Research Article

Modulation of Biochemical Responses in Rats Following Consumption of Some Herbalized Nigerian Alcoholic Drinks

Odey, M. O[1], Ibor, O.R[2]*, Ujong, U. P[3], Chukwuka, A. V[4], Andem, A. B[2]

[1]*Department Biochemistry, Faculty of Basic Medical Sciences, University of Calabar, Calabar, Nigeria*
[2]*Department of Zoology and Environmental Biology, University of Calabar, Calabar, Nigeria*
[3]*Department of Medical Biochemistry, Cross River University of Technology, Calabar, Nigeria*
[4]*National Environmental Standards and Regulations and Enforcements Agency (NESREA), Field Office Osogbo, Osun State, Nigeria*

ABSTRACT

The unconfirmed perception of herbal-based alcoholic beverages as drinks of medicinal, antimalarial and aphrodisiac value have resulted in increased intake of thwiese products in many developing countries. Herein, we investigated biochemical and physiological responses in rats of some commonly consumed herbalized alcoholic beverages in Nigeria. Male Wistar rats were treated with Action bitters (AcB), Alomo bitters (ALB), Origin bitters (OrB), 1960 bitters, Local gin (LG), Local gin plus Vitamin B (LG+VtB) daily at a dose of 2.68 mL/kg bw. A control group was given distilled water. Serum biochemical parameters; cholesterol, triacylglycerol, high and low density lipoprotein (H-LDL), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), gamma-glutamyl transferase (GGT) and electrolytes; potassium (K_+), sodium (Na_+), chloride (Cl_-) and bicarbonate (HCO_{3-}) were measured after 90 days treatment. There were significant increases in serum cholesterol, triacylglycerol, H-LDL, AST, ALT, ALP, LDH and GGT in alcohol treated groups compared with control suggesting hepato-toxicity. The Levels of (K_+), sodium (Na_+), and bicarbonate (HCO_{3-}) significantly decreased in treatments groups compared with control indicating negative physiological consequences on serum electrolytes including hypokalemia, hyponatremia and osmotic balance. Principal component analysis (PCA), revealed a positive relationship between LG treatment group with cholesterol, triacylglycerol, AST, ALT, ALP, GGT, and LDH) indicating that LG induced more pronounced biological effects compared with other tested alcoholic beverages. Overall, the increase in cholesterol, triacylglycerol and LDL with a corresponding decrease in HDL in treatment groups compared with control may suggest a probable mode of action and provide a mechanistic insight by which alcoholic beverages induce coronary liver and heart disease.

Keywords: Alcoholic beverages, Biochemical parameters, Electrolytes, Hepatotoxicity, Wistar rats

Author for correspondence: E-mail: iborrichard@unical.edu.ng; Tel. +234 8034180445

INTRODUCTION

Chronic consumption of locally produced herbal-based alcoholic beverages and it potential downstream physiological and biochemical effects remains a serious problem in many developing countries including Nigeria. This is because, many local populace depend solely on such beverages as a source of medication, deriving pleasure and recreation without considering the potential harmful biological effects of chronic intake. It is generally believed that chronic alcohol consumption can possibly result in several diseases, including coronary heart disease (CHD) (O'Keefe et al., 2007; Mehlig et al., 2014), liver disease (Lewis, 2006; Liamis et al., 2000) and responsible for about 5.9% of global deaths (Clarke et al., 2017). Also, causal relationship has been proposed between chronic alcohol consumption and a number of biological pathways including metabolism, detoxification, electrolyte (ionic regulations including:-Sodium, Potassium, Chloride and Bicarbonate), cholesterol balance and the general low and high density lipoprotein-cholesterol – an important biological pathway regulating reverse transportation of cholesterol (Lewis, 2006; Liamis et al., 2000). Some of the effects of alcoholism such as impairment of cognitive control of behavior (Crews and Boettiger, 2009), activation of

neurodegenerative processes (Vetreno and Crews, 2015), liver disease and damage including steatosis, hepatitis, cirrhosis and hepatocellular carcinoma (O'Shea et al., 2010; Friedmann, 2013; Rehm et al., 2013) have been reported.

Alcohol is a psychoactive drug that provides energy (7.1 kcal/g), however, excessive intake can increase the risk of weight-gain and the development of obesity or malnutrition (Vasanthi et al., 2012). Many biological effects have been related to adverse alcohol consumption including those of the central nervous system, liver, kidney, lipid metabolism and cardiovascular disease (Ruffle, 2014). Also, changes in lipid profile may result in liver dysfunction and hepatocellular damage and these has been linked with long-term alcohol consumption (Gupta et al., 2010; Manjeshwar et al., 2010). Interestingly, the alcohol in alcoholic beverages is ethanol (grain ethanol) which is considered as a toxin in cases of excessive consumption and can result in liver cirrhosis (Ginsberg et al., 2010). Alcoholic drinks (largely water, ethanol and sugar) are often considered empty caloric beverages because they contain none of the essential nutrients that are needed in the body for cellular respiration, and the caloric energy provided by alcohol comes from carbohydrate (Losowsky et al., 2009), implying that calories from alcohol has less value to the body than a calorie from carbohydrates. This is so because, the energy derived from alcohol is completely used up by the body's metabolic processes in the liver, leaving no energy to be used or stored by the body (Nestel, 2015; Castelli, 2010). Chronic alcoholics suffer from malnourishment resulting from lack of essential nutrients in the body and inability to digest and absorb essential nutrients required for cellular metabolism (Priyabrata et al., 2010). It has also been proposed that 50% of the total daily calories is replaced by ethanol in alcoholics (Petticrew et al., 2017) suggesting nutritional deficiencies (Bujanda, 2000) and decreased antioxidant status (Lieber, 2000)

The metabolism of alcohol in the liver by alcohol dehydrogenase and microsomal ethanol-oxidizing system (MEOS) generates the production of toxic metabolites which interferes with the metabolism of the body's essential nutrients, and the accumulation of these toxins processed in the liver may result in alcohol liver disease or hepatic damage (Strogner et al., 2014). It has been proposed that hepatic damage resulting from chronic alcohol consumption may result from the formation of metabolites such as acetaldehyde, formed during the oxidation of ethanol in the mitochondria of the cell (Cahill et al., 2002), and the likelihood of chronic liver damage has been linked with chronic drinking lifestyle and the quantity of alcohol consumed (Collins and Kirouac, 2013). Alcoholic liver diseases resulting from the accumulation of fats in the liver can progress into hepatic fibrosis and cirrhosis (Harrison and Diehl, 2002). Other reports have demonstrated that acute and chronic alcohol consumption can result to intestinal barrier dysfunction with increased translocation of bacterial endotoxin and this can result in the activation of hepatic kuffer cells, formation of reactive oxygen species and increased tumor necrosis factor in the liver (Hoek and Pastorino, 2002).

Despites all these reports on the adverse effects of chronic alcohol consumption, alcohol intake remain on the increase and an issue of human health concern, especially around the African sub region. Recently, several products from different manufacturers of alcoholic beverages, fortified with diverse kinds of herbs and plant products such as alomo bitters, action bitters, origin bitters, 1960 bitters and local gin has gained wide acceptance in the market in Africa and widely consumed with their active components still unknown. These products have unconfirmed claims suggesting their nutritional and medical importance because they are assumed to cure and/or ameliorate several known ailments such as waist pain, menstrual cramps, cardiovascular disorders, digestive difficulties, antimalarial and aphrodisiac including the production spermatocytes in males. Despite all these reports, there is little or no information on the possible biochemical and toxicological effects of the chronic consumption of these products. Therefore this study was aimed at investigating the physiological, biochemical and toxicological effects of chronic consumption of these products in a mammalian model.

MATERIALS AND METHODS

Chemicals and reagents: Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP), Cholesterol, Triacylglycerol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), lactate Dehydrogenase (LDH), gamma-glutamyl transferase (GGT), Potassium, Sodium, Chloride and Bicarbonate kits were purchased from Randox Laboratories Limited, United Kingdom (UK). Alcoholic drinks; Action bitters (AcB), Alomo bitters (ALB), Origin bitters (OrB), 1960 bitters, Local gin (LG) and Vitamin B-complex were all purchased from a commercial store in Calabar, Nigeria. All other chemicals were of the highest commercially available analytical grade.

Experimental animal's acclimation and handing procedure: All experimental procedures were reviewed, approved and performed in accordance with the Cross River University of Technology's Ethics Committee guidelines. A total of forty two (42) male Wistar rats weighing 100 –120 g were obtained from the animal house of the Department of Medical Biochemistry, Faculty of Basic Medical Sciences of Cross River University of Technology. Wistar rats were maintained in the laboratory at 28±2.0 °C, Relative humidity of 50.0±5.0 % under a 12:12 h light and dark photoperiod, fed *ad libitum* with vital feed and tap water and maintained in the laboratory for two weeks. After fourteen (14) days acclimatization period, rats were divided into seven experimental (7) groups of six (6) animals each, the experimental control group received distilled water, while other groups received oral intubation of Action bitters (AcB), Alomo bitters (ALB), Origin bitters (OrB), 1960 bitters, Local gin (LG) and Local gin plus Vitamin B-complex (LG+VtB) daily at a dose of 2.68 mL/kg for ninety days. The dose was determined based on the assumption of four (4) adults with a total body weight of 280 kg (70 kg each) consuming 75 cl of the administered alcoholic beverage.

Sample collection: Body weight of rats was taken weekly with a digital weighing balance (Kerro-BL20001) and at end of the 90 days exposure rats were anaesthesized on chloroform vapour and blood collected via cardiac puncture of the left

ventricle using a 5 mL syringe. Blood samples were immediately preserved on ice and thereafter centrifuged at 3000 rpm for 15 min using the table top centrifuge (0412-1 Cole Medical Instrument Co. Ltd, England), serum was collected and preserved for other biochemical and physiological analysis.

Serum biochemical enzymatic assays: Serum biochemical enzymatic activities of Aspartate aminotransferase (AST), Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) were measured using kits purchased from Randox Laboratories Limited, United Kingdom (UK). Briefly, the AST activity was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenylhydrazine at 546 nm. Alanine aminotransferase activity was measured by monitoring the concentration of pyruvate hydrazine formed with 2,4-dinitrophenylhydrazine at 546 nm. Alkaline phosphatase was determined by measuring the increase in absorbance due to increase in the formation of *p*-nitrophenol reaction in the sample at 405 nm. Also, the levels of total Cholesterol, Triacylglycerol, High Density Lipoprotein (HDL), Low Density Lipoprotein (LDL), lactate Dehydrogenase (LDH) gamma-glutamyl transferase (GGT), Potassium, Sodium, Chloride and Bicarbonate were measured in serum using kits purchased from Randox Laboratories Limited, United Kingdom (UK) following the manufacturer's protocols.

Statistical analysis
Data obtained was tested by One-way ANOVA followed by post-hoc analysis (Duncan's multiple range test) between exposure concentrations and control group, values were considered significantly different at $p<0.05$. Statistical analysis was performed using the Prism GraphPad 5 (GraphPad software, La Jolla, USA). The relationship between biochemical, physiological responses and different alcohol treatment was analyzed using principal component analysis (PCA). Extraction of principal component and biplot were achieved using the Statistica TM for iWindows version 8.0 (Statsoft. Inc. USA)..

RESULTS

Changes in weight of rats
The changes in weight of male Wistar rats exposed to several locally consumed alcoholic beverages after 90 days exposure shows a decrease in body weight of rats in all alcohol treated groups compared with the control (Table 1). However, significant decrease ($p < 0.001$) in weight was only recorded in rats treated with LG (102.8±2.2 g) and LG+VtB (124.5±2.0 g) compared with control (136.2±2.6 g) after 90 days exposure period (Table 1).

Effects on steroid hormone primary precursor (cholesterol) and triacylglycerol levels
We measured the effects of alcohol exposure on the primary precursor of steroid hormones (cholesterol) and triacylglycerol in Wistar rats following a 90 days exposure period and observed a significant increase in the levels of cholesterol across all alcohol exposure groups compared with control (Fig. 1A). While for triacylglycerol, a significant

increase was recorded in all alcohol treated groups except for ALB treatment group which did not increase significantly compared with control (Fig. 1B).

Table 1.
Changes in weight of Wistar rats following a 3 months treatment with different locally consumed alcoholic drinks (n=6 organisms/treatment group).

Treatment group	Weight (g)
Control	136.2±2.6
Action bitters	134.0±2.0
Alomo bitters	131.8±2.5
Origin bitters	132.0±2.0
1960 bitters	130.8±1.5
Local gin	102.8±2.2***
Local gin plus Vitamin B	124.5±2.0**

Values are given as means ± standard error (SEM) of exposure treatment. Asterisk (*) indicates significant differences at p < 0.001 between exposure group and control.
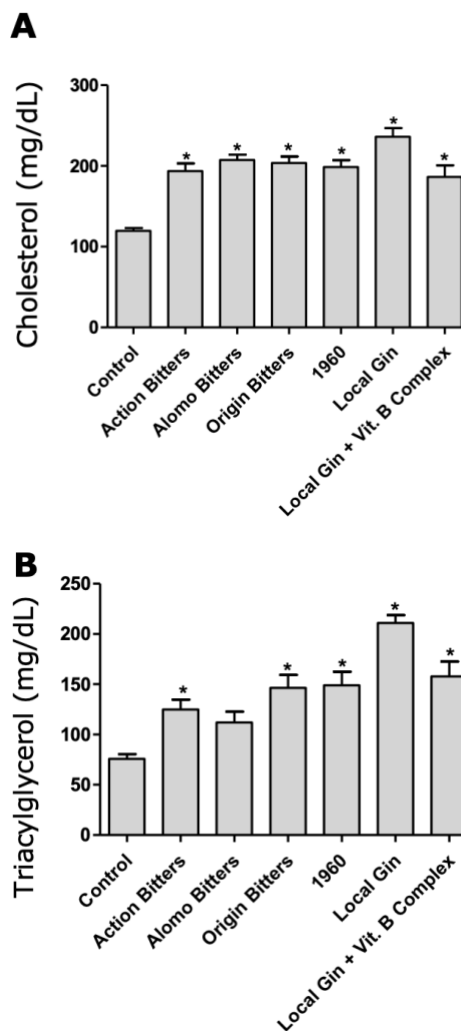
Figure 1.
Changes in steroid hormone precursor and triacylglycerol levels following 90 days administration of several alcoholic beverages in male Wistar rats. (A) Cholesterol, (B) Triacylglycerol. Data is presented as mean values ± standard error of mean (SEM) and different letters indicates significant difference (p<0.05) between exposure concentrations and control (n= 6 per treatment group).
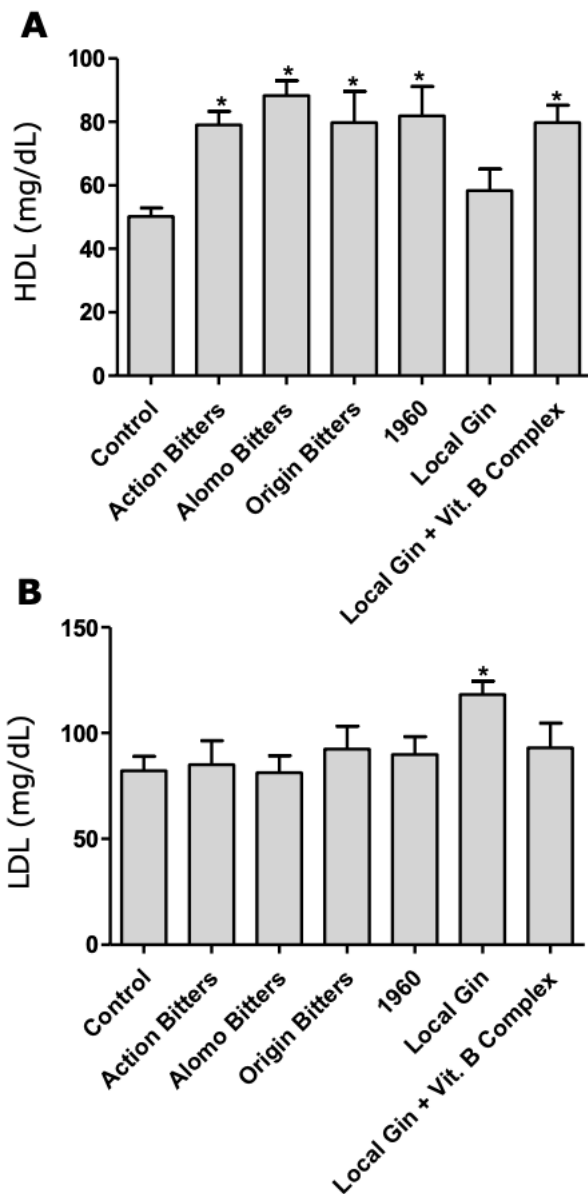
A

B

treatment group which showed a significant increase compared with control (Fig. 2B).   The AST and ALP activities increased significantly in all alcohol exposure groups (Fig. 3A & C), while ALT activity only increased significantly in rats exposed to ALB, OrB and LG with no significant changes in AB, 1960, and LG+VtB exposure groups compared with control (Fig. 4B). For, GGT and LDH activities, we observed a significant increase in both GGT and LDH enzymatic activities in all alcohol treated groups compared with control (Fig 4A & B, respectively).
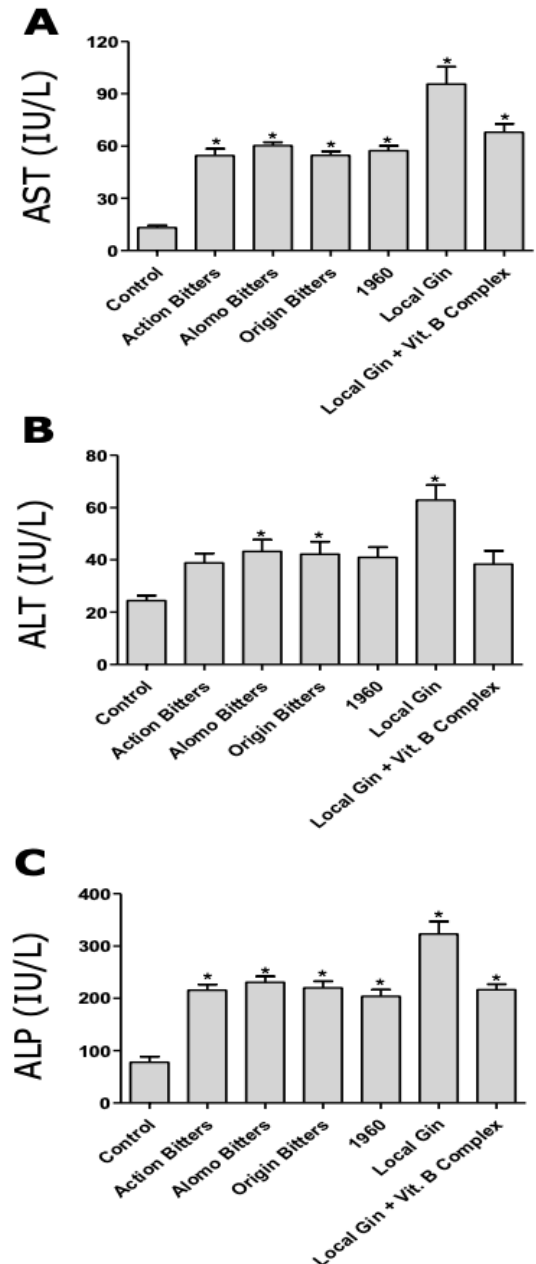
A

B

C

Figure 2.
Alterations in high and low density lipoproteins levels following 90 days administration of several alcoholic beverages in male Wistar rats. (A) High density lipoproteins, (B) Low density lipoproteins (B). Data is presented as mean values ± standard error of mean (SEM) and different letters indicates significant difference (p<0.05) between exposure concentrations and control (n= 6 per treatment group).

Effects on enzymatic activities
Changes in biochemical parameters were assayed by measuring the serum levels of high and low density lipoprotein (HDL and LDH), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), Lactate dehydrogenase (LDH), GGT, K_+, Na_+, Cl_- and HCO_{3-} in male Wistar rats following treatment with alcoholic beverages for over a 90 days exposure period showing that alcohol significantly modulated the levels of physiological and biochemical enzymes in male Wistar rats (Fig. 2-5). Also, the HDL levels were significantly increased in all treatment groups except in LG treated group compared with control (Fig. 2B), while for LDH the reverse was the case with no significant changes in LDH levels except for LG

Figure 3. Changes in serum enzymatic activities of Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT) and Alkaline Phosphatase (ALP) levels following 90 days administration of several alcoholic beverages in male Wistar rats. (A) Aspartate Aminotransferase (AST), (B) Alanine Aminotransferase (ALT), (C) Alkaline Phosphatase (ALP). Data is presented as mean values ± standard error of mean (SEM) and different letters indicates significant difference (p<0.05) between exposure concentrations and control (n= 6 per treatment group).
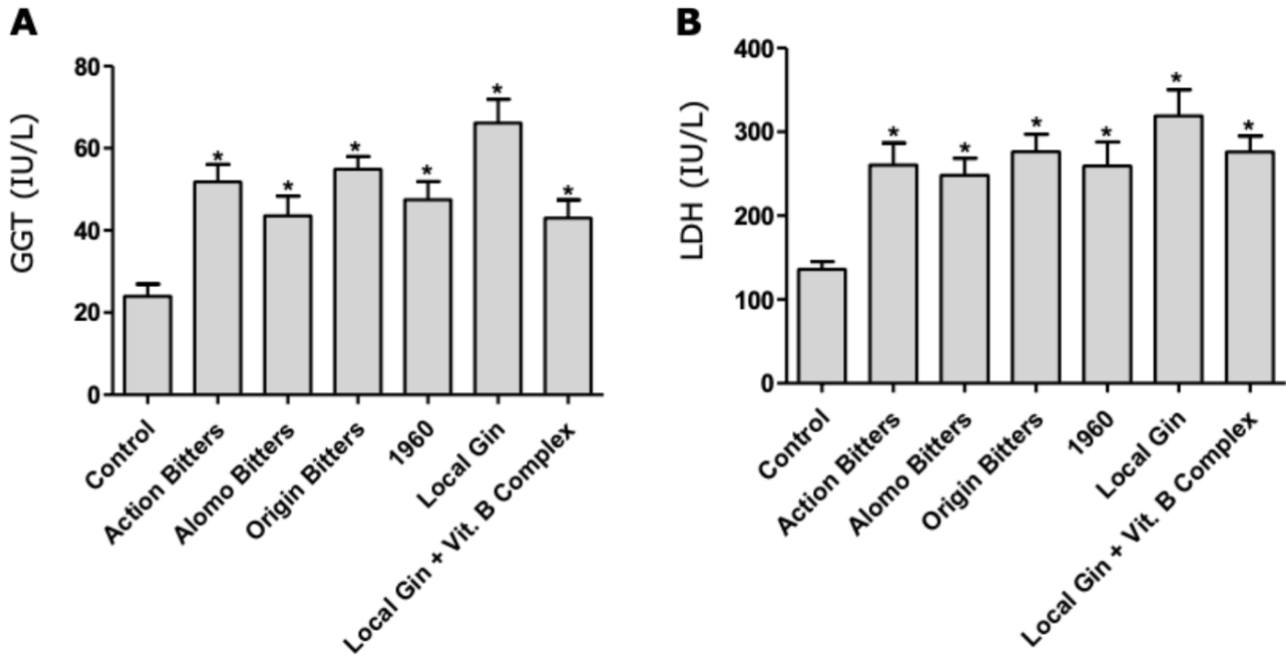
Figure 4.
Modulations in serum Lactate dehydrogenase (LDH) and Gamma-glutamyl transferase (GGT) levels following 90 days administration of several alcoholic beverages in male Wistar rats. (A) Lactate dehydrogenase (LDH), (B) Gammy-glutamyl transferase (GGT). Data is presented as mean values ± standard error of mean (SEM) and different letters indicates significant difference ($p<0.05$) between exposure concentrations and control (n= 6 per treatment group).

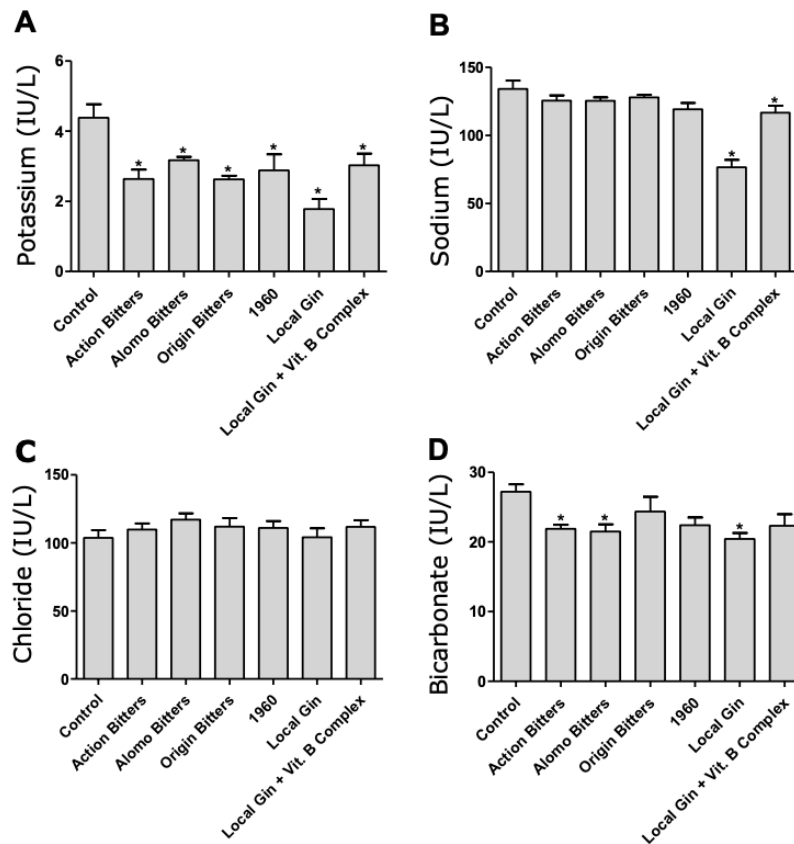**Figure 5.**
Changes in serum electrolytes levels following 90 days administration of several alcoholic beverages in male Wistar rats. (A) Potassium (K_+), (B) Sodium (Na_+), (C) Chloride (Cl_-), (D) Bicarbonate (HCO_{3^-}). Data is presented as mean values ± standard error of mean (SEM) and different letters indicates significant difference ($p<0.05$) between exposure concentrations and control (n= 6 per treatment group).

The levels of K+ in serum of male Wistar rats significantly decreased in all alcohol exposed groups compared with control (Fig. 5A). Generally, the levels of Na+ decreased in all alcohol treated groups, but a significant decrease was observed in LG and LG+VtB treated groups compared with control (Fig. 5B), while no significant changes were recorded in Cl- levels in control and alcohol exposed groups (Fig. 5C). Also, while there was a decrease in the levels of HCO3- in all alcohol exposed rats compared with control, a significant decrease was only recorded in AB, ALB, and LG exposure groups compared with control (Fig. 5D).

Principal Component Analysis (PCA): Correlation between alcohol treatment groups with physiological and biochemical responses in male Wistar rats.
The relationship between physiological and biochemical responses in male Wistar rats and treatments with several alcoholic beverages are shown in the extracted principal components, percentage variation, and PCA biplot (Fig. 6). Three (3) principal components accounting for 85.6% were extracted (SM1) with PC1 and PC2 accounting for 46.8 and

21.4% of the observed correlational variations respectively (Fig. 6). PC1 which accounted for 46.8% of the orientation of variables in the biplot, showed a strong positive relationship between physiological and biochemical responses (Triacylglycerol (0.80), cholesterol (0.85), AST (0.90), ALT (0.73), ALP (0.89), GGT (0.76) and LDH (0.71)) with LG (0.70) treatment group in male Wistar rats, indicating that LG treatment was strongly associated with modulated physiological and biochemical parameters in Wistar rats, suggesting that LG treatment significantly increased the levels of these enzymes in rats. PC1 also showed a positive relationship between biochemical responses (K+ (-0.75), Na (-0.76) and HCO3- (-0.57)) with control group (-0.78), indicating that he levels of these important physiological electrolyte ions increased in the control group compared with treatment regimes. PC2 accounted for 21.4% of the total variance between the interactions in biplot and indicated that HDL (0.73) showed a strong positive correlation with ALB (0.51) treatment group implying that increased HDL levels was associated with ALB treatment in male Wistar rats.
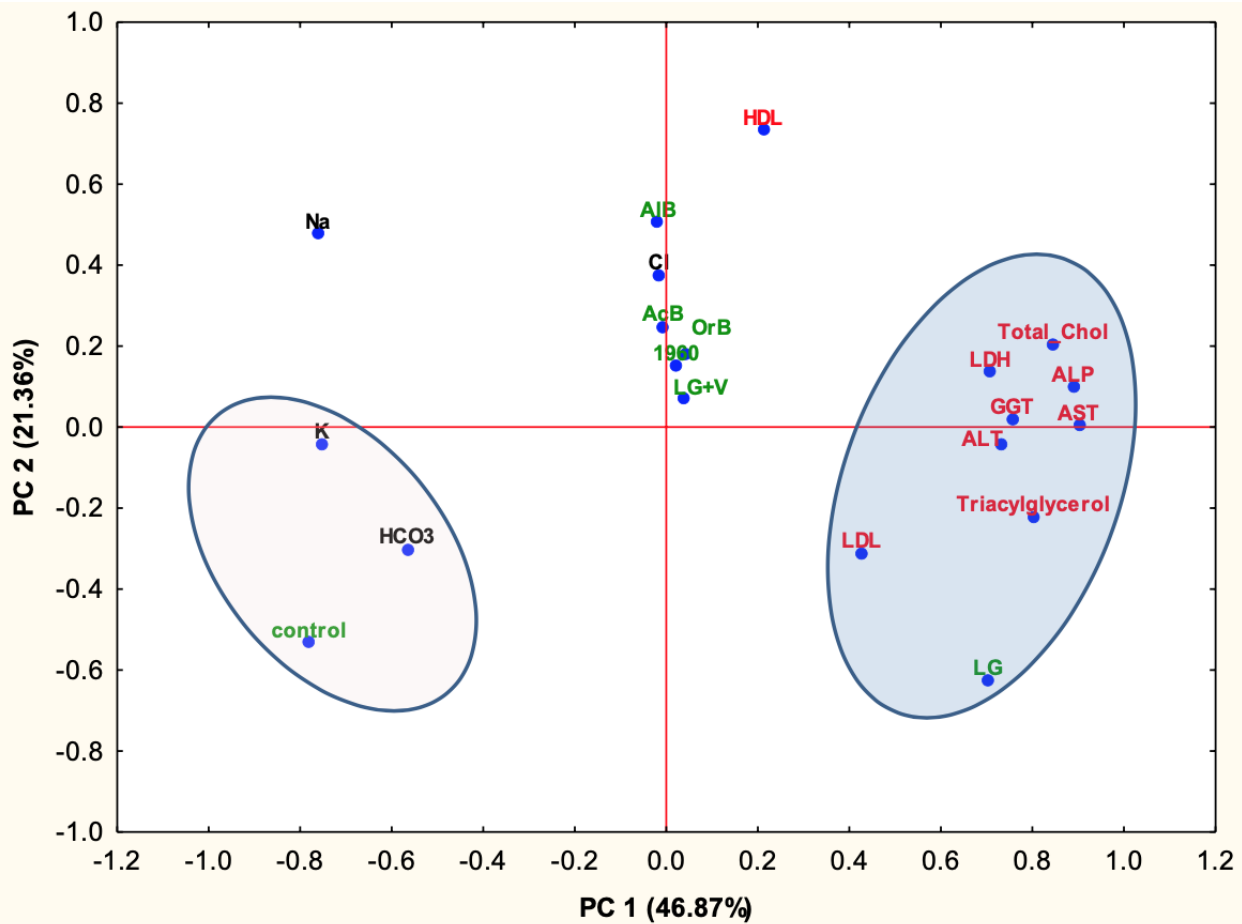

Figure 6.
Biplot of the principal component analysis (PCA), showing relationship between biochemical and physiological responses with treatment of several alcoholic beverages in Wistar rats.

DISCUSSION

In many developing countries, such as Nigeria, there is increased consumption of herbal-based alcoholic beverages due to general speculations suggesting that these products are medicinal, and / or therapeutic to most ailments (including waist pain and menstrual cramps), antimalarial and aphrodisiac, without any confirmed negative physiological and biochemical effects. Herein, we investigated the possible toxicological, biochemical and physiological effects in a mammalian model (Wistar rats) of some commonly consumed alcoholic beverages in Nigeria and demonstrated that treatment of rats with several alcoholic beverages for a 90 days exposure period resulted in a significant increase in levels of cholesterol, triacylglycerol, high and low density lipoprotein, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase and gamma-glutamyl transferase, with a corresponding significant decrease in body weight, K_+, Na_+, and HCO_3- levels. Correlational analysis by PCA showed a strong positive relationship between measured biochemical responses and LG treated groups indicating that LG exposure significantly increased the levels of these biochemical enzymes in Wistar rats. Also, we observed a positive relationship between control and serum electrolytes (K_+, Na_+ and HCO_3-) levels indicating higher levels of these ions in control and suggesting that alcohol treatment significantly decreased electrolytes levels in rats.

Regardless of their origin and function, all steroid hormones have cholesterol as their primary precursor (Nahar et al., 2007), with the high and low density lipoproteins involved in the regulation of cholesterol transportation from the arteries into the liver where they are metabolized. In this study, we observed a significant increase in the levels of cholesterol, triacylglycerol, high density lipoprotein and low density lipoprotein in alcohol treated group compared with control. We believe that such increases may indicate increased fat deposition and buildup within the arteries with resulting downstream negative implications in the development of several coronary liver and heart diseases. This assumption is supported by established knowledge that increase in cholesterol, triacylglycerol, high and low density lipoproteins may result in the development of atherosclerosis (a condition resulting from fat deposition and buildup in the artery) and this may increase the rate of liver and heart diseases including the development of stroke and peripheral artery disease. Also, our results may suggest that the mechanism by which chronic alcoholism induces the development of coronary liver and heart disease may be via the development of atherosclerosis via the increase in cholesterol, triacylglycerol, low and high density lipoproteins levels and their subsequent deposition in the artery. Previously, it has been reported that chronic alcoholism promotes and influence the risk of developing coronary liver diseases (Lewis, 2006). Also, continues increases in cholesterol, triacylglycerol, low and high density lipoproteins levels in alcohol treated rats may promote the rate of fat buildup and deposition and this may promote other disease such as obesity and type 2 diabetes. Similarly, it has been reported that alcohol consumption influences the risk of developing type 2 diabetes (Kyoko et al., 2008). Interestingly, our data indicates that while LDL levels increased significantly in LG treatment group, the reverse was the case

for HDL and this may indicate that the ability of HDL to scavenge and transport LDL to the liver for final metabolism maybe impaired. Furthermore, such significant increase in LDL levels in LG treatment group may also support the continuous and prolonged deposition and buildup of fats within the artery and may provide a novel insight towards understanding the mechanisms underlying the development of coronary liver and heart disease in chronic alcoholics. Such increased deposition of LDH in the liver for metabolism may have other hepatocellular effects including the development of coronary liver and heart disease.

An increase in the activities of some physiological and biochemical enzymes such as aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase are indicators of compromised cellular functions and integrity including hepatocellular damage, necrosis, altered membrane permeability and cholestasis from the toxicological standpoint (Begum, 2004; Adeogun et al., 2013). Specifically, increased AST activity is a specific marker for liver damage and disease, ALT is considered as a marker for liver indicative of degeneration, liver fat accumulation (Westerbacka et al., 2004) and related to hepatic insulin sensitivity (Vozarova et al., 2002). ALP is indicative of tubular degeneration and necrosis. Furthermore, it has been reported that serum activity of LDH are influenced by liver cirrhosis (Luxon, 2011; Gonzalez-Reimers et al., 2015) and as such high serum LDH activities are always associated with liver disease (Kotoh et al., 2011), while increased GGT activity has been suggested as a marker of systemic liver inflammation associated with cardiovascular risk and oxidative stress (Marchesini et al., 2005; Kerner et al., 2005; Lee et al., 2004; Yamada et al., 2006) and has also been suggested as a marker for alcohol consumption. Herein, we observe a significant elevation of AST, ALT, ALP, LDH and GGT serum activity in alcohol treated groups compared with control and such increases may suggest hepatocellular damage via hepatotoxicity. Similarly, administration of alcohol in mouse resulted was reported to induce several liver pathological changes such as liver fibrosis, cirrhosis and steatosis (Bergheim et al., 2006; Landman et al., 2013). It has been suggested that Tumor Necrosis Factor alpha (TNF-α) plays an essential role in alcohol induced liver damage by stimulating the intercellular endothelial adhesion molecule leading to liver damage. Our findings indicating a significant increase in AST, ALT, ALP, LDH and GGT are consistent with previous reports suggesting an increase in the activities of these enzymes in chronic alcoholic patients (Horai et al., 2018). A relationship between alcohol consumption, increased liver enzymes (GGT, ALT and AST) and the risk of type 2 diabetes has also been established previously (Vozarova et al., 2002; Nakanishi et al., 2004; Nannipieri et al., 2005), while other epidemiological reports have demonstrated that alcohol consumption increases the levels of GGT with increased risk of type 2 diabetes (Lee et al., 2003a, b; Nakanishi et al., 2004; Meisinger et al., 2005; Wannamethee et al., 2005). Consistently, we observed a higher level of all measured biochemical enzymes (AST, ALT, ALP, GGT and LDH) in the LG treated group compared with other treatments and control and this may suggested that LG induced a higher biochemical and toxicological effects in rats compared with other alcohol treatments.

The serum electrolyte levels are physiologically and toxicologically important due to their roles in several biochemical process including nerve excitability, their participatory roles in the transmission of electrical impulses along the cell membranes in the neurons and muscles, stabilization of protein structures in enzymes and their contribution in maintaining osmotic balance via regulation cellular fluids (Liamis et al., 200). The osmotic regulation of these ions occurs through the kidney, with lesser amounts lost in sweat and feces. For example, potassium is an intracellular cation that participates in the resting membrane potential in neurons and muscle fibers after membrane depolarization and action potential. Herein, a significant decrease in K_+ levels was observed in alcohol treated groups compared with control and such decreases may have implications in the development of hypokalemia. Also, given that K_+ participates actively in the regulation of Na_+ in the renal tubules under the influence of aldosterone, such decreases in K_+ levels may have negative physiological consequences. Sodium plays a significant role in osmotic balance and herein a significant decrease in Na_+ levels were recorded in alcohol treated groups compared with control and this may suggest hyponatremia. Given that Na_+ is filtered in the glomerular capillaries of the kidney such decrease in Na_+ levels may result in loss of Na_+ in through increased production and elimination of urine, as it has been reported that excessive production of urine and sweating can decrease the levels of Na_+ in organisms. In this study, while the level of Cl_- remain unchanged across all treatment groups and control, a significant decrease in HCO_{3-} levels was observed in treatment groups (AcB, ALB, and LG), compared with control. Such decreases in HCO_3 levels may have implications in the ability of HCO_{3-} to regulate acid-base balance in these organisms. Consistent with our findings, alterations in serum electrolytes levels in alcoholic patients have been reported. Some epidemiological studies have consequently suggested a relationship between the prevalence of hypernatremia and pathogenetic mechanisms in hospitalized chronic alcoholic patients (Liamis et al., 2000). Taken together the present findings and previous reports, the observed decreased in serum electrolytes levels in alcohol treated rats compared with control may suggest that this alcoholic beverages may possess nephron-toxicity effects with resulting effects on the kidney function. Interestingly, correlational analysis study by PCA revealed a strong positive relationship between biochemical responses (cholesterol, triacylglycerol, AST, ALT, ALP, GGT, LDH) and alcohol suggesting an increase in the levels of these biochemical parameters in alcohol treated rats and this may indicate hepatotoxicity with resulting downstream effects on the development of coronary liver and heart disease in organisms exposed to these group of alcoholic beverages. Also, the positive relationship between electrolytes ions such as K_+, Na_+, Cl_- and HCO_{3-} with control indicates that these ions were higher in control group and decreased in treated groups, such decreases in electrolytes levels may indicate nephron-toxicity with resulting physiological, biochemical and toxicological effects. The observed significant decrease in body weight in LG and LG+VtB treated group compared with control suggests that nutrition may be compromised by chronic administration of these alcoholic beverages. Similarly,

malnutrition resulting from chronic alcohol consumption have been related to lack of essential nutrients in the body and inability to digest and absorb essential nutrients required for cellular metabolism (Priyabrata et al., 2010

Our results indicate that exposure of commonly consumed alcoholic beverages to male Wistar rats, resulted in hepatotoxicity (as indicated by increased cholesterol, triacylglycerol, AST, ALT, ALP, GGT, LDH levels), hypokalemia, hyponatremia with resulting negative effects on osmotic balance (as indicated by decreased the level of serum electrolyte ion levels (K_+, Na, and HCO_{3-}) suggesting nephron-toxicity. These may have several biochemical and toxicological consequences in chronic alcoholics who constantly consume any or a combination of these products. Overall, the increase in cholesterol, triacylglycerol and LDL with a corresponding decrease in HDL in treatment groups compared with control may suggest a probable mode of action and provide a mechanistic insight by which alcoholic beverages induce coronary liver and heart disease. Public health awareness, outreaches and enlightenment campaigns are necessary to inform vulnerable individuals with predisposed health conditions on the short, mid and long-term health risk that could come with the chronic intake of these and other similar herbal-based alcoholic beverages.

REFERENCES

Adeogun, O.A., Onocha, P., Oladoyinbo, S. (2013). Variations in Plasma Biochemical Parameters of Clarias gariepinus Exposed to Sub-Lethal Concentrations of the Methanolic Extracts of Raphia Hookeri. Tropical Veterinarian, 30,17-31.

Begum, G. (2004). Carbofuran insecticide induced biochemical alterations in liver and muscle tissues of the fish Clarias batrachus (linn) and recovery response. Aquatic Toxicology, 66, 83-92

Bergheim, I., Guo, L., Davis, M.A., Lambert, J.C., Beier, J.I., Duveau, I. (2006). Metformin prevents alcohol-induced liver injury in the mouse: critical role of plasminogen activator inhibitor-1. Gastroenterology, 130, 099-2112.

Bujanda, L., (2000). The effects of alcohol consumption upon the gastrointestinal tract. American Journal of Gastroenterology, 95, 3374–3382.

Cahill, A., Cunningham, C.C., Adachi, M., Ishii, H., Bailey, S.M., Fromenty, B., Davies, A. (2002). Effects of alcohol and oxidative stress on liver pathology: the role of the mitochondrion. Alcoholism: Clinical Experimental Research 26, 907-915.

Clarke, T.K., Adams, M.J., Davies, G., Howard, D.M., Hall, L.S. (2017). Padmanabhan S. Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N = 112117). Molecular Psychiatry, 22,1376–1384.

Collins, S.E., Kirouac, M. (2013). Alcohol Consumption. In Encyclopedia of Behavioral Medicine Springer, New York, NY. 61-65 pp.

Crews, F.T., Boettiger, C.A. (2009). Impulsivity, frontal lobes and risk for addiction. Pharmacology and Biochemical Behaviour, 93, 237–247.

Friedmann, P.D. (2013). Clinical practice. Alcohol use in adults. N. England Journal of Medicine, 368, 365-373.

Ginsberg, H., Olefsky, J., Farquhar, J.W., Reaven, G.M. (2010). Moderate ethanol ingestion and plasma triglyceride levels. A study in normal and hypertriglyceridemic persons. Annual International Medicine, 80, 143-149.

Gonzalez-Reimers, E., Quintero-Platt, G., Rodriguez-Rodriguez, E., Martínez-Riera, A., Alvisa-Negrín, J., Santolaria-Fernández, F. (2015). Bone changes in alcoholic liver disease. World Journal of Hepatology, 7, 1258-1264.

Gupta, S., Ahirwar, D., Jhade, D., Kumar, S.K., Bharti, A. (2010). Pharmacognostic Standardization, Physico and Phytochemical Evaluation of Aerial Parts of Mentha arvensis Linn. International Journal of Pharmacological Science and Drug Research, 2, 261-264

Harrison, S.A., Diehl, A.M. (2002). Fat and the liver--a molecular overview. In Seminars in gastrointestinal disease 13(1), 3-16.

Hoek, J.B. & Pastorino, J.G. (2002). Ethanl, oxidative stress and cytokine-induced liver injury. Alcohol, 27, 63-68.

Horai, T., Hishimoto, A., Otsuka, I., Mouri, K., Shimmyo, N., Boku, S., Okishio, N., Sora, I. (2018). A cross-sectional study exploring useful indicators for low bone mineral density in male alcoholic patients. Neurological Disease Treatment, 14, 663-669.

Kerner, A., Avizohar, O., Sella, R. (2005). Association between elevated liver enzymes and C-reactive protein: possible hepatic contribution to systemic inflammation in the metabolic syndrome. Journal of Arterioscler Thrombocytes Vascular Biology, 25, 193-197.

Kotoh, K., Kato, M., Kohjima, M. (2011). Lactate dehydrogenase production in hepatocytes is increased at an early stage of acute liver failure. Experimental and Theroetical Medicine, 2, 195-199.

Kyoko, K.S., Tomoshige, H., Yoshiko, N., Nobuko, H., Takeshi, Y., Ginji, E., Hiroshi, K. (2008). Liver enzymes compared with alcoholic comsumption in predicting the risk of type two diabetes. Diabetes Care, 31, 1230-1236.

Landmann, M., Kanuri, G., Spruss, A., Stahl, C., Bergheim, I. (2013). Oral intake of chicoric acid reduces acute alcohol-induced hepatic steatosis in mice. Nutrition 30, 882-889.

Lee, D.H., Blomhoff, R., Jacobs, D.R. (2004). Is serum gamma glutamyltransferase a marker of oxidative stress? Free Radical Research, 38, 535-539.

Lee, D.H., Ha, M.H., Kim, J.H., Christiani, D.C., Gross, M.D., Steffes, M., Blomhoff, R., Jacobs, D.R. (2003a). Gamma-glutamyltransferase and diabetes: a 4 year follow-up study. Diabetologia 46, 359-364

Lee, D.H., Jacobs, D.R., Gross, M., Kiefe, C.I., Roseman, J., Lewis, C.E., Steffes, M., (2003b). Gammaglutamyltransferase is a predictor of incident diabetes and hypertension: the Coronary Artery Risk Development in Young Adults (CARDIA) study. Clinical Chemistry, 49, 1358-1366.

Lewis, G. F. (2006). Determinants of plasma HDL concentrations and reverse cholesterol transport. Current Opinion in Cardiology, 21, 345-352.

Liamis, G.L., Milionis, H.J., Rizos, E.C., Siamopoulos, K.C., Elisaf, M.S. (2000). Alcohol and Alcoholism 35, 612-616.

Lieber, C.S., (2000). Alcohol: its metabolism and interaction with nutrients. Annual Review in Nutrition, 20, 395-430.

Losowsky, M.S., Jones, D.P., Davidson, C.S., Lieber, C.S. (2009). Studies of alcoholic hyperlipemia and its mechanism. American Journal of Medicine, 35, 794-803.

Luxon, B.A. (2011). Bone disorders in chronic liver diseases. Current Gastroenterology and Reproduction, 13, 40-48.

Manjeshwar, S. B., Haniadka, R., Pereira, M.M., D"Souza, J.J., Pallaty, P.L., Bhat, H.P., Popuri, S. (2011). Update on the Chemopreventive Effects of Ginger and its Phytochemicals. Critical Review in Food Science and Nutrition, 51, 499-523.

Marchesini, G., Avagnina, S., Barantani, E.G. (2005). Aminotransferase and gamma-glutamyltranspeptidase levels in obesity are associated with insulin resistance and the metabolic syndrome. J0urnal of Endocrinology, 28, 333-339.

Mehlig, K., Strabdhagen, E., Svensson, P., Rosengren, A., Toren, K., Thelle, D., Lissner, L. (2014). Cetp TaqIB genotype modifies the association between alcohol and coronary heart disease: The intergene case-control study. Alcohol, 48, 695-700.

Meisinger, C., Lowel, H., Heier, M., Schneider, A., Thorand, B. (2005). Serum gamma-glutamyltransferase and risk of type 2 diabetes mellitus in men and women from the general population. Journal of Internal Medicine, 258, 527-535.

Nakanishi, N., Suzuki, K., Tatara, K. (2004). Serum γ-glutamyltransferase and risk of metabolic syndrome and type 2 in middle aged Japanese men. Diabetes Care, 27, 1427-1432

Nannipieri, M., Gonzales, C., Baldi, S,. Posadas, R., Williams, K., Haffner, S.M. (2005). Stern MP, Ferrannini E. Liver enzymes, the metabolic syndrome, and incident diabetes: the Mexico City Diabetes Study. Diabetes Care, 28, 1757-1762

Nestel, P.J., Hirsch, E.Z (2015): Mechanism of alcohol-induced hypertriglyceridemia. J. Lab. Clin. Medicine 2015; 66: 35-365.

Nahar L, Sarker S.D., Turner A.B. (2007): A review on synthetic and natural steroid dimers. Curr Medical Chemistry. 14(12): 1349-1370.

O'Keefe, J.H., Bybee, K. A., Lavie, C.J. (2007): Alcohol and cardiovascular health: the razor-sharp double-edged sword. Journal of American College of Cardiology, 50, 1009-1014.

O'Shea, R.S., Dasarathy, S., McCullough, A.J. (2010): Alcoholic liver disease. Hepatology 51, 307–328

pettcrew M, Douglas N, Knai C.M., Maani H.N., Durand **M.A., Eastmure E and Mays N (2007).** provision of information to consumers about the caloric content of alcoholic drinks: did the Responsibility Deal pledge by alcohol retailers and producers increased the availability of calorie information? Public Health, 149: 159-166.

Priyabrata, P., Pritishova, B., Das, D., Panda, S.K. (2010). *Ocimum Sanctum* Linn. A reservoir plant for therapeutic applications: An overview, Pharmacog. Review, 4, 95-105.

Rehm, J., Samokhvalov, A.V., Shield, K.D. (2013). Global burden of alcoholic liver diseases. Journal of Hepatology, 59, 160 -168.

Ruffle, J.K. (2014). Molecular neurobiology of addiction: what's all the (Δ)FosB about?. American Journal of Drug and Alcohol Abuse, 40: 428-437.

Stogner, J.M., Eassey, J. M., Baldwin, J. M., Miller, B.L. (2014). Innovative alcohol use: Assessing the prevalence of alcohol without liquid and other non-oral routes of alcohol administration. Drug and Alcohol Dependence, 142, 74-78.

Vasanthi, H.R., Parameswari, R.P., DeLeiris, J., Das, D.K. (2012). Health benefits of wine and alcohol from neuroprotection to heart health. Frontiers in Biosciences, 4, 1505-1512.

Vetreno, R.P., Crews, F.T. (2015). Binge ethanol exposure during adolescence leads to a persistent loss of neurogenesis in the dorsal and ventral hippocampus that is associated with impaired adult cognitive functioning. Frontiers in Biosciences, 9:35.

Vozarova, B., Stefan, N., Lindsay, R.S., Saremi, A., Pratley, R.E., Bogardus, C., Tataranni, P.A. (2002). High alanine aminotransferase is associated with decreased hepatic insulin sensitivity and predicts the development of type 2 diabetes. Diabetes, 51: 1889-1895.

Wannamethee SG, Shaper AG, Lennon L, Whincup PH (2005): Hepatic enzymes, the metabolic syndrome, and the risk of type 2 diabetes in older men. Diabetes Care 2005; 28: 2913-2918.

Westerbacka, J., Corner, A., Tiikkainen, M., Tamminen, M., Vehkavaara, S., Hakkinen, A.M., Fredriksson, J., Yki-Jarvinen, H. (2004). Women and men have similar amounts of liver and intra-abdominal fat, despite more subcutaneous fat in women: implications for sex differences in markers of cardiovascular risk. Diabetologia, 47, 1360-1369

Yamada, J., Tomiyama, H., Yambe, M., Koji, Y., Motobe, K., Shima, K., Yamamot, Y., Yamashina, A. (2006). Elevated serum levels of alanine aminotransferase and gamma glutamytransferase are markers of inflammation and oxidative stress independent of metabolic syndrome. Atherosclerosis, 189, 198-205.