

In-Vivo Assessment of Some Haematological and Biochemical Parameters in Normal Wistar Rats Treated with Pineapple (*Ananas Comosus*) Wine

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Abstract

*This study revealed the in vivo assessment of some haematological and biochemical parameters in normal Wistar rats treated with Pasteurized fermented pineapple (*Ananas comosus*) wine with yeast strain *Saccharomyces cerevisiae* (five days fermentation) at room temperature. The wine was administered orally to sets of normal experimental rats for three months at 0.5ml of pineapple wine/g of body weight in alternate days with water in between for three consecutive months, and some haematological, histological and biochemical variables before and after the administration of the wine were assessed in the sera of rats. The haematological studies showed that most parameters studied {HB(12.96 ± 2.0 - 16.52 ± 1.6 g/100ml), RBC (5.28 ± 1.0 - $9.26 \pm 1.6 \times 10^9/mm^3$) and TWBC(3.46 ± 0.6 - $6.54 \pm 1.9 \times 10^3/mm^3$) were significantly higher ($P < 0.05$) only during the second and third month of wine administration. The histological parameters were significantly decreased ($P > 0.05$) for {ALP(101 ± 9.5 - 91.8 ± 2.4 IU/L), AST(68.6 ± 5.0 - 50.0 ± 3.5 IU/L) and ALT(26.4 ± 4.3 - 13.6 ± 4.4 IU/L) and the results of biochemical parameters showed no significant difference (Total Protein, Albumin and Globulin) except for Cholesterol (78.4 ± 4.3 - 67.6 ± 5.6 mg/dl⁻¹) and Bilirubin(0.47 ± 0.0 - 0.36 ± 0.2) which showed a significant decrease ($P > 0.05$) at the end of the third month of pineapple wine administration. We concluded that the oral administration of 0.5ml/g body weight of pineapple (*Ananas comosus*) wine to Wistar rats in alternate days with water for three consecutive months was beneficial in that it increased the haematopoietic effect, served as immune buster as a result of TWBC, and may not lead to the occurrence of liver damage. Therefore, it can be concluded that the moderate consumption of wine may be beneficial to the body.*

Keywords: Pineapple, Wine, *Saccharomyces cerevisiae* . Haematology, Cholesterol.

Introduction

In recent times, home wine production has been practiced with various fruits such as apple, pear and strawberry, cherries, plum, pineapple and oranges (Flee, 1993; Webb, 1984).

Wines are healthful beverages that are seen as a natural remedy for man's illness from early days and are said to aid recovery during convalescent period (Jay, 1996; Okafor, 2007). Fermentation processes are usually done by species of the yeast *Saccharomyces*, whereby the sugars in the fruit juice are converted into alcohol and organic acid, that later react to form aldehydes, esters and other chemical components (Watanabe and Shimazu, 1980).

Studies have shown that alcohol may benefit many bodily organs, including the heart and the brain. However, the benefits are available only when wine is taken in moderation as over consumption of alcohol including wine can cause some diseases including alcohol-induced liver cirrhosis (Jordan, 2002). Alcohol, a major component of wine, is a drug that depresses the central nervous system (Rall, 1990). Alcohol has several biochemical effects after consumption; it alters the intracellular $\text{NAD}^+ / \text{NADH}$ ratio and this affects the equilibrium constant of a number of important metabolic reactions that utilize these two cofactors (Murray *et. al*; 1996). Alcoholism leads to fat accumulation in the liver, hyperlipidemia and ultimately cirrhosis. The exact mechanism of action in the long term is still uncertain; whether or not extra free fatty acid mobilization plays some part in causing the accumulation of fat is not clear, but several studies have demonstrated elevated levels of free fatty acids in the animals after administration of a single intoxication dose of ethanol (Robert *et. al*; 2000).

Dietary components have measurable effect on blood constituents. Examination of blood therefore provides a valuable opportunity to clinically investigate the presence of several metabolites and other constituents in the body of animals (Hafkenschied and Dijt, 1979). Blood examination is also a good way of assessing the health status of an animal as it plays a vital role in the physiological, nutritional and pathological status of the animal (George, 1994). Changes or differences in blood composition could be evaluated if normal values are known. Haematological parameters usually measured are haemoglobin (HB), white blood cell (WBC) and red blood cell (RBC) (Hafkenschied and Dijt, 1979). In view of this effect, this work is aimed at evaluating the toxicological effect of pineapple wine on Wistar rats, using haematological and clinical indices.

Materials and Methods

Fresh fruit of pineapple, *Ananas comosus* (Family Bromeliaceae) was purchased from a local market in Akure, Ondo State. The fruit was examined and authenticated by Mr. Ibitoye A.A of Crop, Soil and Pest department of the Federal University of Technology, Akure, Ondo State, Nigeria. The yeast strain (*Saccharomyces cerevisiae*) was obtained from Federal Institute of Industrial Research, Oshodi, Lagos State, Nigeria.

Preparation of Wort

The pineapple was washed, peeled and cut into chips. 500gm of the chips were blended with a top speed blender. 500ml of water was added and mixed properly. The wort was recovered by filtering with course cheese cloth and the residue removed and dried for animal feed (Fagbamila, 2000).

Fermentation of Wort

100ml of pineapple wort was placed in a 500ml beaker. The pH of the wort was adjusted to 5.5 from an initial value of 4.7 with dilute KOH. 5 gm urea (source of Nitrogen), 5gm potassium dihydrogen phosphate (to maintain the ionic balance of the medium and provide sugar phosphate for the yeast) and 1.0gm. magnesium chloride (enzyme activator) were added to the wort and the mixture was autoclaved at a pressure of 103.4KPa , temperature 121°C for sterilization. After sterilization, the wort was cooled to room temperature and was then seeded with 10gm of *Saccharomyces cerevisiae*. The seeded wort was placed in a 500ml beaker. The pH was adjusted to 5.5 as earlier explained. The wort was also sterilized and cooled. The sterilized wort was poured into a precleaned fermentor and the 100ml wort earlier seeded with *Saccharomyces cerevisiae* was poured into the fermentor as well. All openings on the fermentor were properly closed and fermentation was allowed to continue for 5 days. When fermentation was completed as indicated by complete cessation of carbondioxide evolution, the fermented wort (wine) was filtered and pasteurized. The pasteurized wine was provided to wistar rats as drink to determine the portability of wine on animate objects.

Animals and Treatments

Animals weighing an average of 152g were bred and housed in the Animal house of the Department of Biochemistry, Federal University of Tech, Akure, Ondo State.

Two sets of rats were quarantined (the control and the experimental rats). There were three rats in each set. The control rats were fed with commercial rat feeds (Bendel feeds Nigeria Ltd) twice daily. Each of the rats was given 0.5ml of pineapple wine/g of body weight to drink on alternate days with water in between for three consecutive months. Haematological and biochemical analysis of blood samples of all the rats in both sets were conducted before the commencement of wine administration. Thereafter, analysis on the experimental set of rats were conducted after the first, second and third months of wine administration.

The rats were sacrificed by cervical dislocation. Blood samples were collected by ocular punctures into EDTA bottles for haematological and biochemical analysis.

Determination of Haematological Parameters

Haemoglobin Concentration (HB) Determination

Haemoglobin concentration was determined by the method of Agbede *et.al.*,(1999).

Determination Red Blood Cell Count

RBC count is the number of RBC in a stated volume of the whole blood. It was determined by the improved Neubauer's counting chamber according to the method of Blaxhall (1973).

Determination of Total White Blood Cell (TWBC) Count

The total white blood cell count was determined by the visual method as described by Dacie and Lewis (1995).

Determination of Biochemical Parameters (Albumin, Total Protein and Globulin).

Serum total protein and albumin were analyzed using the biuret and bromocresol green methods, respectively. In both cases, commercially available test kits, products of Randox laboratories, U.K. were used and with the manufacturers instructions strictly adhered to. Serum globulin was determined as the difference between serum total protein and albumin.

Determination of Serum Alanine Aminotransferase (ALT) activity

This was carried out according to the method of Reitman and Frankel (1957). Diluted sample (0.01mL) was mixed with phosphate buffer (100mM, pH 7.4), L-alanine (200mM) and the mixture was incubated for exactly 30 minutes at 37⁰C. 0.5ml of 2, 4 dinitrophenylhydrazine (2mM) was added to the reaction mixture and allowed to stand for exactly 20 minutes at 25⁰C. Then 5ml of NaOH (0.4M) was added and the absorbance was read against reagent blank after 5 minutes at 546nm. Reagent blank was prepared as described above by replacing sample with 0.1ml of distilled water.

Determination of Serum Aspartate Aminotransferase (AST) activity

This was carried out according to the method of Reitman and Frankel (1957). Briefly, 0.1ml of diluted sample was mixed with phosphate buffer (100mM, pH 7.4), L-asparatate (100mM), and α -Oxoglutarate (2mM) and the mixture was incubated for exactly 30 minutes at 37⁰C. 0.5ml of 2, 4 dinitrophenylhydrazine (2mM) was then added to the reaction mixture and allowed to stand for exactly 20 minutes at 25⁰C. Then 0.5ml of NaOH (0.4M) was added and the absorbance was read against reagent blank after 5 minutes at 546nm. Reagent blank was prepared as described above replacing sample with 0.1 of distilled water.

Determination of Alkaline Phosphatase (ALP)

2.2ml of 0.1M carbonate buffer (pH 10.1), 0.1ml of 0.1M MgSO₄. 7H₂O, and 0.2ml of the sample were mixed together and incubated at 37⁰C for 10 minutes. Thereafter, 0.5ml of 19mM of paranitrophenyl phosphate was added and again incubated at 37⁰C for 10 minutes. 2.0ml NaOH was added and mixed, and read against blank at 400nm.

Determination of Total Bilirubin

This was carried out based on the method described by Jendrassik and Grof (1983). 0.2ml of 29mM sulphanilic acid, 0.05ml of 38.5mM sodium nitrite, 1.0ml of 0.25M caffeine and 0.2ml of the sample were mixed together and allowed to stand for more than 10 minutes at 20-25⁰C. Thereafter, 1.0ml of 0.93M tartarate was finally added, mixed and allowed to stand for 5-30 min at 20-25⁰C. The absorbance was taken at 578nm.

Determination of Total Cholesterol

The total cholesterol concentration of the sample was estimated according to the enzymatic method (PAPS Protocol) of Agappe Diagnostics Kit, India.

Analysis of Data

The result of the replicates were pooled and expressed as mean \pm Standard Error (SE) (Zar, 1984). A one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out. Significance was accepted at $P \leq 0.05$.

Table 1: Effects of Pineapple Wine on Haematological Parameters in Wistar Rats

Sets of wistar rats.	HB g/100ml	RBC $\times 10^6/\text{mm}^3$	TWBC $\times 10^3/\text{mm}^3$
Control	12.96 ^b \pm 2.0	5.28 ^a \pm 0.1	3.46 ^a \pm 0.6
Experimental. Before wine Administration	9.90 ^a \pm 0.7	4.36 ^a \pm 0.2	3.36 ^a \pm 0.1
Experimental. 1 st Month of wine Administration.	12.30 ^b \pm 0.6	5.24 ^a \pm 0.3	3.90 ^a \pm 0.3
Experimental. 2 nd Month of wine Administration.	16.12 ^c \pm 1.6	8.98 ^b \pm 1.6	5.58 ^b \pm 0.5
Experimental. 3 rd Month of wine Administration.	16.52 ^c \pm 1.6	9.26 ^b \pm 1.6	6.54 ^b \pm 1.9

Data represent the mean of triplicate readings.

Values with the same uppercase superscript letter along the same column are not significantly different ($P > 0.05$)

Table 2: Effect of Pineapple Wine on Histological Parameters in Wistar Rats

Sets of wistar rats.	ALP I.U/L	AST I.U/L	ALT I.U/L
Control	101.00 ^c \pm 9.5	68.60 ^b \pm 4.98	26.40 ^b \pm 4.3
Experimental. Before wine Administration	101.60 ^c \pm 0.9	80.00 ^c \pm 4.1	26.00 ^b \pm 1.6
Experimental 1 st Month of wine Administration.	71.40 ^a \pm 3.9	60.80 ^c \pm 2.3	17.40 ^a \pm 7.6
Experimental 2 nd Month of wine Administration.	93.20 ^b \pm 2.9	52.00 \pm 5.5	13.60 ^a \pm 4.2
Experimental 3 rd Month of wine Administration.	91.80 ^b \pm 2.4	50.00 \pm 3.5	13.60 ^a \pm 4.4

Data represent the mean of triplicate readings.

Values with the same uppercase superscript letter along the same column are not significantly different ($P > 0.05$).

Table 3: Effects of Pineapple wine on other Biochemical Parameters in Wistar rats.

Sets of wistar rats.	Total Protein (g/dl)	Albumin (g/dl)	Globulin (mg/dl)	Cholesterol (mg/dl ⁻¹)	Total Bilirubin (mg/dl ⁻¹)
Control .	6.90 ^a \pm 0.8	3.08 ^b \pm 0.2	3.82 ^a \pm 0.7	78.40 ^b \pm 4.3	0.47 ^a \pm 0.0
Experimental. Before wine Administration	6.28 ^a \pm 0.1	2.96 ^b \pm 0.2	3.34 ^a \pm 0.2	73.60 ^a \pm 6.2	0.40 ^a \pm 0.0
Experimental 1 st Month of wine Admini.	5.74 ^a \pm 0.2	2.16 ^a \pm 0.3	3.58 ^a \pm 0.3	78.20 ^b \pm 5.6	0.32 ^b \pm 0.0
Experimental 2 nd Month of wine Admini.	6.90 ^a \pm 1.3	3.02 ^b \pm 0.7	3.68 ^a \pm 0.6	67.00 ^a \pm 6.5	0.36 ^b \pm 0.2
Experimental 3 rd Month of wine Admini.	6.84 ^a \pm 1.1	3.12 ^b \pm 0.5	3.82 ^a \pm 0.6	67.60 ^a \pm 5.6	0.36 ^b \pm 0.2

Data represent the mean of triplicate readings.

Value with the same uppercase superscript letter along the same column are not significantly different ($P > 0.05$).

Result and Discussion

Table 1 shows the effects of pineapple wine on Haemoglobin (HB), Red Blood Cells (RBC) and Total White Blood Cells (TWBC) indices of the Wistar Rats. From the results, it was observed that there were significant increase ($P < 0.05$) in the total values of Hb, Rbc and TWBC contents of the experimental rats in the second and third months of the wine administration when compared with the control. Moderate administration of pineapple is advantageous in that it increases the Haemoglobin content (Hb), the erythropoiesis formation and serves as immune buster. This is in contrast to chronic excessive alcohol ingestion, which was reported by Ballard (1989) to reduce the numbers of blood cell precursors in the bone marrow and to cause characteristic structural abnormalities in these cells, resulting in fewer-than normal or non-functional mature blood cells.

From the table 2, the effect of administration of pineapple wine was shown on the toxicological parameters. From the results, the level of Alkaline phosphatase (ALP) Aspartate aminotransferase (AST) and Alanine amino transferase (ALT) showed significant decreased ($P > 0.05$) in the experimental rat for the first, second and the third months of pineapple wine administration when compared with the control which was an indication that effect of moderate wine administration for the whole of three month were not associated with liver damage. Elevation in ALP, AST and ALT are usually secondary to tissue damage. This is because such damage results in the leakage of these enzymes from their intracellular stores into plasma. ALT is most prevalent in the liver whereas AST may also be found in the heart, skeletal muscle and liver to nearly the same extent (Orhue, *et al.*, 2005). Significant increases in the transaminases commonly accompany such liver diseases as toxic hepatitis, acute liver necrosis and hepatic cirrhosis. Increase in AST is often seen in hemolytic anaemia, myocardial infarction and choelstatic diseases of the liver (Mayne, 1994; Mallach, 1996., Jordan, 2002).

Table 3 shows the effect of pineapple administration on the biochemical parameters (Total Protein, Albumin, Globulin, Cholesterol and Bilirubin) of the wistar rats. From the experiments, there were non-significant changes in the result of Total Protein, Albumin and Globulin of the experimental rats when compared with the control. This showed that moderate administration of pineapple wine had not really had any negative effect on the Total Protein, Albumin and Globulin when compared with the control in the Wistar rats. Albumin is produced entirely in the liver and is of great import in regulating the flow of water between the plasma and tissue fluid by its effect on colloid osmotic pressure. A drop in serum albumin level is usually the result of decreased protein synthesis by the liver or increased protein loss through the gut or the kidney (Halsted and Halsted, 1991; Cheesbrough, 1998). Serum albumin levels were within the recommended limits. This is in contrast to heavy alcohol intake, which caused significant reduction in the concentrations of total protein and albumin in humans (Marway *et al.*, 1993; Zakhari, 1997; Sun *et al.*, 2001).

The result of serum concentration of total cholesterol showed a significant decrease ($P > 0.05$) of the experimental rats when compared with the control between the last two months (2nd and 3rd month) of administration of pineapple wine. According to other studies, HDL and LDL are important for assessment of lipid profile. It is well known that hyperlipidemia is one of the major risk factors for atherosclerosis (Fan *et al.*, 1999). An increase in the concentration of lipids results in liberation of lysosome and trigger cell degeneration. Major component of total cholesterol is LDL which is directly related to coronary heart disease (Brousseau and Schaefer, 2000).

It was also revealed from result in table 3 that there was a significant decrease in the level of Bilirubin ($P > 0.05$) of the experimental rats between the first three months of pineapple wine administration as when compared with the control. Data suggest that alcohol consumption may inhibit protein synthesis, especially in the heavy drinkers. The binding and transport of substances by albumin, such as bilirubin could be affected by heavy or moderate alcohol consumption. Bilirubin is transported to the liver by binding non-covalently to albumin (Wardlaw and Kessel, 2002).

Conclusion

Moderate administration of pineapple wine orally for three consecutive months in wistar rats had some haematological and biochemical significances in that it had erythropoetic effects as result of increase in the Red blood cell (Rbc), served as immune buster due to an increase in the total white blood cell (TWBC) and the decrease in ALP,AST and ALT might not result to lever damage since their elevation alone are usually secondary to tissue damage.

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