Chronic exposure to high alcohol concentrations in experimental animals may induce iron overload and oxidative stress

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ABSTRACT: Background: Most studies on preventive or harmful effects of alcohol have some limitations of inability to measure quantity of alcohol consumed by study participants. Objectives: This study investigates the concentration of alcohol consumed that can lead to increased levels of iron indices and impact on some markers of oxidative stress in experimental rabbits. Materials and methods: Thirty male rabbits divided into 5 groups of 6 animals each were used for the study. They were fed with 5mL of 4%, 12%, 20% and 40% food grade ethanol daily for 6 weeks by gavage while the group 5 (control) was given water. Thereafter, they were anaesthetized using chloroform and blood collected by cardiac puncture. Serum iron, total iron binding capacity (TIBC), uric acid, albumin, malondialdehyde (MDA) and ferritin were assayed by colorimetric and ELISA techniques. Percentage transferring saturation (%TS) was calculated as serum iron divided by TIBC. ANOVA was used to compare the means between the groups while Pearson correlation coefficient was used to test the association between the measured variables with alcohol concentrations. Results: Serum iron, %TS, ferritin, MDA and uric acid levels were higher while TIBC and albumin were lower with increasing concentrations of alcohol. All measured variables except TIBC and albumin correlated positively while TIBC and albumin correlated negatively with concentrations of alcohol. Conclusion: This study suggests that chronic alcohol consumption >12% increases serum markers of iron status and oxidative stress in experimental animals. Caution may be applied in the consumption of higher concentrations of alcohol in order to avoid the consequences of iron overload and oxidative stress.

KEYWORDS: Chronic alcohol consumption, Iron status, oxidative stress, rabbits.
INTRODUCTION
Indices of iron stores have been reported to be higher in alcoholics and heavy drinkers (Moirand et al., 1991; Leggett et al., 1990; Irving et al., 1988; Chick et al., 1987; Kristenason et al., 1981), individuals drinking small amounts of alcohol than abstainers (Morojele, 2010; Robinson et al., 1998; Milman and Kirchnoff, 1996). Iron overload was reported in patients with alcoholic liver cirrhosis (Gavens et al., 2016) and iron overload was suggested to be the main mechanism involved in alcoholic liver disease (Ishii et al., 1997; Tsukamoto et al., 1995; Shaw and Jayatilleke, 1992). There is evidence that iron and alcohol could initiate the generation of free radicals and exacerbate oxidative stress especially in the liver (Niemela, 1999; Ren et al., 2016). The association between chronic alcohol consumption, iron status and production of oxidative stress is the main interest of this study.

Iron is an essential trace element that plays a vital role in both oxygen transport and energy metabolism. Iron is carried in the body either in association with proteins such as haem (haemoglobin, myoglobin, cytochromes) or as non-haem containing proteins such as ferritin, transferrin and flavoproteins. Substantial amount of iron is present in the form of haemoglobin and the majority of the remaining iron is stored by an intracellular protein called ferritin. Since iron is required for various vital functions of the body, iron deficiency results in anaemia and haemochromatosis if the levels of iron are below and above the normal range respectively. In addition, iron is an essential trace metal for hemoglobin synthesis of erythrocytes, oxidation–reduction reactions and cellular proliferation, whereas excess iron accumulation causes organ dysfunction through the production of reactive oxygen species (ROS) (Schliep et al., 2016; Potz et al., 2016; Plunk et al., 2016). Body iron metabolism is a semi closed system, and is critically regulated by several factors including the newly identified peptide hepcidin. In the circulation, iron is usually bound to transferrin and most of the transferrin bound iron is utilized for bone marrow erythropoiesis. As there is no active mechanism to excrete iron from the body, a progressive accumulation of body iron may easily occur (Sergent et al., 2005).

There is no consensus as to what constitutes mild, moderate or heavy alcohol consumption. Most epidemiological studies on preventative uses or harmful effects of alcohol have some limitations of measuring alcohol consumed since they rely on surrogate information such as interviews. Such information may lead to invalid estimates of alcohol consumption pattern (Emokpae and Irobosuna, 2016; O'Shea et al., 2010). Here, we investigate the amount of ethanol consumed that can lead to increased levels of iron indices and impact on some markers of oxidative stress in experimental rabbits.

MATERIALS AND METHODS
Experimental animals
Thirty male rabbits, Oryctolagus cuniculus, aged 10-15 months and weighing 1.2-2.0kg were used for the study. They were purchased at the Cattle market in Benin City and housed in ventilated cages at the animal house, Faculty of Pharmacy, University of Benin, Benin City. The animals were allowed to acclimatize for two weeks during which they were fed with growers’ mash (Bendel feed and Flower Mill Limited) for six weeks. After which the animals were divided into five groups of six animals each. In other to allow for easy administration of alcohol, the animals were grouped based on similarities in weight and were fed with the appropriate food and tap water provided ad libitum for drinking which was renewed every day.

Experimental procedure
Food grade dry gin (Chelsea, London bottled in Ogun state, Nigeria) was used for this study. The ethanol (stock concentration of 43%) was diluted in to 4%, 12%, 20% and 40% and 5mL each of the different concentrations were administered (group 1-4), while the animals in group 5 were given water by gavage daily in the morning for six consecutive weeks.

Sample collection and preparation
After six weeks of ethanol treatment, the animals were anaesthetized using chloroform with each of them placed on their back on solid surface. A V-shaped cut was made in the abdomen in order to have an easy access to the heart. Blood samples were thereafter collected by cardiac puncture using 25G needle and syringe. Blood samples were collected into plain containers, which were allowed to clot at room temperature. The blood samples were centrifuged at 1000 g for 10 minutes and sere separated into plain containers. The separated sera were kept frozen at -20°C until they were analyzed.

Sample analyses
Serum iron, uric acid and albumin were assayed by colorimetric method using reagents supplied by BioLabo Diagnostics (Kandivali, Mumbai, India). The unsaturated site on apotransferrin was saturated by the addition of sufficient ferric iron, after which the excess was removed by adsorption with basic magnesium carbonate powder. After centrifugation, the bound iron remaining in supernatant was measured spectrophotometrically and the absorbance directly proportional to the concentration of iron binding capacity present in the sample.
Total iron binding capacity (TIBC) was calculated as iron concentration multiply by 5, while percentage transferrin saturation (TS) was calculated as serum iron divided by TIBC expressed in percent. Malondialdehyde (MDA) and ferritin were assayed by ELISA technique using reagent supplied by Northwest Life Science specialties, Vancouver, Canada and Monobind Inc, Lake Forest, CA, USA respectively.

**Statistical Analysis**

The statistical package for social sciences (SPSS) version 16.0 was used for statistical analysis. All values were expressed as Mean ± Standard error of the mean. Analysis of variance (ANOVA) was used to compare the mean values of the observed measured variables between the groups.
Pearson correlation coefficient was used to test the association between measured parameters and concentrations of alcohol ingested by the animals. A p-value of ≤0.05 was considered as statistically significant.

RESULTS AND DISCUSSION

The results are as presented in Tables 1 and 2. Serum iron, %TS, ferritin, MDA and uric acid levels were higher while TIBC and albumin were lower with increasing concentrations of alcohol ingested by the experimental animals. Table 2 shows the association between the measured variable and concentrations of alcohol ingested by the animals. All measured parameters except TIBC and albumin correlated positively while TIBC and albumin correlated negatively with the concentrations of alcohol.

The main findings of this study are that serum levels of iron, %TS, ferritin, MDA and uric acid were higher while TIBC and albumin were lower in the animals fed with different concentrations of alcohol than controls. The levels of the measured variables were significantly altered at concentrations (>12%) of alcohol ingested by the experimental animals.

A number of studies over time have reported both beneficial and detrimental effects associated with alcohol consumption. Moderate alcohol consumption has been observed to decrease the risks of several diseases like coronary heart disease, type 2 diabetes, ischaemia stroke and hypertension (Tolstrup et al., 2006; Xin et al., 2001; Carmago et al., 1997). There are however conflicting reports regarding the concentrations of alcohol that may be beneficial or otherwise in consumers. Most previous studies on the impact of alcohol consumption on iron status have some limitations, because measurements of alcohol by researchers depended on surrogate information obtained through interview with patients or their families. Therefore, quantification of alcohol consumption is subject to biases. We observed from this study that concentration of alcohol above 12% may lead to increased iron status and oxidative stress.

Whereas, some studies reported no adverse effect (Knight et al., 2003), others observed an increased risk for the development of renal disease (Schaeffner et al., 2006), abdominal fat (Greenfield et al., 2003), tuberculosis, oesophageal cancer (Sticker et al., 2002; Mandishona et al., 1998; Moyo et al., 1997), weight gain and obesity (Wannamethee and Shaper, 2004) as well as alcoholic liver disease (Whitfield et al., 2001).

The observed increase in the levels of serum iron in chronic alcohol consumption is consistent with previous studies (Otunola and Afolayan, 2016; Ioannou et al., 2004; Whitfield et al., 2001; Ganne-Carrie et al., 2000). It was reported that alcohol intake at low concentrations increase iron status which may either be beneficial or deleterious depending on the circumstances (Whitfield et al., 2001). Ioannou et al (2004) reported that consumption of up to 2 alcoholic drinks per day seems to be associated with reduced risk of iron deficiency and iron deficiency anaemia without a concomitant increase in the risk of iron overload. They also stated that the consumption of more than 2drinks per day is associated with risk of iron overload (Ioannou et al., 2004). The possible explanation for the observed increased iron status are that alcohol causes increased iron absorption as a result of down regulation of hepcidin expression in the liver. Hepcidin is an important protein that regulates iron absorption in the gut (Harrison-Findik, 2007). Some authors suggested that there may be impairment in iron utilization by red blood cells in alcoholics (Celada et al., 1979; Waters et al., 1966). A study of tissue culture reported a suppressed colony formation by erythroid progenitor cells by concentrations of ethanol (Meagher et al., 1982). Apart from these, alcohol can also affect iron utilization through deficiency and impaired metabolism of folic acid and pyridoxine (Ford et al., 1995; Celada et al., 1979; Eichner and Hillman, 1971).

Percentage transferrin saturation refers to the proportion of iron-binding sites of transferrin loaded with iron and values above 50% for males and 45% for females indicate iron overload or haemochromatosis. At these levels, there is accumulation of non-transferrin bound iron or labile iron which is involved in the generation of reactive oxygen species via the Fenton reaction or the Haber-Weiss reaction (Casu and Rivella, 2014; Papanikolaou and Pantopoulos, 2005). The observed higher levels of %TS is also in agreement with previous studies (Dawson, 2000).

Ferritin, an iron storage protein is present in the liver and reticulo-endothelial cells and usually reflects the quantity of iron in the body (Lieb et al., 2011; Lipshitz et al., 1974). Even though serum level of ferritin was increased in chronic alcohol consumption, it may not necessarily leads to high serum iron levels. High serum levels of ferritin in alcoholics may occur due to alcohol induced liver damage (Wish, 2006).

Statistically significant differences in the measured oxidative stress markers were observed in animals fed with >12% alcohol. This observation is consistent with previous study (Yuksel et al., 2005). This increase in oxidative stress markers could result in cell destruction or cell death. We earlier reported a non-significant decrease in the level of
albumin in experimental animals fed with increasing concentrations of alcohol (Emokpae and Irabonosa, 2016). Chronic consumption of high alcohol concentrations could lead to chronic liver disease and inhibition of protein synthesis. These changes in the measured oxidative stress markers may be occasioned by increased levels of serum iron status due to increased concentrations of alcohol consumed. It was suggested that metabolism of alcohol generates acetyl-CoA, leading to the breakdown of adenine nucleotide, resulting in the formation of adenosine monophosphate which is a precursor of uric acid (Choi et al., 2004). Chronic alcohol consumption may precipitate gouty arthritis in susceptible individuals. Studies have also suggested that increased uric acid levels observed in chronic alcohol consumption may be beneficial because of it’s antioxidant properties (Emokpae and Irabonosa, 2016; Emokpae, 2013; Bartimaeus and Eno-Eno, 2002).

In conclusion, data presented in this study suggest that chronic alcohol consumption >12% increases serum markers of iron status and oxidative stress in experimental animals. Caution may be applied in the consumption of higher concentrations of alcohol in order to avoid the consequences of iron overload and oxidative stress.

REFERENCES


