Original Research Article

REPRODUCTIVE TOXICITY & BIOMARKER RESPONSE OF MALE ALBINO RATS *(Rattus norvegicus)* TO A DAILY DOSE OF LOCAL GIN (OGOGORO)

Abstract

This study evaluates the effect of local gin (ogogoro) on Hepato-renal parameters such as aspartate amino transferase, alanine amino transferase, sodium, potassium, chlorine and bicarbonate, haematological parameters such as total protein, packed cell volume, red blood cell, white blood cell haemoglobin, platelet and lymphocytes and sperm count parameter.
The results show that: The mean serum electrolytes were for week 1 (Na 165.0, K 5.27, Cl 99.67 and HCO_3 19.67), week 2 (Na 138, K 5.77, Cl 89.67 and HCO_3 20), week 3 (Na 126.67, K 3.67, Cl 87.67 and HCO_3 19) and week 4 (Na 117.67, K 2.70, Cl 73.67 and HCO_3 22) and showed a significant difference in Na, Cl and HCO3 only when compared with the average control at ($P<0.05$), AST had a mean of 30.0 in week 1 which increased to 45.0 in week 4 while ALT had a mean of 15.0 in week 1 and increased to 30.67 in week 4. The mean serum protein reduced from 51.15 in week 1 to 42.53 in week 4 with significant difference ($P<0.05$). Mean PCV reduced from 36.0 in week 1 to 24.40 in week 4, Hb from 12.07 in week 1 to 8.80 in week 4 with a significant difference ($P<0.05$) when comparing the test with the average control, WBC from 6.17 in week 1 to 5.40 in week 4, Platelet increased from a mean of 255 on week 1 to 683 on week 4 with significant difference ($P<0.05$), RBC had a mean of 5.27 in week 1 and 5.25 on week 4 with no significant difference ($P>0.05$). Lymphocyte reduced from a mean of 69.0 week 1 to 45.50 in week 4 but when the test was compared with the average control it had a significant difference ($P<0.05$). While the mean sperm count was 275 in week 1 and 325 in week 4. These investigations demonstrated that local gin changes blood parameters which could lead to anaemia in mammals when constantly taken and also cause a detrimental effect on sperm count which could cause infertility in males as well as kidney and liver disease.

INTRODUCTION

Local gins (ogogoro in Nigeria) are a traditional alcoholic beverage consumed by millions of people in West Africa. It is usually produced from the distillation of fermented oil palm wine or raphia palm wine, and its percentage alcohol by volume varies from 40% to 60% depending on the source [1]. The production of the local gin was prohibited by the colonial masters in Nigeria prior to independence. Its production however, is no longer illegal as it holds great promise as a substitute for the imported spirits used as raw materials in the local production of distilled alcoholic beverages. The process of producing local gin from palm

wine is one of the flourishing industries amongst the Ijaws and Urhobos in Nigeria [2]. Basically, the effects alcohol will have in the body depend on how much alcohol builds up in the bloodstream. The Blood Alcohol Concentration (BAC) and the rate at which it rises and falls depends on how much alcohol is consumed, how fast it is absorbed from the stomach and small intestine into the blood, how it is distributed into the body and then how quickly it is eliminated from the body [3]. Even one-time (acute) alcohol consumption, such as binge drinking, can temporarily alter the activity of many organ systems [4]. Consumption of local gin has be reported to be associated with an increase lethal effect on the liver cells [5]. The liver damage caused by alcohol is attributed to alcohol metabolism and the by-product of that metabolism [6]. The high acid value and percentage alcohol content of ogogoro as a source of alcoholic drink is very dangerous as this could be associated with conditions of high level of acid in the liver (acidosis), a condition that leads to cirrhosis of the liver if not treated [2]. Palm wine and local gin has been reported to cause considerable liver damage through induction of peroxidation of lipids and finally inhibits the protein synthesis [7]. Raised activity of glutamyl transpeptidase (GGT) has also been reported to be very high in cirrhotic individuals and also in alcoholic individuals which is related to structural liver damage. [5] showed that the degree of alcoholic liver disease is related to the duration of consumption of local gin (ogogoro). Massive fatty changes, necrosis and broad infiltration of the lymphocytes were recorded in the livers of ethanol treated rats [8]. [9] proved that the observed endocytosis and vesicle protein content in alcoholic fatty liver disease animals are most likely effects of ethanol metabolism in the liver, which is not seen in Non-alcoholic fatty liver disease. Alcohol, one of the numerous factors that can compromise kidney health can interfere with kidney function through acute or chronic consumption or indirectly as a consequence of liver disease [10]. According to [11] consumption of alcohol was inversely associated with the risk of developing end-stage renal disease among approximately 65000

Chinese men aged 40-65 years. According to [12], treatment of rats with alcohol may have adverse effect on the bone marrow, kidney and haemoglobin metabolism since it has been reported that only substances which significantly affect the values of red blood cells and associated parameters would have effects on the bone marrow, kidney and haemoglobin metabolism. Sperm DNA damage has also been reported to be caused by local gin which can lead to the reduction of male reproductive capacity [13]**.** [14] in his findings showed that heavy drinking (alcoholism) affects some biochemical haematological parameters. This study is therefore designed to evaluate the Hepato-renal and haematological response and also possible adverse effect on the sperm of male Wistar rats exposed to a daily dose of local gin.

MATERIALS AND METHOD

Experimental design; Twenty four (24) healthy male eight (8) weeks old albino rats (*Rattus norvegicus*) of weight ranging from 220grams - 250grams, were used for the study. The animals were weighed at the end of each week using a Mettle (MT-501) weighing balance and were randomly divided into six (6) groups of four (4) rats each before housing them in a wire-meshed cage with the 12 hours light-darkness cycle for one week so as to acclimatize to the conditions of the environment. The study was generally conducted in accordance with recommendation from the 2013 declaration of Helsinki on guiding principles in the care and use of animals for research [15]. The local gin were administered orally with the aid of an oral canula mounted on a 1ml syringe and delivered directly into the oesophagus of the animals 1.45ml/kg daily for three weeks and on the last fourth week no administration was given to the test animals to check the withdrawal effect and control group was given distilled water.

Sample Analysis: Standard procedures were ensured during the collection of the blood, sperm and liver samples prior to biochemical analysis. Epididymal sperm count was determined with the Neubauer haemocytometer (Deep 1/10 mm, LABART, Munich, Germany) and light

microscope at 40× magnifications. The plasma activity of Alkaline Phosphatase (ALP) was determined using Radox kit (colorimetric method) of [16]. Biuret method was used to determine the level of total protein in the samples according to the method of [17]. The plasma activity of aspartate transaminase AST and alanine transaminase ALT was determined using Reitman and Frankel method [18]. The serum electrolytes were determined using ISO 4000 Automated electrolyte analyser. SFRI, France.

*Method of Data Analysis:*Data were analyzed using Tukey test at a level of 5% probability, using Assitat Software Version 7.7 en (2017).

RESULTS

4.1. Effects of Local Gin on Hepato-renal Parameters of Albino Rats

The results of kidney and liver analysis Table 1 revealed that Na level reduced from a mean value of 165 in week 1 to 138in week 2, to 126.67 in week 3 to 117.67 in week 4 having a control of 133.67, 157, 136 and 149.67 in week 1, 2, 3 and 4, with an average control of 142.50 there was a significant difference across the week ($P<0.05$). K and Cl reduced from 5.27 and 99.67 in week 1 to 2.70 and 73.67 in week 4 with an average control of 5.36 and 98.83 respectively, and only Cl had a significant difference ($P<0.05$). HCO3 had a mean of 19.67 in week 1 which increased to 22.0 but was still lower than the average control of 23.83 but there was a significant difference ($P<0.05$) across the week. AST and ALT increased from 30 and 15 in week 1 to 45 and 30.67 in week 4 having an average control of 25.17 and 12.17 respectively with a significant difference ($P<0.05$) across the week for both AST and ALT. Protein level decreased from a mean of 51.15 to 42.53 in week 4 with an average control of 69.09, there was a significant difference ($P<0.05$) across the week.

4.2. Effects of Local Gin on the Haematological Parameters and sperm count of Albino rats.

The results for haematological analysis (Table 2) revealed that PCV reduced from a mean value of 36.0 in week 1 to 24.40 in week 4 having an average control of 30.63 but there was no significant difference ($P<0.05$) across the week. Hb also decreased from 12.07 in week 1 to 8.80 in week 4 with an average control of 9.75 but also with no significant difference ($P<0.05$) across the week. RBC and WBC also reduced but not significantly ($P<0.05$) from 5.27 and 6.17 in week 1 to 5.32 and 8.77 in week 4 having an average control of 5.32 and

8.77 respectively. The platelet level increased from a mean of 255 in week 1 to 683 in week 4 with an average control of 342.83 with a significant difference ($P<0.05$) across the week. The results of sperm analysis in Table 3 shows that the sperm count was generally in the treated group was lower than the control, week 1 was 275, week 2 425, week 3 625 and week 4 325 while the control group was 475 in week 1, 575 in week 2, 475 in week 3 and 650 in week 4 with an average control 508.33. There was a significant difference ($P<0.05$) across the week.

Table 1: Effects of Local Gin on Hepato-renal Parameters of a Male Albino Rats

		Na(mmol/l)	K(mmol/l)	Cl(mmol/l)	HCO_3(mmo/l)	AST(U/L)	ALT(U/L)	Protein
Week 1	Control	133.67±2.52^{b}	4.07±0.25^{a}	100.67±4.51^{a}	23.67±0.58^{a}	17.67±3.51^{b}	10.67±1.53^{a}	65.77±12.1^{a}
	Test	165.0±4.0^{a,A}	5.27±1.45^{a,A}	99.67±1.53^{a,A}	19.67±0.58^{b,B}	30.0±6.0^{a,B}	15.0±3.0^{a,B}	51.15±3.94^{a,BC}
Week 2	Control	157.67±22.50^{a}	7.27±2.55^{a}	109.67±18.50^{a}	23.67±1.53^{a}	34.67±3.51^{a}	10.0±2.0^{a}	72.31±3.36^{a}
	Test	138.0±6.0^{a,B}	5.77±1.05^{a,A}	89.67±7.51^{a,A,B}	20.0±0.0^{b,B}	28.67±0.58^{b,B}	8.67±0.58^{a,C}	61.33±5.19^{b,AB}
Week 3	Control	136.67±10.50^{a}	5.0±0.6^{a}	86.67±4.51^{a}	24.67±3.51^{a}	27.0±5.51^{a}	11.0±4.0^{a}	69.27±2.15^{a}
	Test	126.67±0.58^{a,BC}	3.67±0.55^{b,A}	87.67±1.53^{a,AB}	19.0±0.0^{b,B}	27.67±1.53^{a,B}	12.67±0.58^{a,BC}	57.93±4.35^{b,A}
Week 4								
	Control	149.67±0.58^{a}	5.10±0.1^{a}	106.0±1.0^{a}	23.0±1.0^{a}	23.0±1.0^{b}	13.0±1.0^{b}	73.27±2.16^{a}
	Test	117.67±0.5^{b,C}	2.70±0.1^{b,A}	73.67±2.52^{b,B}	22.0±3.0^{a,AB}	45.0±5.518^{a,A}	30.67±2.52^{a,A}	42.53±1.94^{b,C}
	Average	142.50±11.84^{B}	5.36±1.13^{A}	98.83±9.17^{A}	23.83±1.87^{A}	25.17±4.18^{B}	12.17±2.591^{B,C}	69.09±5.90^{A}
	Control							

$^{a-b}$Different letters in the same column indicate significant difference ($p<0.05$) within the weeks

$^{A-B}$Different letters in the same column indicate significant difference ($p<0.05$) across the weeks

158 Table 2 Effects of local gin on haematology of male albino rats.

		PCV %	Hb	RBC (x 10^{12})	WBC (x 10^{9})	Platelet	Lymphocytes(x 10^{9})
Week 1	Control	26.67 ± 1.53^{b}	9.0 ± 0.3^{b}	4.23 ± 0.1^{b}	9.0 ± 2.5^{a}	270.0 ± 0.0^{a}	70.0 ± 5.0^{a}
	Test	$36.0\pm2.0^{a,A}$	$12.07\pm0.65^{a,A}$	$5.27\pm0.25^{a,A}$	6.17 ± 1.3^{aA}	$255.0\pm75^{a,A}$	$69.0\pm11^{a,A,B}$
Week 2	Control	32.57 ± 2.95^{a}	9.90 ± 0.9^{a}	5.56 ± 0.7^{a}	9.87 ± 5.6^{a}	335.67 ± 105^{a}	84.40 ± 1.4^{a}
	Test	$36.0\pm6.5^{a,A}$	$10.17\pm2.65^{a,A}$	$6.14\pm1.47^{a,A}$	4.90 ± 0.4^{aA}	$305.67\pm158^{a,A}$	$77.87\pm2.05^{b,A}$
Week 3	Control	32.85 ± 3.95^{a}	10.37 ± 1.15^{a}	6.04 ± 0.64^{a}	7.47 ± 2.8^{a}	423.0 ± 108^{a}	78.20 ± 1.4^{a}
	Test	$24.77\pm10.75^{a,A}$	$7.75\pm3.35^{a,A}$	$4.34\pm1.96^{a,A}$	$9.05\pm6.0^{a,A}$	$454.0\pm374^{a,A}$	$37.80\pm29.1^{a,B}$
Week 4	Control	39.07 ± 2.35^{a}	13.87 ± 0.45^{a}	6.90 ± 1.6^{a}	6.27 ± 0.0^{a}	416.67 ± 3.51^{b}	84.0 ± 0.7^{a}
	Test	$24.40\pm4.3^{b,A}$	$8.80\pm2.4^{b,A}$	$5.25\pm1.15^{a,A}$	$5.40\pm0.7^{a,A}$	$683.0\pm99^{a,B}$	$45.50\pm0.7^{b,A,B}$
	Average	30.63 ± 2.81^{A}	9.75 ± 0.78^{A}	5.32 ± 0.49^{A}	8.77 ± 3.67^{A}	342.83 ± 71.17^{A}	77.73 ± 2.60^{A}
	Control						

159 $^{a-b}$Different letters in the same column indicate significant difference ($p<0.05$) within the weeks

160 $^{A-B}$Different letters in the same column indicate significant difference ($p<0.05$)across the weeks

Table 3. Effects of local gin on sperm count of male albino rats.

Week	Test	Sperm count(x 10^6)
Week 1	Control	475.0 ± 125^{a}
	Test	$275.0\pm175^{a,C}$
Week 2	Control	575.0 ± 25^{a}
	Test	$425.67\pm221.9^{b,B}$
Week 3	Control	475.0 ± 175
	Test	$625.0\pm25^{a,A}$
Week 4	Control	650.0 ± 50^{a}
	Test	$325.0\pm25^{b,B,C}$
	Average control	$508.33\pm108.33^{A,B}$

[a-b]Different letters in the same column indicate significant difference ($p<0.05$) within the weeks

[A-B]Different letters in the same column indicate significant difference ($p<0.05$) across the weeks

DISCUSSION

This study revealed that the administration of local gin at a dose of 1.45ml/kg lead to a significant increase of sodium ion in the kidney and decreased the concentration of bicarbonate in kidney on the first week and also when compared with mean control, which implies that alcohol consumption reduces the amount of sodium ion excreted by kidney. It agrees with the report that potassium losses stimulate ADH activity, thereby increasing amount of fluid reabsorbed and causing the body's sodium concentration to decrease [19]. In week two, administration of local gin at dose of 1.41ml/kg also decreased the level of bicarbonate and protein significantly. The low serum bicarbonate is associated with a poor renal function and can cause chronic kidney disease. Which is in conformance with the report of presented by [20]. This signifies that renal hyper filtration (RHF) is one of renal adaptive

responses to an acidogenic diets which is believe to be the main cause factor of low serum bicarbonate in subjects with preserved renal function [20]. On the third week, the dose of local gin also caused a decrease on level of potassium, bicarbonate and protein which was statistically significant ($P<0.05$) when compared to the control groups. Due to withdrawal effects on the fourth week, it was found that the level of sodium, potassium, chlorine and protein was reduced with a high level of statistical significant ($P<0.05$).This high level of decreased could as a result of withdrawal effects on the animals which caused an increase in alpha4 subunit containing GABA receptors thereby causing difficulty in sleeping, sweating, heart arrhythmias and kidney or liver dysfunction, delirium. This findings agrees with the work of [21] that alcohol withdrawal causes seizures and delirium. While analysis of aspartate amino transferase (AST) and alanine amino transferase (ALT) levels on week one showed an increase in AST & ALT which were statistically significant. This agrees with this present finding that raised activity of glutamyl transpeptidase (GGT) has been reported to be very high in cirrhotic individuals; so also in alcoholism which is related to structural liver damage [22]. Also in the second week the AST and protein level reduced which was statistically significant ($P<0.05$). This implies that reduction in protein level may be as a result of protein inhibition by alcohol intake. This agrees with research done by [7]. Palm wine and local gin caused considerable liver damage through induction of peroxidation of lipids and finally inhibits the protein synthesis [7]. There was no significant difference ($P>0.05$) on the third week in AST and ALT when compared to the control. While on the last week, the result of this investigation showed increased level of AST and ALT respectively. This fluctuation in the level of AST and ALT could be as a result of alcoholic liver disease, viral hepatitis (hepatitis B and C), and hemochromatosis. The result is in accordance with the finding by [23], who reported that alcoholic liver disease is brought about by deficiency decrease in ALT serum activity which contributed to the increase in the AST/ALT ratio. The

results from haematological parameters of this study obtained from the rats on the first week showed an increase on platelet level at statistical significant ($P<0.05$). This abnormality could be as a result underlying condition or disease such a thrombocythemia (ET) which is a rare disease in which bone marrow produces to many platelet. It could also be as a result of abnormal haemoglobin molecule which may lead to anemia, leukemia and cancer in the rat which might be why there is a reduction in the blood PCV, HB and RBC from 36, 12.07 and 5.27 in week 1 to 24.40, 8.80 and 5.25 in week 4. This finding is in conformity with [12, 24 27], who reported that treatment of rats with alcohol may have adverse effect on the bone marrow, kidney and haemoglobin metabolism. Also on the second week, the result showed decreased in platelet which was statistically significant ($P<0.05$).This may be due to direct and indirect effects of local gin on the hematological system most especially leukocyte, erythrocyte and thrombocyte. This is in agreement with the findings of [2] that leukocyte, erythrocyte and thrombocyte production and functions are affected directly effect of local gin consumption. On the third the test showed significant effect decrease level of lymphocytes when compared with respective mean control the blood parameters. This is also in accordance with [2]. While on the last week the effect of withdrawal was highly noticed. The sperm count obtained from rats tested with administration of local gin in this present research as showed in Table 3 indicated drastic significant reduction on the number of sperm cells on second and fourth week and also on week one and two when compared with the mean control at ($P<0.05$). This implies that the reduction may be caused by high level of abnormality which was caused by DNA damage and death in the sperm cells due to alcohol intake thereby reducing viable population. This result agrees with the findings of [13, 25, 26]**,** which asserted that local gin can induce a considerable damage to the sperm DNA thus capable of reducing male reproductive capacity.

CONCLUSION

In this work, it was noticed that constant consumption of local gin (active constituent is ethanol) has an adverse significant effects on haematological, sperm and hepato-renal parameters. This study therefore implies that daily intake of local gin at these relative concentrations may be associated with high level of infertility, kidney and liver disease in mammals a class to which man belongs.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

Ethical Approval: As per international standard or university standard written ethical permission has been collected and preserved by the author(s).

Consent: NA

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