EFFECTS OF VARIOUS VOLUMES OF RED WINE ON LIVER FUNCTION OF MALE WISTAR RATS

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Abstract: This study investigated the effects of various volumes of red wine on liver function of male Wistar rats. The essence of this research is to determine, if the potential toxicity observed in consumption of red wine can be reduced by lowering the volume of red wines consumed using experimental animals. Thirty (30) male Wistar rats (weighing 190 ± 5.0 g) were bought and randomly distributed into five groups (n=6). Red wine (14%) of various volumes: 400, 350, 300, 250 ml/70kg body weight and distilled water (control) were administered orally to Groups 1, 2, 3, 4 and 5, respectively for fourteen days. The rats were later sacrificed and subjected to selected biochemical and histological analysis. The results show that the concentrations of TG, ALB, TP, TB, ALP, ALT and AST in the rats administered red wine of different volumes were significantly higher ($p<0.05$) than the control. The significant levels of AST, ALT, ALP and TB in the test animals show a possible toxicity of red wine at the different volumes administered. This shows that lowering the volume of red wine and its regular consumption could still predispose animals to toxicity associated with red wine. However, the high level of TP and ALB shows that moderate consumption of red wine may encourage protein biosynthesis, thereby supporting certain liver functions. This is in agreement with the histoarchitectural state of the liver tissues of the test animals compared to the liver tissue of the control group, which showed no obvious alterations.

Keywords: Antioxidant, histology, liver, protein synthesis, red wine, toxicity

Introduction

The earliest knowledge about wine cultivation came from ancient Egypt, where wine making process was represented on tomb walls dating to 2600 BCE (McGovern *et al.*, 1996). The real scientific identity of wines emerged in 1995, when Vinson and Hontz from the Department of Chemistry, University of Scranton published a paper titled "Phenol Antioxidant index: comparative antioxidant effectiveness of red and white wines" (Vinson and Hontz, 1995). This study showed that even though red wines had a higher phenolic content than white wines, white wines had a significantly lower IC50 (which is the concentration for inhibiting 50% low density lipoprotein or bad cholesterol) and thus were better antioxidants than red wines (Jung *et al.*, 1999).

The antioxidant in vitamin E plateaus at 20%, whereas wines' antioxidants will plateau at 100% after a couple of glasses. The fermentation process of converting grapes into wine enhances the antioxidant level, it also produces alcohol, which helps the absorption of antioxidants. Wine is believed to be more beneficial to health than concentrated grape extract, while red wine contains more antioxidants than white wine, with the amount varying according to the grape variety, region and vintage climate (Skurray, 1998). Red wine showed the highest concentration of resveratrol (4 mgL^{-1}) compared to rose and white wine which was at least 10-fold lower (Michele *et al.*, 2009).

Red wines are made from black or red grapes. The skins of the grapes are added to the juice during fermentation to add pigments to the wine. The main difference between red wines and others is the presence of the skin throughout the entire fermentation process (Serafini *et al.*, 1998). The body converts ethanol into harmless substances, but this is not accomplished immediately. In a case where alcohol is consumed at a faster rate than the body can handle, ethanol builds up in the system and begins to interfere noticeably with brain and liver functions (Montoliu *et al.*, 1994). The liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds; any injury to the liver or impairment of its functions may lead to many adverse effects on one's health (Subramaniam *et al.*, 2015). Popular red wines include Beaujolais, Cabernet Sauvignon, Chianti, Merlot and Pinot (Serafini *et al.*, 1998). The decision to choose red wine in this study was due to the conflict presented between the above known antioxidant qualities of red wine and recent discoveries of its potential dangers (Kukoyi *et al.*, 2018). This necessitated the reason for administering various volumes of red wine to experimental animals.

Materials and Methods

Experimental animals

Thirty (30) healthy male Wistar rats of seven weeks old (weighing 190 ± 5.0 g) were bought from National Veterinary Research Institute, Vom, Jos, Plateau State, Nigeria. They were randomly distributed into five groups (n=6). All animals were allowed access to feed and water *ad libitum* throughout the experiment. Standard laboratory protocols for animal studies were maintained.

Experimental design

Red wine (14%) of various volumes: 400, 350, 300, 250 ml/70kg body weight, and distilled water (for control) were administered orally to Groups 1, 2, 3, 4 and 5, respectively for fourteen (14) days. The administration was done in accordance with Pedro *et al*. (2006).

Collection of samples for biochemical and histological analysis

After the 14-day administration of the red wine, the rats were sacrificed after anesthesia with chloroform. Blood samples were collected through cardiac puncture and dispensed into plain plastic sample tubes. The blood samples were allowed to clot and centrifuged at 4000 rpm for 10 minutes. The serum was collected for the biochemical analysis.

The liver of each animal was carefully removed using dissecting scissors and stored in 10 percent formalin for further analysis (Geetha, 2011). They were cut into thin slices of 5 x 2 x 1 mm thickness and then processed using the SPIN tissue processor, according to the specification of Avwioro (2011) and Choji *et al*. (2015).

Determination of selected biochemical parameters

The concentrations of triglycerides (TG), albumin (ALB), total protein (TP), total bilirubin (TB), alkaline phosphatase (ALP), alanine amino transferase (ALT) and aspartate amino

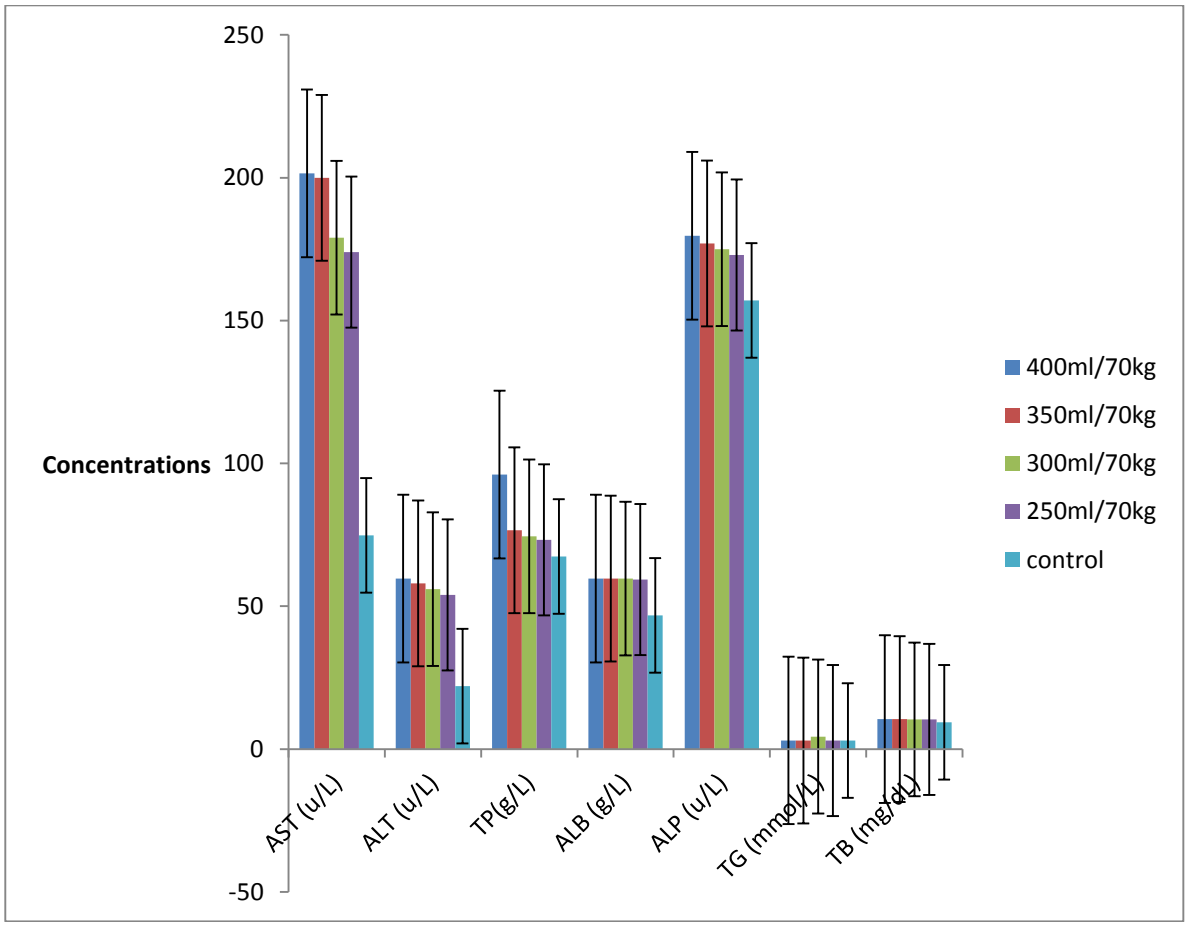
transferase (AST) were determined using Randox kits, according to the principle explained by Geetha (2011).

Statistical analysis

All the grouped data were statistically evaluated with Statistical Package for Social Sciences (SPSS) software, version 21. Hypothesis testing methods included one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range Test. The means were compared for significance at $p \leq 0.05$ and the results expressed as mean ± SEM.

Results and Discussion

The result of the study shows there were significant increase ($P<0.05$) in the levels of TG, ALB, TP, TB, ALP, ALT and AST for the various volume administrations compared to the control (Fig. 1). The significant elevation of AST, ALT, ALP and TB in the test animals show that red wine may potentially lead to hepatocellular damage if it is consumed regularly for a long period of time. Serum levels of selected biochemical indices like transaminases, alkaline phosphatase, total bilirubin, triglycerides and cholesterol has been reported to be elevated in liver diseases (Subramaniam *et al*., 2015). This report is in agreement with the result of this current study. Liver diseases pose a serious challenge to international public health (Ahsan *et al*., 2009).



Values are expressed as mean ± SEM

Fig. 1: Concentrations of selected biochemical parameters in rats administered different volumes of red wine

Ginjom *et al*. (2011) reported that moderate alcohol consumption, particularly red wine, can reduce incidence of mortality from cardiovascular diseases (CHD). The research attributed the health benefits of red wine consumption to the alcohol and phenolic compounds present in red wine. Similarly, grape juice contains many of the same biologically active phenolic compounds found in red wine and may also contribute to the prevention of many diseases related to oxidative stress, such as atherosclerosis and Parkinson's disease (Ribereau-Gayon, 2010).

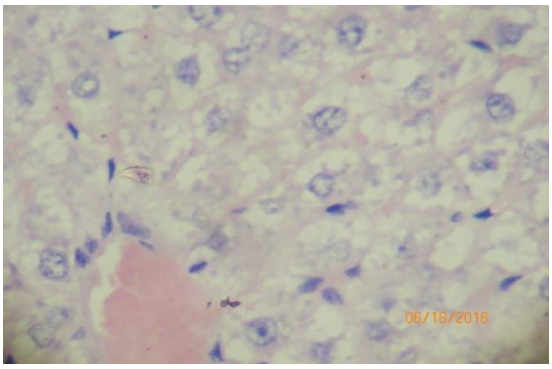


Fig. 2: Photomicrograph of liver section from rat in control group administered distilled water

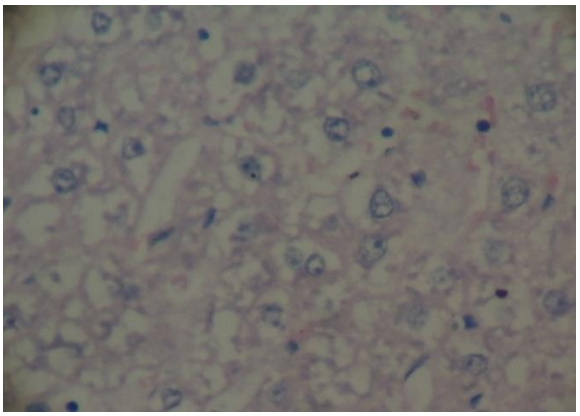


Fig. 3: Photomicrograph of liver section from rat administered red wine: 400 ml/70kg bodyweight

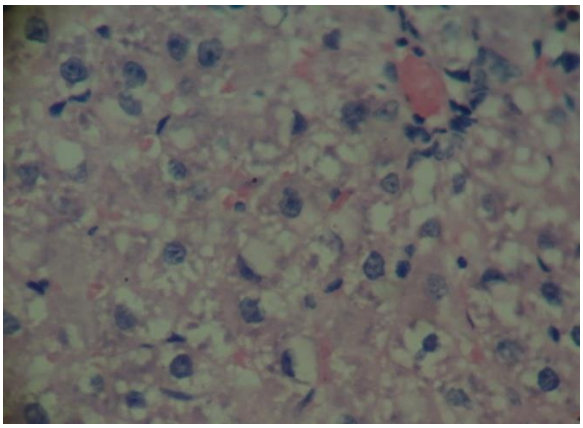


Fig. 4: Photomicrograph of liver section from rat administered red wine: 350 ml/70kg bodyweight

The phenolic composition of grape juice can be significantly affected during grape juice production, especially when grapes are crushed and pressed (Ginjom *et al.*, 2011). The commercial grape juice-making process uses clarifying agents and filters which can decrease resveratrol levels. Resveratrol is also likely to be destroyed during commercial pasteurization because it is unstable to light and heat (Ginjom *et al.*, 2011). The occurrence of phenolic substances in wines such as red wine is not only a consequence of their extraction from grapes during winemaking. During fermentation, the complex polymeric and glycosidic phenolic substances present in grapes break down into monomeric forms and thus become relatively easier to be degraded by digestive juices in human intestine. Therefore, wine may likely have an increased antioxidant capacity when compared to grape juice (Revilla and Ryan, 2000).

Dietary polyphenolics are substrates for a number of enzymes in small intestine and liver. They are usually ingested in larger forms as glycosides. Some glycosides can be absorbed intact in the small intestine using the sodium-dependent glucose transporter 1, although most dietary polyphenolics need to be transformed into smaller aglycones by various intestinal enzymes before passing the gut barrier. Conjugation will then immediately occur in the gut barrier (Prior *et al.*, 2005). These conjugates are further metabolized in the liver. On the other hand, the polyphenols left in the intestinal lumen are subjected to hydrolysis and degradation in the colon due to the activity of enzymes of the colonic microflora, causing the destruction of the flavanol structure and the formation of simpler phenolic compounds (Prior *et al.*, 2005). These complex breakdown, conjugation, and degradation activities that happen after ingestion of dietary polyphenolics influence the bioactivities of the compound and their subsequent effect.

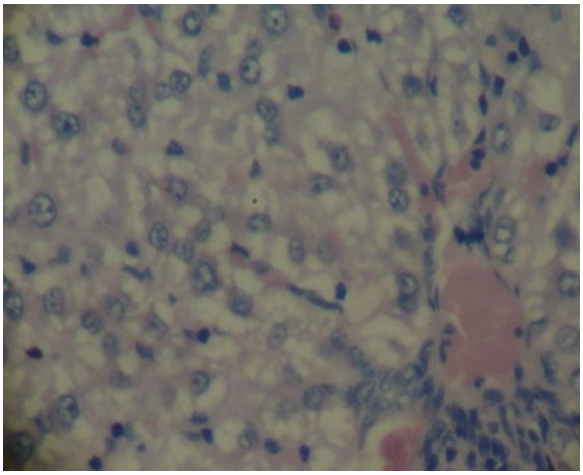


Fig. 5: Photomicrograph of liver section from rat administered red wine: 300 ml/70kg bodyweight

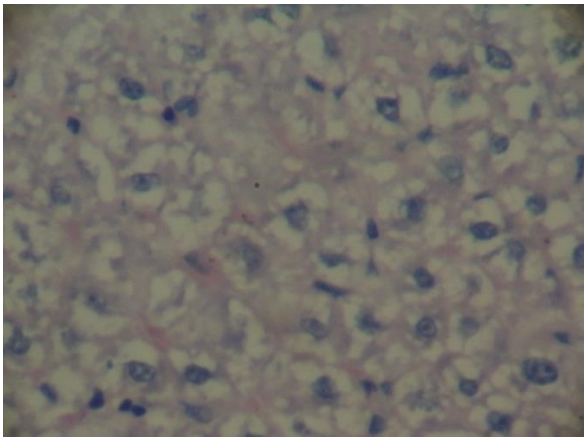


Fig. 6: Photomicrograph of liver section from rat administered red wine: 250 ml/70kg bodyweight

A review by Manach *et al.* (2004) highlighted that polyphenols found in the fruits and vegetables most commonly consumed in human diet are not necessarily the most active within the body due to the absence of ethanol. Their antioxidant properties may be exhibited more in the presence of alcohol. The result of this study shows there could be a potential damage to the liver. This finding is in accordance with the report of Kukoyi *et al.* (2018) which reported that red wine consumption is potentially dangerous to the liver. The result also shows that administration of the red wine encouraged protein synthesis. Imo *et al*. (2015) reported that most proteins that are found in the plasma are synthesized by the liver cells (hepatocytes) and secreted into circulation. A reduction in the protein levels may be a result of possible damage to the hepatocytes. The serum protein level is a known marker of the synthetic function of the liver and a good guide to ascertain the severity of liver damage (Imo *et al*., 2015). If protein synthesis is encouraged, the hepatocytes may therefore be protected from damage. The elevation of the selected biochemical parameters in this current study is believed to be as a result of daily administration of the red wine. The histoarchitectural state of the liver tissues of the test animals compared to the liver tissue of the control group (Figs. 2-6) show no obvious alterations. However, based on the volumes used in this study, red wine has a tendency of interfering with some liver functions. Therefore, anytime red wine is served, it will be good to drink moderately. Good health belongs to those who drink moderately (Grønbaek *et al.*,1995).

Conclusion

This research revealed that regular consumption of red wine for a long period of time may have the potential of interfering with some liver functions. However, it may also have the ability to aid protein synthesis. Moderate consumption of red wine is therefore recommended. More research is required to determine the specific alcohol percentage and volume at which red wine will pose no risk to the liver of experimental animals.

Conflict of Interest

The authors declare no conflict of interest.

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