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Toxicological Effects of Kaikai - An Experimental Study in Wistar Rats

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Abstract: ***The public has witnessed several health and social challenges including chronic liver diseases, different organ cancers, death, and distortion of social status as a result of prolonged consumption of home brew local gin called Kaikai. The health challenges due to prolong consumption of Kaikai in Nigeria have assumed significant public health dimension requiring further investigation. This situation has been worsened by the apparent lack of data, information and study about the toxicological constituents and respective effects of the local gin. The aim of this study was to evaluate the toxicity of Kaikai in Rats which could be extrapolated to gain the picture of its effect in human. An experimental study which involved the administration of Kaikai to Wistar Rats under strict monitoring and observation. This was followed by histological investigation of harvested liver and blood. Histology result of Rat's organ showed the presence of macronodular or macrovesicular steatosis, microvesicular steatosis, fibrosis and serum electrolyte imbalance. By inference, the duration of consumption of Kaikai or local gin, the dosage of alcoholic constituent and impurities would determine the nature of impact.***

Keywords: Kaikai, biological markers, alcohol, Wistar Rat

1. Introduction

Local gin also called Kaikai in Nigeria is a clear, colourless, volatile liquid that undergoes oxidation, dehydration, reaction with metals, halogen acids or inorganic halides and esterification [1]. Traditional alcoholic beverages have been consumed in Nigeria and other West African communities for centuries, and western commercial spirits, beer and wine have been available since pre-colonial days [2], [3]. Kaikai is an alcoholic drink distilled from palm wine; it is usually brewed through a process of local fermentation and distillation in forested settlements due to the illicit status conferred on it by government agencies. In the rural communities of Nigeria, local brewing of alcohol is a major occupation and consumption of the finished product (Kaikai which is a surrogate alcohol) is also common.

Just like other countries, local gin abuse is a major public health problem in Nigeria and one of the leading causes of preventable death, illness and injury throughout the world. Alcohol is responsible for 170,000 deaths in sub-Sahara Africa in 1990 and is the fourth leading cause of disability amongst men in the developing countries [4], [5]. Epidemiologic studies of the last decades have unequivocally identified chronic alcohol consumption as an important risk factor for the development of various types of cancers, including cancers of the organs and tissues of the respiratory tract and the upper digestive tract, liver, colon or rectum (colorectal), and breast [6]. The brewing of traditional local gin on a small scale, and commercial purposes, have turned into one of the primary sources of income for many households, especially single parent or female headed units. This study therefore leveraged on identified chemical constituents of the alcoholic drinks and relates them to the symptoms of alcohol related diseases.

The scientific study of alcohol related mortality began in the 1926 with Pearl's studies on the death rates among various categories of drinkers. He and others found that heavy drinkers had higher rates of overall mortality and of mortality from cirrhosis than did lighter drinkers or abstainers. Since then, mortality studies have continued to demonstrate that heavy drinkers and alcoholics die from cirrhosis at a much higher rate than the general population [6].

One of the most influential efforts to summarize research in this area was undertaken in 1975 by an international group of scientists sponsored by the World Health Organization [7], [8]. The resultant book, Alcohol Control Policies in Public Health Perspective [4], reviewed studies of clinical and nonclinical populations of heavy drinkers. All studies found that a greater proportion of heavy drinkers died of cirrhosis than would be expected based on rates of cirrhosis deaths in the general population (i.e., liver cirrhosis deaths among heavy drinkers ranged from 2 to 23 times higher than the rate that would be expected in the general population). Several researches established a clear connection between heavy alcohol consumption and liver disease. Investigators also have observed that the price of alcohol is a significant determinant of alcohol consumption and thus of cirrhosis mortality rates [4].

Alcohol contributes to or is the sole cause of chronic and acute health problems because of its direct toxic effects on organs (as in alcohol liver cirrhosis), its intoxicating properties (as in accidents and injuries), and because it is a dependence producing substance [6]. Concerned by the recent cases of blindness and eventual death from the consumption of local gin (Kaikai) in Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC), alerted Nigerians on the danger of the local gin. The erstwhile Director-General of NAFDAC, Professor Dora Akunyili, who gave the warning, opined that these deaths might not be unconnected with the mixing of unknown substances with local gin to make it more

intoxicating [3]. Alcohol has been associated with numerous health and social problems for centuries, but it is only in recent years that the extent of such problems has been quantified. According to World Health Organization (WHO) estimates, alcohol is the fourth leading risk factor for death and disability globally, almost at par with tobacco [8]. Since Kaikai is available at a low cost and its consumption is not regulated it may be associated with both long- and short-term health consequences.

Hence this research will attempt to identify the chemical constituents of Kaikai and relate them to the symptomatology of alcohol related diseases seen in this environment.

In 2008, the National Agency for Food and Drug Administration and Control (NAFDAC) of Nigeria, reported that their laboratory analysis indicated that the Kaikai contained high concentrations of methanol in excess of 20g/100l and contaminants which include toluene and benzene and these chemicals can cause severe organ damage. NAFDAC also reported that 22 people died in Ilaje community in Aja Local Council Area of Lagos State following the consumption of local gin; they were reported to have gone blind before their eventual death [3]. The objective of this study to uncover toxicological effects of kaikai in rats and make extrapolations on its potential effects in human.

2. Materials and Methods

An experimental study which involved the administration of calculated dosages of Kaikai to 30 healthy looking Wistar rats for 2 months. Firstly, Kaikai was collected from Rumuiji in Rivers State of Nigeria and analysed to establish alcohol content at Fugro Laboratory in Port Harcourt [9]. Secondly, 30 Wistar rats were identified at the University of Port Harcourt, Zoology department and acclimatized at the department of pharmacology animal laboratory. Thirdly, the rats were fed with using special feed formula from Pfizer Nigeria Limited and calculated amount of Kaikai for 2 months. Fourthly, the rats were monitored for behavioural changes and sacrificed with the liver and blood samples harvested for histological examination.

This experiment, transportation and care of rats were performed in compliance with the relevant institutional guidelines at the department of pharmacology, University of Port Harcourt, Nigeria with approval obtained from the postgraduate ethics committee.

Research design - Healthy rats made of both sexes were acclimatized in the laboratory using animal cages. They were shared into 6 groups with similar body weights of five test rats per group and incubated in metabolic cages for two weeks for acclimatization. They were all fed ad libitum with animal feed (Pfizer Nigeria Ltd). Samples of local gin with same constituents and concentrations were administered to rats for 8 weeks at varying calculated doses. The dosages were deduced from extrapolation of the average consumption of a physiologic man. These rats were grouped into five experimental and 1 control groups according to their weights. Thereafter, calculated dosages of local gin sample were administered to the entire groups for eight weeks with concurrent monitoring. The local gin was administered by oral droppings using micro-sized Ryle tube attached to a graduate syringe.

Dosage Calculation of Kaikai - Calculated dosages were deduced from extrapolation of the average consumption of a physiologic man. i.e. if a normal physiologic man with an average weight of 70 kg consumes 3 cups (assuming a cup is 33 mls) of kaikai several times in a day as deduced from several interviews; then respective weights of rats would consume varying amount of doses as calculated below.

If a 70kg man consumes 99ml of kaikai in a day, then

A 120-gram rat would consume: 0.12 x 99 divided by 70 = 0.17 mls.

In other to ensure even proportionality, the dosages for each group was varied by a factor of 2 both above and below the average calculate consumption of 0.17mls.

This implies that dosages of groups A, B, C, D, E shall be 0.1ml, 0.17 ml, 0.34ml, 0.68 ml and 1.36 ml respectively with group F being the control group that was not given any alcohol sample.

Monitoring- These rats were observed and monitored for abnormal features and presentations adjudged as caused by the local gin. Some of the presentations watched for include hyperactivity, voracious eating, drowsy, sluggish, abnormal sounds, drowsiness, excessive sleep, sluggishness, increases salivation and bloody discharge to name but a few. Tissue Harvest - Each group was sacrificed in stages i.e. half of each group at the end of 4weeks and the remainder at the end of 8 weeks. Their tissues (blood and organs) were sent to the University of Port Harcourt Teaching Hospital laboratory for biochemical and histopathology analysis. The blood samples were collected by scooping of blood from dissected rat's heart using containers with anticoagulants (EDTA and Heparin). Organ samples, namely the liver, stomach, spleen, heart, and intestine were carefully collected and stored in Formalin using large sample containers to avoid squeezing of tissues. These organ tissues were fixed and used to prepare slides for view using high powered microscope. Blood samples were analysed for glucose levels, biochemical markers, liver enzymes and electrolyte in blood samples; organ tissues were viewed for morphological aberrations and any histopathology abnormalities adjudged as a toxicological effect caused by the local gin.

3. Result and Discussion

Samples of Kaikai collected and analysed at the Fugro Laboratory, Port Harcourt yielded high content of alcohol and other constituents (see Table 1).

Table 1: Constituents of Analysed local gin

Parameter	Method	Rumuji – Rivers
Alcohol content (%)	AOAC 942.06	91.0
Alcohol content (mg/dl)	AOAC 942.06	910,000
Water content (%)	AOAC 942.06	7.35
Impurities as suspended particles (mg/l)	AOAC 940.99	4.00
Nitrates (mg/l)	EPA 352.1	0.15
Chloride (mg/l/)	AOAC 966.09	3.74
Copper (mg/l)	AOAC 967.08	< 0.05

Source [10]

Rat Monitoring: With the administration of the calculated Kaikai to the rats, several manifestations were observed.

First week: Close monitoring revealed occasional hyperactivity and voracious eating of some rats in groups C, D and E with 0.34 ml, 0.68 ml, and 1.36ml dosages.

Second week: Occasional hyperactivity, voracious eating and fighting between some rats continued.

Third week: Some rats became very drowsy, sluggish and made abnormal sounds.

Fourth week: One rat died in group C, 2 died each in groups D and F. Their organs were harvested and sent for histopathology. At the end of the fourth week two rats were sacrificed in each group with their organs harvested and sent for haematology and histopathology

Fifth week: Drowsiness, excessive sleep, sluggishness, and increases salivation was noticed
Sixth week: Drowsiness, excessive sleep, sluggishness, and increase salivation continued

Seventh week: Drowsiness, excessive sleep, sluggishness, increase salivation continued with 2 deaths in control group.

Eight week: Appreciable increase in average weight of about 10gm, small bloody discharge found in their mouth and nostril. 2 rats were sacrificed in each group with their organs harvested for haematology and histopathology.

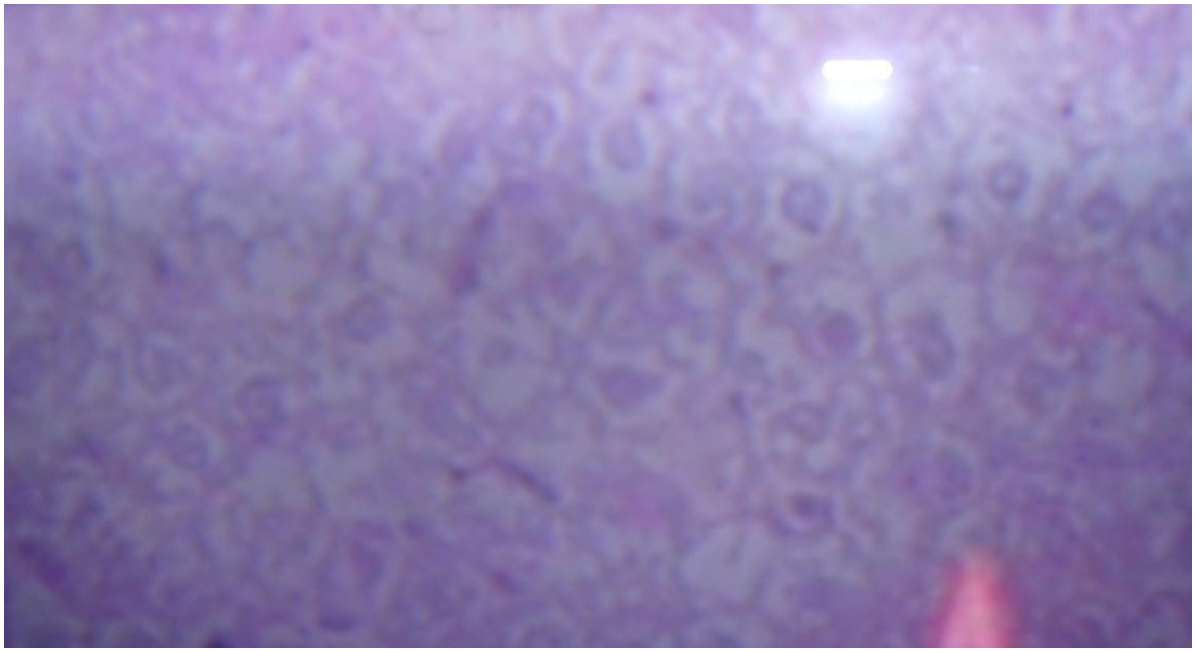
Haematological analysis revealed mild electrolyte disturbance, blood dyscrasia and biochemical aberration as evidenced by mild elevation of sodium, potassium, gamma-glutamyltranferase and aspartate aminotransferase, coupled with blood film showing few, scattered, hypochromatic and macrocystic red cells. The most frequent electrolyte aberrations found were hypernatremia, hyperchloremia, hypermagnesemia, hypocalemia, and biochemical changes such as increased gamma-glutamyltransferase, aspartate aminotransferase, alanine amino-transferase and lactic dehydrogenase.

Significant histopathological changes were found in the hepatic tissues of the rats. These changes include macronodular or macrovesicular steatosis; microvesicular steatosis; and fibrosis.

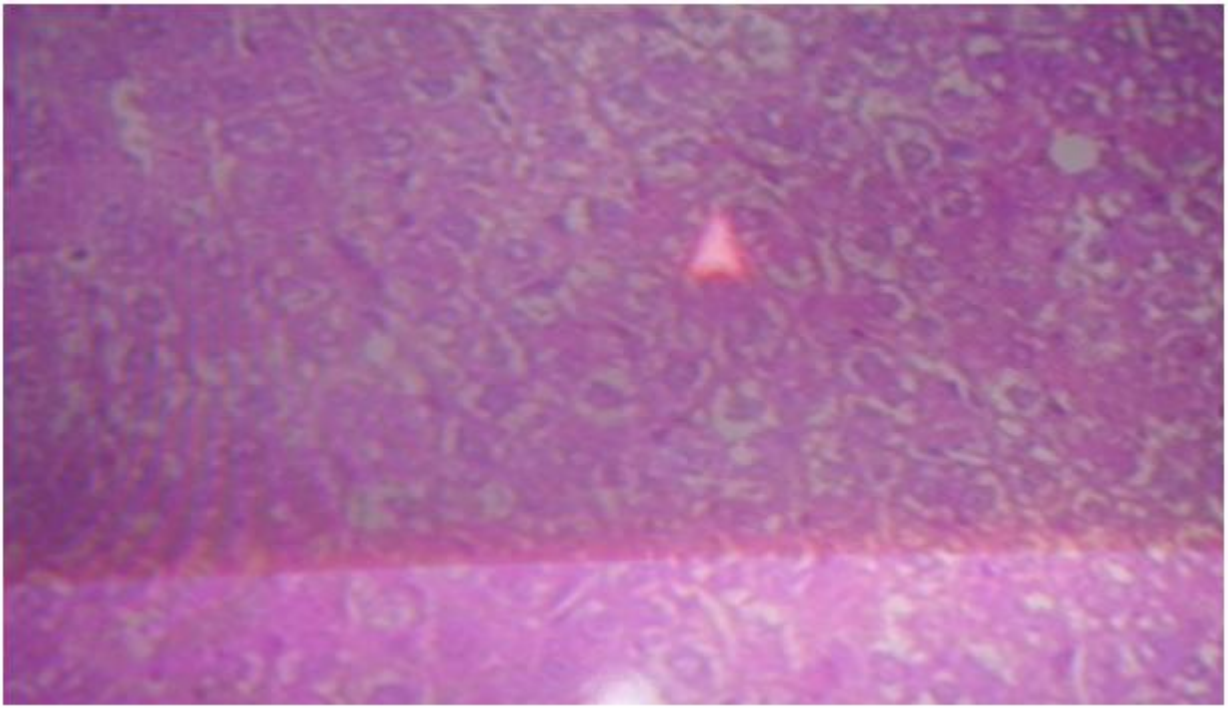
Table 2: Haematological assay data

Alcohol Study Parameters																								
Groups	A (5 Rats)				B (5 Rats)				C (5 Rats)				D (6 Rats)				E(6 Rats)				F (6 Rats)			
Quantity of Alcohol	0.1 ml				0.17ml				0.34ml				0.68ml				1.36ml				CONTROL			
Duration of Administration	2nd week		6th week		2nd week		6th week		2nd Week		6th week		2nd Week		6th week		2nd week		6th week		2nd week		6th week	
SGOT (u/l)	124	94			143	81	46	151	94	98	61	98	91	143	91	94	95	154	172		123	91	115	77
SGPT (u/l)	32	19			28	2	18	30	24	25	4	25	18	44	INS	32	15	104	56		20	26	15	27
YGT (IU/l)	1	1			Neg	4	INS	INS	3	2	5	2	1		INS		3				1	5	INS	2
ALK Phosph.(IU/l)	INS	711			Neg	636	INS	207	699	443	229	443	602	35	INS	255	389	34	215		671	725	311	286
Glucose(mmol/l)	4.1	4.2			3.1	4.9	3	2.5	3.5	5.3	2.5	5.3	5	3.3	3.2	3.4	5.2	8.4	4.4		4	5.2	4	5.7
Na (mmol/l)	137	133			132	138	104	133	138	130	147	130	137		145		138				135	134	144	139
K (mmol/l)	4.4	3			68	3.8	60	6.3	3.7	6.6	18	6.6	2.7		5.2		4.4				5.3	3.5	5.5	4
Urea (mmol/l)	6.2	3.6			INS	3.3	5.2	3.9	4	5.8	5.7	5.8	3.6	4.8	2.7	3.1	2.7	7.1	2.9		INS	4.5	4.9	5.4
Creatinine (mmol/l)	INS	INS			INS	INS	NA	NA	INS	INS	NA	INS	INS		NA		INS				INS	INS	NA	NA
T.Protein (g/l)	61	INS			INS	71	51	51	55	64	58	64	63	78	58	81	75	92	82		NA	62	53	*47*
Albumin (g/l)	NA	NA			NA	37	30	27	30	34	36	34	31	39	33	37	55	47	38		INS	33	37	37
Bicarbonate (mmol/l)	NA	NA			NA	NA	NA	NA	NA	NA	2	NA	NA								NA			

Note: Highlighted data were gotten from second run of studies



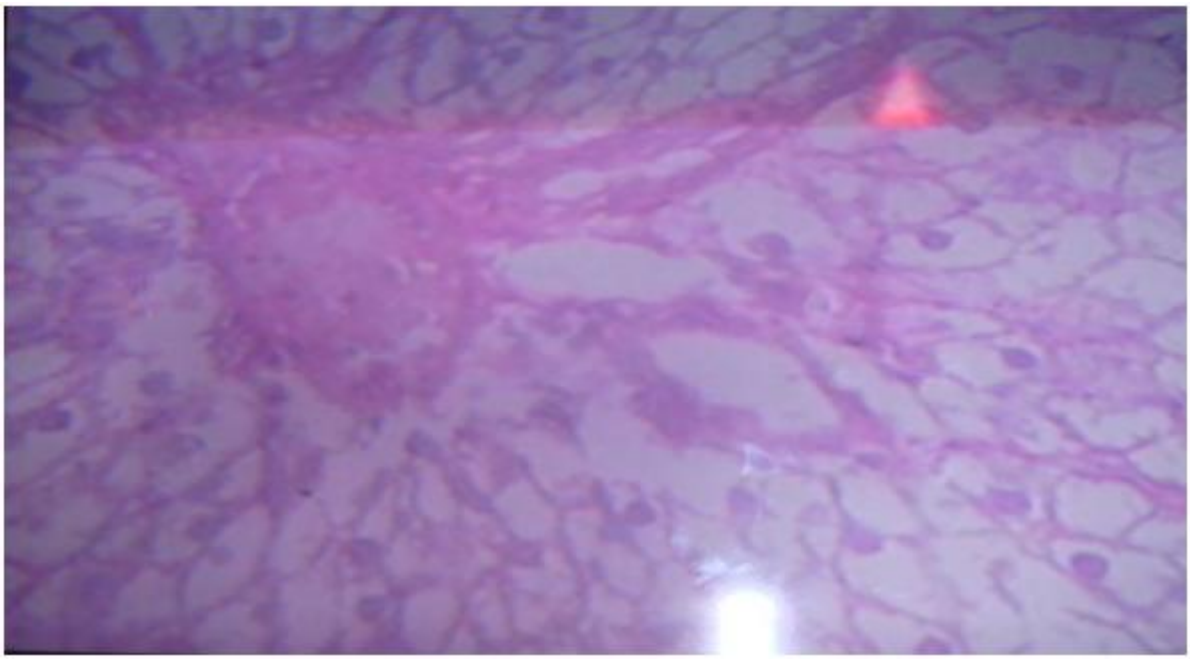
A1 - Image magnification of 3072 x 2304
Figure 1: Central macrovesicular steatosis. Showing early formation of fibrous tissue



B2 Image dimension 3072 x 2304
Figure 2: Microvesicular steatosis

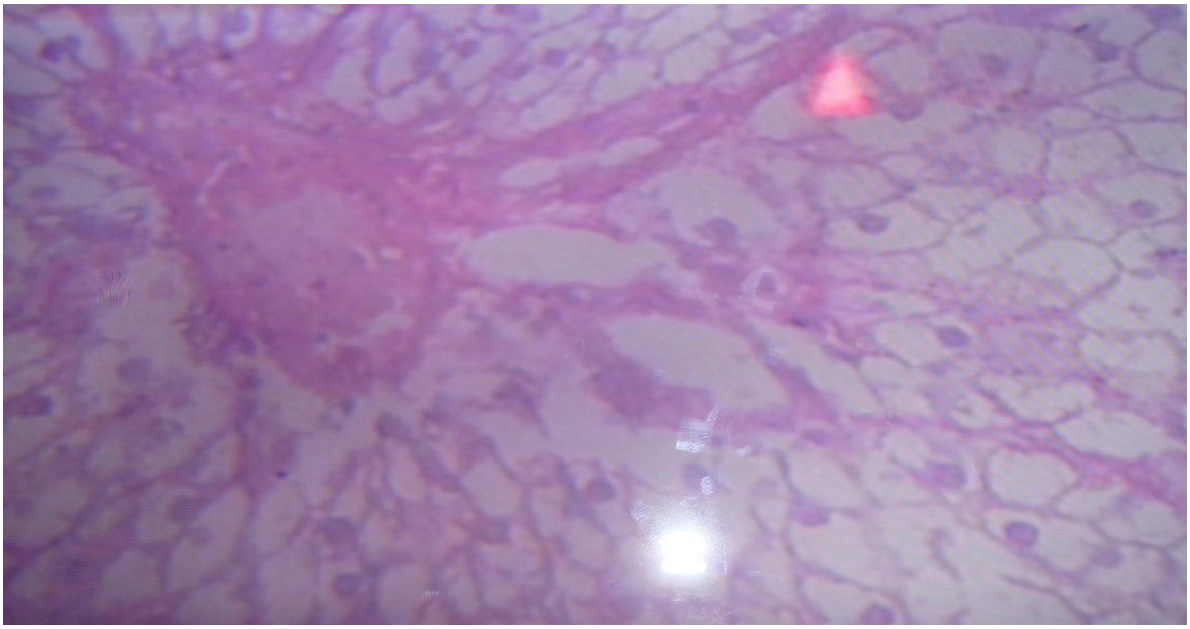
Figure 2 above shows microvesicular steatosis with fine fibrous strands.

There is hypertrophy of the perisinusoidal cells. Alcohol abuse has been known to cause microvesicular steatosis, a lesion ascribed to impaired mitochondrial function. Because alcohol abuse leads to reactive oxygen species in the hepatic mitochondria, it may damage mitochondrial DNA with the 4977 base pair deletion. The common deletion is frequent in the hepatic DNA of alcoholic patients with microvesicular steatosis. Alcohol-induced mitochondrial DNA damage may contribute to the occurrence of this lesion in some alcoholics. Because alcohol abuse leads to reactive oxygen species in the hepatic mitochondria, it may damage mitochondrial DNA. Alcoholic liver disease remains one of the most common causes of chronic liver disease in the world.



F1 Image dimension 3072 x 2304
Figure 3: Macrovesicular steatosis

Shows generalized macrovesicular steatosis (fatty liver) with fibrosis around the portal tracts. The fibrosis is not bridging (Stage 1 fibrosis).



F 2 Image dimension of 3072 x 2304
Figure 4: Early fibrosis

While consumption of alcohol gives pleasure, it is noteworthy that the manifestations by the study rats showed signs of alcohol toxicity. These signs include sluggishness, hyperactivity, voracious eating, abnormal sleep, abnormal sound and bloody discharges from orifices. Signs of significant hepatocellular toxicity were also discovered as evidenced by the histopathology aberrations such as macronodular or macrovesicular steatosis, microvesicular steatosis, and fibrosis (see Figures 1, 2, 3, 4). Prolonged alcohol consumption has been known to cause microvesicular steatosis (see Figure 2), an aberration attributed to altered mitochondrial function [10], [11]. The biochemical breakdown of alcohol gives rise to Acetaldehyde which in turn leads to the production of cytochrome P-4502E1 that generates reactive oxygen species (ROS) and activates pro-carcinogens causing various types of cancer on prolong consumption [10]. The induction of CYP2E1 also increases the conversion of various xenobiotics, including pro-carcinogens (nitrosamines, aflatoxin, vinyl chloride, polycyclic hydrocarbons, and hydrazines) to cancer causing form [11], [12], [13], [14], [15], [16],[17] [19]. Of all the organs in human body, the liver commonly present structural and functional changes due its susceptibility to prolonged alcohol exposure [13],

[18],[19], [20], [21], [22],
[23].

This study further revealed varying degrees of electrolyte disturbance, blood dyscrasia and biochemical aberration. Biochemical markers assessed in this study are presented in Table 2. Electrolyte disturbances and biochemical changes were reported by Rauchenzauner in Austria. The most frequent electrolyte disturbances found were hypernatremia (41%), hyperchloremia (21%), hypermagnesemia (17%), and hypocalcemia (15%), whereas hypokalemia and hypophosphatemia were observed quite rarely (5% and 3.4% respectively). The commonest biochemical derangement observed were consistent with features of cellular toxicity such as elevate liver enzymes (gamma glutamyl transferase, aspartate aminotransferase, alanine aminotransferase and lactic dehydrogenase) as well as signs of pancreatitis (increased serum lipase and amylase) and muscle damage (elevated creatine kinase). The most frequent changes in blood counts were leucocytosis (23%), thrombocytopenia (14%), and anemia (12%). While the duration of consumption is a determinant factor to the toxicological effects, it is noteworthy that the dosage of the alcohol content and other impurities in the alcohol is critical to the development of associated pathologies.

The strength of this study, however, lies in the use of calibrated laboratory equipment, internationally accredited laboratory and certified laboratory scientist in the analysis of tissue samples ensured validity of results. The limitations include the paucity of related studies exploring the histological presentations Kaikai fed rats, which could be a valuable reference resource; the lack of nationally published statistics, sustenance of rats during bench work and fund for the research. Further work would be required to identify the socio-economic and self-reported health impacts of the excessive consumption of Kaikai on humans.

4. Conclusion

This study has revealed the toxicological effects of the local gin in rats which enabled extrapolations to the potential impacts in human health. It showed that several health challenges could be inflicted by prolonged consumption of Kaikai as evidenced by the disorders of the rat's liver (macronodular or macrovesicular steatosis, microvesicular steatosis, fibrosis and serum electrolyte imbalance). The trio factors of duration, dosage of Kaikai and presence of impurities or contaminants are critical to developing health effects. It is therefore important that prolonged consumption of Kaikai or by extension any local gin should be viewed as flash point for negative public health issues.

5. Acknowledgements

The author would like to thank Professor Omotayo Ebong and the Department of Pharmacology, Nigeria for the approval of the study and providing the academic ambience to undertake this research.

6. Ethical Statement

The authors declare that due diligence and compliance with high ethical standards was observed in the course of this study. Ethical clearance and supervision on the use of rats for this study was secured from the Department of Pharmacology at the University of Port Harcourt, Nigeria.

7. Conflict of Interest

The authors declare that they have no conflict of interest or funding for this project

8. Funding Source

There was no funding for this study. All cost was borne by the author.

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