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Original Research Article

Effect of *Phoenix dactylifera* fruit wine produced by *Saccharomyces cerevisae* on the haematological and some biochemical parameters in albino rats

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Abstract: The effect of date palm fruit wine on the haematological and some biochemical parameters (lipid profile, lipid peroxidation and protein) was investigated Date fruit wine was produced by fermenting the fruit must with *Saccharomyces cerevisiae* from Guangxi Danbaoli, China aerobically for 6 days and anaerobically for four weeks at 28 $\pm 2^{O}$C. During both fermentation processes alcohol content, total yeast counts and total heterotrophic counts were monitored. A total of 30 albino rats were divided into six (6) groups of 5 animals each. Group 1 (control) received the normal rat feed and water; while groups 2 – 5 were administered date fruit wine at 0.3ml/kg, 0.6ml/kg, 0.9ml/kg and 1.5ml/kg body weight respectively and group 6 (standard) received white wine (carlorossi) 1.5mg/kg body weight for a period of 28days.During the aerobic fermentation. Alcohol content increased from 0 to 5.5%, total yeast counts increased from 4.69×10^2 to 15.39×10^2 cells/ml while total heterotrophic bacterial count ranged from 2.0 to 9.0 cfu/ml. *Lactobacillus casei* and *Lactobacillus spp.* were encountered. During anaerobic fermentation .Yeast population dropped from 8.90×10^2 cells/ml to 0.92×10^2 cells/ml. The final percentage alcohol was 9.2%. The result after the experiment showed a general decrease in body weight across the groups compared to the control. Haematological indices showed no significant difference ($p<0.05$) in PVC, RBC, WBC and Hb of the groups treated with *Phoenix dactylifera* (PD) compared to the control. There was also a significant decrease ($p<0.05$) in lipid peroxidation in the liver homogenate across the groups and also in the serum of the PD treated animals relative to the standard control. There was no significant difference ($p<0.05$) in total cholesterol, LDL-C but elevated HDL-C in the PD administered groups compared to the control. Triglyceride was significantly reduced ($p<0.05$) in the PD treated groups compared to the standard control. The results of the present study showed that date fruit wine has no harmful effect on the blood tissue and vital organs such as liver etc. Thus, if properly processed and refined, it could be a good source of commercial fruit wine, with immense nutritional and health benefits.
Keywords: *Phoenix dactylifera*, wine, hematology, lipid peroxidation, lipid profile.

INTRODUCTION

Phoenix dactylifera (PD), also known as date palm is a monocotyledonous plant found mostly in North Africa and Middle East region [1]. They are among a few plants that could survive the harsh arid environment. In these arid regions where foods are scarce, date palm serves as a good source of food as they are rich in carbohydrates [1]. It is also rich in water soluble vitamins of the B group, many minerals specially potassium, calcium and iron, along with many antioxidants, and flavonoids [2].

Aside from serving as a common food source, date palm fruits have been used traditionally to treat various types of ailments and it has been reported that consumption of the fruit is good for health [3]. Its high content of dietary fibers often makes it a good food supplement for hyperglycemic patients [4]. Besides these, the dates are cholesterol free, fat free and sodium free. They are also used as animal feed; especially for camels in the Middle Eastern countries which are given fodder supplemented with dried date pieces [5].

Fruit wine can be made from virtually any plant matter that can be fermented [6]. Wine drinkers have a 34 percent lower mortality rate than beer or spirits drinkers. Furthermore, it reduces Heart-Attack Risk [6].

In the present study, the effect of date fruit wine on the haematological parameter and lipid profile of albino rats was investigated.

MATERIALS AND METHODS

Reagents

Chemicals used were of analytical grade and were products of Randox Laboratory Limited UK.

Preparation of sample

The PD fruit was bought from International Market, Lokoja, Nigeria and was authenticated in Salem University Herbarium Unit with Specimen Voucher Number SU 10354.

Preparation of Date fruit Wine

Good quality, fresh and dried date palm fruits were sliced using a sterile sharp knife to remove the seed and macerated using a clean sterilized grinding engine to produce a small granular texture of the date palm fruit so as to increase the surface area of the fruit for the activity of yeast for fermentation. After grinding, 6.5kg of crushed date palm fruit was gotten, Twenty seven (27) litres of water boiled at 100^{o}C for 30minutes and allowed to cool to about 45^{o}C was mixed with the 6.5kg of the crushed date palm fruit. After even mixing using a sterilized stirrer, the fruit pulp produced was evenly divided between two sterilized fermentors. Each fermentor held about 13.5ltrs of the fruit must. The must was sterilized with Sodium metabisulphate solution to remove microbial contaminants by introducing the Standardized solution into the must 24h before addition of pitching yeast [7].

Fermentation Process

Standardized amount of yeast *Saccharomyces cerevesia*e was added to the must in a fermenting Jar by sprinkling it over the surface of the juice then stirred; the inoculated must was covered with sterile muslin cloth and incubated at room temperature (28±2°C); it was aerated daily by stirring twice to encourage yeast multiplication [7]. Aerobic fermentation was terminated after 6 days and the must was sieved to remove the shaft and debris of the crushed fruits. During the Anaerobic phase of fermentation, the filtrate obtained after sieving the must was transferred into anaerobic fermentation jar and incubated at room temperature. An air trap was fixed to the fermenting jar. Sodium metabisulphate solution was added to the filtrate to supply sulfur dioxide gas. Fermentation was terminated after four weeks; the wine was then stored to allow the yeast to flocculate. The resulting wine was then aged for two months. The aged wine was then filtered using pressurized filtering kit, decanted into sterile bottles and corked.

Fermenting organism monitoring

The population of yeast in the fermenting must during aerobic and anaerobic phases was monitored by microscopic counting using Haemocytometer [8].

The alcohol content of the must was determined using Vinometer for beer and wine (Model VNM 110).

Enumeration of total heterotrophic bacterial count in the wine

Populations of bacteria in the wine were assessed by standard pour plate method using nutrient agar. Ten fold serial dilution of the wine were made and 1ml of desired dilution plated according to Awe [7]. Isolated organisms were characterized and identified using a series of biochemical test and identification keys by Barnett *et al*. [9].

Experimental animals

Eight (8) weeks old male albino rats of average weight (150-250g) were purchased from Biochemistry Animal House, Salem University Lokoja, Nigeria. They were divided into six groups and acclimatized for one week prior to commencement of experiment which were maintained at 12 hours light/darkness condition and were fed *ad libitum* with rat chow and water for a period of 28 days. Ethical approval was obtained from the institutional animal ethics committee in order to carry out this research.

Animal grouping

Group 1 (control) received the normal rat feed and water

Groups 2 – 5 were administered date fruit (PD) wine at 0.3ml/kg, 0.6ml/kg, 0.9ml/kg and 1.5ml/kg body weight respectively

Group 6 (standard) received white wine (carlorossi) 1.5mg/kg body weight

Preparation of serum

Blood were collected into a non-heparinized tubes, allowed to clot and centrifuged at 3000rpm (using a bench centrifuge, Model 90-2) for 10 minutes. The sera were then decanted into other sample tubes and stored in the refrigerator for subsequent use. The blood for hematological study was also collected into EDTA bottles for further analysis

Preparation of tissue homogenates

The tissues from each animal (liver, kidney and heart) were rapidly excised during the sacrifice, washed with cold normal saline to remove excess blood, weighed and stored immediately at - 4^{o}C. Subsequently, the liver, was homogenized (using a BOSCH PSB 570-2 homogenizer) in ice-cold phosphate buffer (pH 7.4). The homogenates were centrifuged at 3000rpm for 15 minutes and the supernatant decanted and kept in the refrigerator for further use.

Haematological analysis

The haematological parameters of the blood samples were estimated by standard procedures using Cell Counter (Medonic M- Series). The haemoglobin concentration (Hb), packed cell volume (PCV), red blood cell count (RBC), and white blood cell count (WBC) were thus determined.

Assessment of lipid peroxidation

Thiobarbituric acid reacting substances (TBARS) in tissue was estimated by the method of Torres *et al.* [10].

Procedure:

2ml of thiobarbituric acid (TBA) and 2ml trichloroacetate (TCA) was added to 50µl of the tissue homogenates. The mixture was incubated for 30 minutes at 80^0c. The tubes were allowed to cool immediately under ice and centrifuged at 3000rpm for 15 minutes. The supernatant was measured using spectrophotometer at 535nm wavelength.

Protein Determination

Procedure

450µl distilled water was pipette into test-tubes (duplicate). 50µl of the sample was added and 1.5ml of biuret reagent was finally added. The absorbance was read at 540nm.

Assessment of lipid profile

The enzymatic colorimetric determination of total cholesterol, triglyceride, and HDL-C was done according to the method of Allein *et al,* [11]; LDL-C as described by Friedewal *et al*. [12].

Statistical analysis

The results are expressed as mean ± SD of five animals from each group, the data were evaluated by one-way ANOVA, P values < 0.05 were considered statistically significant.

RESULTS

The relationship between number of days and total viable count, yeast count and percentage alcohol produced during aerobic and anaerobic fermentations are shown in Table 1 and 2. During aerobic fermentation there is a notable decrease in the number of viable organism from 9.0 cfu/ml to 2.0 cfu/ml while a constant value of 2.0 cfu/ml was recorded during anaerobic stage. The yeast counts increased from 0.0 $\times 10^2$ cells/ml to 35.0×10^2 cells/ml during aerobic stage while there was a drop in the yeast count from $2.0.\times10^2$ cells/ml to 0.0×10^2 cells/ml during anaerobic fermentation. The percentage alcohol content increased from 0.0% to 5.6% during aerobic and from 5.5% to 9.2% during anaerobic fermentation.

Table 3 shows the effect of date fruit wine on body weight of the treated animals. There was an observed decrease in the body weights across the groups.

Table 4 shows the effect of date fruit wine on haematological parameters of treated albino rats. There was no significant difference ($p<0.05$) in RBC, PCV, Hb, WBC, neutrophil, eosinophil, basophil and monocytes of the date fruit wine treated groups compared to the control. Conversely, significant decrease ($p<0.05$) exists in the lymphocytes of the treated groups relative to the control.

Table 5 shows the effect of date fruit wine sample on the lipid profile of the treated rats. There was a significant decrease ($p<0.05$) in the triglyceride level of the treated groups compared to the control. LDL-C was observed to be relatively similar in the treated groups compared to the control. HDL-C was also found to be higher in the animals treated with the standard and higher doses of the date fruit wine compared to the control. However, there was significant difference ($p<0.05$) in some of the lipid profile parameter of the animals administered the standard wine (white wine) relative to the control (water).

Fig.1 shows the effect of the date fruit wine on lipid peroxidation in the serum and liver of the treated rats. Lipid peroxidation was significantly ($p<0.05$) reduced in groups given 0.3ml/kg, 0.6ml/kg, 0.9ml/kg, 1.5ml/kg PD wine when compared to the control and standard group in the serum; which is also the same in all the treated groups when compared with the control in the liver tissue.

Table 1: Relationship between percentage alcohol, total yeast count and total viable count during aerobic fermentation of must

Period of fermentation (Days)	TVB Count (cfu/ml)	Total yeast count ($x10^2$ cells/ml)	% Alcohol produced
0	5.0	0.0	0.0
1	3.0	5.0	0.0
2	3.0	20.0	3.1
3	2.0	35.0	4.5
4	2.0	28.0	5.0
5	2.0	10.0	5.5
6	2.0	8.0	5.6

Table 2: Relationship between percentage alcohol, total yeast count and total viable count during anaerobic fermentation of must.

Period of fermentation (Weeks)	TVB Count (cfu/ml)	Total yeast count (x10^2 cells/ml)	% Alcohol produced	
0	2.0	8.0	5.6	
1	0.0	2.0	8.0	
2	0.0	2.0	8.2	
3	0.0	0.0	8.5	
4	0.0	0.0	8.7	
5	0.0	0.0	9.0	
6	0.0	0.0	9.2	

Table 3: Effect of date fruit wine on body weights of the treated albino rats

Groups	Initial weight	Week 1	Week 2	Week 3	Week 4 (final weight)
Control	246±6	237.2±6.5	235.4±6	238.5±6.3	243±6.1
0.3ml/kg PD	232±4	216.2±3.4	194.2±4	166.6±4	156.8±3.6
0.6ml/kg PD	266.8±5	240.4±6.1	229.1±5.4	206.6±5	149.9±5
0.9ml/kg PD	231.8±7	224.6±6.7	193.3±5	186.8±6	144.9±6
1.5ml/kg PD	197.6±2	182.9±3	157.2±2.1	133.9±3	113..7±2.5
White wine	220±5	212±7	184.2±3	133.9±4	119±9

Results are mean of five readings ± SD. * means significant difference from the control (water) at p<0.05

Table 4: Effect of date fruit wine on haematological parameters of treated albino rats

PARAMETERS	CONTROL	0.3ml/kg PD	0.6ml/kg PD	0.9ml/kg PD	1.5ml/kg PD	STANDARD (WHITE WINE)
PCV%	70±0.1	70±1	72±1	70±0.2	72.5±0.1	68±0.2
Hb(g/dl)	23.1±0.2	23.03±0.3	25.6±0.12	24.8±0.03	26.1±0.2	25±0.1
WBC×10^9g/L	5.7±0.4	6.8±0.2	6.8±0.23	6.3±0.02	6.7±0.7	5.6±0.23
RBC×10^{12}g/L	13.9±0.09	15±0.12	15.1±0.6	14.9±0.1	16.9±0.7*	15.7±0.21*
Neutrophils%	52±0.1	48±0.1*	55±0.9*	48.5±0.5*	52.5±0.4*	53±0.7*
Lymphocytes%	54.5±0.2	47±0.1	40.5±0.12*	48.5±0.4	43.5±0.2*	52±.1.0
Monocytes%	1.5±0.001	1.5±0.02	1.0±0.01	1.0	1.5±0.02	1.2±0.01
Eosinophil	2.0±0.001	2.0±0.01	2.0±0.01	1.5±0.01	2.0±0.01	1.3±0.02*
Basophils	1	1±0.02	0.5	1	0.5±0.003	0.7±0.001

Results are mean of five readings ± SD. * means significant difference from the control (water) at p<0.05

Table 5: Effect of date fruit wine on the lipid profile of treated albino rats.

Groups(doses) Parameter	Water	0.3ml/kg PD	0.6ml/kg PD	0.9ml/kg PD	1.5ml/kg PD	White wine
Cholesterol Mg/dl	10.1±.080	10.3±.010	11.9±.060	11.5±.040	11.6±.030	12.7±.020*
Triglyceride Mg/dl	3.1±.001	1.8±.003*	1.9±.060*	2.0±.070*	2.3±.007*	1.86±.090*
HDL-C Mg/dl	0.13±.009	0.06±.001	0.14±.001	0.22±.006*	0.33±.004*	0.3±.001*
LDL-C Mg/dl	10.5±.006	11.2±.007	10.7±.001	11.5±.009	11.3±.004	12.1±.003*

Results are mean of five readings ± SD. * means significant difference from the control (water) at p<0.05

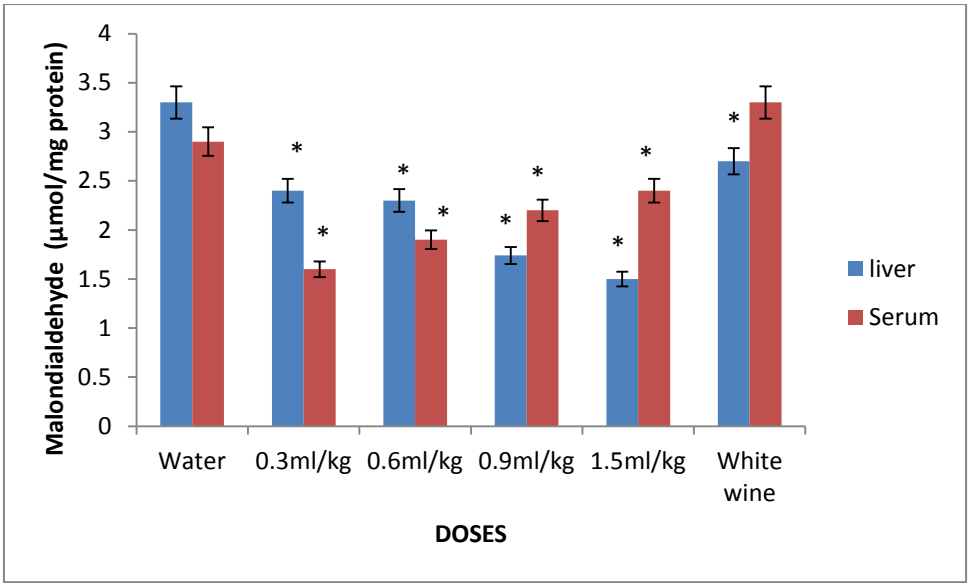

Fig. 1: Effect of date fruit wine on lipid peroxidation in serum and liver of treated albino rats

DISCUSSION

Dates are the sweet fruits of the tree *Phoenixdactylifera* or the date palm. These fruits are being consumed all over the world primarily as a source of energy. Numerous studies have been conducted to study the benefits of the date palm, either from its fruit or seed, and it has been found that the date palm possesses several highly beneficial properties such as antiviral, antifungal, antioxidant, anti-hyperlipidemic activity and hepatoprotective activity [13]. The whole date fruits are traditionally used to prepare a wide range of products such as date juice concentrates (spread, syrup and liquid sugar), fermented date products (wine, alcohol, vinegar, and organic acids) and date pastes for different uses (e.g. bakery and confectionary) besides their direct consumption. The alcohol content of the fermenting must increased progressively both aerobic and anaerobic fermentation. The increase in the alcohol content can be attributed to yeast metabolism by continuous utilization of the sugar content, ethanol is produced and thus there is an increase in the alcohol content of the fermenting must, this continued until all the available sugar in the fermenting must has been utilized. The final alcohol content of the wine (9.2%) ranks it among good table wines. A good table wine must have alcohol content between 8 and 14% [14].

In the present study, the effect of the date fruit wine on blood hematology and some biochemical parameters was investigated.

The results showed a significant decrease in the final body weights of the animals treated with date fruit wine. Alcohol has been reported to increase metabolic rate significantly, thereby causing more calories to be burnt rather than be stored in the body as fat [15].Thus the decreased body weights could be attributed to the increased metabolic rate in the treated groups.

The results of the hematological study shows that treatment of the rats with date fruit wine caused no significant changes on the RBC count, Hb, PCV and WBC when compared with the control and standard wine. This indicates that there was no destruction of matured red blood cells, neither is there any change in the rate of erythropoiesis, although a relative increase was observed at a dose of 1.5ml/kg.

High levels of total cholesterol (TC), low density lipoprotein-cholesterol (LDL-C) and triglyceride (TG) leads to cardiovascular diseases and atherosclerosis; as they are deposited in the arterial walls causing plaques and lesions. In this study, the observed reduction in triglycerides and relatively similar LDL-C level in the groups administered PD wine compared to the control may be an indication of the ability of the wine to maintain normal lipid status in the living system. This is also corroborated by the observed elevated high density lipoproteins (HDL-C) of the treated groups (at 0.9ml/kg and 1.5ml/kg) compared to the control. It has been reported that high levels of triglyceride is associated with high levels of smaller/denser LDL-C particles (pattern B) while low levels of triglyceride is associated with high levels of the larger/ less dense LDL-C particles (pattern A) [16, 17].

Thus, in the present study, the significantly lower levels of TG observed in the treated groups is linked to the pattern –A form of the LDL-C which is relatively harmless. Moreso, the observed increase in HDL-C indicates a protective effect of the date fruit wine.

The excessive production of free radicals and lipid peroxides could induce lipid peroxidation of cell membrane structure leading to ionic leakage through

cellular membranes and excessive calcium influx with incident cellular dysfunction and death from calcium overload [18]. Furthermore, increased lipid peroxidative status in the membrane indicates membrane and tissue damage because the end products of lipid peroxidation may be mutagenic and carcinogenic [19]. However the administration of date fruit wine as observed in this experiment was able to reduce lipid peroxidation in both serum and liver homogenates indicating a protective effect on the tissues against oxidative damage.

CONCLUSION

The present study revealed similar results in both the hematological and biochemical parameters of the rats treated with date fruit wine and standard white wine, indicating that date fruit wine if properly processed and refined could be another good source of commercial fruit wine with immense nutritional and health benefits.

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