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Research Article

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THE ROLE OF AQUEOUS AZADIRACHTAINDICA LEAF EXTRACT ON ALCOHOL-INDUCED KIDNEY TOXICITY IN ADULT WISTAR RATS

***Edobor Obayuwana, Endurance Osakioduwa Imafidon, JosephRaymonEnoghase, Samuel Monday Nweke**

Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.

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ABSTRACT

Introduction: Alcohol abuse is a major cause of disease with serious social and economic consequences. It causes various health problems, including mental health problems, kidney disease, liver cirrhosis and cardiovascular diseases. *Azadirachtaindica* (Neem), a pantropical plant with multiple uses has been described by some researchers for its potential renal protective effects. **Aim:** This study was aimed to evaluate the effect of *Azadirachtaindica* on alcohol-induced kidney damage in Wistar rats. **Methods:** Thirty Wistar rats weighing between 120 and 170 grams were divided into six groups(I-VI)of 5 rats per group and received, 1 ml of distilled water daily for 14days, 1 ml of 50% alcohol daily for 14days, 250 mg/kg body weight of *Azadirachtaindica* daily for 14 days, 500 mg/kg body weight of *Azadirachtaindica* daily for 14days, 1 ml of 50% alcohol daily for 14daysfollowed by 250 mg/kg body weight of *Azadirachtaindica* daily for 14days, 1 ml of 50% alcohol daily for 14days followed by 500 mg/kg body weight of *Azadirachtaindica* daily for 14daysrespectively. At the end of treatment, blood and kidney tissues were collected for analysis of serum Sodium ion (NA^+), Potassium ion (K^+), Urea, Creatinine (Cr) and histology of the kidney respectively. **Result:** There was no significant change in serum NA^+, K^+, Urea and Cr levels in alcohol and *Azadirachtaindica* treated groups when compared to the untreated control. Histological findings showed heavy interstitial infiltrates of inflammatory cells, interstitial oedema and vascular dilatation and congestion in alcoholic treated rats when compared to the kidneys of untreated control. However, treatment with *Azadirachtaindica* particularly the 500 mg/kg dose caused a reversal of the inflammatory process induced by alcohol on the rats kidneys while at same time inducing interstitial vascular congestion. **Conclusion:** *Azadirachtaindica* possesses anti-inflammatory properties against alcohol-inducednepritis but induces vascular congestion in rats kidneys.

Keywords: Alcohol abuse, Azadirachtaindica, Kidney toxicity.

INTRODUCTION

Alcohol is a psychoactive substance that can become habit-forming. It has been used for ages in many different cultures [1].One of the main causes of illness, alcoholism has serious social and economic repercussions[2]. Abuse of alcohol can also cause harm to others, such as strangers, coworkers, relatives, and friends[3]. Over 200 illnesses, traumas, and other health issues are linked to alcohol use. Drinking alcohol raises the risk of major noncommunicable diseases such liver cirrhosis, numerous cancers, and cardiovascular disease, as well as mental and behavioral disorders, including alcohol dependency[4].Alcohol is one of the numerous factors that can compromise kidney function. It can interfere with kidney functions directly through acute or chronic consumption or indirectly, as a consequence of liver disease[5]

Azadirachtaindica, a pantropical plant, has received international attention in recent years. It is regarded as a "miracle tree" with countless applications. According to study by an ad hoc advisory panel, it is "the tree to meet the challenges of the world"[6]. The tree is significant from a religious, commercial, medical, and aesthetic standpoint and nearly every part of it— including the roots, trunk, bark, leaves, flowers, fruits, and seeds—has multiple uses [7].Neem, or *A. indica,* is a plant that grows well in a range of soil types and climates. It generates oil, pharmaceuticals, insecticides, firewood, fodder, and wood. It is mentioned that it serves as a "community pharmacy" and that the value of its by-products outweighs the worth of its wood[6].The oral LD50 of the aqueous extract of *Azadirachtaindica* leaves is 6200 mg/kg [8]

***Corresponding Author: Edobor Obayuwana,**
Department of Anatomy, School of Basic Medical Sciences, College of Medical Sciences, University of Benin, Benin City, Nigeria.

MATERIALS AND METHODS

Experimental rats

Thirty Wistar rats weighing between 120g and 170g were used. The rats were given a two-week acclimatization period before the administration method begun. They were given free access to conventional rat feed and water. The research ethics committee's guidelines for animal handling and treatment at the University of Benin's College of Medicine were espoused and fully implemented.

Collection and identification of plant material and preparation of aqueous extracts

Azadirachtaindica leaves was obtained from the wild in Benin City, Egor Local Government Area, Edo State, Nigeria's. They were identified in the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria, and then air-dried for seven (7) days before being ground into powder and weighed on an electrical weighing scale. Extraction was carried out utilizing proven methods [9]

Preparation of aqueous leave extract of *Azadirachtaindica* was conducted in the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, Benin City. Before being macerated in distilled water in a jar, the leaves were pulverized in a British milling machine. 500 g of powder was soaked in 2 litres of distilled water in a conical flask. After twenty-four (24) hours, the solution (a mixture of leaf extract powder and distilled water) was filtered with a filter rag and funnel. Before decanting the supernatant, the filtrate was allowed to settle for a time. At 60^{o}C, the supernatant was steamed to dryness in an evaporating dish (Royal Worcester, England) using an H-H

Digital Thermometer Water Bath (Mc Donald Scientific International – 22050Hz1.0A). The extracts were kept refrigerated at 4°C in plastic vials until needed.

Experimental Protocol

Group I: Control group; Rats received distilled water (2ml) for 14 days

Group II: Rats received 1 ml of 50% alcohol only for 14 days.

Group III: Rats received 250 mg/kg (low dose) of *Azadirachtaindica* only for 14 days.

Group IV: Rats received 500 mg/kg (high dose) of *Azadirachtaindica* only for 14 days.

Group V: Rats received 1 ml of 50% alcohol for 14 days and treated with 250 mg/kg of *Azadirachtaindica* for 14 days.

Group VI: Rats received 1 ml of 50% alcohol for 14 days and treated with *Azadirachtaindica*500 mg/kg for 14 days.

All administrations were done by gavage, using orogastric tube and lasted for fourteen (14) and twenty-eight (28) days respectively. The rats were carefully handled to minimize oral or oesophageal injuries.

Blood and Tissue collection, processing and histological staining

The rats were sacrificed using chloroform anaesthesia, Blood and the Kidney tissues were taken at the end of the two weeks and four weeks study. Blood was collected in sterile bottles for analysis of serum Sodium ion, Potassium ion, Urea and Creatinine levels and were immediately sent to the Chemical Pathology laboratory of the University of Benin Teaching Hospital for biochemical analysis. The kidney tissues were preserved for 72 hours in 10% buffered formalin before being histologically processed and stained with Haematoxylin and Eosin using standard procedures [10]. The sections obtained were examined and photomicrographs were taken using a Leica DM750 research microscope with an attached digital camera (Leica CC50). The tissues were photographed digitally at magnifications of 400x.

Statistical analysis

Results obtained were expressed as Mean ± SEM (standard error of means). Differences between the means were determined by one-way analysis of variance (ANOVA). Values were considered statistically significant if P value is less than 0.05 ($p < 0.05$). LSD Post Hoc test was used to determine where the significance lay. Statistical package Graph Pad Prism Version 9.0 for Windows (GraphPad Software Inc.) was used to analyze the data obtained in this study.

RESULTS

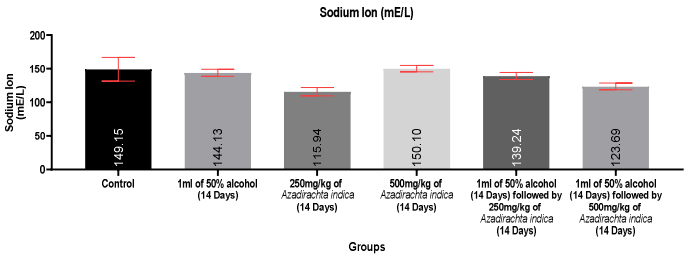

Fig.1. Showing Sodium Ion Levels

There was no statistically significant difference ($p>0.05$) in Sodium Ion Levels in treated rats when compared to control.

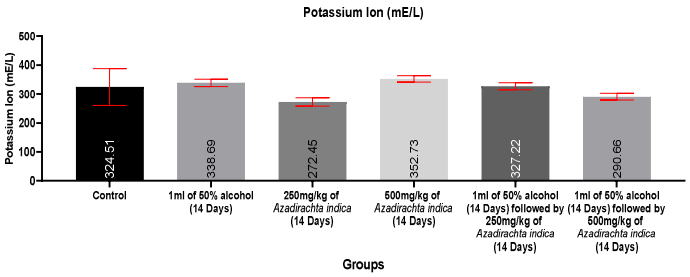

Fig.2. Showing Potassium Ion Levels

There was no statistically significant difference ($p>0.05$) in Potassium Ion Levels in treated rats when compared to control.

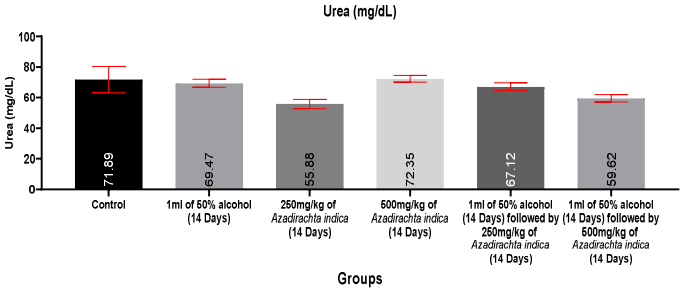

Fig. 3. Showing Urea Levels

There was no statistically significant difference ($p>0.05$) in Urea Level in treated rats when compared to control.

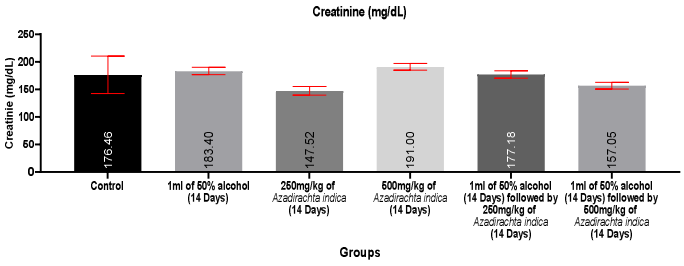

Fig.4 Showing Creatinine Levels

There was no statistically significant difference ($p>0.05$) in Creatinine Level in treated rats when compared to control.

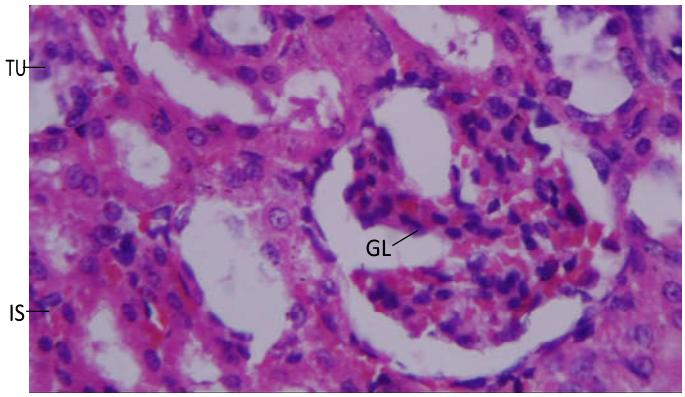

Plate 1.Rat kidney. Control.

Composed of normal tissue architecture: tubules (TU), interstitial space (IS), glomeruli (GL) : H&E 400x

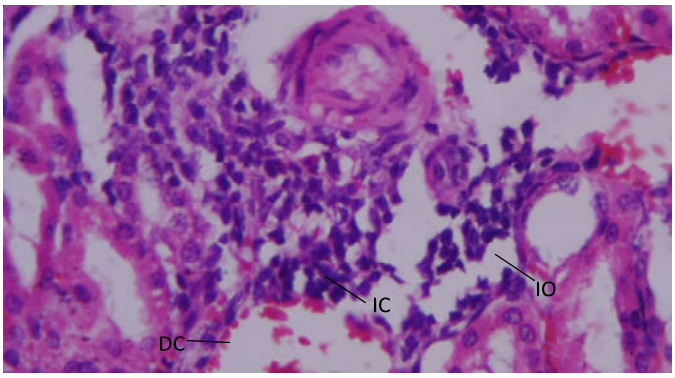

Plate 2. Rat kidney given Alcohol only

Showing: heavy interstitial infiltrates of inflammatory cells (IC), interstitial oedema (IO), vasodilatation and congestion (DC): H&E 400x

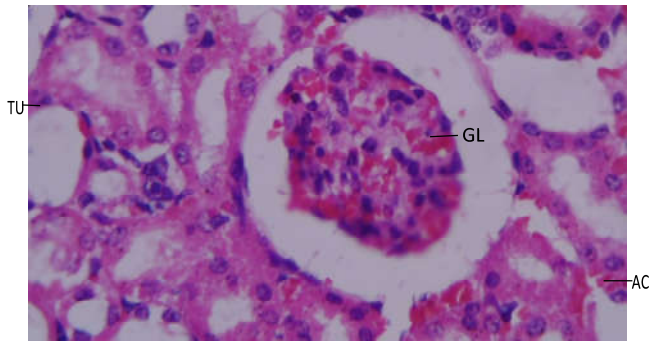

Plate 3. Rat kidney given 250mg/kg *Azadirachtaindica* only

Showing normal architecture: tubules (TU), active interstitial congestion (AC), glomeruli (GL) : H&E 400x

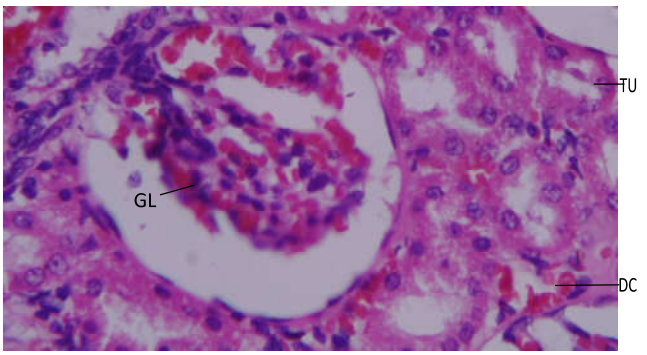

Plate 4. Rat kidney given 500mg/kg *Azadirachtaindica* only

Showing normal architecture: active interstitial congestion and vasodilatation (DC), tubules (TU), glomeruli (GL): H&E 400x

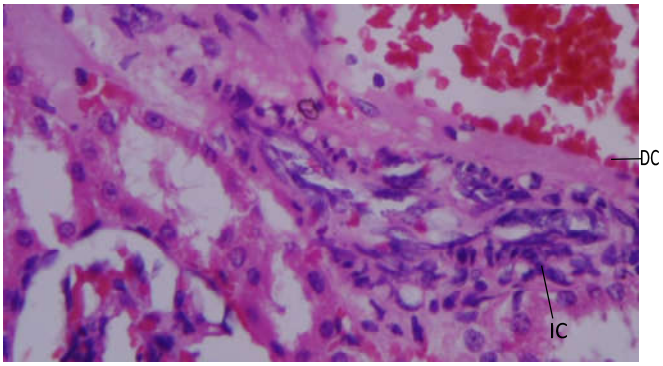

Plate 5. Rat kidney given Alcohol followed by 250mg/kg *Azadirachtaindica*

Showing: vasodilatation and congestion (DC), interstitial infiltrates of inflammatory cells (IC): H&E x 400x

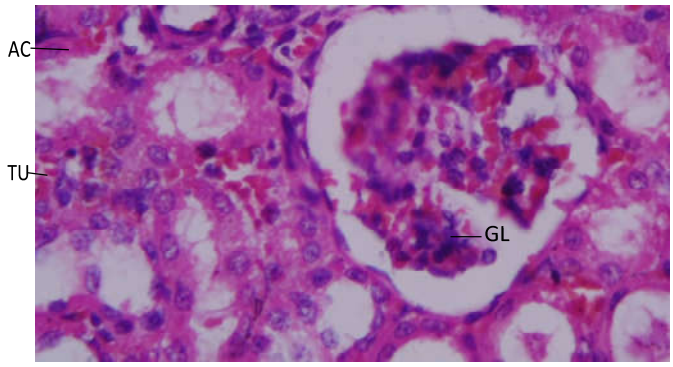

Plate 6. Rat kidney given Alcohol followed by 500mg/kg *Azadirachtaindica*

Showing normal tissue architecture: tubules (TU), active interstitial congestion (AC), glomeruli (GL): H&E 400x

DISCUSSION

The study of natural medicines and their physiological consequences is a growing area of biomedical research. This discourse explores the findings of an experimental investigation concerning the effects of *Azadirachtaindica*, popularly named Neem, on renal functions in rats that were given alcohol. The impact of Neem after alcohol intake was determined by assessing some renal function markers such sodium, potassium, urea, and creatinine levels. The results offer fascinating perspectives on the possible medicinal uses of neem and its function in preserving homeostasis in spite of the stresses that alcohol introduces.

The results showed that there was no statistically significant change in sodium ion, potassium ion, urea and creatinine levels in rats treated with alcohol and Neem when compared to the untreated control. One of the main functions of the kidneys is to regulate both the volume and the composition of body fluid, including electrolytes. However, alcohol's ability to increase urine volume (its diuretic effect) alters the body's fluid level (hydration state) and produces disturbances in electrolyte concentrations. These effects vary depending on factors such as the amount and duration of drinking, the presence of other disease conditions, and the nutritional status of the alcoholics[5]. The duration and pattern of ingestion alcohol play a key role in the pathogenesis of alcohol-induced organ damage. Previous studies on chronic administration of alcohol have reported varied effect on the electrolytes. Some researchers reported hyponatremia with chronic consumption of alcohol[11]. Others reported hyperkalemia or hypokalemia[12], hyperuricemia[13] and no significant change in creatinine [14] levels in chronic alcohol consumption. However, researchers have also reported hypernatremia in subjects with acute alcohol consumption [15]. Few works have done on acute or subacute alcohol toxicity on the kidneys.

The histology of the kidneys in the alcohol treated rats showed heavy interstitial infiltrates of inflammatory cells, interstitial oedema, vascular dilatation and congestion when compared to rats kidneys of untreated control group. Alcohol is known to have a nephrotoxic effect, which can lead to kidney damage. It can induce changes in renal function by affecting the glomerular filtration rate (GFR), blood urea nitrogen (BUN), and serum creatinine levels. Its impact on the kidney may involve oxidative stress and inflammation, leading to cellular damage and death, as well as changes in blood flow due to vasodilatation [16]. The presence of inflammatory cells indicates inflammatory response

due to alcohol consumption. This acute insult on the kidneys could possibly be a cause of acute interstitial nephritis.

The 500 mg/kg dose of *Azadirachtaindica* was observed to reverse the inflammatory process induced by alcohol in rat's kidneys when compared to the alcohol only group. Neem (Azadirachtaindica) is known for its medicinal properties, including anti-inflammatory, antioxidant, and immunomodulatory effects[17] However, it was observed that the two doses of *Azadirachtaindica* in this present study (250 and 500 mg/kg doses), induced interstitial congestion and vascular dilatation in rat's kidneys. An indication that prolonged or excessive consumption of *Azadirachtaindica* leaves may have some toxic effect on the kidney.

CONCLUSION

Azadirachtaindica possesses antiflammatory potentials against alcohol-induced inflammatory process in the rats kidneys. However, interstitial congestion and vascular dilatation associated with its use may potentially be injurious to the kidneys after prolonged consumption

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