British Journal of Medicine & Medical Research
20(9): 1-11, 2017; Article no.BJMMR.25624
ISSN: 2231-0614, NLM ID: 101570965

SCIENCEDOMAIN *international*
www.sciencedomain.org

Comparative Safety Profiles of Pure and Alcohol Beverages in Wistar Rats

O. G. Dawodu[1,2*], O. A. T. Ebuehi[2] and O. S. Odesanmi[2]

[1]Department of Science Laboratory Technology, Federal Polytechnic Ede, Osun State, Nigeria.
[2]Department of Biochemistry, College of Medicine, University of Lagos, Lagos, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/BJMMR/2017/25624
Editor(s):
(1) Chan-Min Liu, School of Life Science, Xuzhou Normal University, Xuzhou City, China.
(2) Alex Xiucheng Fan, Department of Biochemistry and Molecular Biology, University of Florida, USA.
(3) Chan Shen, Department of Biostatistics, MD Anderson Cancer Center, University of Texas, USA.
Reviewers:
(1) B. O. Ajiboye, Afe Babalola University, Ekiti State, Nigeria.
(2) Mesfin Yimam, Unigen, Inc., Seattle, USA.
(3) Peter Clapp, Regis University, USA.
Complete Peer review History: http://www.sciencedomain.org/review-history/18481

Original Research Article

Received 12th March 2016
Accepted 13th February 2017
Published 3rd April 2017

ABSTRACT

Objective: Alcohol consumption is readily acceptable worldwide, and despite warnings and billions of dollars spent yearly on its deleterious effects; people still to the present day take copious amounts of it. The study investigated the comparative effects of pure and alcoholic beverages on Wistar rats to ascertain their level of safety.
Methods: Thirty five male albino Wistar rats divided into 7 groups, were administered daily (v/v) pure and beverage alcohol comprising of; 5% (v/v) ethanol, 5.1% beer; 15% (v/v) ethanol, 13% red wine; and 40% (v/v) ethanol, 40% spirit; while the control group was administered saline, by intra-gastric route (IG) for 28 days. On the 29th day, the animals were sacrificed and blood collected for biochemical analysis. The rat brain, liver, kidney and lungs were excised for histopathological examinations and aliquots of the beverage alcohols were subjected to Gas chromatography-mass spectrometry (GC-MS) analysis.
Results: The activity of ALT was not significantly different in treatment groups when compared with controls. The GGT and AST activities of the treatment groups were significantly increased ($p<0.05$). The rat organ photomicrographs showed that the lungs was most adversely affected,

**Corresponding author: E-mail: dawgrace@yahoo.com;*

followed by the liver, kidney and brain. The GC-MS chromatograms of the respective beverages contained the following: beer 19, red wine 10 and spirit 16 constituents.
Conclusion: The plasma ALT, AST, GGT activities however, did not indicate excess alcohol consumption in the animals although unusual values were observed. The histological profile on the rat organs showed that there was some form of organ damage implying that these beverages may be injurious to health. In addition, the GC-MS spectroscopy revealed that these alcoholic beverages had different proportions of the chemical constituents which may portend some futuristic threat to health.

Keywords: Alcoholic beverages; liver enzymes; safety profiles; GC-MS.

1. INTRODUCTION

It has been reported that well over 2 billion people consume alcohol worldwide and this may result in health implications which affect work, family life productivity etc. [1]. Globally, alcohol consumption has increased in recent decades, with all or most of that increase in developing countries [2]. In industrialized countries, heavy intake of alcohol is a leading cause of preventable mortality and morbidity, second only to cigarette smoking [1].

An alcoholic beverage is a drink containing ethanol, commonly known as alcohol. Alcohol is consumed in the society in 3 main forms either as beer, wine or spirit. These forms of alcoholic consumption pattern represents low alcoholic content of less than 10% in the beer group, moderate alcoholic content of less than 20% in the wine group, and high alcoholic content in the of 40% and more in the spirit group, with variations within these specified groups, [3]. It is a known fact that advertisers make a lot of effort in promoting the alcoholic beverages consumed in the society and this has a vital impact in the lives of young people as they becomes frequenters of such beverages as their awareness is piqued by such interesting displays [4].

Testino [5], reported from a human perspective, that continuous use of alcohol over time is capable of damaging almost all the organs in the body. The objective of this study was to ascertain the safety levels of beverage alcohol and pure alcohol in Wistar rats.

2. MATERIALS AND METHODS

2.1 Animals

Thirty five inbred 6-week-old male Wistar rats (80.64± 2.77 g) obtained from the Animal House Department, College of Medicine, University of Lagos, were used for this study. The animals were housed under standard laboratory conditions (temperature 22±1°C and relative humidity of 45-55%; natural light and dark cycle), and had free access to standard rat chow and water.

2.2 Chemicals

All chemicals and reagents used for this study were of analytical grade and purchased from Sigma Chemicals, USA. The different alcoholic beverages used were purchased from their distribution centres here in Nigeria; Star beer from NBL Nigerian breweries, Red wine from Davide Campari-Milano S.p.A and Seaman's Schnapps from Nigerian Distilleries Limited.

2.3 Experimental Design

The experiment was conducted for 28 days. The animals were randomly allocated to seven experimental groups of 5 rats each; (1) a control group, which received saline (0.9% (w/v) NaCl), (2) an ethanol (EtOH) group, which received 5% (v/v) EtOH, (3) an alcohol beverage group, which received 5.1% beer, (4) an EtOH group, which received 15% (v/v) EtOH, (5) an alcohol beverage group, which received 13% red wine; (6) an EtOH group, which received 40% (v/v) EtOH; (7) an alcohol beverage group, which received 40% spirit. For sample size calculation the "resource equation method" was used [6,7].

The ethanol (alcohol) used in the study was reagent grade 200% proof. The volume of alcohol administered to the animals was calculated using the Widmark [8] formula modified by Bouwer, [9]. The means of administration was intra-gastric (IG) between 8.00am and 10.00am daily; this was done with sterilized needles and catheters. The alcoholic concentrations used for this study were determined from the alcohol beverages consumed by man; beer has alcoholic concentrations 2-8% (in Nigeria majorly beer is between 5 and 6%), wines has alcoholic concentrations 10-20% (hence the median 15% was used), while spirits generally are between

30% and above (40% was taken as Schnapps is a favourite refined drink in Nigeria). Physical parameters such as feed, water intake and body weight were determined daily.

2.4 Animal Handling and Experimentation

The research protocol was approved by the Animal Care Committee of College of Medicine, University of Lagos, Idi-Araba, Lagos, while animal usage itself, followed the animal guidelines for the protection and usage of animals for experiments of the same institution adapted from the animal care guidelines of the National Academy of Sciences-National Research Council (NAS-NRC).

2.5 Analytical Procedures

Alanine amino transferase (ALT), aspartate amino transferase (AST) and gamma glutamyl transferase (GGT) activities were determined in the serum:- Whole blood was collected immediately into plain tubes and spun at 3000g for 5 min for serum separation, after which it was stored at -20°C for marker enzymes analysis. AST and ALT were determined using the method of Reitman and Frankel [10] modified by Hammed, [11]. The activity of GGT in the serum was also determined using the method of Szasz, [12] modified by Marghoob Hassan et al. [13]. All analysis was done with commercially available Randox kits from Randox Laboratories, U.K.

2.5.1 Histopathological studies

Selected organs (liver, kidney, brain and lungs) were excised, cleaned of blood and other extraneous materials and fixed in 10% neutral buffered formalin, dehydrated in ethanol, cleared in xylene, embedded in paraffin; 5-6µm sections were routinely stained with haematoxylin and eosin (H&E) and assessed with a light microscope Nikon Eclipse E400 model, [14].

2.5.2 GC-MS analysis

The 3 alcoholic beverage samples were concentrated to 1ml in vial bottles, and taken for gas chromatography mass spectrometric (GC-MS) analysis for the determination of their chemical composition. The gas chromatographic Model: 7890A (GC) analysis was performed on Agilent Technologies interfaced with mass selective detector model: 5975C (MSD). The electron ionization was at a 70V with an ion source temperature at 250^{o}C. Highly pure helium gas (99.9% purity) was used as carrier gas, while HP-5ms (30 mm X 0.25 mm X 0.320 µm) was used as the stationary phase. The oven temperature was at 80^{o}C held for 4 min and ramped to 270^{o}C at the rate of 3.5^{o}C/min holding for 6 min. 1 µ/l was auto injected [15].

2.6 Data Analysis

The SPSS version 20 computer software package (SPSS Inc. Chicago, U.S.A) was used for the computation of results obtained from this study. Data are presented as mean ± standard error of the mean (SEM) and comparing data with respect to significant difference was evaluated using ANOVA, for comparison between sample means with level of significance assessed at 5% confidence interval also for multiple comparisons LSD was used.

3. RESULTS

The comparative effect of pure and alcohol beverage on physical parameters is shown in Table 1. The body weight of rats administered beer, 40% pure alcohol and spirit were significantly higher than the control. There was no significant difference ($p<0.05$) observed with the feed intake irrespective of alcohol consumed. The water intakes however, all the rats in the paired alcoholic groups were significantly lower than the control group administered saline.

The comparative effect of alcohol consumed on the plasma activities of ALT, AST and GGT is shown in Table 2. There was no significant difference ($p<0.05$) was observed with serum ALT activity, but significant difference was observed with serum GGT and AST activities when compared with controls ($p<0.05$).

The comparative liver to body weight ratios of animals administered alcohol and their beverage counterparts is shown in Table 3. The control rats administered saline, had the least liver weight to body weight ratio, while all paired alcohol groups had increase in weight, with the alcoholic beverage groups having decrease in weight, the highest increase was observed in the 5% ethanol to 5.1% beer group.

The lung of control rats showed normal structure with no signs of toxicity (Plate 1A), however the rats administered 40% ethanol had inflammatory cells with few eosinophils indicating infection of the lungs, (Plates 1B, 1C) this was more pronounced with the rats administered spirit, (Plates 1D and 1E).

Table 1. Body weight, feed and water intake of rats administered alcohol, beer, red wine and spirit for 28 days

Group	Body weight (g)	Feed intake (g)	Water intake (ml)
Control	102.33±4.99	50.58±1.34**	123.50±10.82
5% EtOH	106.81±4.44	51.38±1.19	76.12±3.01[b]
5.1% Beer	122.43±6.70[a]	55.58±1.30	85.15±4.95[b]
15% EtOH	126.38±5.51	55.35±0.94	96.19±4.12[b]
13% Red Wine	108.19±3.19	41.31±1.83	99.00±3.71[b]
40% EtOH	111.84±3.12[a]	43.15±1.56	76.46±4.17[b]
40% Spirit	128.56±3.55[a]	29.96±2.00	61.85±5.40[b]

**Data are expressed as mean ± standard error of the mean (SEM).*
*[a,b]p<0.05, **no significant difference*

Table 2. Serum activities of ALT, AST & GGT of rats administered alcohol, beer, red wine and spirit for 28 days

Group	ALT (U/L)	AST (U/L)	GGT (U/L)
Control	31.21±6.11	27.76±9.89	93.36±18.80
5% EtOH	32.79±4.21	138.01±41.44[b]	98.14±10.02
5.1% Beer	30.45±9.69[a]	79.68±20.52[b]	6.61±16.17
15% EtOH	30.58±2.02	57.46±4.64	67.74±19.98
13% Red Wine	32.39±3.05	35.43±2.08	8.10±2.41[c]
40% EtOH	27.77±3.29	17.15±6.91	82.80±5.30
40% spirit	26.92±1.47	34.71±11.41	91.48±6.95

**Data are expressed as mean ± standard error of the mean (SEM). [a,b,c]p<0.05*

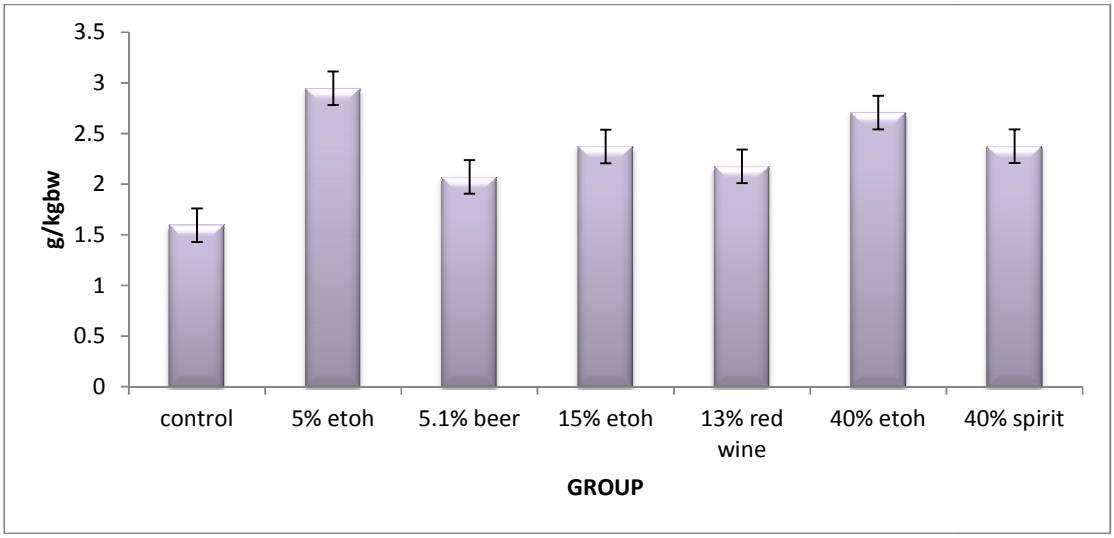

Fig. 1. Percentage liver weight of rats administered alcohol, beer, red wine and spirit for 28 days

Light microscopic evaluation of liver tissues shows that control group had normal liver architecture (Plate 2A), this was also reflected in the rats administered 5% EtOH and beer (Plate 2B, 2C). However, rats administered red wine and 15% ethanol had well preserved liver architecture with inflammatory cells around the portal tract, also observed was a congested central vein.

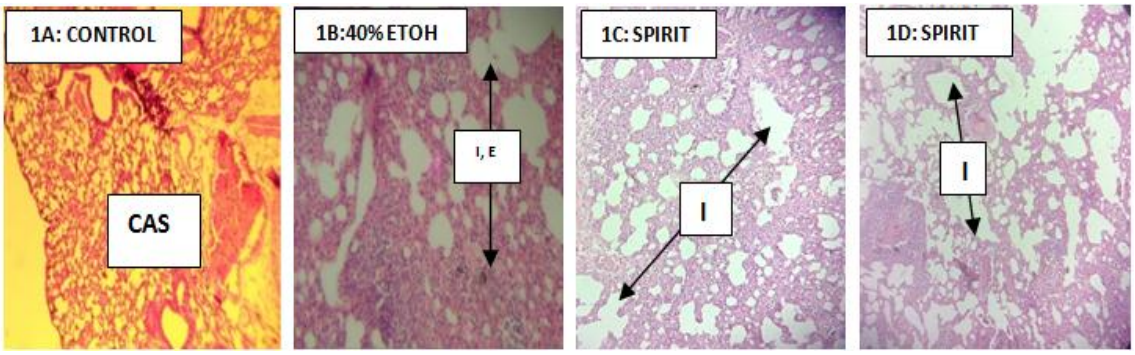

Plate 1. Photomicrographs of cross sections of rat lung (×100)
1A: Normal lungs showing clear alveolar spaces 1B: Inflammatory cells I, few eosinophils E are noted, 1C: Moderate inflammatory cells and congestion within the interstitium. 1D: Severe inflammatory cells infiltrate within the interstitium

Table 3. Comparison between organ (liver) and body weights of rats administered alcohol, beer, wine and spirit for 28 days

Group	Body weight (g)	Liver weight (g)	% liver weight (g/kgbw)
Control	102.33±4.99	1.63±0.38	1.60
5% EtOH	106.81±4.44	3.15±1.35	2.95
5.1% Beer	122.43±6.70	2.53±0.28	2.07
15% EtOH	126.38±5.51	3.00±0.52	2.37
13% Red Wine	108.19±3.19	2.35±0.32	2.18
40% EtOH	111.84±3.12	3.03±0.51	2.71
40% Spirit	128.56±3.55	3.05±1.76	2.37

**Data are expressed as mean ± standard error of the mean (SEM)*

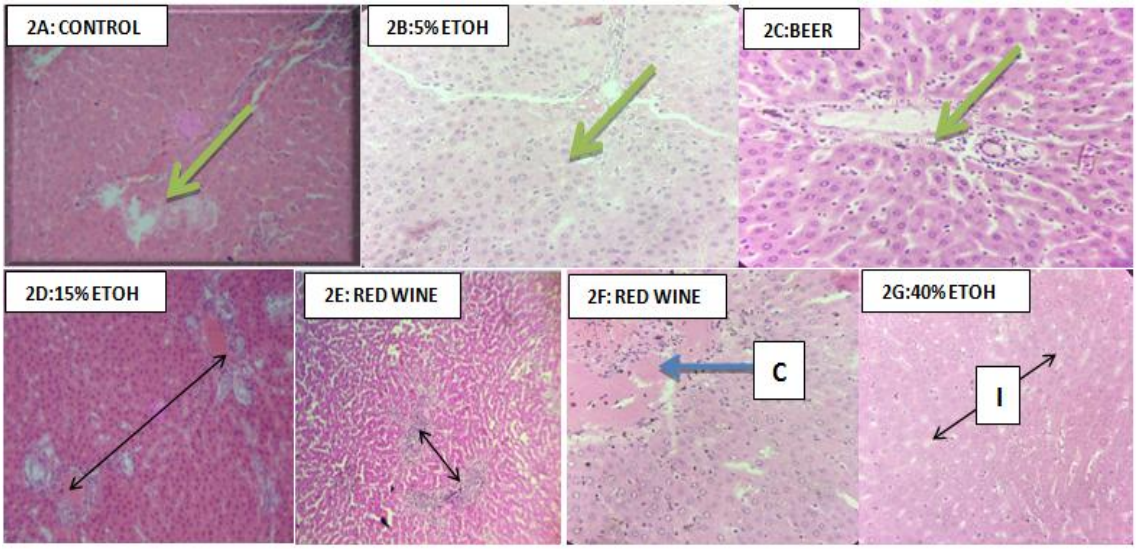

Plate 2. Photomicrographs of cross section of rat liver (×100)
2A, 2B, 2C: Showing normal study i.e. normal liver architecture i.e. normal portal vein. 2D, 2E: well preserved Liver architecture with inflammatory cells around the portal tract, while 2F: another rat in the same group had inflammatory cells with Congested central vein C. 2G: Well preserved liver architecture with few inflammatory cells I within the portal tract PT

Normal structure of the cortex and medulla was observed in the kidney of control rats (3A), and also with the animals administered beer, 5% ethanol and red wine (3B, 3C, and 3D). There was hypertrophy of the epithelial cells of the animals administered 40% alcohol and spirit, i.e. acute tubular necrosis (ATN), (3F and 3G).

In Plate 4, almost all the animals had normal tissue architecture in the brain irrespective of type or concentration of alcohol administered. Animals administered 15% ethanol had mild edema with perinuclear halo, (Plate 4D).

Figs. 2-4 are chromatographic profiles of the 3 alcoholic beverages administered to the rats. Interpretation of these chromatograms on Table 4 showed that beer gave 19 constituents; red wine gave 10 constituents; while the spirit had 16 constituents. All 3 chromatograms had azetidine, a heterocyclic compound and oxalic acid as common constituents.

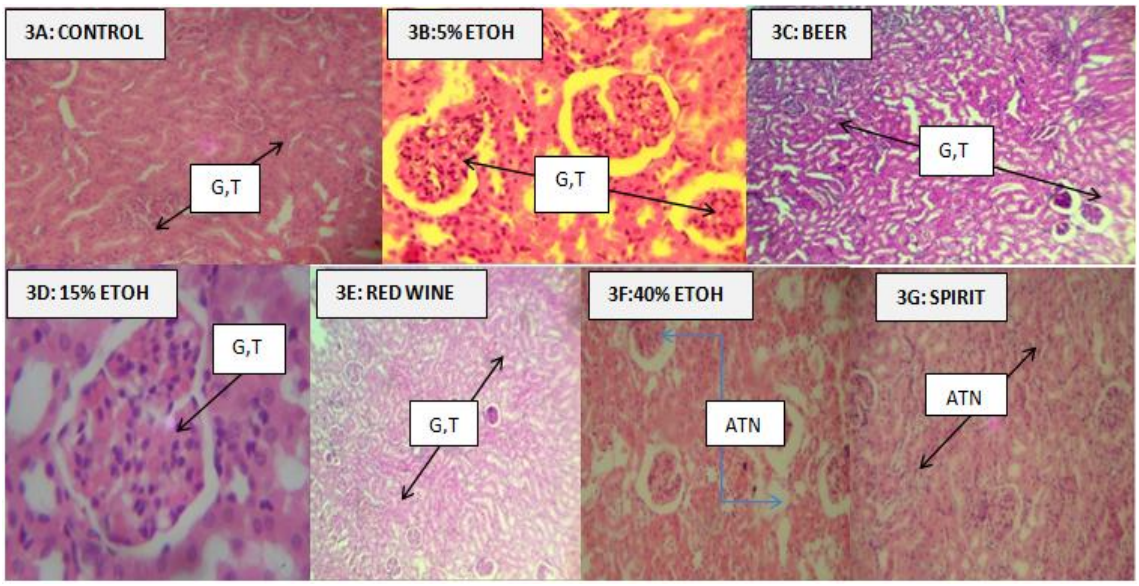

Plate 3. Photomicrographs of cross section of rat kidney (×100)
3A, 3B, 3C: Normal glomeruli G and tubules T in Kidney i.e. normal study, i.e. The glomeruli G appears normal with obvious central vein. 3D, 3E: Normal glomeruli G and tubules T in Kidney i.e. Normal Study3F, 3G: Ghost like appearance of the tubules T with sloughing off of the epithelial cells: Acute Tubular Necrosis

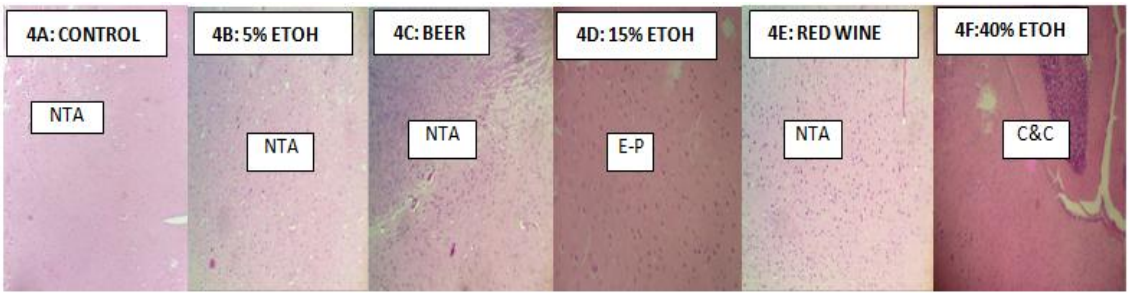

Plate 4. Photomicrographs of cross section of rat Brain (×100)
4A, 4B, 4C, 4E: Rats administered saline and 5% ethanol, beer, red wine, showing normal tissue architecture i.e. normal neuronal bodies on a fine textured neutrophil. while 4D: Rats administered 15% ethanol showed mild edema E, with perinuclear halo P, and those of rats administered 40% ethanol , 4F: Had Cerebrum and cerebellum i.e. neuronal bodies on a fine eosinophillic Neutrophil N

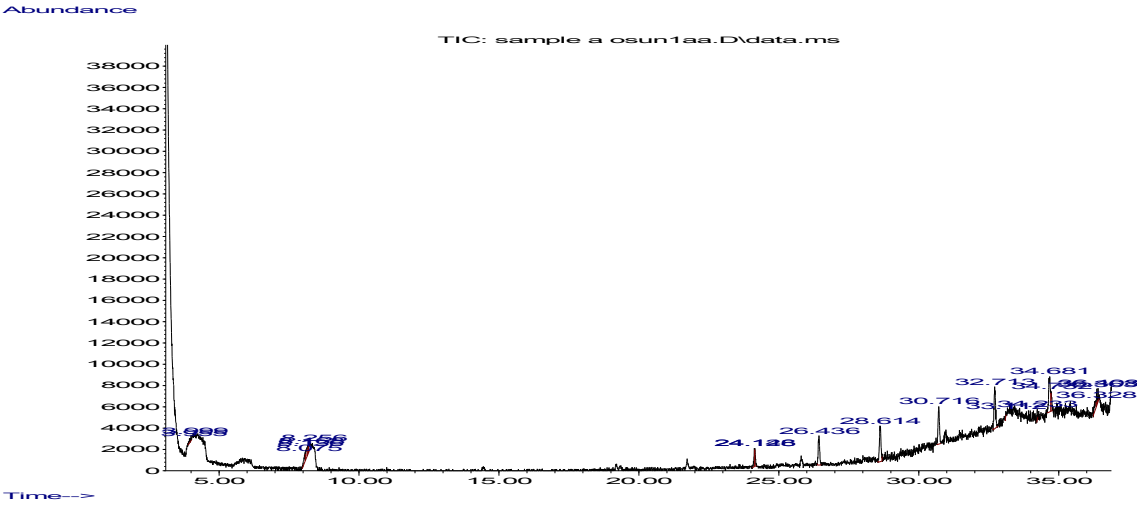

Fig. 2. GC-MS chromatogram of beer

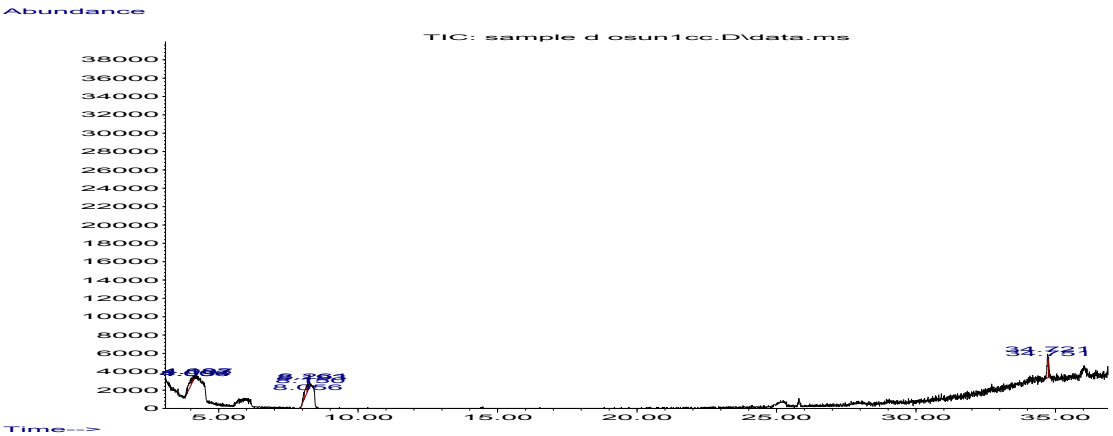

Fig. 3. GC-MS chromatogram of red wine

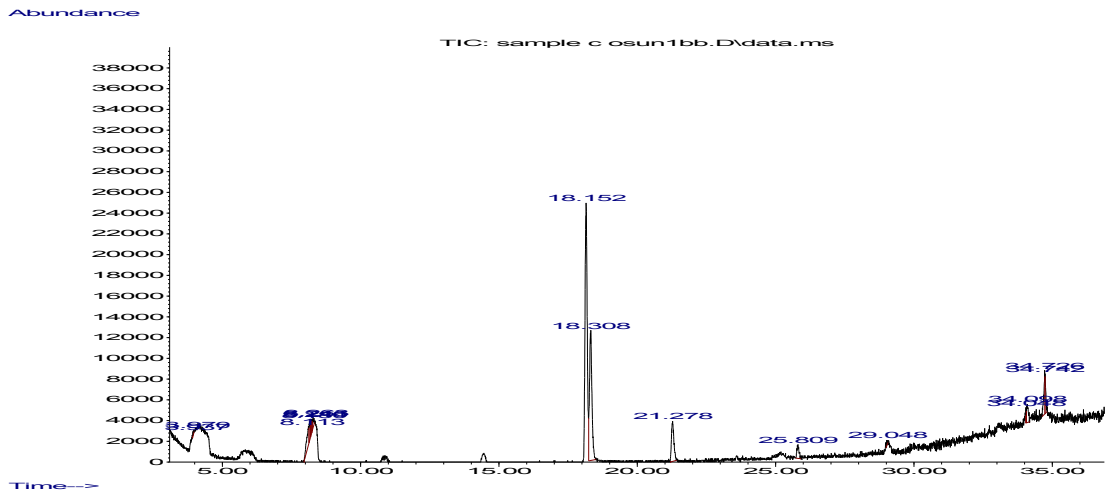

Fig. 4. GC-MS chromatogram of spirit

Table 4. Gas chromatography –mass spectrometry peaks and components of beer, wine and spirit

S/N	Sample A: Beer				Sample B: Red wine				Sample C: Spirit			
	Library ID	**RT(MINS)**	**Area (%)**	**Nature of compound**	**Library ID**	**RT (MINS)**	**Area (%)**	**Nature of compound**	**Library ID**	**RT (MINS)**	**Area (%)**	**Nature of compound**
1.	Azetidine	3.997	4.57	Heterocyclic	Azetidine	4.105	28.83	Heterocyclic	Azetidine	3.970	0.16	Heterocyclic
2	Oxalic acid	34.684	68.79	Organic acid	Oxalic acid	8.261	30.45	Organic acid	Oxalic acid	8.263	4.36	Organic acid
3	1H-Tetrazol-5-amine	8.168	7.78	Amine	1H-Tetrazol-5-amine	8.053	5.25	amine	5H-Tetrazol-5-amine	29.047	0.24	Amine
4	Hexane	8.256	2.89	Hydrocarbon	1-dodecanol	34.72	26.90	alcohol	Benzyl benzoate	21.277	7.86	Ester
5	Tetradecanol	34.235	1.02	Alcohol	1-eicosanol	34.75	12.59	alcohol	2-methyl octane	8.111	3.49	Hydrocarbon
6	Tetradecanal	34.328	3.748	Aldehyde					5-keto2-2-dimethyl heptanimine	18.153	46.28	Amine
7	9-octadecanal	36.363	2.133	Aldehyde					Decanoic anhydride	18.307	25.79	Organic acid
8	19-Eisosadiene	36.408	1.470	Unsaturated hydrocarbon								
9	Eicosanol	34.732	4.322	alcohol								

4. DISCUSSION

The body weight feed and water intakes of the alcohol paired groups, showed that the rats reacted to alcohol differently. The feed intake showed that there was no adverse increase or decrease between all groups as animals almost ate the same amount of food irrespective of their initial body weight. The water intake there was almost no significant difference observed across the groups except with red wine. The weight gain revealed significant differences observed when the groups were compared in pairs with the highest increase observed in the animals that took beer. This was in agreement with the work of Sayon-Orea et al. [16] who indicated that the calories obtained from alcohol consumption could lead to body weight gain. In the present study, sub-acute ethanol administration (for a period of 28 days), significantly increased the levels of the hepatic enzymes - AST. A rise in the AST level is usually accompanied by an elevated ALT level [17]. The present results were not in agreement with the clinical findings of Pari and Karthikesan [18] who showed that chronic alcohol intake leads to many cellular and tissue abnormalities including alterations in liver enzymes (ALT, AST). These changes may indicate increased permeability, damage and /or necrosis of hepatocytes [19]. In this study, there was a rise in AST level not accompanied with the rise in ALT level. The rats administered beer, 5% ethanol and red wine had AST levels higher than the control which was indicative of the extent of organ damage in their tissues as their livers though normal had inflammatory cells, and a rat administered red wine even had congested central vein. Hence for this study extent of organ damage was not seen from the ALT activity but from AST activity. This could also stem from the fact that, the rats had lower alcoholic administrations ranging from 5-13%. Hence, for this study AST activity was a more sensitive marker of liver damage than ALT [20]. This difference could also be explained with the AST to ALT ratios of beer, 5% ethanol and red wine groups, as it was greater than 2 [21]. In agreement with Ruppin et al. [22] on his studies of ethanol treatment in rats, there was also a significant increase in the serum level of gamma-glutamyl transferase (GGT), even though from this study, there was no correlation with the different alcohol concentrations administered to the rats, as the results varied irrespective of the alcoholic consumed. The liver weights in the comparative alcohol beverage groups, from this study, showed that the type of alcohol beverage consumed could have gross changes on the its weight. All groups in this study had higher liver weights when compared with the control, with the 5% alcohol group having the highest weight [23]. The GC-MS analysis of the 3 alcoholic beverages showed that beer contained 19 volatile constituents, red wine had 10, while spirits had 16 volatile constituents. The compounds present in the distillates were identified by their mass spectra available in the spectrum library. They were mainly esters and fatty acids in agreement with the work of Plutowska and Wardencki [15]. A particular constituent of all the 3 beverages was azetidine, a known heterocyclic compound, it's possible that its detoxification in the liver may be incomplete and may lead to damage of these tissues. Most beverages are accepted mainly through tastory evaluations and not the constituents themselves.

The liver according to literature is the main organ responsible for the detoxification of alcohol in the body. The present study revealed that liver damage is not subject to level of alcohol consumed as any level could have adverse effect. The kidney as an excretory organ is known to be central to total body homeostasis, regulating extracellular water and electrolytes as well as acid base balance, among other critical functions. Renal damage could occur as a result of acute intoxication or chronic alcoholism [24].

From this study it was observed that there was more lung tissue damage recorded with alcohol consumption of 40% and above, this was in agreement with the work of Kershaw and Guidot, [25] who observed chronic alcohol consumption could lead to alcoholic lung disease and also alcoholic lung disease could be comparative to liver disease following the onset of chronic alcohol usage.

5. CONCLUSION AND RECOMMENDATION

This study showed that alcohol consumption poses a threat to health due to the level of organ damage observed from the paired alcoholic groups and the unusual activities of the liver enzymes. This finding was further corroborated with the GC-MS results which showed a wide variety of individual constituents. From the present study, increased health awareness and education, should be recommended to the consumers of alcoholic beverages on the

possible health risks associated with ready and excessive consumption of such beverages.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. WHO. Global status report on alcohol and health. WHO Library Cataloguing-in-Publication Data Global status report on alcohol and health. WHO Press, WHO, Geneva, Switzerland; 2014.
2. Mayowa MO, Chikere EIC. Prevalence and perceived health effect of alcohol use among male undergraduate students in Owerri, South-East Nigeria: A descriptive cross-sectional study; 2011.
   Available:www.bmc.org
3. Buglass AJ. Handbook of alcoholic beverages: Technical, analytical and nutritional aspects. Wiley Online Library Publishers; 2011.
4. Saffer H. Alcohol advertising and youth. Journal on Studies on Alcohol. 2002; Supplement No. 14:173-181.
5. Testino G. Alcoholic diseases in hepato-gastroenterology: A point of view. Hepatogastroenterology. 2008;55(82-83): 371-7.
6. Festing MF, Altman DG. Guidelines for the design and statistical analysis of experiments using laboratory animals. ILAR J. 2002;43:244–58.
7. National centre for replacement, refinement, and reduction animals in research. Experimental design / statistics; 2012.
   Available:http://www.nc3rs.org.uk/category.asp?catID=7
8. Widmark EMP. Principles and applications of medicolegal alcohol determination. Davis, CA: Biomedical Publications. 1981; 107-108.
9. Brouwer IG. The Widmark formula for alcohol quantification. Journal of the South African Dental Association. 2004;59:427–428.
10. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. American Journal of Clinical Pathology. 1957;28:56-63.
11. Hammed MA. Metabolic Profile of rats after one hour of intoxication with a single Oral dose of ethanol. Journal of Pharmacology and Toxicology. 2011;6:158-165.
12. Szasz G. A kinetic photometric method for serum gamma glutamyl transpeptidase (GGT). Clinical Chemistry. 1969;15(2): 124-36.
13. Mohd Azam Hyder. Marghoob Hasan, Abdelmarouf Hassan Mohieldein. Comparative levels of ALT, AST, ALP and GGT in liver associated diseases. European Journal of Experimental Biology. 2013;3(2):280-284.
14. Jaijoy K, Soonthornchareonnon N, Lertprasertsuke N, Panthong A, Sireeratawong S. Acute and chronic oral toxicity of standardized water extracts from the fruits of *Phyllanthus emblica* Linn. International Journal of Applied Research in Natural Products. 2011;3(1):48-58.
15. Plutowska B, Wardencki W. Application of gas chromatography-olfactometry (GC-O) in analysis and quality assessment of alcoholic beverages. A review. Food Chemistry. 2008;107(1):449–463.
16. Sayon-Orea C, Martinez-Gonzalez MA, Bes-Rastrollo M. Alcohol consumption and body weight: a systematic review. Nutrition Reviews. 2011;69(4):419-431.
17. Nyblom H, Berggren U, Balldin J, Olsson R. High AST/ALT ratio may indicate alcohol liver disease rather than heavy drinking. Alcohol and Alcoholism. 2004; 39(4):336-9.
18. Pari L, Karthikesan K. Protective role of caffeic acid against alcohol-induced biochemical changes in rats. Fundamental and Clinical Pharmacology. 2007;21(4): 355-61.
19. Saravanan R, Viswanathan P, Pugalendi KV. Protective role of usolic acid on ethanol mediated experimental liver damage in rats. Life Science. 2006;78(7):713-8.

20 Panteghini M, Falsetti F, Chiari E, Malchiodi A. Determination of Aspartate aminotransferase isoenzymes in hepatic disease-preliminary findings. Clinica Chimica Acta. 1983;128: 133-40.

21. Shivaraj GS, Prakash BD, Vinayak VH, Avinash AKM, Sonal NV, Shruthi SK. A review on laboratory liver function tests. Pan African Medical Journal. 2009;3(17): 1-11.

22. Ruppin DC, Frydman MI, Lunzer MR. Value of serum gamma-glutamyl transferase activity in the diagnosis of hepatobiliary disease. Medical Journal Australia. 1982;1(10):421-4.

23. Crabb DW. The liver. In: Galanter, M., ed. Recent developments in alcoholism. Ten Years of Progress. New York: Plenum Press. 1993;11:207-230.

24. Vamvakas S, Bruening T, Thomasson B, Lammert M, Baumueler A, Bolt HM, Dekant W, Birner G, Henscler D, Ulm K. Renal Cell Cancer correlated with occupational exposure to trichloroethene, Journal of Cancer Research Clinical Oncology. 1998;126(3):178-80.

25. Kershaw CD, Guidot DM. Alcoholic lung disease. Alcohol Research and Health. 2008;31:(1):66-75.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/18481