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ORIGINAL ARTICLE

Comparative Effects of Some Alcoholic Beverages on Hepatic Parameters in Experimental Animals

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ABSTRACT
The relationship between exposure to a variety of alcoholic beverages and the concomitant effects on the liver was monitored applying Spectrophotometric technique; to achieve this, the activities, in international units, IU, of serum alkaline phosphatase (ALP), alanine aminotransferase (ALT) were assayed; similarly, the concentrations of albumin, total proteins (g/litre) and bilirubin (mMole/litre) were determined. Animals were exposed to the alcoholic beverages for seven (7) and fourteen (14) consecutive days respectively. Results obtained, except for ALT activity in both Protocols where palm wine was administered ($p>0.05$), indicated time-dependent increases in both serum enzymes' activities and serum concentrations of albumin, bilirubin and total proteins as values for the test groups were higher and statistically significant ($p<0.05$) compared to control groups values. Base on the results obtained from this work, these alcoholic drinks have deleterious effects on the liver albeit to different magnitude: Gin, Ogogoro and Goskolo ranked topmost followed by beer and then palm wine in that order. SPSS Statistical Software15.0 Windows was used and the student's t-test was chosen to analyse the data; P value 0.05 was considered significant.
***Key Words**: Dry gin, Goskolo, Ogogoro, Beer, Palm wine, Ethanol, Liver.*

INTRODUCTION

Alcoholic beverages vary considerably in ethanol content and in foodstuffs they are produced from. Most alcoholic beverages are broadly classified as fermented beverages; beverages made by the action of yeast on sugary foodstuffs or distilled beverages by distillation. Comparatively, dry gin contains higher ethanol content than palm wine and beers. The knowledge of ethyl alcohol (ethanol) consumption and its relationship to overall health is essential to the study of nutrition given its usage [1]; ethanol-containing products are prepared from appropriate substrates, usually cereals and other starch containing foods, just as tapping of palm-wine is common in Africa [2]. *Goskolo* and *Ogogoro* are locally distilled beverages without a standard scientific method of preparation which makes them extremely dangerous to health of the drinkers [3], *goskolo* in particular has low ethyl alcohol content but has caused the death of many drinkers to the extent that its preparation, sell and drinking has been banned within *Jos* metropolis; gin is usually a re-distilled ethyl alcohol of agric origin with initial strength of at least 96% [4] and is also consumed routinely by residents. Ethanol is rapidly absorbed by diffusion without a specific transport mechanism and therefore is the most efficiently absorbed of all energy sources [5]. Although often consumed traditionally and for "beneficial" purposes, it has deleterious effects on nutrient availability and on the heart, liver[6], brain, cardiovascular system, gastro-intestinal tract and other organs [7]; they also reported that ethanol leads to depletion of glutathione, hepatic vitamin A and its toxicity including that of β-carotene; furthermore, they reported that β-carotene supplementation in cigarette smokers that consume alcohol promoted pulmonary cancer as well as cardiovascular complications. Ethanol affects erythrocyte ion transport system and changes the fluidity of the erythrocyte membrane [8]; such changes to the properties of the membrane may be caused by direct effect of ethanol on the membrane or by formation of reactive metabolites of ethanol, which can damage membrane components oxidatively. Furthermore, ethanol produces many of its damaging effects by exerting specific actions on molecules that regulate key developmental processes (e.g., L1 cell adhesion molecule, alcohol dehydrogenase and catalase); it

interferes with the early development of midline serotonergic neurons and disrupts their regulatory-signaling function for other target brain structures. This way, it affects trophic factors that regulate neurogenesis and cell survival; it induces excessive cell death via oxidative stress or activation of caspase-3 proteases. As ethanol is broken down, dangerous highly reactive intermediates are generated for instance acetaldehyde; also, chronic ethanol consumption promotes absorption of iron from food in the intestine and facilitates storage of iron in the liver. Iron is an important catalyst of free radicals generation [9]. This study was therefore designed to monitor the effects of these alcoholic beverages with respect to volume of beverage and duration of exposure, on the liver of experimental animals.

MATERIALS AND METHODS

All chemicals used were of analytical grade obtained from BDH, OHAUS digital balance, refrigerated ultra centrifuge (Mistral-2L, MSE).

Spectrophotometric techniques were applied thus: [10] methods to assay for the transaminases, [11] method for the analysis of total proteins; albumin was analysed using [12] method, [13] method used for the determination of total and direct bilirubin, whereas [14] method used to assay for the activity of alkaline phosphatase.

EXPERIMENTAL ANIMALS

Thirty white male albino Wistar rats with mean weight 200.00g were used; they were orally fed once daily with standard feeds obtained from Grand Cereals Nigeria PLC, Jos, over a period of seven and fourteen consecutive days respectively.

Treatment

Animals were subjected to treatments based on the ethics and practices of the scientific community of Nigeria. The rats were divided into five groups of six each as follows: group I was the control, group II administered palm wine, group III fed dry gin, group iv fed bottled beer and group v was fed *ogogoro;* in all instance, samples were orally administered once daily continuously for seven and fourteen days according to body weight; hence mean volume of 4.3ml of appropriate samples were administered to each group except those in the control set up in which case they were fed 4.3ml distilled water. On the seventh day, 3 rats each were randomly picked and sacrificed after prior inducement of anaesthesia using chloroform. Blood samples were collected by direct cardiac puncture after longitudinal dissection and then allowed to stand for 5 minutes before spinning at 2,000 rpm for 10 minutes; thereafter, sera were collected using plastic droppers and used for analysis. This constituted protocol A. The remaining animals were fed continually with the beverages until the fourteenth day before subjecting them to the aforementioned treatment – this set constituted protocol B.

Statistical Analysis

The student's t-test was applied to analyse the results obtained; P value of 0.05 was considered significant

RESULTS AND DISCUSSION

Ethyl alcohol has pharmacological effect on the central nervous system which leads to incoordination, slowed reaction time and impaired judgment all of which last for several hours after its consumption [15]; [6] have reported that peroxidative damage to membrane lipids and oxidation of membrane protein thiols potentially harmful to membrane fluidity and flexibility is responsible for decreased resistance to haemolysis as demonstrated in women who consume alcohol. Its consumption is linked to a higher incidence of traumatic wounds and increases the risk for morbidity and mortality just as its acute exposure also impairs the proliferative response during healing, causing delays in epithelial coverage, collagen synthesis, and blood vessel regrowth [16]. Generally, the formation and degradation of reactive oxygen species occurs during normal cellular respiration and following toxic injury. Highly reactive species are generated and in the presence of electrons, oxygen forms the free radical: (O_2^{-})**.** Superoxide can be rapidly converted by superoxide dismutase to peroxide which, in turn, can generate highly reactive hydroxyl radicals in the presence of iron. Superoxide can also combine with nitric oxide to form hydroxyl and nitrogen dioxide radicals ($NO_2^{\cdot}$) via peroxynitrite anion ($OONO^{-}$). Macromolecular damage will result if reactive oxygen molecules are not neutralised. Catalase and glutathione remove reactive oxygen species (ROS) via enzymatic mechanisms that convert hydrogen peroxide to water and oxygen. Vitamins E and C prevent damage by scavenging ROS and lipid peroxyl radicals. Ethyl alcohol produces many of its damaging effects by exerting specific actions on molecules that regulate key developmental phenomena by interfering with the early development of midline serotonergic neurons and disrupting their regulatory-signaling function of other target brain structures, interfering with trophic factors that regulate neurogenesis and cell survival or inducing excessive cell necrosis through oxidative stress or activation of

caspase-3 proteases [17]. Raised activity of gamma-glutamyl transpeptidase (GGT) has been reported to be very high in cirrhotic individuals; so also in alcoholism which is related to structural liver damage [18]. Furthermore, it facilitates glutamine support of gut integrity; glutamine has protective effects against induced paracellular hyper permeability [19]. GGT is responsible for the extracellular catabolism of glutathione, the main thiol intracellular antioxidant agent in mammalian cells. It occurs, linked through a small lipophillic sequence of its larger subunit on the cell surface membrane of most cell types; although the same protein is produced in all tissues, differences in sugar moieties allow that only the liver GGT is detectable in serum [20]. Generally, the tissue-dependent level of enzyme in the blood is a function of the flux of extrication from the cell and that of inactivation, degradation and elimination in the blood plasma. Enzyme assay is of value in the diagnosis and monitoring of liver, bone, prostatic carcinoma and muscle disorders [21]; furthermore, contribute greatly to monitoring the effects of ethanol on body organs in both human and experimental research.

Table 1 gives the result for protocol A. The levels of all the parameters but ALT in the test groups were higher than the control group – indicating some degree of adverse effects on the liver which was also as for protocol B in table 2. From table 1, the level of albumin for the test groups indicated a more severe effect for group fed dry gin than the group fed palm-wine. Biosynthesis of albumin occurs exclusively in the liver. This protein serves as a regulator of osmotic equilibrium and animal plasma normally contains 25-35g/litre of albumin which constitutes about 56% of total protein concentration. Plasma and serum proteins act as anions in acid-base balance, coagulation reactions and as carriers for many compounds. Since the level of albumin for test groups was higher ($P<0.5$) than control values, it follows that consumption of these beverages could predispose to poor acid-base balance and hence shock, acidosis, impaired coagulation and transport of many compounds. In the case of total protein, the values of test groups were significantly ($P<0.05$) higher than control values with the magnitude being higher for group fed palm-wine than the group fed dry gin; furthermore, severity of effect was higher in protocol B: fourteen days of treatment than protocol A, seven days of treatment – the longer the duration of exposure to these beverages the more severe the effects on the liver.

Table 1: Results of the concentration of serum albumin (g/dm^3), bilirubin (µmol/l) including the activities of alanine amino transferase and gamma glutamyl transferase (IU) for the tests and control groups respectively after seven days of treatment

Type of Alcohol	Albumin	Bilirubin(Total)	ALP	ALT	GGT
Control	29.02±0.8	9.23±1.1	46.62±0.1	38.41±0.8	4.23±1.4
Dry gin	43.34±1.6 [a]	10.04±0.4[a]	106.34±0.9[a]	82.11±0.5 [a]	4.42±2.2[b]
Palm-wine	39.60±0.7 [a]	9.65±0.8[b]	124.33±0.8 [a]	37.74±0.9[b]	4.21±1.6 [b]

[a] significant increase ($p<0.05$) compared to control values
[b] increase not statistically significant ($p>0.05$) compared to control values
All values are means of three determinations (±SEM); n = 6

Sustained elevated activity of alkaline phosphatase could indicate disorders of the liver, bone or both and production is increased in response to cholestasis [22] and thus a sensitive marker/indicator of obstructive and space-occupying lesions of the liver which include neoplastic and infiltrative diseases. The group fed palm-wine had higher activity values over that fed dry gin when compared to control values ($P<0.05$) – implying both drinks could contribute, in conjunction with other factors, to the aforementioned disorders. In the case of ALT, whose activity could indicate hepatocellular damage and necrosis, hepatocyte proliferation or hepatocellular degeneration, with the exception of groups fed palm wine ($p>0.05$), tests groups had higher enzyme activity values ($P<0.05$) compared to control; groups fed dry gin having higher enzyme activity than group fed palm-wine. Bilirubin is an endogenous anion derived from haemoglobin degradation from erythrocytes and is elevated in jaundice and liver disease. From the result obtained, the group fed dry gin had higher concentration value than the group fed palm-wine albeit both were statistically

significant (P<0.05) with respect to the control group value; this could serve as a possible indicator, along with other parameters, of anaemia, haemolytic jaundice or defect in conjugation phenomenon even though most mild elevations of total bilirubin in asymptomatic individuals are due to increased levels of unconjugated or unprocessed bilirubin [23].

Table 2 gives the results where animals were also fed for seven days consecutively but with beer, *ogogoro* and *goskolo* in this case. Results indicated significant (P<0.05) increases over values for control group for all the parameters but ALT whose activity, though higher than control for the group fed beer, was not statistically significant (p>0.05). All others however were significantly higher (p<0.05) than control. Furthermore, the extents of increases were higher than for table 1 implying possible synergy between duration of exposure to these drinks and adverse effects on the liver; this could imply that the alcoholic beverages had different capacities to damage the liver----more so that they have different ethanol content. Considering albumin, group fed conventional beer had the highest impact followed by *ogogoro* and then *goskolo*. In the case of bilirubin, only group fed *goskolo* resulted in increase value compared to control. This could be a signal that continuous administration of *goskolo* and hence drinking it could result or predispose drinkers to jaundice and hepatic diseases. In the case of ALP, all treatments resulted in increased activity relative to the control albeit severities of effects were different; *goskolo* had the greatest effect followed by *ogogoro* and beer in that order. As for GGT, results obtained were all similar with that of the control.

Table 2: Results of the concentration of serum albumin (g/dm^3), bilirubin (µmol/l) including the activities of alanine amino transferase and gamma glutamyl transferase (IU) for the tests and control groups respectively after seven days of treatment.

Type of Alcohol	Albumin	Bilirubin(Total)	ALP	ALT	GGT
Control	42.46±0.2	11.36±0.1	46.33	33.4±0.2	5.03±0.2
Beer	45.30±0.6 [a]	10.86±0.2[a]	107.41 [a]	33.3.28±0.5 [b]	5.03±0.1 [b]
Ogogoro	45.22±0.8 [a]	8.53±0.7 [a]	126.02 [a]	105.25±0.2 [a]	5.03±0.2 [b]
Goskolo	44.46±0.6 [a]	20.11±0.7 [a]	141.11 [a]	117.27±0.5 [a]	5.03±0.3 [b]

[a] significant increase (p<0.05) compared to control values
[b] increase not statistically significant (p>0.05) compared to control values
All values are means of three determinations (±SEM); n = 6

Table 3 gives the result for fourteen days of administering the drinks. In all instances, treatment resulted in increased levels of the parameters in test groups relative to control (p<0.05); dry gin appeared to be more deleterious relative to group fed palm wine. This did not however include GGT whose activity values were constant in all the groups.

Table 3: Results of the concentration of serum albumin (g/dm^3), bilirubin (µmol/l) including the activities of alanine aminotransferase and gamma glutamyl transferase (IU) for the tests and control groups respectively after fourteen days of treatment.

Type of Alcohol	Albumin	Bilirubin (Total)	ALP	ALT	GGT
Control	40.62±0.4	12.21±0.2	74.01±0.7	23.60±0.4	2.83±2.3
Dry gin	180.10±0.8 [a]	13.91±1.2[a]	554.04±0.8 [a]	25.41±1.3[a]	2.84±1.2 [b]
Palm-wine	133.01±0.6 [a]	12.01±1.1[b]	119.44±0.8 [a]	24.01±0.9 [b]	2.85±1.1 [b]

[A] significant increase (p<0.05) compared to control values
[b] increase not statistically significant (p>0.05) compared to control values
All values are means of three determinations (±SEM); n = 6.

In table 4, the group fed beer had values less than even the control value in the case of ALT but *ogogoro* resulted in significant increase ($p<0.05$) over control except for GGT. Group fed *goskolo* had values higher ($p<0.05$) than the control excluding GGT and albumin.

Table 4: Results of the concentration of serum albumin (g/dm^3), bilirubin (µmol/l), alanine amino transferase and gamma glutamyl transferase (IU) for the tests and control groups respectively after fourteen days of treatment.

Alcohol	Albumin	Bilirubin (Total)	ALP	ALT	GGT
Control	30.75±0.4	10.17±0.1	75.21	30.52±1.7	2.85±0.1
Beer	29.51±2.8[a]	10.45±0.5[a]	122.68[a]	16.25±1.6[b]	2.85±0.1[b]
Ogogoro	45.34±2.5[a]	19.92±0.3[a]	298.44[a]	44.55±2.2[a]	2.85±0.6[b]
Goskolo	27.39±0.4[a]	10.32±0.2[a]	498.25[a]	31.72±0.8[a]	2.85±0.6[b]

[a] significant increase ($p<0.05$) compared to control values
[b] increase not statistically significant ($p>0.05$) compared to control values
All values are means of three determinations (±SEM); n = 6.

CONCLUSION

Whereas there are several other factors: markers, isoenzymic nature of the enzymes assayed for in this study and therefore the need to explore them to have a concrete basis for categorical inferences, those who consume these drinks, especially *goskolo*, dry gin and *ogogoro* which have higher ethanol content than palm-wine, stand the risk of depletion of hepatic vitamin A and hence impaired vision [24], toxicity of β-carotene and vitamin A [7], poor absorption of amino acids from the intestines [5] and impaired cellular metabolism of lipids which contribute to hepatic damage [9]. However, due to their isoenzymic nature, increase in the activities of these enzymes may not necessarily indicate a disease affecting one tissue or organ; but this does not obliterate the fact that consumption of these drinks could lead to malnutrition as nutrients such as amino acids and lipids become more unavailable to the cell owing to poor absorption from the stomach/intestines in the presence of ethyl alcohol as well as liver diseases.

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