It was concluded that tiger nut has an ameliorative effect on AlCl3 induced histomorphological changes in the histology of kidney and it has no any negative effect on the histology of kidney.

Keywords: Ameliorative, Tigernut, Aluminium Chloride, Kidney, Wistar Rats

INTRODUCTION

Tiger nut (Cyperus esculentus) is known in Nigeria as aya in Hausa, ofio in Yoruba and akihausa in Ibo. Cyperus esculentus grows mainly in the middle belt and northern regions of Nigeria where three varieties (black, brown and yellow) are cultivated. Among these, only two varieties, yellow and brown are readily available in the market. The yellow variety is preferred to all other varieties because of its inherent properties like its bigger size, attractive colour and flesher body (Raphael et al., 2010). Tigernut also contributes to the reduction of cholesterol, it reduces the risk of coronary heart disease, arteriosclerosis and is recommended for those who have heavy digestion, flatulence and dysentery (Gambo & Da’u, 2014). Study has shown that there was no significant effect on serum cholesterol and protein and on total and differential white blood cell, red blood cell, haemoglobin, packed cell volume and erythrocyte sedimentation rate in the rat that were feed with tigernut (Raphael et al., 2010). Cyperus esculentus is also used for the treating urinary tract and bacterial infection and assist in reducing the risk of colon cancer when eaten (Adejuyitan et al., 2009). Aluminum (Al) is the third most abundant element in the earth’s crust (Abdel-Moneum & Gaafar, 2016; Klein, 2019; Sargazi et al., 2001) after oxygen and silicon (Sargazi et al., 2001), amounting to an estimated 8% of total earth mass (Priest, 1992) and it is widely distributed (Starkey,
Aluminium (Al) is an environmental and industrial pollutant that induces a broad spectrum of toxicity (Liu et al., 2016). Aluminium is presents in many manufactured foods and medicines and is also added to drinking water for purification purposes (Buraimoh & Ojo., 2013).

**MATERIALS AND METHODS**

**Chemicals:** Aluminium chloride (AlCl₃) was used as solution for oral administration. It was collected from Department of Biochemistry, BUK. About 30g of AlCl₃ was used in this research.

**Reagents:** Hematoxylin and Eosin stains (H&E), 10% Neutral Buffered formalin (NBF), graded base and apex which project into minor calyx (Saldarriaga 2008). Renal sinus consist of renal pelvis, major calyx, 8 minor calyces, branches of nerves and blood vessels, loose connective tissue and fat (Bergmen et al 1992).

The nephron is the functional unit of kidney, there are 0.8 to 1.2 million nephron in each kidney and greatly varies in its structure among different vertebrate; also the formation of nephrons shows a variable degree of differences among species. In birds, the kidney has two kind of nephrons. One is reptilian type of nephron and small sized, without loops of Henle, and other mammalian type large in size with long or intermediate length loops (Lentile 2017).

Aluminium is absorbed by several routes (oral, intranasal, transdermal and parenteral) (Saad et al., 2018). The bioavailability of aluminium from drinking water is approximately 0.1% (Walton, 2007; Walton, 2009). The absorption of aluminium from the diet is reported to be between 0.01% and 0.04% (Greger et al., 1992). Transdermal absorption of aluminium has been reported after a single underarm application of Al hexahydrate, the absorption was found to be 0.012% of Al applied (Flarend et al., 2001).

Aluminium that has accumulated in the body is thought to have a generalised cytotoxic effect (Nordal et al., 1998). Aluminium accumulation is a potential hazard of end stage chronic renal failure (Opelz et al., 1973). Aluminium toxicity is indicated by the accumulation of aluminium in bone and by symptoms and signs from several organs (Boyce et al., 1982). It has also been reported that aluminium accumulates in all tissues of mammals such as the heart, liver, kidneys, blood, bones and brain (Al-Kahtani, 2014) and it was found that one of the main organs targeted by aluminium exposure is the kidneys which play a major role in preventing accumulation of aluminium by excreting it throughout urine (Stoehr, 2006).

Aluminium (Al) is an environmental and industrial pollutant that induces a broad spectrum of toxicity (Liu et al., 2016). Aluminium is presents in many manufactured foods and medicines and is also added to drinking water for purification purposes (Buraimoh & Ojo., 2013).
alcohol (absolute ethanol, 90% ethanol, 80% ethanol, 70% ethanol and 50% ethanol), xylene, 1% acid alcohol, chloroform and paraffin wax were obtained from Department of Human Anatomy Bayero University Kano. Normal saline was purchased from pharmacy and distilled water was obtained from department of pharmacy, and tween eighty was obtained from Department of Microbiology.

**Animals:** Twenty adult males and females wistar rats weighing 80 – 170g were purchased from department of pathology in Aminu Kano Teaching Hospital (AKTH), Bayero University Kano (BUK).

**Plant Collection and Extraction:** Brown tiger nut was purchased from the Rimi market. Then it was taken to department of plant biology for identification (voucher no: BUKHAN367). The department of pharmacy for extraction. 4L of tiger nut was macerated using ethanol to give the ethanolic tiger nut that was use in this experiment. The weight of the tiger nut after the extraction was 205.31g.

**METHODS**

**Study Design and Experimental Procedure**

After the animals were acclimatized for one week, they were divided in to four groups on descending order of weight, each group has five rats. The groups are;

**Group 1** - Control group, received normal saline.

**Group 2** – Cyperus Esculentus (C.E) group, received liquid tiger nut extract 25% of LD50 daily by oral route.

**Group 3** – Aluminium chloride (AlCl₃) and Cyperus Esculentus (C.E) 01 group, liquid tiger nut extract 50% of LD50 and aqueous aluminium chloride 500mg/kg daily by oral route.

**Group 4** – Aluminium chloride (AlCl₃ ) and Cyperus Esculentus (C.E) 02 group, liquid tiger nut extract 75% of LD50 and aqueous aluminium chloride 500mg/kg daily by oral route.

**Dose of the Substances that were Administered to each Rat.**

**Dose of normal saline for group 1**

Normal saline has no any case of toxicity, therefore the selected dose for each rat is same as the stock.

Dose = \( \text{Selected dose} \times \frac{\text{weight of rat}}{\text{Stock}} \)

□ the dose of normal saline for each rat in the control group is same as it is body weight in ml

**Dose of aluminum chloride for groups 3 and 4**

Aluminum chloride was reported to induce renal toxicity at 500mg (Ajibade et al., 2019).

□ 500mg is our selected dose for each rat in the groups that AlCl₃ will be administered.

LD50 = 3470mg/ml .

Dose = \( \text{Selected dose} \times \frac{\text{weight}}{\text{Stock}} \)

Selected dose = 500mg/ml

Stock = 500mg/ml

□ the dose of AlCl₃ for rat is base on its weight in ml.

**Dose of tiger nut extract for group 2**

Since there is no any case of toxicity associated with tiger nut, the LD50 of tiger nut can be above 5000ml/kg. Therefore, 5000ml/kg is the LD50 of tiger nut used in this study.

Dose = \( \text{Selected dose} \times \frac{\text{weight}}{\text{Stock}} \)

Selected dose = 25%LD50 = 1250mg/ml

Stock = 1000mg/ml

**Dose of tiger nut extract for group 3**

Dose = \( \text{Selected dose} \times \frac{\text{weight}}{\text{Stock}} \)

Selected dose = 50%LD50 = 2500ml/kg

Stock = 1000mg/ml

**Dose of tiger nut extract for group 4**

Dose = \( \text{Selected dose} \times \frac{\text{weight}}{\text{Stock}} \)

Selected dose = 75%LD50 = 3750mg/ml

Stock = 1000mg/ml

**Animal Sacrifice:** Twelve rats three from each group were sacrificed. The rats were sacrificed at the last day of the experiment under chloroform anesthesia. A midline incision was done through the ventral abdominal wall and the kidney tissue was collected immediately and fixed in 10% formal saline (fixative) for the minimum of 24 hours. The tissue was processed using routine histological techniques and stained with hematoxylin and eosin stains for general tissue architecture.
RESULTS

The photomicrograph of the kidney stained with hematoxylin and eosin (H&E) has shown that;

Group 1 – Control group that receive normal saline for 14 days present normal kidney with normal glomerulus and normal tubules.

Group 2 – *Cyperus esculentus* group that received 1250mg/kg of tiger nut for 14 days present normal kidney with normal glomerulus and normal tubules.

Group 3 – *Cyperus esculentus* and *AlCl₃* group 01 that received 2500mg/kg of tiger nut followed by 500mg of *AlCl₃* for 14 days; they present a kidney with normal glomerulus, normal tubules and 3-4 foci of lymphocytes.

Group 4 – *Cyperus esculentus* and *AlCl₃* group 02 that received 3750mg of tiger nut followed by 500mg of *AlCl₃* for 14 days; they present a kidney with normal glomerulus, normal tubules and foci of lymphocytes.

The above result is shown in the photomicrographs below;

**Plate I:** Photomicrograph of group 1 kidney stained with H&E, ×100 magnification. Showing normal glomerulus, Bowman's capsule and renal tubules. They received normal saline.


**Plate II:** Photomicrograph of group 2 kidney stained with H&E, ×100 magnifications. Showing normal glomerulus, Bowman's capsule and renal tubules. Received 1250mg/kg bwt of liquid tiger nut.

Plate III: Photomicrograph of group 3 kidney stained with H&E, ×100 magnification. Showing normal glomerulus, bowmans capsule and renal tubules. They received 2500mg/kg bwt of liquid tiger nut extract, followed by 500mg of AlCl3.

Plate IV: Photomicrograph of group 3 kidney ×100 magnification. Showing lymphocytic aggregates (1) due interstitial nephritis and distal convoluted tubules. They received 2500mg/kg bwt of liquid tiger nut extract, followed by 500mg of AlCl3.
1. Lymphocytic aggregates 2. Distal convoluted tubules

Plate V: Photomicrograph of Group 4 kidney ×100 magnification. Show normal glomerulus, bowmans capsule and renal tubules. They received 3750mg/kg bwt of liquid tiger nut extract, followed by 500mg of AlCl3.
Plate VI: Photomicrograph of group 4 kidney ×100 magnification. Showing lymphocytic aggregates (1) due interstitial nephritis and distal convoluted tubules. They received 3750mg/kg bwt of liquid tiger nut extract, followed by 500mg of AlCl$_3$. 

1. Lymphocytic aggregates  
2. Distal convoluted tubules

**DISCUSSION**

This study was designed to assess the ameliorative effect of tiger nut extract on AlCl$_3$ induced histomorphological changes in the kidney. Some of the damaging effect of AlCl$_3$ was established on the kidney like the interstitial nephritis around the distal convoluted tubules in group 3 and 4 leading to lymphocytic aggregates. In this study, there were no any alterations in the histomorphology of the kidney of the control and Cyperus esculentus groups (1&2). But there was a little sign of inflammation (lymphocytes aggregates) in group 3 and 4 due to interstitial nephritis. Interstitial nephritis with severe inflammatory cells infiltration as sign of aluminum induced histological change is the only change that was observed in the experimental groups (3&4) and this corresponds to the findings of Saad et al. (2018) when 2.5mg/kgbwt was administered to some group of rabbits five times per week for 3 months even though the duration of their study was 6 times (90 days) the duration of this study (14 days), and their duration is approximately 61 days of administration. But the concentration of the AlCl$_3$ in this study (500mg/kg) is 200 times the concentration of AlCl$_3$ in their study (2.5mg/kg), and this is indicating that the histological changes that were observed in their study which include; widening of the Bowman’s space, increased urinary spaces and necrosis of glomerular capillary tufts, vacuolar degeneration, tubular epithelium attenuation to necrosis, enlargement of epithelial cells toward the tubular lumen, congested blood vessel, interstitial nephritis with severe inflammatory cells infiltration, mesangiolysis of mesangium, ischemic glomerular necrosis are supposed to be observed in the experimental groups that received both tiger nut and AlCl$_3$, but due to the protective effect of tiger nut against aluminium chloride, only interstitial nephritis with severe inflammatory cells infiltration was observed.

Mohammed et al. (2017) has administered 37 mg/kg bwt of AlCl$_3$, for 60 days in his study and his result showed that the kidney of the treated group showed inflammatory cell infiltration particularly neutrophils, macrophages and lymphocytes between renal tubules, with the congested blood vessels and vacuolar degeneration of epithelial cells and severe congested blood vessels between renal tubules, and also the kidney showed inflammatory cells infiltration in the wall of collecting tubules with hyperplasia of epithelial cells of collecting ducts and these cells aggregated as hyperchromatic pleomorphic cells arranged as mass or sheath or glandular structure and atrophy of glomerular tufts with dilated Bowman’s space ,congested blood vessels and acute cellular degeneration. But in this study, only the inflammatory cell infiltration was observed in the experimental groups. Even though their duration was 4 times (60 days) higher than this study’s duration, but this study dose (500mg/kg) was 13 times their dose (37mg/kg), and the remaining pathologic changes were not observed in the experimental groups of this study. This is showing the powerful protective effect of tiger nut against AlCl$_3$.

Ajibade et al.(2019) has administered same dose of AlCl$_3$, as in this study (500mg/kg) to some groups of rats for 31days.
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And their result has shown that there was mild disarrangement of kidney architecture with decreased capsular space and mild degeneration of glomerulus, but none of these pathologies was observed in our experimental groups. This is also proving the protective effect of tiger nut against AlCl₃ induced histological changes. Berlyne et al (1972) has subjected some group of rats to 2/3 nephrectomy that was done on the other side under chloroform anaesthesia. The animals were allowed to recover for 1-2 weeks from the operation and were then divided into control and test groups. The test groups received 180mg of AlCl₃ and the control received distilled water. The rats of the test groups died within 8 days and that of the control group recovered from their injury. The concentration of the AlCl₃ dose in this study is 2.5 times their dose (180mg) which means the effect supposes to be high in this study’s experimental groups, but our rats were healthy so there was no death. But due to the protective effect of tiger nut, there was no dangerous effect talk less of death, which means tiger nut was the ameliorating agent.

REFERENCES


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