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Research Article

Ameliorative Role of Tiger Nut (*Cyperus Esculentus*) on Aluminum Chloride Induced Histopathological Changes on The Liver of Adult Wistar Rats

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Abstract

Tigernut (Cyperus esculentus), belongs to the Cyperaceae family and is also found to be a perennial crop of the same genus as the papyrus plant. It is widely distributed in the temperate zones within south Europe and also grows naturally in Ghana, Nigeria and Sierra Leone. It is known in Nigeria as "Aya" in Hausa, "Ofio" in Yoruba and "AkiHausa" in Igbo. The aim of the study was to investigate the ameliorative effect of tigernut on aluminum chloride induced histopathological changes on the liver of adult wistar rats. The objectives of the study were to induce liver damage in wistar rats using aluminium chloride, and to determine whether tiger nut has ameliorative effect on the histopathological change induced by aluminium chloride on liver of wistar rat. Twenty five wistar rats weighing between 80g and170g were randomly grouped into five rats each (A, B, C, D and E). After two weeks of acclimatization, group A was administered with normal saline, group B was administered with 500 mg/kg bwt of aluminum chloride, group C was administered with 1250 mg/kg bwt of cyperus esculentus, group D was administered with 500mg/kg of aluminum chloride and 2500mg/kg of cyperus esculentus, group E was administered with 500 mg/kg of aluminum chloride and3750mg/kg of cyperus esculentus.

After two weeks of administration three rats from each group were sacrificed humanely and the liver of the rats were harvested for histological studies. The results showed that oral administration of aluminum chloride caused histopathological changes manifested with congested central vein, inflammation and fibrosis on the liver tissue, the group treated with cyperus esculentus showed normal histology of the liver, and the group treated with aluminum chloride and cyperus esculentus showed decrease in histopathological changes and partially restored live rtissue. In conclusion aluminum chloride induced histopathological changes on the liver tissues of adult wistar rats was ameliorated using cyperus esculentus with restoration of the degenerative changes which were time-dependent and dose-dependent respectively.

Keywords: Ameliorative; *Cyperus esculentus*; histopathological; liver; Wistar rat

Introduction

Tiger nut (*Cyperus esculentus*), belongs to the Cyperaceae family and is also found to be a perennial crop of the same genus as the papyrus plant. It is widely distributed in the temperate zones within south Europe and also grows naturally in Ghana, Nigeria and Sierra Leone (Anon, 1992). It is known in Nigeria as "Aya" in Hausa, "Ofio" in Yoruba and "AkiHausa" in Igbo where these three varieties (black, brown, yellow) are cultivated (Umerie et al., 1997) among these the yellow variety is preferred over others because of its inherent properties such as large size, attractive color and fleshier nature. Cyperus esculentus can be eaten raw, roasted, dried, baked or be made into a refreshing beverage called Kunu (Oladele and Aina, 2007).

Tigernut tubers are rich in starch, fats, sugars, proteins, oleic acid, and vitamins B, C and E (Omode et al.,1995; Okwu, 2005; Dhouha et al., 2016). It is reported that they are rich in minerals such as phosphorous, potassium, calcium, magnesium and iron (Belewu and Belewu, 2007; Oladele and Aina, 2007; Arafat et al., 2009; Dhouha et al., 2016). Their antioxidant capacity is known to be relatively high because reports from previous studies have shown that they contain considerable amounts of water-soluble flavonoids and glycosides which are known natural antioxidants (Pietta, 2000; Oloyede et al., 2014).

Cyperus esculentus was reported to help in preventing thrombosis and activate blood circulation. It helps in preventing cancer, due to its high content of soluble glucose. It was also found to assist in reducing the risk of colon cancer (Adejuyitan et al., 2009).

Human and animals interacts daily with their environment and exposed to broad spectrum of chemicals and heavy metals like aluminum, mercury, lead and cadmium; all belongs to the most important hazardous substances that can bioaccumulate in the tissues with low excretion (Ali and Amin, 2006).

The liver is the largest solid organ in the body (Kuntz and Kuntz, 2006; Saukonnen et al., 2006). It is involved in the metabolism of numerous substances (including bilirubins, pophyrins, bile, amino acids and proteins, carbohydrates, lipids and lipoproteins, hormones and vitamins), biotransformation, detoxification and acid-base balance (Kuntz & Kuntz, 2006). Its multi-various functions constantly expose it to injury that may lead to different forms of liver disorders/diseases (Ihedioha, 2005; Saukonnen et al., 2006). Liver diseases have a worldwide distribution, and a major cause of morbidity and mortality globally (Blachier et al., 2012; Lozano et al., 2012; Nwokediuko et al., 2013; Sarine and Maiwal, 2018). Toxic liver diseases constitute a large proportion of liver disorders/diseases, and its occurrence has been steadily increasing over the years (Rehme et al., 2013; Nwokediuko et al., 2013).

The largest source of airborne Al-containing particles is the dust from soil and rocks (Sorenson et al., 1974). Human activities, such as mining and agriculture, contribute to the dust in winds (Filipek et al., 1987). Cigarette smoke may contribute to the concentration of Al in the air (Pappas, 2011; Afridi et al., 2015). Al occurs ubiquitously in natural waters due to weathering of Al-containing rocks and minerals and mobilization from terrestrial to aquatic environment (Campbell et al., 1992). Al is present in foods naturally or from the use of Al-containing food additives (Hayacibara et al., 2004; Yokel et al., 2008). The use of Al cookware, utensils and wrappings can increase the amount of Al in food (Liukkonen-Lilja and Piepponen, 1992; Pennington and Schoen, 1995). Various intravenously administered pharmaceutical products were reported to contain 684–5977 µg/g of Al (Sedman et al., 1985). Many antacids contain 104-208mg of Al per tablet, capsule or 5ml of suspension (Zhou and Yokel, 2005).

Materials and Methods

Materials

The materials used during this study include; the tigernut (*Cyperus esculentus*), Wistar rat (n=25), animal feed, aluminum cages, plastic bottles for drinking water of the rats, syringes (1ml, 2ml and10ml), digital weighing machine, distilled water, Aluminum chloride, tween 80, normal saline, plain bottles, disposable hand gloves, chloroform, surgical blade and small size plastic bucket, glass slides, stains, dissection kit, metallic mold, metallic canula, white plastic cassettes, transparent tissue containers, cotton wool, water bath, oven refrigerator, glasss lides, camera, microtome and microscope.

Experimental Animals

Twenty five healthy adult wistar rats of both sex weighing between 80-170g were purchased from animal house of Department of Biological Sciences, Bayero University, Kano. The animals were housed in the animal house of Anatomy Department Bayero University, Kano enclosed in steel cages and fed with commercial grower feed purchased from Agro Feed Mills and water at a standard room temperature of 20 to 25 degree Celsius under a 12hour light-dark cycle before administration of the extract throughout the experimental period. All experimental protocols were in compliance with ethical committee Faculty of Basic Medical Sciences, College of Health Sciences Bayero University Kano, and Ethics on Research in Animals as well as internationally accepted principles for laboratory animal use and care, were strictly adhered to.

Grouping of Animals

The experimental animals were weighed and randomly divided into five groups, with five rats per group. Groups were labeled as A (control group) and the treatment groups B, C, D and E.

Group A: received normal saline.
Group B: received 500mg/kg of aluminum chloride only.
Group C: received1250 mg/kg of Cyperus esculentus using a metallic canula.
Group D: received 500 mg/kg of Aluminum chloride + 2500 mg/kg of Cyperus esculentus.
Group E: received 500mg/kg of Aluminum chloride + 3750mg/kg of Cyperus esculentus.

All treatments were administered via gavage and lasted for a period of 14 days.

Plant (Sample) Collection, Extraction and Preparation

Dried tubers of tigernut was purchased from Rimi market Kano, Nigeria. They were identified and authenticated in the Department of Biological Sciences, Bayero University, Kano. Tubers of *Cyperus esculentus* (tigernuts) were cleaned, washed and air dried. Thereafter, the tubers were milled to fine powder using manual engine grinder (Modelcorene, A.5 lander YCIA S.A). The milled sample was macerated in 5L of 80% ethanol for 48hours. Thereafter, it was filtered with Whatman No. 1 filter paper. The filtrate was then concentrated under reduced pressure in a vacuum at 45^{o}C using a rotary evaporator (Searl Instruments Ltd. England) into a colloid form and stored at 4^{o}C. The ethanol extract was weighed and dissolved using drop of tween 80 and distilled water were dissolved to obtain the solution needed for administration.

Chemical Substance

The Aluminum chloride was obtained from Biochemistry Department, Faculty of Basic Medical Sciences, Bayero University, Kano.

Animal Sacrife and Sample Collection

Fifteen 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 liver 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

Plate I: H&E photomicrograph of liver of an adult Wistar rat seen in magnification (×100) that was administered with normal saline and used as a control group. The photomicrograph indicated the norma histology of a liver presenting radiating hepatocytes, (HC) around the central vein (CV) and normal sinusoids (S).

Plate II: H&E photomicrograph of liver of an adult Wistar rat administered with 500 mg/kg of Aluminum chloride seen in magnification (×100) shows CCV: congested central vein, F: fibrosis and I: inflammation.

Plate III: H&E photomicrograph of liver of an adult Wistar rat administered with 12500 mg/kg of Cyperus esculentus seen in magnification (×100) shows Normal hepatocytes architecture (H), central vein (CV) and normal blood sinusoids (S).

Plate IV: H&E photomicrograph of liver of an adult Wistar rat administered with 500mg/kg of Aluminum chloride and 2500 mg/kg Cyperus esculentus seen in magnification (× 100) shows normal hepatocytes architecture (H), normal sinusoids (S) with congested central vein (CCV).

Plate V: H&E photomicrograph of liver of an adult Wistar rat administered with 500 mg/kg of Aluminum chloride and 3750mg/kg of

Cyperus esculentus seen in magnification (×100) shows normal hepatocytes architecture (H), central vein (CV) normal sinusoids (S) with mild inflammation.

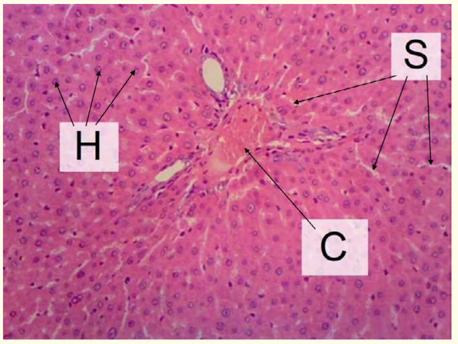

Plate I: Paraffin sections stained with heamatoxylin and eosin (H&E, x100) for the control group showing normal HC: Hepatocytes, CV: Central vein, and S: Sinusoids.

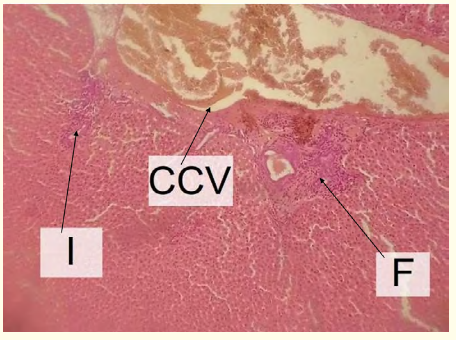

Plate II: Paraffin sections stained with heamatoxylin and eosin (H&E, x100) administered with 500mg/kg body weight of Aluminum chloride showing CCV: Congested Central vein, F: Fibrosis, and I: Inflammation.

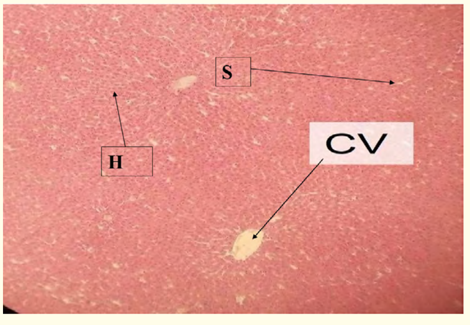

Plate III: Paraffin sections stained with heamatoxylin and eosin (H&E, x100) administered with 1250mg/kg body weight of Cyperus esculentus showing normal hepatocytes architecture (H), Central vein (CV) and normal blood sinusoids (S).

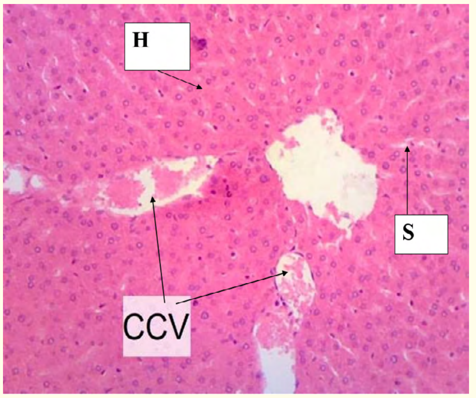

Plate IV: Paraffin sections stained with heamatoxylin and eosin (H & E, x100) administered with 500mg/kg of Aluminum chloride and 2500 mg/kg of Cyperus esculentus showing normal hepatocytes architecture (H), normal sinusoids (S) with congested central vein (CCV).

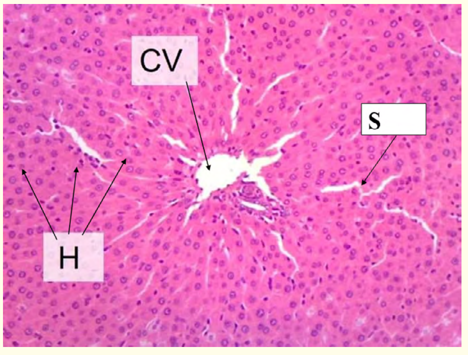

Plate V: Paraffin sections stained with heamatoxylin and eosin (H & E, x100) administered with 500mg/kg of Aluminum chloride and 3750 mg/kg of Cyperus esculentus showing normal hepatocytes architecture (H), central vein (CV), and normal sinusoids (S) with mild inflammation.

Discussion

From the result of the study, the control and group treated with tigernut showed normal hepatocytes radiating from the central vein were separated by sinusoids, the liver cells were polyhedral cells with eosinophilic cytoplasm and each cell had a rounded pale stained nucleus.

Aluminum is a heavy metal that occurs in nature as oxide or salts. It is one of the most hazardous and cumulative environmental pollutants (WHO, 2007). It is absorbed from gastrointestinal tract, bind to erythrocytes and is widely distributed initially to soft tissues such as liver, kidney, and spleen. Liver is the major site of aluminum accumulation in the experimental animals (Buraimoh et al., 2012). The lesions in the liver may be due to the action of aluminum chloride on hepatic glycogen and imbalance in the antioxidant protective mechanisms leading to oxidative stress in the cells which also has effect on DNA content and the ability to incorporate amino acid into protein (Bogdanovi et al., 2008). Treatment with aluminum chloride induced the increase in reactive oxygen species accumulating in rat brain leading to lipid peroxidation, protein degradation, and finally to cell death (Abubakar et al., 2003). The changes observed in the hepatocyte in the present study showed that aluminum chloride is hepatotoxic as seen in the liver section of Wistar rats treated with a dose of 500 mg/kg as it resulted in congestion of the central vein, mild fibrosis of the portal triad, inflammation, hyperplasia of cuboidal cells lining the bile duct, multifocal lymphocytic infiltration. From this result, features were consistent with the findings of (Saad et al., 2018) but congestion of central vein revealed was in line with the findings of (Buraimoh et al., 2012).

Group 4 and 5 which are aluminum chloride (500 mg/kg) and *Cyperus esculentus* treated groups administered 2500 mg/kg and 3750 mg/kg respectively showed *Cyperus esculentus* caused a significant decrease in the histopathological changes induced by aluminum chloride in the liver as it partially restored most of the changes. This means tigernut has hepatocurative properties. This corroborates findings by Hassan (2007) who studied the potential effect of tigernut oil on some haemato-biochemical blood indices in male albino rats. This effect was exhibited by tigernut due to its phytochemical composition reported by Oguwike et al. (2017). It could also be due to its nutritional constituent reported by Roselló-Soto et al. (2019) who investigated the nutritional and microbiological quality of tiger nut tubers (*Cyperus esculentus*), derived plant-based and lactic fermented beverages.

Conclusion

The study revealed that aluminum chloride caused liver damage while tigernut alone had no harmful effect on the liver. Conclusively, tigernut should be eaten regularly and is cheap, and had been observed to have ameliorated the histopathological effects of aluminum chloride on the liver tissue of adult wistar rats, because we do eat aluminium metal in our cooked foods using aluminium cooking utensils which accumulates over time, and this ameliorative effect was dose-dependent and time-dependent respectively. Furthermore, we should embrace natural foods and herbs as they have medicinal importance and are cheaply affordable.

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