Original article

NEPHROTOXIC EFFECTS OF ODOGWU BITTERS HERBAL DRINK IN ADULT MALE WISTAR RATS

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ABSTRACT

Context: Herbal alcoholic drinks just like every other alcoholic beverage are intoxicating and could be harmful to the kidneys and all the vital organs of the body when abused. This study aimed to investigate the nephrotoxic effects of graded doses of Odogwu Bitters, an alcoholic herbal drink in adult male Wistar rats. **Materials and Methods:** The study procured 16 adult male Wistar rats weighing between 195 and 230 grams which were divided into four groups (A – D) of four rats. The control group was Group A while the other groups were the

experimental groups. The control group received only feed and water while Group B received 0.45ml of Odogwu Bitters daily, Group C received 0.75mls of Odogwu Bitter solutions daily, and Group D received 1.05mls of the Odogwu Bitters daily. **Results:** The histology result showed no pathology in group B while that of C and D showed mild mesangial hypercellularity. The kidney function test results – creatinine, urea, and uric acid showed a non-significant difference between the control group and experimental groups. **Conclusion:** Findings from this study showed that moderate and high doses of Odogwu Bitters consumed over five weeks mildly affected the kidney; but did not affect the kidney when taken for five weeks at a mild dose.

KEYWORDS: Creatinine, Hyperuricemia, Mesangial hypercellularity, Urea, Uric acid.

INTRODUCTION

The consumption of various herbal drinks has become an integral part of modern lifestyles, with individuals often opting for drinks that cater to their taste preferences.[1.2] Many people use herbal drinks for the treatment of so many ailments because of the claims of their efficacies by the manufacturers.[3.4.5.6] People have different purposes for consuming herbal drinks. Alcoholic herbal bitters provide multipurpose service to many people,[7] and are often consumed without considering its possible side effects. Some people drink it socially, some use it as an aphrodisiac and others use it as an immune booster. People who socially take herbal drinks believe that they are natural and as such are less hazardous to the body.[4.8.9] However, just like every other alcoholic drink, alcoholic herbal drinks can be addictive and could cause injury to some vital organs of the body.[10]

Herbal drinks are very popular in Nigeria.[4.11.12.13.14] There are different brands of herbal drinks in Nigeria, and Odogwu Bitters is one of the most popular among

them.[15] Odogwu Bitters is an herbal alcoholic beverage that contains many phytochemical constituents derived the different herbs and roots.[15.16] Odogwu Bitters is prepared from well- and carefully selected herbs. It contains ginger and honey which helps in fighting inflammation in the body.[16] It also aids in boosting the immune system and may also help in the relief of stress; regulation of blood sugar level and promotion of weight loss.[15.16] Odogwu Bitters have gained popularity among consumers worldwide,[15] it offers a range of sensory experiences and potential health benefits,[16] however, the impact on kidney and general human health remains a subject of concern and scientific inquiry.

The rate at which herbal alcoholic drinks are consumed in Nigeria within the last five years has become a cause for concern because of the increased cases of kidney diseases and their related complications.[17] There is a need for public health intervention strategies or plans to reduce the abuse of herbal alcoholic drinks and their underlying consequences.[18.19.20.21] However, these consequences have not been fully investigated and documented the effects of some of the Nigerian popular alcoholic herbal drinks sold in Nigerian markets. Therefore, this study aimed to investigate the potential implications of excessive consumption or abuse of Odogwu Bitters herbal drink on the kidneys of adult male Wistar rats.

MATERIALS AND METHOD

Ethical consideration and approval for the study

The ethical approval was obtained from the Health Research Ethics Committee of Nnamdi Azikiwe University Teaching Hospital, Anambra State, Nigeria. The reference number is NAUTH/CS/66/VOL.16/VER.3/257/2022/087 dated 1st March 2023.

Materials

Animals

The animal models used for this study were 20 male adult Wistar rats. The rats weighed between 195g to 230g. The rats were supplied by a local farm (in Nsukka, Enugu state, Nigeria) and were allowed to acclimatize for one week. The rats fed and drank water freely without interference. The health status of the animals was certified by a veterinarian before it was humanely transported to the research facility, and when the animals were housed at the research facility. The rats were housed in comfortable spacious cages with 12-hour dark / light cycle. The health status of the rats was closely monitored during the experiment. All the procedures followed the ethics guidelines of the Ethics Committee of the Faculty of Basic Medical Sciences, Nnamdi Azikiwe University.

Feed, herbal drink and reagents

The following materials were used to carry out this study: Odogwu Bitters (supplied by the sole distributor in Nkwo Nnewi market, Nnewi); Top Feeds Grower's mash Super-Deluxe Animal Feed (produced by Eastern Premier Feed Mills Ltd, a subsidiary of Premier Feeds Mills company limited, Plateau state, Nigeria); 10% Formal saline, normal saline, distilled water, alcohol (100%), xylene, sodium citrate, ethanol (100%), and paraffin wax supplied by the Department of Anatomy, Nnamdi Azikiwe University; Haematoxilin (produced by Number Laboratory Chemicals, India); Eosin (produced by Kem Light Laboratory, India); Dragendorff's reagent; Benedict's reagent; Analytical grade reagents (produced by Syntron Bioresearch Incorporated, United States of America).

Methods

Acute Toxicity Test

The median lethal dose (LD-50) of Odogwu Bitters was determined by using Dietrich Lorke's method.[22]

Experimental Protocol

The rats were divided into four groups of five rats each; and then housed in four big, meshed cages. Group A served as the control group while groups B, C and D served as the experiment groups. Group B received 0.45ml of Odogwu Bitters solution daily, group C received 0.75mls of Odogwu Bitter solutions daily, and Group D received 1.05mls of the Odogwu Bitters daily.

The rats were weighed before the commencement of experiment using a 6000g capacity weighing scale (with model number WT6000GT produced by WANT balance instrument company limited, China). The rats were fed *ad-libitum* on standard pelleted mash and a clean tap-water during the entire course of the acclimatization and experiment periods. The cages and environment were kept clean and disinfected daily.

Animal Euthanasia

The rats were humanely euthanized via cervical dislocation.

Blood collection and organ harvesting

Blood samples were collected via ocular puncture (using a standard heparinized micro-hematocrit capillary tube) into plastic plain tubes. The blood samples were allowed to stand for 30 minutes at room temperature to complete clotting. The clotted blood sample were centrifuged (using 800D Electric Centrifuge Machine with 4000RPM W/6X20Ml Rotor capacity) at 2500rpm (rotary per minutes) for 10 min and clear serum samples were aspirated off and stored frozen at -2^0C until required for biochemical analysis.

The rats were placed on a plane board, and the limbs were humanely held firm to the boards with optical pins. The abdomen of each rat was carefully dissected with the aid

of a dissecting kit. The liver was harvested, washed in a plain tube containing normal saline, and then placed in a 10% formal saline for histology analysis.

Phytochemical analysis

The following constituents were qualitatively and quantitatively evaluated – saponins, tannins, flavonoids, steroids, alkaloids, cardiac glycosides, reducing sugars, proteins, carbohydrates, and terpenoids. The study employed standard phytochemical analyses procedures as documented by Mathivha et al.[23]

Histology analysis

The liver samples were fixed, dehydrated, cleared, embedded, sectioned, and stained using standard tissue processing procedures documented by Onyejike et al.[24] Photomicrographs were taken with Amscope 14MP digital microscope camera fitted on NOVEX compound microscope with Hi-PLAN objectives. The labelling was done with Photoscape v3.7.

Kidney Function Test

The biochemical parameters determined on the sera specimen include urea, creatinine and uric acid. The study carried out kidney function test following procedures documented by Onyejike et al.[25]

Statistical Analysis

Data obtained from this study were analysed using the Statistical Package for Social Sciences (SPSS) International Business Machines Corporation (IBM) series version 29. The confidence level for the hypothesis test was set at 95%. Data were descriptively and inferentially analyzed. The difference between the control group and the experiment groups were analyzed using a two-way ANOVA. Independent samples t-test was employed to compare between groups.

Duration of the study

The entire study duration lasted for 10 weeks. The rats were acclimatized for one week. The

experiment lasted for five weeks. Data analyses lasted for four weeks.

RESULTS

Histopathological effects of Odogwu Bitters on the Kidney

The histology result of the control group showed evenly distributed glomeruli, of similar size, with normal mesangial cellularity (Figure 1). It also showed numerous open glomerular capillaries, and normal endothelium. The tubules were of normal density and tubular epithelium is viable. There was no significant pathology in the interstitium.

The histology results of rats in group B showed that the glomeruli are evenly distributed, of similar size, and with normal mesangial cellularity (Figure 2). It also showed that there are numerous open glomerular capillaries, and normal endothelium. The tubules were of normal density and tubular epithelium is viable. There was no significant pathology in the interstitium.

The histology result of rats in group C showed evenly distributed glomeruli, which are of similar size, with mild mesangial hypercellularity (Figure 3). There were numerous open glomerular capillaries, and normal endothelium. The tubules were of normal density and tubular epithelium is viable. There was no significant pathology in the interstitium.

The histology result of rats in group D showed evenly distributed glomeruli, which were of similar size, with mild mesangial hypercellularity (Figure 4). There were numerous open glomerular capillaries, and normal endothelium. The tubules were of normal density and tubular epithelium is viable. There was no significant pathology in the interstitium.

Phytochemical analysis results of Odogwu Bitters

The results of the qualitative and quantitative phytochemical analyses of Odogwu Bitters showed that it contained traces of saponin (0.13%), alkaloid (0.09%), terpenoid (0.30), flavonoid (0.12), carbohydrate, cardiac glycosides, and reducing sugar (0.11%) (Tables 1 and 2). However, the study could not quantify the amount of cardiac glycoside and carbohydrate in the herbal drink (Table 2).

Effects of Odogwu Bitters on creatinine, urea, and uric acid level

The result on the effect of Odogwu Bitters on serum creatinine level showed that there was a statistically non-significant difference ($p = .096$) between the control group and experimental groups (Table 3).

The result on the effect of Odogwu Bitters on serum urea level showed that there was a statistically non-significant difference ($p = .237$) between the control group and experimental groups (Table 3).

The result on the effect of Odogwu Bitters on serum uric acid level showed that there was a statistically non-significant difference ($p = .217$) between the control group and experimental groups (Table 3).

Table 1: Result of Qualitative analysis of Odogwu Bitters

Phytochemical constituents	Odogwu Bitters	Interpretation
Saponin	+	Present in trace
Alkaloid	+	Present in trace
Terpenoid	+	Present in trace
Cardiac glycoside	+	Present in trace
Flavonoid	+	Present in trace
Carbohydrate	+	Present in trace

Reducing sugar	+	Present in trace
Protein	-	Absent
Steroid	-	Absent
Tannin	-	Absent

Table 2: Result of quantitative analysis of Odogwu Bitters

Phytochemical	Quantity (% w/v)
Saponin	0.13
Alkaloid	0.09
Terpenoid	0.30
Flavonoid	0.12
Reducing sugar	0.11

Table 3: ANOVA result of the difference between the control group and the experimental groups for urea, creatinine and uric acid.

Tests		Sum of Squares	df	Mean Square	F	Sig.
Creatinine (mg/dl)	Between (Combined) Groups	.034	3	.011	2.991	.096
	Within Groups	.030	8	.004		
	Total	.065	11			
Urea (mg/dl)	Between (Combined) Groups	41.554	3	13.851	1.733	.237
	Within Groups	63.936	8	7.992		

	Total	105.490	11			
Uric acid (mg/dl)	Between (Combined) Groups	10.641	3	3.547	1.519	.282
	Within Groups	18.681	8	2.335		
	Total	29.322	11			

*. The mean difference is significant at the 0.05 level.

Figure 1: Photomicrograph of the kidney of rats in the control group (group A)

Key: M – Mesangium. G – Glomerulus, T – Tubule

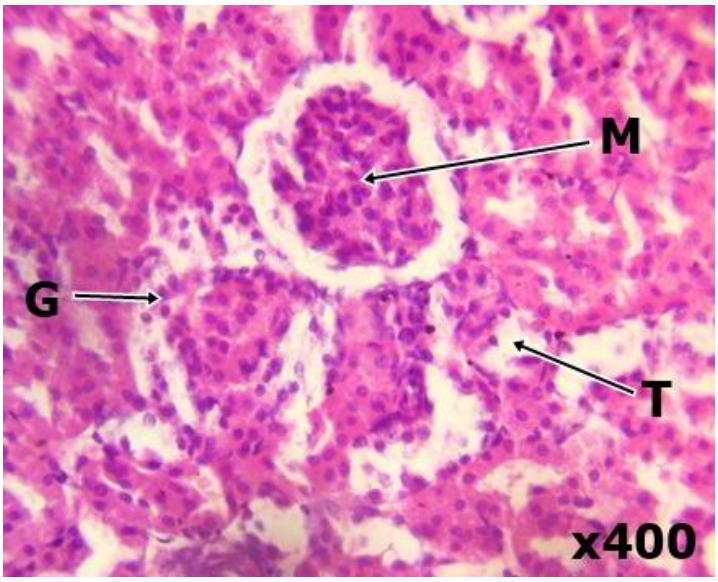

Figure 2: Photomicrograph of the kidney of rats in group B

Key: M – Mesangium, G – Glomerulus, T – Tubule

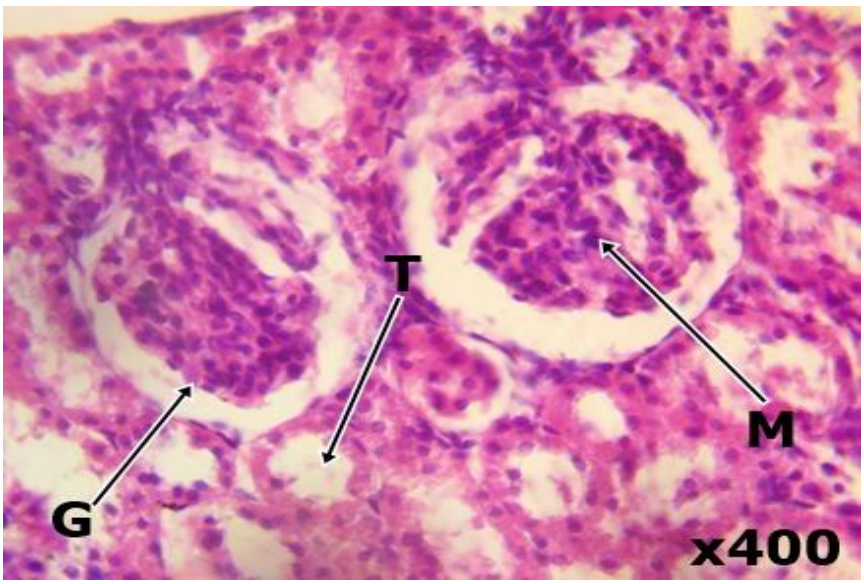

Figure 3: Photomicrograph of the kidney of rats in group C

Key: M – Mesangium, G – Glomerulus, T – Tubule

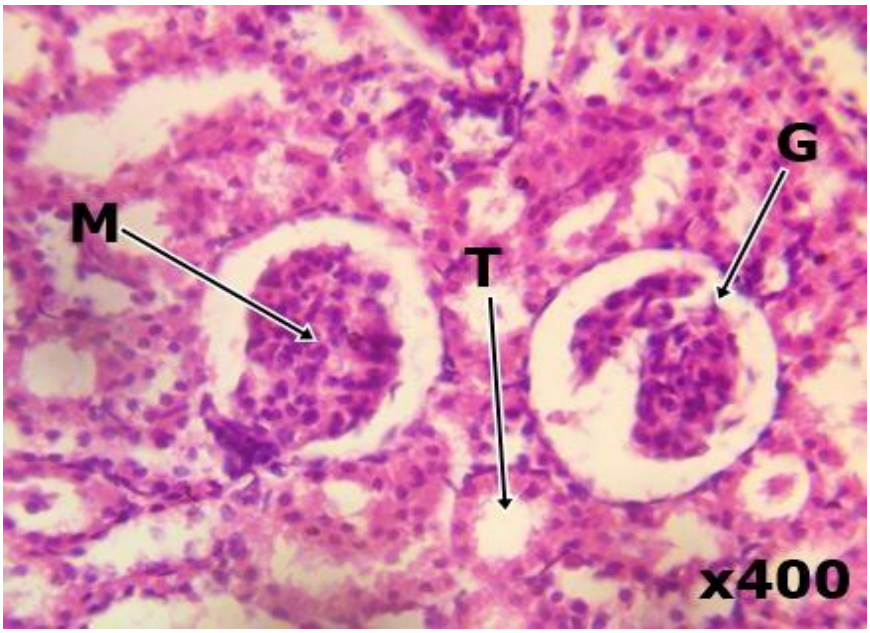

Figure 4: Photomicrograph of the kidney of rats in group D

Key: M – Mesangium, G – Glomerulus, T – Tubule

DISCUSSION

In Nigeria, alcoholic herbal bitters provide multipurpose service to many people,[7] and are often consumed without considering its possible side effects.[26] Findings from this study have shown some of the renal pathologies that may arise from prolonged intake of Odogwu Bitters. The histological findings showed that the rat groups administered with moderate and high doses of Odogwu Bitters had mild mesangial hypercellularity, but the rat group that received mild doses of Odogwu Bitters did not show any pathology. This means that mild doses of Odogwu Bitters do not affect the kidneys of adult male Wistar rats over five weeks. This assertion was also correlated with the results of creatinine, urea, and uric acid levels also showed that there was no difference between the control and the group that received mild dosage (see Table S1).

It is important to note that mesangial hypercellularity can progress to chronic kidney disease especially if not treated.[27.28] In addition, high uric acid levels also referred to as hyperuricemia can cause kidney stones.[29.30] Low creatinine level, high urea level and high uric acid level mean that the kidney functions at a suboptimal rate. This could be because of an injury or disease. However, the changes observed in the creatinine, urea, and uric acid levels for the groups that received moderate and high doses of Odogwu Bitters were statistically non-significant. Hence, this confirms the assertion that the moderate and high doses of Odogwu Bitters administered to the rats caused mild pathology on the kidney.

CONCLUSIONS

Odogwu Bitters herbal alcoholic drink causes mild mesangial hypercellularity on adult male Wistar rats when administered in moderate and high doses over a five-week period. It does not cause any pathology on the kidney of adult male Wistar rats when administered in low (mild) doses over a five-week period. Hence, abuse of this herbal drink should be discouraged.

SUPPLEMENTARY MATERIALS

Table S1: Descriptive statistics of the rat groups for creatinine, urea and uric acid analyses.

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SUPPLEMENTARY FILES

Table S1: Descriptive statistics of the rat groups for creatinine, urea and uric acid analyses

Test	Groups	Mean	N	Std. Deviation
Creatinine (mg/dl)	Control group	.6213	3	.07078
	Group B	.6780	3	.05889
	Group C	.5613	3	.05742
	Group D	.5420	3	.05889
	Total	.6007	12	.07668
Urea (mg/dl)	Control group	16.8497	3	1.87750
	Group B	17.7887	3	2.07760
	Group C	20.2167	3	3.92386
	Group D	21.5127	3	2.95468
	Total	19.0919	12	3.09677
Uric Acid (mg/dl)	Control group	3.6233	3	.96837
	Group B	2.5433	3	2.55823
	Group C	4.8767	3	.91571
	Group D	4.7267	3	1.00977
	Total	3.9425	12	1.63268