Original Article

Comparative Safety Profiles of Pure and Beverage Alcohols in Wistar Rats

A R T I C L E I N F O

Keywords:
Alcoholic beverages
liver enzymes
safety profiles
GC-MS

A B S T R A C T

Background: Alcohol consumption is readily acceptable worldwide with little or no concern for its adverse effects. Objective: 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 were administered daily (v/v) pure and beverage alcohol composing 5% alcohol, beer, 15% alcohol, wine and 40% alcohol (spirit), control (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 histological examinations and aliquots of the beverage alcohols were subjected to 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 different (P<0.05). The rat organ photomicrographs showed that the lungs was most adversely affected, 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.

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. (WHO, 2004, Nwoye, 2013). Globally, alcohol consumption has increased in recent decades, with all or most of that increase in developing countries. (Ebuehi and Asonye, 2006, Mayowa and Chikere, 2011). In industrialized countries, heavy intake of alcohol is a leading cause of preventable mortality and morbidity, second only to cigarette smoking (WHO, 2014).

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. (https://en.wikipedia.org/wiki/List_of_alcoholic_beverages)

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. (WHO, 2004, Nwoye, 2013). Globally, alcohol consumption has increased in recent decades, with all or most of that increase in developing countries. (Ebuehi and Asonye, 2006, Mayowa and Chikere, 2011). In industrialized countries, heavy intake of alcohol is a leading cause of preventable mortality and morbidity, second only to cigarette smoking (WHO, 2014).

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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. (Saffer, 2002)

However, long-term use of alcohol in excessive quantities is capable of damaging practically every organ system in the body with the most important effects, from a clinical point of view, relating to diseases of the circulatory, nervous and hepato-gastrointestinal systems (Testino, 2008). The objective of this study was to ascertain the safety levels of pure alcohol and beverage alcohol in Wistar rats.

MATERIALS AND METHODS

Animals

Thirty five inbred 6-week-old male Wistar rats (80.64±2.77g) bred in the Laboratory Animal Centre, College of Medicine, University of Lagos, 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 rat chow and water.
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.

Experimental Design

The rats were administered the respective diets and drinks for 28 days. The animals were randomly allocated to seven experimental groups of 5 rats each: (1) a control group D, which received saline (0.9%(w/v) NaCl); (2) an EtOH group A, which received 5% (v/v) EtOH; (3) an alcohol beverage group A*, which received 5.1% beer; an EtOH group B, which received 15% (v/v) EtOH; (3) an alcohol beverage group B*, which received 13% red wine; an EtOH group C, which received 40% (v/v) EtOH; (3) an alcohol beverage group C*, which received 40% spirit. The volume of alcohol administered to the animals was calculated using the Widmark (1981) formula modified by Bouwer (2004). The means of administration was intragastric (IG) between 8.00am and 10.00noon daily, this was done with sterilized needles and catheters. Physical parameters such as feed, water intake and body weight were determined daily.

Animal Handling and Experimentation

The research protocol was approved by the Animal Care Committee of College of Medicine University of Lagos, IIdi-Arabá, 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).

Analytical Procedures

Alanine amino transferase (ALT), aspartate aminotransferase (AST) and gamma glutamyl transferase (GGT) activities in serum:- Whole blood was collected in plain tubes, spun at 3000g for 5 min for serum separation after which it was stored at -20°C for marker enzymes analysis, AST, ALT, were determined colorimetrically using the method of Reitman and Frankel (1957) modified by Hammed (2011) carried out with commercial Randox Kits, (Randox Laboratories Ltd. UK). GGT was determined colorimetrically using the method of Szasz, (1969) modified by Marghoob Hassan et al., (2013) also carried out with commercial Randox Kits.

Histopathological Assay

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). Jaijoy et al., (2011).

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°C. Highly pure helium gas (99.99% purity) was used as carrier gas, while HP-5ms (30mm X 0.25mm X 0.320µm) was used as the stationary phase. The oven temperature was at 80°C held for 4 min and ramped to 270°C at the rate of 3.5oC/min holding for 6 min. 1µl was auto injected.

Statistical Analysis

The SPSS v. 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 were evaluated using ANOVA, for comparison between sample means with level of significance assessed at 5% confidence interval also for multiple comparisons LSD was used.

RESULTS

Table 1 shows the comparative effect of pure and beverage alcohol on physical parameters of rats. Significant difference was observed with the body weight of animals administered beer, 40% pure alcohol and spirit. There was no difference observed with the feed intake irrespective of alcohol consumed. The water intake however, of animals administered red wine was significantly higher when compared with controls.

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

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>BODY WEIGHT (g)</th>
<th>FEED INTAKE (g)</th>
<th>WATER INTAKE (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D (SALINE)</td>
<td>102.3±5.49</td>
<td>50.5±1.34</td>
<td>123.5±10.82</td>
</tr>
<tr>
<td>A(5% PURE ALC)</td>
<td>106.8±5.94</td>
<td>51.3±1.12</td>
<td>76.12±3.01</td>
</tr>
<tr>
<td>A*(5.1% BEER)</td>
<td>122.4±6.70*</td>
<td>55.5±1.30</td>
<td>85.15±4.95</td>
</tr>
<tr>
<td>B(15% PURE ALC)</td>
<td>126.3±5.51</td>
<td>55.3±0.94</td>
<td>96.19±4.12*</td>
</tr>
<tr>
<td>B*(13% RED WINE)</td>
<td>108.1±3.19</td>
<td>41.3±1.83</td>
<td>99.0±3.71</td>
</tr>
<tr>
<td>C(40% PURE ALC)</td>
<td>111.8±4.12*</td>
<td>43.1±1.56</td>
<td>76.4±4.17</td>
</tr>
<tr>
<td>C*(40% SPIRIT)</td>
<td>120.5±5.35*</td>
<td>29.9±2.00</td>
<td>61.8±5.40</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± standard error of the mean (SEM). *P<0.05

Table 2 shows the comparative effect of alcohol consumed on the plasma activities of ALT, AST and GGT. No significant difference was observed with ALT activity , but significant difference was recorded with GGT and AST activities when compared with controls, (P<0.05).

Table 2. Plasma Activities of ALT, AST & GGT of rats administered alcohol, beer, wine and spirit for 28 days.

<table>
<thead>
<tr>
<th>TREATMENT GROUP</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SALINE</td>
<td>31.2±16.11</td>
<td>27.76±9.89</td>
<td>93.36±18.80</td>
</tr>
<tr>
<td>5% PURE ALC</td>
<td>32.7±4.21</td>
<td>138.0±14.44</td>
<td>98.14±10.02</td>
</tr>
<tr>
<td>5.1% BEER</td>
<td>30.45±9.69</td>
<td>79.68±20.52</td>
<td>6.61±16.17</td>
</tr>
<tr>
<td>15% PURE ALC</td>
<td>30.58±2.02</td>
<td>57.46±4.64</td>
<td>67.74±19.98</td>
</tr>
<tr>
<td>13% RED WINE</td>
<td>32.39±5.05</td>
<td>35.43±2.08</td>
<td>8.10±4.21</td>
</tr>
<tr>
<td>40% PURE ALC</td>
<td>37.77±3.29</td>
<td>17.15±6.91</td>
<td>82.80±5.30</td>
</tr>
<tr>
<td>40% SPIRIT</td>
<td>26.92±1.47</td>
<td>34.71±11.41</td>
<td>91.40±6.95</td>
</tr>
</tbody>
</table>

P-value          | 0.968     | 0.007*    | 0.011*    |

*Data are expressed as mean ± standard error of the mean (SEM), *P<0.05
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).

**Plate 1: Photomicrographs of cross sections of rat lungs (x100, 400)**

1A: normal lungs showing clear alveolar spaces 1B: show inflammatory cells I, few eosinophils E are noted, 1C: Moderate inflammatory cells and congestion within the interstitium. 1D, 1E: Severe inflammatory cells infiltrate within the interstitium.

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 (Plates 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 2: Photomicrographs of Cross section of livers of rats (x100)**

2A, 2B, 2C: showing normal study i.e. normal liver architecture i.e. normal portal vein normal glomeruli and normal tubules. 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 (Plate 3A), and also with the animals administered beer, 5% ethanol and red wine (Plates 3B, 3C, 3D). There was hypertrophy of the epithelial cells of the animals administered 40% alcohol and spirit, i.e. acute tubular necrosis (ACN), (Plates 3F and 3G).

**Plate 3: Photomicrographs of cross section of kidney of rats (x100, 200)**

3A, 3B, 3C: Normal glomeruli G and tubules T in Kidney i.e. normal study. 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 Study 3F, 3G: Ghost like appearance of the tubules T with sloughing off of the epithelial cells: Acute Tubular Necrosis

In Plate 4 below, 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).

**Plate 4: Photomicrographs of cross section of the Brain (x100, 400)**

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 with perinuclear halo and those of rats administered 40% ethanol, 4F: had Cerebrum and cerebellum i.e. neuronal bodies on a fine eosinophilic Neuropil N.

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Fig 2: GC-MS Chromatogram of Redwine

Fig 3: GC-MS Chromatogram of Spirit

Figures 1-3 are the chromatographic profiles of the 3 alcoholic beverages administered to the rats. Interpretation of these chromatograms on Table 3, 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.

Table 3. Gas Chromatography–Mass Spectrometry Peaks and Components of Beer, Wine and Spirit

<table>
<thead>
<tr>
<th>S/N</th>
<th>LIBRARY ID</th>
<th>RT(MINS)</th>
<th>AREA (%)</th>
<th>NATURE OF COMPOUND</th>
<th>LIBRARY ID</th>
<th>RT(MINS)</th>
<th>AREA (%)</th>
<th>NATURE OF COMPOUND</th>
<th>LIBRARY ID</th>
<th>RT [MINS]</th>
<th>AREA (%)</th>
<th>NATURE OF COMPOUND</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Azetidine</td>
<td>3.997</td>
<td>4.57</td>
<td>Heterocyclic</td>
<td>Aspartate</td>
<td>4.105</td>
<td>28.93</td>
<td>Heterocyclic</td>
<td>Aspartate</td>
<td>3.975</td>
<td>0.16</td>
<td>Heterocyclic</td>
</tr>
<tr>
<td>2</td>
<td>Oxalic acid</td>
<td>34.684</td>
<td>66.79</td>
<td>Organic acid</td>
<td>Oxalic acid</td>
<td>8.263</td>
<td>30.45</td>
<td>Organic acid</td>
<td>Oxalic acid</td>
<td>8.263</td>
<td>4.36</td>
<td>Organic acid</td>
</tr>
<tr>
<td>3</td>
<td>1H-Tetrazole-5-amine</td>
<td>8.168</td>
<td>7.78</td>
<td>Amine</td>
<td>1H-Tetrazole-5-amine</td>
<td>8.053</td>
<td>5.25</td>
<td>Amine</td>
<td>1H-Tetrazole-5-amine</td>
<td>29.047</td>
<td>0.24</td>
<td>Amine</td>
</tr>
<tr>
<td>4</td>
<td>Tetracenone</td>
<td>8.256</td>
<td>2.89</td>
<td>Hydrocarbon</td>
<td>1-Dodecanol</td>
<td>34.72</td>
<td>26.90</td>
<td>Alcohol</td>
<td>Benzy1 betanate</td>
<td>21.277</td>
<td>7.86</td>
<td>Ether</td>
</tr>
<tr>
<td>5</td>
<td>Tetracenol</td>
<td>34.235</td>
<td>3.02</td>
<td>Alcohol</td>
<td>1-Dodecanol</td>
<td>34.75</td>
<td>12.59</td>
<td>Alcohol</td>
<td>2-Methyl furfural</td>
<td>8.111</td>
<td>3.49</td>
<td>Heterocyclic</td>
</tr>
<tr>
<td>6</td>
<td>Tetracenol</td>
<td>34.328</td>
<td>3.748</td>
<td>Alcohol</td>
<td>1-Dodecanol</td>
<td>34.75</td>
<td>12.59</td>
<td>Alcohol</td>
<td>2-Methyl furfural</td>
<td>8.111</td>
<td>3.49</td>
<td>Heterocyclic</td>
</tr>
<tr>
<td>7</td>
<td>9- Octanone</td>
<td>36.363</td>
<td>2.133</td>
<td>Alcohol</td>
<td>1-Dodecanol</td>
<td>34.75</td>
<td>12.59</td>
<td>Alcohol</td>
<td>2-Methyl furfural</td>
<td>8.111</td>
<td>3.49</td>
<td>Heterocyclic</td>
</tr>
<tr>
<td>8</td>
<td>Eicosadiene</td>
<td>36.408</td>
<td>1.470</td>
<td>Unsatuated</td>
<td>Hydrocarbon</td>
<td>18.153</td>
<td>46.28</td>
<td>Amine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Elsinol</td>
<td>34.732</td>
<td>4.322</td>
<td>Alcohol</td>
<td>1-Dodecanol</td>
<td>34.75</td>
<td>12.59</td>
<td>Alcohol</td>
<td>2-Methyl furfural</td>
<td>8.111</td>
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<td>Heterocyclic</td>
</tr>
</tbody>
</table>

DISCUSSION

The body weight feed and water intakes of the alcohol paired groups, revealed that the animals 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., (2011) 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 (Nyblom et al., 2004). The present results were not in agreement with the clinical findings of Pari and Karthikesan (2007) 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 (Saravanan et al., 2006). In agreement with Ruppin et al. (1982) 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 an abnormality was recorded as the results varied irrespective of the alcoholic consumed. The GC-MS analysis of the 3 alcoholic beverages showed that beer contained 19 volatile...
concentrations, 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 Plutowoska et al., (2010). Most beverages are accepted mainly through taste 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 (Vamvakas et al., 1998).

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 report of Kershaw and Guidot, (2008), 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.

CONCLUSION

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 in these alcoholic beverages. 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.

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REFERENCES


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