AMELIORATIVE EFFECT OF RED WINE ON ETHANOL-INDUCED TOXICITY OF SOME BIOMOLECULES IN WISTAR ALBINO RATS

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Abstract: Alcohol is produced in different forms and its consumption has been abused, leading to various social and health challenges. This work investigated the ameliorative effect of red wine on ethanol-induced toxicity of some biomolecules in wistar albino rats. Equal concentrations (6%) of ethanol and red wine were administered to two separate groups of rats. Distilled water was served to the control rats. After 8 wks, an immune response was elicited by an intraperitoneal injection of lipopolysaccharide (LPS). The concentrations of both biochemical (glucose, total protein, albumin, AST, and ALT) and haematological parameters (immune cells) were found to be significantly lower ($P < 0.05$) in ethanol-consuming rats compared to the controls. In the red wine-consuming rats, there were no statistically significant ($P > 0.05$) differences in the concentrations of the parameters compared to the control group. There were significant ($P < 0.05$) negative changes observed in relation to haematological parameters when LSP was administered to the ethanol-consuming rats, but these were unchanged in LPS-fed rats that consumed the same amount of ethanol in form of red wine. Although, a recent study observed that no consumption limit can be said to be safe for alcohol consumption, the present study revealed that red wine has clear non-toxic benefits on biochemical and haematological parameters in wistar rats.

Keywords: Alcohol, acetaldehyde, biochemical, haematological, red wine

Introduction

Ethanol is the most common consumable form of alcohols (Wu and Zaman, 2015); its consumption and consequences of its abuse is well documented. Alcohol use is a leading risk factor for global disease burden and causes substantial health loss (Griswold *et al*., 2018). It is estimated that alcohol is responsible for about 2.5 million deaths each year and for ~4.5% of the global burden of disease and injury (World Health Organisation, 2014). Alcohol is an established causal factor for road traffic accidents, violence, cirrhosis of the liver, epilepsy, poisoning and some types of cancer. With regard to cancer, alcohol consumption was estimated to have caused ~500,000 cancer deaths worldwide in 2004 (Rehm *et al*., 2009, 2017); 4.4% of cancer deaths in China in 2005 (Liang *et al*., 2010) and 3.5% in the United States in 2009 (Nelson *et al*., 2013). In Europe, alcohol consumption varies from country to country (Boniol and Autier, 2010; La Vecchia *et al*., 2014), with proportion of cancer cases attributable to alcohol varying accordingly (Boffetta *et al*., 2006).

Ethanol abuse causes a variety of health issues (Corrao *et al*., 2004; Datta *et al*., 2012). Ethanol is a potent hepatotoxin that can cause liver stress and degenerative liver disease like, alcoholic liver disease (ALD) (Datta *et al*., 2012). Ethanol metabolism generates harmful products (Israel *et al*., 2015) that are directly involved in the production of reactive oxygen species, ROS (Bagnardi *et al*., 2015), thereby creating a conducive environment for oxidative stress (Datta *et al*., 2012) and other diseases such as fatty liver and neuropathy (De and Jillian, 2013; O'Shea *et al*., 2010). Ethanol is metabolized in the body by enzymes like alcohol dehydrogenase and the microsomal ethanol oxidizing system (MEOS), releasing toxic acetaldehydes and highly reactive, and potentially damaging, oxygen-containing molecules that can interfere with the normal metabolism of other nutrients, particularly lipids, and contribute to liver cell damage (Israel *et al*., 2015; Lieber, 2003). Ethanol is also associated with several diseases like cardiovascular damages and renal problems (Fernández-solà, 2015; Room, *et al*., 2005).

Various types of alcoholic beverages are known (CDC, 2007; Ambler *et al*., 2003; Bosetti *et al*., 2000); these include; wine, beer, neutral spirit, gin, rum, tequila, whiskey, cider, vodka, etc (Cleophas, 1999; Ruidavets *et al*., 2002; Takkouche *et al*., 2002). These beverages have different concentrations of alcohol and varying toxicities that may be ameliorated by some phytochemicals (Sataieva and Zukow, 2014). Although, a recent study (Griswold *et al*., 2018) observed that no alcohol consumption level is safe, alcoholic drinks like, red wine have been shown to contain beneficial substances including phytochemicals (Corder *et al*., 2001; Tresserra-Rimbau *et al*., 2015; Wallerath *et al*., 2002). Therefore, the aim of this study was to investigate the ameliorative effect of red wine on ethanol-induced toxicity of selected biomolecules in wistar albino rats.

Materials and Methods

Materials

Fifty-eight (58) albino rats, between 8-12 wks old were used as experimental subjects.

Red wine and ethyl alcohol

The red wine was produced and bottled by Stellen wine services, Lyndoch, South Africa, with alcoholic content 13.5% v/v; batch No. L1127702 06; 19, manufacturing date 04/10/2011.The ethyl alcohol was obtained from absolute ethanol (99.99%). The alcoholic concentration of both ethanol and red wine were respectively adjusted to 6% volume and administered to the experimental animals for eight (8) weeks as described by Percival *et al.*, 2000. Other chemicals used including, bovine serum albumin (BSA), sodium potassium tartrate, 2,4-dinitrophenyl, and α-ketoglutarate were obtained from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and reagents used were of analytical grade.

Methods

The experimental animals were randomly divided into three groups A, B and C. Each group was subdivided into three groups (A_1, A_2, A_3, B_1, B_2, B_3 and C_1, C_2, C_3). Each sub-group had six experimental animals. The animals were kept in cages in a well-ventilated animal house. The rats were fed rice bran and beans husks obtained locally. All the animals were given equal volumes of fluids *ad libitum*: Group A (control) was given distilled water, Group B, 6% ethanol and Group C, 6% red wine. The body weights and feed intake were monitored weekly but fluid intake was monitored daily. After 3 wks, 6 animals from each group were injected with 5 mg of

lipopolysaccharide (LPS) to induce immune response. Four (4) animals from each group were injected with normal saline alone to serve as non-stimulated controls. The rats were anaesthetized with an intraperitoneal injection of xylazine, ketamine and water in the ratio of 1:1:4 (Percival *et al.*, 2000). The animals were deprived of feed overnight before sacrificing by cervical dislocation. Blood samples were collected from the jugular veins of the rats by use of needles and syringes, put in EDTA test-tubes and stored in the refrigerator for analysis.

Measurement of glucose (glucose oxidase method)

Glucose concentration was determined by glucose oxidase method (Barham and Trinder, 1972; Lott and Turner, 1975) which uses the colour reagent (sulphonated 2,4-dichlorophenol + 4-aminophenazone). The absorbance of the chromogen was measured against the blank and the concentration of the glucose in each sample calculated using equation 1;

$$Concentration\ of\ glucose\ g/dl = Absorbance\ of\ Test \times Concentration\ of\ Standard \quad (1)$$

Measurement of total protein

Total protein concentration was determined using the biuret method of Karchmar (1970). The absorbance of peptide coloured complex was measured at 540 nm against the reagent blank. Total protein was calculated using equation 2;

$$Total\ protein\ (g/dl) = Absorbance\ of\ Test \times Concentration\ of\ Standard \quad (2)$$

Measurement of albumin

The bromocresol green (BCG) method (Doumas *et al*. 1997; Spencer and Price, 1977) was used to quantify albumin in the test samples. BCG was mixed with samples at pH 4.15, allowed to stand for 3 min and the resulting green coloured product was measured spectrophotometrically at 630 nm in both standard and test samples. Albumin concentration was calculated using equation 3:

$$Albumin\ Concentration\ (g/dl) = Absorbance\ of\ Test \times Concentration\ of\ Standard \quad (3)$$

Measurement of serum transaminases (SGOT and SGPT)

The activity of serum glutamic oxaloacetic transaminase (SGOT) also, known as aspartate transaminase (AST) was measured using the method described by Reitman and Frankel (1957), where SGOT activity was determined by monitoring the concentration of oxaloacetate hydrazones formed with 2,4-dinitrophenyl hydrazone (chromogen) and oxaloacetate. The absorbance of the test samples was read at 505 nm and the activity of the enzyme extrapolated from a calibration of the standard curve.

The same method was also used to measure serum glutamic-pyruvic transaminases (SGPT), also called alanine transaminase (ALT), except that the incubation time upon addition of serum was 30 minutes, as opposed to 60 min for SGOT. The activity of the enzyme was also extrapolated from a calibration of the standard curve.

Differential white blood cell count

Blood films/smears on slides were stained and examined under the microscope to establish the number and morphology of the red blood cells, white blood cells {granulocytes (i.e. neutrophils, eosinophils, basophils), monocytes, lymphocytes and plasmacytes}, platelets and the relative frequency of different types of granulocytes. The appearances of white blood granules were colour-matched as follows: eosinophils granules (orange red), basophil granules (dark blue), neutrophils granules (deep blue), and lymphocytes (clear purple nuclei). Staining of the blood film with Giemsa stain was done by the method described by Dunning and Safo (2011). The new improved Neubauer counting chamber was used for the counting of the white blood cells.

Statistical analysis

The collected data was subjected to analyses of variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 21.

Results and Discussion

Ethanol consumption may cause the production of free radicals during its detoxification process producing acetaldehyde in the liver. But polyphenols in red wine and their antioxidant properties may counteract the potentially damaging effects of acetaldehyde.

Weight of experimental animals

The weight of experimental animals was monitored weekly. The average weight of the experimental animals in group A (fed with distilled water) was found to be 135.96 g whereas those of groups B (fed with 6% ethanol) and C (fed with 6% red wine) decreased by 1.26 g and 0.32 g, respectively when compared to the control group, similar to the report of Štrbák *et al*. (1998). The observed decrease in weight might be as a result of inadequate feeding which may lead to loss of water (dehydration). Ethanol inhibits antidiuretic hormone, i.e. arginine vasopressin (AVP) which leads to voluminous urination (Bartlett *et al*., 2017; Godino and Renard, 2018). The minimal decrease in weight with red wine might result from the protective nature of the antioxidant phytochemicals in it (Stephan *et al*., 2017) .

.***Biochemical parameters from experimental animals***

Each value shown in Table 1 is a mean of three determinations for the biochemical parameters (glucose, total protein, albumin AST, and ALT). Plasma glucose concentration decreased by 0.19 g/dl in animals fed with ethanol (group B) and 0.04 g/dl in animals that were fed with wine (group C). The decrease might be due to the inhibitory effect of ethanol on glucose absorption (Person, 1991; Steiner *et al*., 2015). Since glucose is found to accumulate in body tissues with high water content, loss of water (fluid) by an animal could possibly affect glucose concentration and hence its decrease. The effect of ethanol on glucose level is dependent on ethanol concentration and acute ethanol intake causes hypoglycemia (Al-Balool *et al*., 1989; Steiner *et al*., 2015). It has also been reported that ethanol consumption causes poor absorption of food (Person, 1991; Steiner *et al*., 2015). Intake of high concentration of ethanol, however, increases the level of glucose (Shanmugam *et al*., 2011), indicating that ethanol abuse is harmful to organs that participate in regulation of blood sugar such as pancreas and liver, and affect other organs like kidneys, heart and immune cells (Shanmugam *et al*., 2011). However, some phytochemicals have been shown to significantly reduce hypoglycemia (Bala *et al*., 2011), and so, the small decrease in glucose concentration (0.04 g/dl) in animals that consumed red wine might result from the wine photochemicals' ability to counteract ethanol's deleterious effects (Stephan *et al*., 2017).

Table 1: Effects of ethanol and red wine on biochemical parameters of wistar rats

Animal group	Weight (g)	Parameters*				
		Glucose (g/dl)	Total Protein (g/dl)	Albumin (g/dl)	AST (u/l)	ALT (u/l)
A. Water	135.96 ± 1.44	4.36 ± 0.40	3.40 ± 0.01	1.05 ± 0.01	7.83 ± 0.00	5.33 ± 0.00
B. 6% Ethanol	134.70 ± 0.00	4.17 ± 0.00	3.10 ± 0.00	1.00 ± 0.00	8.25 ± 0.01	10.65 ± 0.01
C. 6% Red wine	135.64 ± 0.15	4.32 ± 0.13	3.30 ± 0.01	1.04 ± 0.00	7.81 ± 0.02	5.29 ± 0.03

* Each value is a mean of three determinations ± standard error mean (SEM)

Total protein and albumin

Total proteins represent different classes of biomolecules that serve indispensable structural and functional roles in the body. Total protein concentration decreased by 0.03 g/dl in animals that consumed ethanol and 0.01 g/dl in those that consumed red wine when compared with the control. The decrease may be as a result of poor feeding (malnutrition), denaturation, defective anabolism and dehydration, processes precipitated by ethanol. A decrease in protein concentration may result to a corresponding decrease in albumin especially gamma globulin by albuminuria (Lieber, 1990). Acute exposure to ethanol may possibly lead to decreased capacity of the liver to synthesize albumin (Lieber, 1990; Preedy *et al*., 1999). Ethanol consumption is known to increase nitrogen excretion and inhibit protein synthesis which may be indirectly mediated by the reactive metabolite acetaldehyde (Preedy *et al*., 1999). The smaller decrease in protein concentration observed in animals that consumed red wine might again probably be due to polyphenols (resveratrol, quercetin) which protect the liver by scavenging reactive oxygen species (ROS) and other free radicals and enhance protein synthesis (Aguirre *et al*., 2014; Kulkarni & Cantó, 2015; Preedy *et al*., 1999; Weng *et al*., 2011). Changes in skeletal muscle protein metabolism have profound implications for whole body physiology, while protein turnover changes in organs such as the heart (exemplified by complex alterations in protein profiles) have important implications for cardiovascular function and morbidity (Kulkarni & Cantó, 2015).

Transaminases

AST and ALT are intracellular enzymes found in heart, liver, skeletal and muscular tissues. Their concentrations were markedly high in ethanol- consuming rats- 0.42 and 5.32 iu/l respectively, probably due to the destruction of the parenchyma or cell membrane of the cells by ethanol. As a result of this destruction, these enzymes could leak into extracellular fluids or serum (Ebuehi and Asonye, 2007). The increase in the concentration of these enzymes in group B animals was statistically significant ($P < 0.05$) when compared to the control group. Research have shown that conditions of ill- health precipitated by ethanol such as myocardial infarction, inflammation of the heart and muscular tissues and necrosis of the liver can cause such increase in concentrations of the enzymes (Ruhl and Everhart, 2005). Nutritional cardiomyopathy due to malnutrition, cardiac arrhythmia (ectopic heart beat or atrial fibrillation) do elevate levels of AST and ALT in plasma. Hepatic ischaemia, alcoholic cirrhosis, cryptogenic cirrhosis and secondary biliary cirrhosis, all of which ethanol induces, are reportedly leading causes for increased plasma ALT (Ruhl and Everhart, 2005; Stryer, 1996). Other conditions of ill- health precipitated by ethanol that could cause the release of these enzymes are myocardial infarction, necrosis of the liver and muscle tissues, inflammation of the heart and nutritional cardiomyopathy (Ruhl & Everhart, 2005). Alatalo *et al*. (2008) reported that alcohol causes metabolic aberration and steatosis in the liver.

In the red wine consuming- rats, the observed decreases in concentration of both enzymes were relatively small, 0.02 and 0.04 iu/l for AST and ALT, respectively. Although, these decreases were statistically not significant ($P > 0.05$) when compared to control, it probably shows red wine had protective effect on the parenchyma or membrane of cells and thus, prevented efflux of these intracellular enzymes. Antioxidant activity of red wine due to presence of thiols, polyphenols, beta carotene, quercetin, resveratrol, vitamins A and E, and ellagic acid may act as a protective cover for parenchyma or membrane of cells of tissues (Aguirre *et al*., 2014; Percival and Sims, 2000).

Haematological parameters from experimental animals

Total white blood cells (TWBC)

The white blood cells form part of the body's defence mechanism. However, their concentration seemed to be affected by ethanol intake as it was observed to reduce by 1.43 and 0.01% for groups B and C, respectively, when compared with the control. This reduction in TWBC in the group C rats was statistically not significant ($P > 0.05$) when compared with the control group. The reduction however, may be due to poor nutritional status (frequently triggered in alcohol abusers) to boost white blood cells production (Diaz *et al*., 2002). Ethanol is known to damage immune cells by preventing nutrients absorption by the immune system. The consequence is lower concentration of immune cells, and depletion of immunity/defence against various bacterial and viral infections (Barr *et al*., 2016). However, red wine consumption favours the production of immune cells (Percival and Sims, 2000) and hence the marginal reduction of the white blood cells in group C animals when compared with the control. It has also been ascertained that consumption of red wine significantly increases the plasma antioxidant activity maintaining a fairly constant level of white blood cells (Percival and Sims, 2000).

Lymphocytes

Lymphocytes (B cells, helper T-cells, cytotoxic T-cells, suppressor T-cells and natural killer cells) play vital roles in the body's immunity, such as, secretion of antibodies and destruction of virus-infected and tumour cells (Schreiber, 2011). The concentration of lymphocytes was found to decrease on consumption of ethanol (Table 2) probably due to generation of free radicals from detoxification of ethanol (Barr *et al*. 2016). No statistically significant ($P > 0.05$) change in concentration of lymphocytes in animals fed with red wine was observed in the present study. This could probably be due to antioxidant activity of polyphenols in red wine which reportedly antagonizes ethanol's immunotoxicity. In fact, red wine antioxidants like ellagic, β-carotene and catechin have been found to prevent immune suppression by increasing the antioxidant activity in the blood (Percival and Sims, 2000).

Granulocytes (neutrophils, eosinophils and basophils)

The concentrations of granulocytes in the experimental groups were greatly reduced when compared with the control upon administration of ethanol. The concentrations reduced by 1.69% for neutrophils, 3.33% for eosinophils, and 2.30% for basophils, respectively (Table 2). These may have resulted from ethanol's immunotoxicity. Depletion of granulocytes,

natural killer (NK) cells, and T-lymphocytes after short term or prolonged ethanol consumption has been reported (Barr *et al*., 2016; Diaz *et al*., 2002; Szabo and Saha, 2015). Granulocytes are important immune cells known to target bacteria, large parasites and modulate allergic inflammatory responses. Interestingly, the rats that consumed red wine recorded statistically insignificant ($P > 0.05$) reduction in granulocytes when compared with the control: 0.31, 2.33 and 3.59% for neutrophils, eosinophils and basophils, respectively. This may be possible because red wine contains compounds that offset the detrimental effects of ethanol on the immune system (Percival and Sims, 2000).

Table 2: Effects of ethanol and red wine on haematological parameters of wistar rats

Animal group	Weight (g)	Parameters*				
		TWBC (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
A. Water	135.96 ± 0.00	8.43 ± 0.03	66.59 ± 0.00	29.63 ± 0.01	3.65 ± 0.01	3.64 ± 0.00
B. 6% Ethanol	134.70 ± 0.00	7.29 ± 0.07	64.55 ± 0.01	27.94 ± 0.03	1.32 ± 0.01	1.34 ± 0.00
C. 6% Red wine	135.64 ± 0.00	8.42 ± 0.01	65.58 ± 0.00	29.31 ± 0.01	2.33 ± 0.01	3.59 ± 0.03

*Each value is a mean of three determinations ± SEM

Table 3: Effects of lipopolysaccharide (LPS) on haematological parameters of ethanol and red wine- consuming wistar rats

Animal group	Weight (g)	Parameters*				
		TWBC (%)	Lymphocytes (%)	Neutrophils (%)	Eosinophils (%)	Basophils (%)
A. Water	135.93 ± 0.00	8.48 ± 0.02	66.70 ± 0.03	29.63 ± 0.01	3.65 ± 0.01	3.80 ± 0.00
B. 6% Ethanol	134.70 ± 0.00	7.17 ± 0.02	64.31 ± 0.02	28.65 ± 0.03	1.33 ± 0.00	1.20 ± 0.01
C. 6% Red Wine	135.26 ± 0.15	8.48 ± 0.01	66.70 ± 0.00	29.63 ± 0.01	2.65 ± 0.01	3.80 ± 0.01

*Each value is a mean of three determinations ± SEM

Effects of lipopolysaccharide (LPS) on haematological indices

The effect of challenging the immune cells of the rats was measured as shown in Table 3. A slight decrease in the weight of group B animals (6% ethanol) was observed when compared to the control group. Also, in group B, lower values of haematological parameters were observed compared to the control on the administration of LPS. But, in the group C rats (6% red wine), the immune response was not inhibited and therefore the values of the haematological parameters (TWBC, lymphocytes, neutrophils, eosinophils and basophils) were normal, compared with the control values. The difference between the two groups when compared to control was statistically significant ($P < 0.05$). These results further lay credence to the observation that red wine contains compounds that possibly counteract the immunosuppressive effects of ethanol.

Conclusion

Ethanol has been shown to have clear negative effects on the immune cells and proteins of wistar rats. These effects are possibly due to the reactive intermediates, such as acetyldehydes generated during its metabolism in the body. Although, a recent study observed that no consumption limit can be said to be safe for alcohol consumption, the present study revealed that red wine has clear non-toxic benefits on biochemical and haematological parameters in wistar rats.

Conflicts of interest

The authors declare no conflict of interest.

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