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EFFECTS OF VARIOUS ALCOHOLIC BITTERS ON THE HEPATOLOGICAL INDICES OF ALBINO WISTAR RATS

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ABSTRACT

The liver is one of the vital organs in the body and it is known to perform well over 500 physio-biochemical roles. The consumption of various forms of alcohol is known as one of the major causes of plethora of liver diseases globally. This study investigated the effect of various alcoholic bitters on the liver using the hepatic enzymes panel. Forty two (42) albino rats of wistar strains were used for the studies and were divided into seven (7) groups of six (6) animals each. Group 1 served as normal control and were treated with distill water, group 2 were treated with action bitters (AB), group 3 were treated with alomo bitters (ALB), group 4 were treated with origin bitters (OB), group 5 were treated with 1960 bitters, group 6 which was the alcoholic control were treated with Local gin (LG) and group 7 were treated with Local gin + B complex. The animals were treated with 2.68ml/kg body weight of alcohol equivalent of a 70kg man consuming one bottle daily. The treatment was done twice daily and it lasted for the period of thirty (30) days after which the animals were sacrifice and blood was obtained via cardiac puncture. The blood was allowed to stand and clot and then centrifuge to obtain serum. The serum was use for the biochemical assay and results obtained shows that the AST activities of animals in group II (54.5±3.96), III (60.24±1.98), IV (54.67±2.41), V (57.33±2.80), VI (95.52±9.95) and VII (67.96±4.81) shows a significant ($p<0.05$) increased when compared with the normal control (13.22±1.20). More so, the ALT activities in group II (38.83±3.67), III (43.22±4.99), IV (42.14±4.81), V (40.95±3.90), VI (62.81±5.73) and VII (38.37±5.12) also recorded a significant ($p<0.05$) increased compared with the normal control (24.41±1.98). The ALP activities were also significantly ($p<0.05$) higher in group II (215.99±10.82), III (202.36±37.44), IV (220.25±12.56), V (204.09±12.36), VI (323.32±23.61) and VII (216.59±10.45) compared with the normal control (77.67±11.33). The GGT activities shows a significant ($p<0.05$) increased in group II (51.81±4.31), III (43.55±4.84), IV (54.88±3.07), V (47.57±4.37), VI (66.20±5.75) and VII (43.04±4.37) when compared to the normal control (23.95±2.99). The Total serum protein level also showed a significant ($p<0.05$) decreased in group II (4.80±0.52), III (4.46±0.50), IV (4.91±0.74), V (4.37±0.63), VI (2.31±0.55) and VII (3.03±0.48) when compared with the normal control (6.56±0.48), while serum albumin level of animals in group II (3.87±0.38), III (2.58±0.50), IV (3.69±0.49), V (3.61±0.28), VI (1.52±0.33) and VII (2.77±0.29) showed a significant ($p<0.05$) decreased compared with the normal control (5.53±0.63). The results suggest therefore that consumption of alcoholic bitters has unpleasant effects on the liver just like with every alcoholic beverages. Aside the immediate effects of too drinking, such as nausea, vomiting, blackouts, memory loss and anxiety, continuous and heavy drinking over longer periods of time may results in hepatic damage which may then affect other vital biochemical process which is dependent on the liver functions. Hence, these bitters should be consumed with great caution.

KEYWORDS: Hepatological parameters, Cardiac puncture, Albino rats, Alcohol abuse, Brain damage.

INTRODUCTION

The therapeutic use of herbal medicine started about 5000 years ago by the Indian, Egyptians, Greek, Chinese, Roman and Syrian (Emaleku, 2018). They presumably became aware that strange change (metamorphosis) occurred when many fruits, cereals, berries and other plants materials were mixed with water and left in the warmth of the sun. The product made was the first meads; perhaps the earliest beverages known to humans (Abugri and Pritchett, 2013). These products were made from fermentation of honey (meads), fruits and berries (wines) and those from cereals were the first beers (Adedara and Farombi, 2013). These beverages were known, accepted and prized as foods, as magic fluids and as medicines (Alabi *et al., 2013)* until the 19th and early 20th century when there was a steady decline in the therapeutic use of herbal medicine. Plants serves as a basic for medical treatment and different parts have been

a source of herbal medicine which has been shown to be effective to about 80% of population as primary healthcare (Adeyemi *et al., 2012*). Therefore, the medicinal plants are the most important source of life saving drugs for the majority of the world's population.

According to World Health Organization (WHO) herbal medicine is a therapeutic practice that have been in existence for hundreds of thousands of years ago before the development and spreading of modern medicine and are still in use today (Tripathi and Tripathi, 2003). Traditional medicine including herbal drugs played a vital role in the past and may continue to do so in the future (Igbokwe *et al., 2017*). Recently, consumers of alcohol preferred local herbal concoctions in the name of bitters which mimic alcoholic beer of international standard; the age-long consumption of beer was on its way out of fashion. This is because consumers of bitters believe that it contains body purifiers, anti-malaria and contain ingredients that strengthen the virility of men, anti-diabetic, hypo-lipidemic and non-toxic to the kidney (Chineke *et al.*, 2015), Alcoholic beverages such as Alomo bitters, Action bitters, Origin bitters, Pasa bitters, Osomo bitters etc. makes an impact in the alcoholic beverages market despite the initial fears over the hygiene level of their product and their Composition (*Chineke et al., 2015*). The claim that it is restorative and sex energy boosting, continue to lure customers to patronize them in mass. The term 'bitters' as it is used today, is a beverage, often alcoholic, flavored drink with herbal essences that gives it a bitter or bittersweet flavor (Bella *et al*., 2012). The generic term applies for all bitter liquors and herbal bitters. Bitters are produces from root extracts and herb, from the narcotic content of (primarily) tropical and subtropical plant and spices (Bella *et al.*, 2012). Bitters are also made up of numerous groups of chemical compounds extracted from the herbs and roots (medicinal plant) (Oluyomi and Bukola, 2014).

Alcoholic bitters with individuals with an alcoholic bitters use disorder will often complain of difficulty with interpersonal relationships, problems at work or school, and legal problems. Additionally, people may complain of irritability and insomnia (fuster and jeffrey, 2018). Alcohol abuse is also an important cause of chronic fatigue (alchmuller and soykam, 2015).

Signs of alcoholic bitters abuse are related to alcohol's effects on organ systems. However, while these findings are often present, they are not necessary to make a diagnosis of alcohol abuse. Signs of alcoholic abuse shows drastic effect on the central nervous system including g inebriation and poor judgment; chronic anxiety, irritability, and insomnia (alchmuller and soykam, 2015). This study evaluated the effect of various alcoholic bitters on hepatological indices of albino wister.

Traditional medicine in Nigeria like in other developing countries where medical facilities cannot satisfy their national demands plays an important role in combating both human and animal diseases (Nerukar *et al.*, 2006). It was estimated that about 80% of the people who live in the rural areas, rely on the traditional medicine healers for the treatments of their medical problems, using medicinal herbs. (Teoh *et al*., 2010). However, the liver and kidney and haemato-biochemical effects of this plant have not yet been fully studied, tested or documented. Most of the information is still in the hands of the traditional healers, therefore this study becomes necessary because there is excessive abuse of *various alcohol bitters* plant by the rural dwellers in different parts of Nigeria for many of their health problems, and there is no documented information about the effect safety of these plants available for the rural dwellers. Findings from this study have provided vital information about the effects of *various alcohol bitters* (bitter melon) on the hepatogical safety parameters.

MATERIALS AND METHODS

Chemicals and reagents

Biochemical assay kits used for this analysis were obtained from three separate laboratories: Dialab, Randox and Teco diagnostics. Other chemicals include Action bitters, Alomo bitters, Origin bitters and 1960 bitters obtained from Lagos, Nigeria, while the Local gin was obtained from a local drinking joint in Otuoke, and the vitamin B-complex was obtained from Otuoke pharmacy, Otuoke, all in Bayelsa State. Chloroform, Normal saline and distilled water of analytical grades were also used.

Experimental Animals

Forty two (42) male rats of albino strain weighing 80-120g were used for this study. The rats were obtained from the pharmacology Department, Faculty of Basic Medical Sciences of the University of Port Harcourt, and were used for the study. The animals were allowed one week acclimatization period after which they were reweighed and housed in plastic cages with plastic bottom and wire-mesh top, under controlled environmental conditions of temperature (28 ± 2^0C), relative humidity (50±5%) and a twelve hour light/dark cycle. The animal facility was adequately ventilated and the animals maintained regularly on the commercial rat chow. Tap water and food were provided *ad libitum* throughout the experimental period.

Experimental Design and Treatment of Animals

The experimental design employed consisted of 42 wistar rats of albino strain divided into 7 groups of 6 animals each. Group 1 is the normal control, and received distilled water, group 2 received Action bitters, group 3 received Alomo bitters, group 4 received Origin bitters, group 5 received 1960 bitters, while group 6 is the alcoholic control and received local gin while group 7 received local gin plus vitamin B-complex twice daily at a dose equivalent of one bottle per day (70kg body

weight each) at a time. The experimentation lasted for thirty days (1 month).

Blood Sample Collection and Preparation

At the end of the experiment, the animals were sacrificed 12 hour after the last administration and blood (5ml) obtained from each of them via cardiac puncture into non-heparinized plane test tubes. The blood sample (5ml) in non-heparinized plane test tubes were allowed stand for two (2) hours and clot after which were centrifuged with the table top centrifuge (Englan MNIS 105310) and the serum collected with a pasture pipette into a sterile container and stored in the refrigerator from where the biochemical parameters of interest were analyzed.

Table 1: Experimental Design and administration of alcoholic bitters.

Groups	Number of animals	Administration
1(Normal Control)	6	Normal saline
2	6	Action bitters (2.68ml/kg bw)
3	6	Alomo bitters (2.68ml/kg bw)
4	6	Origin bitters (2.68ml/kg bw)
5	6	1960 bitters (2.68ml/kg bw)
6 (alcoholic control)	6	Local gin (2.68ml/kg bw)
7	6	Local gin (2.68ml/kg bw) + vit. B-complex

Biochemical assay

All biochemical assays; Aspartate Aminotransferase, Alanine Aminotransferase, Alkaline Phosphatase, Gamma glutamyl-transferase activities, total protein and albumin concentrations were carried out using standards kits, methods and an AJ-Semi-auto Biochemical Analyzer.

Statistical Analysis

Data obtained was expressed as mean ± SEM and analysis was done Statistical package for Social Scientists (SPSS version 21.0). Values at $p<0.05$ were considered significant in comparison with appropriate control.

RESULTS

Effect of treatment on Liver enzymes

The effect of alcoholic bitters on the hepatocellular indices was evaluated and the results are presented in table 2 and 3.

The result reveals that the AST activity (iu/l) of animals in group II (54.5±3.96), III (60.24±1.98), IV (54.67±2.41), V (57.33±2.80), VI (95.52±9.95) and VII (67.96±4.81) showed a significant ($p<0.05$) increase when compared to the normal control (13.22±1.20). However, the result also shows that in all the treated groups no significant ($p>0.05$) difference when compared with each other.

Results also showed that the ALT activity of animals in groups II (51.81±4.31), III (43.55±4.84), IV (54.88±3.07), V (47.57±4.37), VI (66.20±5.75) and VII (43.04±4.37) have a significant ($p<0.05$) difference when compared to the normal control (23.95±2.99). Furthermore, the result also shows that all the groups have a great increase when compared with the normal control.

The ALP results obtained showed that the activity of ALP in groups I (77.67±11.33), II (215.99±10.82), III (202.36±37.44), IV (220.25±12.56), V (204.09±12.36) and VII (216.59±10.45) have a great decrease when compared to group VI (323.32±23.61). Moreover, the result showed that groups II (215.99±10.82), III (202.36±37.44), IV (220.25±12.56), V (204.09±12.36), VI (323.32±23.61) and VII (216.59±10.45) have a significant ($p<0.05$) difference when compared to the control.

The TP for AB, ALB, OB, 1960, LG and LGB with values 4.80±0.52g/dl, 4.46±0.50g/dl, 4.91±0.74g/dl, 4.37±0.63g/dl, 2.31±0.55g/dl and 3.03±0.48g/dl respectively have a significant difference at $p<0.05$ when compared with the normal control 6.56±0.48g/dl.

For the Albumin, the values for AB, ALB, OB, 1960, LG and LGB are 3.87±0.38 g/dl,2.58±0.50 g/dl, 3.69±0.49 g/dl, 3.61±0.28 g/dl, 2.77±0.29 g/dl and 1.52±0.33 g/dl respectively were observed to have a significant difference at $p<0.05$ when compared to the normal control 5.53±0.63 g/dl.

Table 2: Effect of hepatological Parameters of albino rats (AST, ALT, ALP).

GROUP	AST (iu/l)	ALT (iu/l)	ALP (iu/l)
NC	13.22±1.20	24.41±1.98	77.67±11.33
AB	54.5±3.96*	38.83±3.67*	215.99±10.82*
ALB	60.24±1.98*	43.22±4.99*	202.36±37.44*
OB	54.67±2.41*	42.14±4.81*	220.25±12.56*
1960	57.33±2.80*	40.95±3.90*	204.09±12.36*
LG	95.52±9.95*^{abcd}	62.81±5.73*^{abcd}	323.32±23.61*^{abcd}
LGB	67.96±4.81*^{e}	38.37±5.12* ^{e}	216.59±10.45* ^{e}

Values are expressed as: Mean ± standard error of mean (SEM), n=6, * significant at p<0.05 compared with group 1: (NC), a = significant at p<0.05 compared with group 2: (AB), b = significant at p<0.05 compared with group 3: (ALB), c = significant at p<0.05 compared with group 4: (OB), d = significant at p<0.05 compared with group 5: (1960), e = significant at p<0.05 compared with group 6.

Key: AB = Action Bitters, ALB = Alomo Bitters, OB = Orign Bitters, LG = Local Gin, LGB = Local Gin + B complex, NC = Normal control, AST = aspartate aminotransferase, ALT = Alanine aminotransferase, ALP = Alkaline phosphatase.

Table 3: Effect of hepatological Parameters of albino rats (GGT, Total protein, Albumin).

GROUP	GGT (iu/l)	TP (g/dl)	ALB (g/dl)
NC	23.95±2.99	6.56±0.48	5.53±0.63
AB	51.81±4.31*	4.80±0.52*	3.87±0.38*
ALB	43.55±4.84*	4.46±0.50*	2.58±0.50*
OB	54.88±3.07*	4.91±0.74*	3.69±0.49*
1960	47.57±4.37*	4.37±0.63*	3.61±0.28*
LG	66.20±5.75*^{abd}	2.31±0.55*^{abcd}	1.52±0.33*^{acd}
LGB	43.04±4.37* ^{e}	3.03±0.48*^{ac}	2.77±0.29* ^{e}

Values are expressed as: Mean ± standard error of mean (SEM), n=6, * significant at p<0.05 compared with group 1: (NC), a = significant at p<0.05 compared with group 2: (AB), b = significant at p<0.05 compared with group 3: (ALB), c = significant at p<0.05 compared with group 4: (OB), d = significant at p<0.05 compared with group 5: (1960), e = significant at p<0.05 compared with group 6.

Key: AB = Action Bitters, ALB = Alomo Bitters, OB = Orign Bitters, LG = Local Gin, LGB = Local Gin + B complex, NC = Normal control, GGT = Gamma Glutamyl Transferase, TP = Total Protein, ALB = Albumin.

DISCUSSION

The liver is the target organ of most toxins and primary site for detoxification. It is also an organ for intense metabolism and is therefore prone to various disorders as a consequence of exposure to toxins of extrinsic as well as intrinsic form. The liver plays an important role in metabolism to maintain energy level of structural stability of the body. (Guyton and kensler, 2002). It is also a site of biotransformation by which a toxic compound has been transformed to less harmful form to reduce toxicity (Hodgson, 2004).

The liver is particularly susceptible to alcohol-related injury because it is the primary site of alcohol metabolism. As alcohol is broken down in the liver, a number of potentially dangerous by-products are generated, such as acetaldehyde and highly reactive molecules called free radicals. (Smart *et al*., 2002.).

Most of the alcohol that are consumed are metabolized in the liver. The major pathway for alcohol metabolism involves the enzyme alcohol dehydrogenase (ADH). This enzyme converts alcohol to acetaldehyde through a chemical process called oxidation (Tsutsumi *et al*., 2004). Acetaldehyde is highly toxic to the body, even in low concentrations. Normally, however, the enzyme aldehyde dehydrogenase (ALDH) rapidly oxidizes acetaldehyde to acetate. Most of the acetate travels through the bloodstream to other parts of the body, where it can enter other metabolic cycles (Lieber, 2004) that produce energy or useful molecules. The usual biological role of both ADH and ALDH is to metabolize vitamin A (i.e., retinol). In this study, prolonged administration of the alcoholic bitters significantly increased the alanine aminotransferases, aspartate aminotransferases, gamma glutamyl transferase and alkaline phosphatase but show decrease in total protein and total albumin when compared to their respective control.

The serum activities of aminotransferases is a measure of the concentration of intracellular hepatic enzymes leaked into the circulation, thus serving as a marker of

hepatocyte injury (Shelli, *et al*., 2013). Liver contains aspartate amino transaminases (AST) and alanine amino transaminases (ALT) as hepatobillary enzymes in high concentrations which are released into the blood circulation following hepatic damage and necrosis. This results in raised serum concentration of these enzymes as a result of cellular breakage and loss of functional integrity of cell membranes in liver tissues, making both enzymes' indicators of possible liver damage/toxicity (Attiah *et al*., 2013).

The finding of the study shows that there is a significant increase in AST, ALT, GGT and ALP observed with the administration of AB, ALB, OB, 1960, LG and LGB when compared with the control and LG of the treated group. This can lead to many liver diseases including cirrhosis, alcohol related liver disease (ARLD) for chronic consumers.

Nevertheless, the activities of ALT outside the liver cell are very low and therefore ALT is considered more specific for the hepatocellular damage than other enzymes (Emeka *et al*., 2009) isolated significant increase in AST may have been extrahepatic as this enzyme is also found in other tissues such as kidney, brain, pancreas, lungs, cardiac and skeletal muscles (Kasper *et al*., 2005).

Furthermore, the total protein and serum albumin level were generally reduced. There are significant difference observed with the administration of AB, ALB, OB, 1960, LG and LGB in all the treated groups as compared with the control and LG.

Data suggest that alcohol consumption may inhibit protein synthesis, especially in the heavy drinkers. The binding and transport of substances by albumin, such as bilirubin, drugs and other metabolites could be affected by heavy or moderate alcohol consumption (Wardlaw *et al*., 2002).

Finally, there are many health risks of chronic alcohol abuse, ranging from high blood pressure to stroke. People are most familiar with alcohol's negative effects on the liver. Heavy drinkers have an increased risk of jaundice, cirrhosis, liver failure, liver cancer, and many other conditions.

CONCLUSION

Heavy and long-term alcoholic bitters consumption may results in plethora of liver diseases in susceptible individuals. However, the fact that only a portion-albeit a relatively large one of heavy drinkers develop alcohol hepatitis or cirrhosis indicates the importance of heredity, gender, diet, and other forms of liver disease as influences over the risk for alcoholic liver diseases. Most of the liver damage caused by alcohol is attributed to alcohol metabolism and the by-products of that metabolism. Progressive liver injury whether toxic, inflammatory, or both can lead to fibrosis and ultimately to cirrhosis. Hence, alcoholic bitters should be consumed with great caution.

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