International Scholars Journals

African Journal of Internal Medicine ISSN 2326-7283 Vol. 3 (10), pp. 256-261, November, 2015. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Impact of a multi-fruit wine on some blood parameters of male and female rats

***Moshood O. Ujah, Onyeka C.A and Abubakar L. M**

Department of Medical Biochemistry, Faculty of Medicine, University of Benin, Edo State, Nigeria.

Accepted 19 October, 2015

This study was aimed at evaluating the impact of wine obtained from a blend of five fruit extracts on some haematogical indices of male and female albino Wistar rats. Seventy male and female rats were randomly divided on the basis of body weight and sex into seven study groups. Group I (normal control) received normal diet and distilled water only, while the other groups except VI and VII (which had low doses of standard red and cashew wines respectively) received either low (corresponding to four standard drinks) or high (six standards) doses of the respective multi-fruit wine samples. Haematological indices determined showed that Hb and RBC values were not altered in both sexes compared with the control. The WBC values of all the test groups of female and male rats decreased significantly (P<0.05) compared with the normal control except H-MFWA (high dose of multi-fruit wine with additives) in females which compared well with the normal control. General decrease in MCV values of females and increase in males were significant (P<0.05) in some cases when compared with the normal control. Results of the investigation showed that sex differences associated with the handling of alcohol toxicity in the serum exist among male and female rats with the administration of multi-fruit wine. It also suggests the possible role of phytochemicals and wine additives in influencing the outcome of certain haematological parameters.

Key words: Haematological parameters, multi-fruit wine, phytochemicals, standard wines, wine additives.

INTRODUCTION

Wine is a term associated mainly with alcoholic beverage made from the fermentation of grape juice (Alais and Linden, 1999). However, wine in general is the juice of fruits, tubers, leaves of plant etc. that has been subjected to alcoholic fermentation (Amerine et al., 1980). Such wines are always classified. In other words, wines made from fruits other than grape are classified as fruit wines (Jacobs, 2001).

Wine has enormous health benefits similar to those of fruits from which they are derived (Jacobs, 2001). There is an inverse relationship between moderate alcohol consumption and cardiovascular diseases as seen in the decreased risk of peripheral arterial disease in apparently healthy men (Camargo et al., 1997) and also in women and non-smoking men who are already suffering from the

*Corresponding author. E -mail: ujah_m@yahoo.com

disease (Mingardi et al., 1997).

Wines have been known for their medicinal effect and Physicians believe that wine consumption can aid digestion and help relief stress. Several attributes have been attached to the consumption of alcoholic beverages as they are found for example to be inversely associated with the risk of cholecystectomy (Leitzman, 2003). The consumption of red wine is known to have a remarkable protective effect against oxidative stress in blood plasma (Nakamura et al., 2009).

It delays tumorigenesis due to the presence of specific dietary polyphenols, such as catechins (Ebeler et al., 2002). Alcoholic beverages especially wine produce decreased levels of inflammatory biomarkers (Imhof et al., 2004).

There is an inverse correlation between alcoholic beverage consumption and lymphocyte levels of 8-hydroxydeoxyguanosine, the primary marker for cancer (Bianchini et al., 2001).

However, wine and other alcoholic beverages have also been associated with some negative effects including decreased levels of WBC which shows an independent association (Nakanishi et al., 2003; Estruch et al., 2004; Imhof et al., 2004), though some studies have reported otherwise (Shaper et al., 1985; Oduola et al., 2005) and have also reported on the highly positive and negative association of alcohol with other haematological parameters.

It is necessary to see in this study, whether the aforementioned reports on the haematological parameters considered in previous studies apply to the administration of multi-fruit wine produced, to what extent it does and most importantly if variations exist in these indices among male and female sexes. The display of the inherent characteristics of the multi-fruit wine such as phytochemicals and wine additives in influencing certain haematological indices are also of interest.

MATERIALS AND METHODS

Preparation of multi-fruit wine

The Multi-fruit wine was prepared from a blend of five fruit extract namely, *Lycopersicon esculentum* (tomato), *Citrus sinensis* (orange), *Citrus lemon* (lemon), *Chrysophyllum africanum* (African star apple) and *Prunus amygdalus var dulcis* (almond). The samples collected were processed for the production of the multi-fruit wine as described by Asuk et al. (2011). The fermentation media was obtained and 'must' prepared. Briefly, 20 balls of tomato weighing 1 kg were thoroughly washed with clean water and ground with a blender, 70 g of the almond nut was weighed and also ground with a blender, while 20 balls of orange weighing 3 kg, 10 balls of lemon weighing 800 g and 20 balls of African star apple weighing 2 kg were all squeezed out manually to obtain their respective juice and then covered in pre-sterilized containers. The respective fruit blends of tomato, orange, almond, African star apple and lemon were all mixed together in a 20 litre fermentation jar which was filled to the mark with distilled water. Seven (7 g) of instant baker's yeast, 3.06 g of sodium metabisulphite, 3.45 g of ammonium sulphate were all added and finally, 3.90 kg of granulated sugar was also added to induce fermentation by the baker's yeast. The specific gravity of the resultant mixture in the fermentation jar containing the above mixture of the 'must' was taken at 1.085 at the start of the experiment. On the sixth day of the experiment, the primary fermentation stopped when the specific gravity dropped to 1.000 and remained unchanged. The young fruit-wine was racked (decanted) every week after the primary fermentation stopped, to separate sediments of the wine in order to achieve clarity. The multi-fruit wine was bottled and allowed to age for about six (6) months. It was later stored in the refrigerator at 10-15°C until when required for use. This method is a subject of an earlier report described by Asuk et al. (2011).

To four (4) liters of the multi-fruit wine were added 0.4% citric acid, 6% caramel, 0.003% potassium metabisulphite and 3% sugar. The wine was then filtered (using a Whatman No. 1 filter paper) and bottled to obtain a standard wine referred to as 'wine with additives'.

Another set of wine not treated with additives was left as plain wine. The standard wines vis-à-vis cashew wine and red wine were obtained from CRIN Ibadan and Rabana Supermarket, Calabar respectively. The multi-fruit wine was then characterized or standardized, the alcohol content was determined as 12% with a corresponding value for the red and cashew wines (which were indicated on their bottles). The phytochemical screening was also done for all the wine samples (including red and cashew wines) as indicated in Asuk et al. (2011).

Experimental animals

A total of 70 albino Wistar rats used for this study were obtained from the animal house of the Faculty of Agriculture, University of Calabar, Calabar, Cross River State, Nigeria. The study was given the go ahead by the Animal House Committee of the University. All conditions of animal use were also as approved by United States National Institute of Health (NIH) guide for Care and Use of Laboratory Animals and in accordance with the recommendation of IASP (Zimmermann, 1983). The animals were reared with a commercial stock diet – guinea feeds rat chow (Guinea Feeds Nigeria Ltd, Benin) until they weighed 100 – 250 g when they were used for the experiment. They were housed in wooden box cages (size 1m × 0.5m × 0.2m). The beddings were changed regularly. The animals were kept in the Department of Biochemistry animal house under adequate ventilation with a temperature and relative humidity of 26 ± 2°C and 46%, respectively to acclimatize for 7 days. Feed and

water were provided *ad libitum.*

Experimental design

This involved animal experimentation and treatment with multi-fruit wine obtained from a blend of tomato, orange, African star apple, lemon and almond to determine some physiological changes.

Seventy (70) albino Wistar rats were randomly assigned on the basis of body weight and sex into 7 study groups of 10 rats per group (5 males and 5 females), and treated according to the doses schedule below:

Group I - C: Control group given normal diet + water
Group II - L- MFWA: Low dose of wine produced with additives administered at 5.71 ml /kg body weight for male and females rats.
Group III - H – MFWA: High dose of wine produced with additives administered at 8.57ml/ kg body weight for male and female rats.
Group IV - L – MFWP: Low dose of wine produced without additives administered at 5.71ml/kg body weight for male and female rats.
Group V – H – MFWP: High dose of wine produced without additives administered at 8.57ml/kg body weight for male and female rats.
Group VI – STD 1: Low dose of standard red wine administered at 5.71 ml/kg body weight for male and female rats.
Group VII – STD 2: Low dose of standard cashew wine administered at 5.71 ml/kg body weight for male and female rats.

The animals were administered low and high doses of wine with additives and without additives, while low doses only of the standard wines 1 (red wine) and 2 (cashew wine) were given to their respective groups orally. In addition, they were allowed free access to rat chow and drinking water *ad libitum*. The control was given normal diet and drinking water only. The dose levels used for the animal wine administration was based on the AGDHA on 'Standard drinks guide' for levels considered to be low-risk for a 70 kg man (AGDHA, 2009). The experimental regime lasted for 2 weeks.

The body weights were monitored every two days throughout the duration of the experiment, all the experimental animals was finally denied access to feed for 18 h prior to sacrifice.

Collection of blood samples

Blood samples were collected via cardiac puncture after anesthesia with chloroform (5%) inhalation. They were placed in sterile EDTA sample bottles and briskly agitated to prevent clotting. The samples were used for the estimation of the different haematological parameters within 12 h of collection.

Estimation of haematological parameters

Packed cell volume (PCV) was estimated by the capillary method of Dacie and Lewis (2001). Haemoglobin was estimated based on the principle that it oxidizes to methaemoglobin by ferricyanide. The methaemoglobin is converted to cyanmethaemoglobin by addition of KCN, with absorption maxima at 540 nm. The test samples and the controls were measured against standard cyanmethaemoglobin (BDH Laboratory supplies, Poole BH 151 TD, England).

The red and white blood cells were counted by microscopic visual identification using appropriate diluting fluids according to the methods of Dacie and Lewis (2001), while the absolute red blood cell indices, MCV, MCH and MCHC were derived by calculation from values obtained from RBC count, Hb and PCV using standard formula, thus:

MCV (fL) = (PCV % / RBC count) x 10
MCH (pg) = (Hb conc. in g/dL / RBC count) x 10
MCHC (g/dL) = (Hb conc. in g/dL / PCV %) x 100

Statistical analysis

Data obtained were subjected to statistical analysis using standard computerized Statistical Package for Social Science (SPSS) version 11. ANOVA, post hoc (least significant difference - multiple comparison) test were carried out and values expressed as mean±SEM. Statistical significance was accepted at $p<0.05$.

RESULTS

The haematological values of the female and male albino Wistar rats are given in Tables 1 and 2.

In the female rats, Hb concentration was not significantly altered following administration of the different fruit wine. In the male rats, Hb concentration of all the test samples compared well with the control, but H-MFWA was significantly ($p<0.05$) lower compared with the standard groups (Table 1).

The female PCV values of only H-MFWA showed significant ($P<0.05$) increase compared with both the normal control and the standard controls. The rest showed no significant ($P\geq0.05$) difference (Table 1). The male PCV values also showed a general increase in the wine administered groups, but the increase was only significant in L-MFWA when compared with the normal control (Table 1).

The RBC of the female experimental animal showed no significant ($P\geq0.05$) difference when compared with either the control or the standard controls (Table 1). The RBC count of the male rats increased significantly ($p<0.05$) in L-MFWA compared with standard-1, but a significant ($p<0.05$) reduction was observed in H-MFWA and L-MFWP compared with control and standard-2 (Table 1).

Table 1. Wine administration on haemoglobin concentration, packed cell volume and red blood cell count of female and male albino Wistar rats.

Group	Hb (g/dl)		PCV (%)		RBC (Nx10^6/mm^3)	
	Females	Males	Females	Males	Females	Males
I (C)	11.58 ±0.34	11.65 ±0.28	40.25±0.59	42.50±1.89	5.05 ±0.07	5.03±0.03
II (L- MFWA)	11.74±0.22	12.29±0.38	40.75±1.08	47.56±1.36^{a}	5.61±0.16	5.03±0.19^{b}
III (H- MFWA)	11.86±0.42	10.90±0.58^{bc}	44.25±0.94^{abc}	44.25±0.83	5.47±0.13	4.25±0.26^{abc}
IV (L- MFWP)	11.63±0.09	12.10±0.32	39.00±0.96	41.94±1.19	5.40±0.04	3.85±0.06^{ac}
V (H- MFWP)	12.03±0.13	12.26±0.36	41.63±1.36	42.31±1.92	5.72±0.12	4.91±0.22^{bc}
VI (STD 1)	12.01±0.14	12.00±0.28	40.38±1.38	44.69±1.21	5.33±0.06	3.73±0.03^{a}
V II (STD 2)	11.85±0.09	12.70±0.45	40.25±0.62	45.50±0.59	5.41±0.05	5.29±0.06

Values are mean ± SEM (n=5); a = p<0.05 vs control (C); b = p<0.05 vs STD 1; c = p<0.05 vs STD 2.

Table 2. Wine administration on WBC count and absolute red blood cell indices of female and male albino Wistar rats

Group	Parameter							
	WBC (Nx10^6/mm^3)		MCV (fL)		MCH (pg)		MCHC (g/dL)	
	Females	Males	Females	Males	Females	Males	Females	Males
I (C)	9.22±0.07	9.04±0.05	79.83±1.52	84.59±1.58	23.02±0.93	23.19±0.58	28.81±0.96	27.74±1.21
II (L- MFWA)	8.67±0.13^{abc}	6.86±0.04^{a}	72.93±2.52^{a}	94.53±2.81^{b}	20.99±0.52^{a}	24.42±0.77^{b}	28.97±1.05	25.83±0.34
III (H- MFWA)	9.13±0.05^{bc}	8.34±0.40^{abc}	81.17±2.37^{c}	107.06±7.36^{ac}	21.76±0.88	26.58±2.58	26.80±0.76^{bc}	24.71±1.31^{ac}
IV (L- MFWP)	8.33±0.08^{a}	6.83±0.02^{a}	72.21±1.62^{a}	109.7±3.99^{ac}	21.54±0.20	31.51±1.14^{ac}	29.93±0.77	28.91±0.60
V (H-MFWP)	8.38±0.05^{a}	7.34±0.38^{a}	72.79±1.95^{a}	88.34±7.39^{b}	21.06±0.33^{a}	25.45±1.64^{b}	29.09±0.94	29.37±1.54
VI (STD 1)	8.28±0.09^{a}	6.81±0.03^{a}	75.88±2.95	119.84±3.68^{a}	22.56±0.48	32.18±0.87^{a}	29.98±1.06	26.91±0.52
VII (STD 2)	8.44±0.06^{a}	6.86±0.04^{a}	74.57±1.72	86.19±1.54	22.41±0.40	24.11±1.07	30.10±0.47	27.92±0.91

Values are mean ± SEM (n=5); a = p<0.05 vs control (C); b = p<0.05 vs STD1; c = p<0.05 vs STD 2.

The female WBC values of all the test groups including the standard groups decreased significantly (p<0.05) compared with control except H-MFWA which compared well with the control and had a significant (p<0.05) increase in WBC count when compared with the standard groups (Table 2). The WBC values for all the male groups showed significant (p<0.05) decrease compared with the control, however only H-MFWA showed significant (p<0.05) increase when compared with both standard controls (Table 2).

The MCV values of female rats decreased significantly (p<0.05) in L-MFWA, L-MFWP and H-MFWP compared with control, whereas H-MFWA compared well with control. In the male, MCV values of L-MFWA and H-MFWP compared well with control but not with STD 1 as they were significantly (p<0.05) lower. H-MFWA, L-MFWP as well as STD 1 showed significant (P<0.05) increase when compared with the control. H-MFWA and L-MFWP were also significantly (p<0.05) higher than STD 2 (Table 2).

The female MCH values also showed a decrease in all the groups, which was only significant (p<0.05) in L-MFWA and H-MFWP compared with the control. There was no significant (p≥0.05) difference compared with the standard controls. The male MCH values of only L-MFWP and STD 1 showed significant (p<0.05) increase compared with the control (Table 2).

The MCHC values of the female and male rats compared well with control. However, there was a decrease in H-MFWA of female but significant (p<0.05) in male.

DISCUSSION

The haematological indices of female and male albino Wistar rats have been assessed with graded doses of multi-fruit wine. Alcohol has been reported to have a highly significant positive association with haemoglobin, PCV and WBC and a highly significant negative association with RBC (Shaper et al., 1985; Oduola et al., 2005). The Haemoglobin measurements have been used to estimate the oxygen carrying capacity of blood, in addition the assessment of the reticulo-endothelial status. It has been documented that a decrease could mean anaemia, while an increase polycythemia (Baker and Silverton, 1985).

The haemoglobin values of the female and male rats administered multi-fruit wine did not show any sex

difference or any dose related effect. However, only the PCV values of H-MFWA of female and L-MFWA of male showed significant increase that is not dose- dependent which agrees partially with the reports of Shaper et al. (1985) and Oduola et al. (2005). The most likely reason for this is that wine does not respond directly as alcohol does because it contains phytochemicals which also exhibit some protective action (Olas et al., 2002; Erhardt et al., 2005; Nkondjock et al., 2005; De Lange, 2007). The interaction of these phytochemicals among sexes varies and it is unclear how these may act. Flavonoids and Saponins have been found to have varied responses in female and male organisms, respectively (Frigo et al., 2002; Tin et al., 2007).

The RBC of female showed general increase, while the male showed decrease in some cases which were significant compared with the control. Moreover, administered STD 1 was also significant compared with control. This negative association of alcohol with RBC, as earlier reported, is seen only in males administered H-MFWA, L-MFWP and STD 1.

Apart from H-MFWA which compared well with the control, there was a general significant decrease in the MCV values of females and increase in males which were not dose-dependent. The increase in male was found to be significant in some cases. Increase in MCV has been associated with excessive alcohol consumption (Chick et al., 1981). The result is suggestive of the direct effect of alcohol on the serum of male but does not show dose-dependent effect.

There was a general decrease in MCH of female rats which was significant for H-MFWP and general increase in MCH of males which was significant for L-MFWP as well as STD 1. This suggest that female had tendency towards microcytosis while the male macrocytosis. Microcytosis is caused by iron deficiency resulting in low RBC, while macrocytosis may be due to vitamin B_{12} and folate deficiency resulting in low haemoglobin levels. However this was not the case as the female had normal RBC. Though there was decreased RBC in male, the haemoglobin level were normal.

The MCHC value administered H-MFWA decreased in female, but was significant in male. The rest showed normal response. MCHC has been used as a guide in the classification of anaemia (Baker and Silverton, 1985). This decrease could be due to iron deficiency or hypochromia.

Macrocytosis is frequently linked with alcoholism, with or without liver disease. Alcohol is purported to be the main causes of non megaloblastic macrocytosis (Savage et al., 2000). Large circulating erythrocytes are not always associated with a pathologic process or condition. In fact, RBCs of newborns and infants tend to be larger (mean MCV = 108 fl) than normal adult RBCs and large erythrocytes can be seen during pregnancy in the absence of an obvious etiology (Aslinia et al., 2006). Macrocytosis without anemia may be a normal variant and is only noted as a result of repeated peripheral RBC indices in the absence of any known or existing clinical problems. In some instances, this variation from normal can be found in other family members, suggesting a genetic predisposition and requires no therapeutic intervention or further investigation (Aslinia et al., 2006).

This report however appears to support the present study that the male rats may have exhibited macrocytosis without anaemia, while the female microcytosis without anaemia.

The WBC values for both female and male rats registered a significant decrease including the standard groups except H-MFWA of female which compared well with control. H-MFWA of the male rats was closer to control than the standard groups. This suggests that not only was there absence of foreign bodies on administration of multi-fruit wine, but that the immune system was not agitated. An agitated immune system usually results in increased WBC as observed in cigarette smoking, overall obesity etc, which has a positive correlation with coronary heart disease (Nakanishi et al., 2003).

It is not quite clear at this stage why this varied responses of different doses of MFWA and MFWP on female and male rats on certain haematological indices, but phytochemical studies already carried out, indicate the presence of glycosides, reducing compounds, flavonoids and polyphenols in all the test samples, while alkaloids were only present in STD 2, saponins present in all except MFWP, while tannins were present only in MFWA and MFWP. Anthraquinones were conspicuously absent in all the test samples with phlobatanins and hydroxymethyl anthraquinones present only in STD1 (Asuk et al., 2011). These phytochemicals such as flavonoids (which are estrogenic) and saponins as stated by Frigo et al. (2002) and Tin et al. (2007), most likely may have influenced certain cell signaling pathways that regulate the outcome of certain haematological indices in the female and male rats.

Alcohol has been associated with significant increase in Haemoglobin, PCV and WBC and a significant decrease in RBC (Shaper et al., 1985; Oduola et al., 2005). In this study, using multi-fruit wine, this association is not clear- cut even among female and male rats. The study agrees with the reports of Estruch et al. (2004) and Imhof et al. (2004) that wine and other alcoholic beverages produce decreased levels of WBC. There are also reports that if wine administration is given above two weeks and at not less than three standard drinks, there would be positive effect on immune function (Guilford and Pezzuto, 2011). It is unsure if that would have been the case in this study as the administration was done for two weeks. They had been varied response in some haematological parameters which mostly were not dose-dependent but varied among sexes.

Two standard drinks have been appropriated for male and one for female as moderate intake. However, the low

dose which corresponds to four drinks irrespective of sex was applied and a high dose equivalent to six standards was used. There appear to be a better adaptive response of female to alcohol toxicity to protect the integrity of the serum than male. Generally speaking, some of the multi-fruit wine samples are comparable with the standard wines. For example L-MFWA compares well with cashew wine (STD 2), while L-MFWP compares well with red wine (STD 1) irrespective of sex. Some wine samples have shown better haematological indices compared to standards.

The multi-fruit wine collectively, that is, MFWA and MFWP irrespective of dose and sex, have shown better haematological indices than the red wine. It is unclear why the female in response to all the test samples at the various dose levels, showed microcytosis without anemia and the male macrocytosis without anemia. This indeed indicates that there are sex variations when the serum is exposed *in vivo* to alcoholic beverages.

Conclusion

This research shows clear evidence that sex differences associated with the handling of alcohol toxicity in the serum exist among females and males. The use of multi-fruit wine in this research has brought to the fore the protective nature of multi-fruit wine especially in cases of alcoholic toxicity. It is evident that the varied responses to phytochemicals present in the wine and the role played by these phytochemicals have brought varied adaptive mechanisms to alcoholic beverage consumption among female and male rats. However, the mechanisms of the phytochemicals and that of the additives present in the multi-fruit wine on the outcome of haematological indices of female and male rats appear unclear. Further research is required to elucidate these mechanisms.

ACKNOWLEDGEMENTS

The authors are grateful to Prof. F. Maifo of the Department of Biochemistry, University of Calabar, Cross River State, Nigeria, for making available some materials on wine making. We are also grateful for the technical support provided by Dr. Janifa Maduka, Department of Biochemistry, University of Benin, Edo State, Nigeria.

REFERENCES

Alais C, Linden G (1999). Food Biochemistry. Maryland: Aspen Publishers.

Amerine MA, Berg HW, Kunkee RE, Ough CS, Singleton VL, Webb AD (1980).The technology of wine making, 4th edition. Connecticut : The AVI Publishers.

Aslinia F, Mazza JJ, Yale SH (2006). Megaloblastic anemia and other causes of macrocytosis. Clin. Med. Res., 4(3): 236-241.

Asuk AA, Umoh IB, Eteng MU, Jayeola CO, Akpanyung EO (2011). Production, characterization and safety of wine obtained from a blend of tomato, almond, orange, lemon and African star apple extract. Ann. Biol. Res., 2(5): 492-503.

Australian Government Department of Health and Ageing (2009). Standard drinks guide. Canberra: Woden.

Baker FJ, Silverton RE (1985). Introduction to medical laboratory technology, 6th edition. London: Butterworths.

Bianchini F, Jaeckel A, Vineis P, Martinez-Garcia C, Elmstahl S, van Kappel A, Boeing H, Ohshima H, Riboli E, Kaaks R (2001). Inverse correlation between alcohol consumption and lymphocyte levels of 8-hydroxydeoxyguanosine. Carcinogenesis, 22(6): 885-890.

Camargo CAJr, Stampfer MJ, Glynn RJ, Grodstein F, Gaziano JM, Manson JE, Buring JE, Hennekens CH (1997). Moderate alcohol consumption and risk for angina pectoris or myocardial infarction in U.S male physicians. Arch. Intern. Med., 126(5): 372-375.

Chick J, Kreitmann N, Plant M (1981) Mean cell volume and gamma-glutamyltranspeptidase as markers of drinking in working men. Lancet, 1: 1249-1251.

Dacie JV, Lewis M (2001). Pratical haematology, 9th edition. London: Churchill Livingstone.

De Lange DW (2007). From red wine to polyphenols and back: a journey through the history of the French paradox. Thromb. Res., 119(4): 403-406.

Ebeler SE, Brenneman CA, Klim G (2002). Dietary catechins delay tumor onset in transgenic mouse model. Am. J. Clin. Nutr., 76(4): 865-872.

Erhardt JG, Meisner C, Bode JC, Bode C (2005). Lycopene, beta-carotene and colorectal adenomas. Am. J. Clin. Nutr., 8(6): 1219-1224.

Estruch R, Sacanella E, Badia E, Antunez E, Nicolas JM, Fernandez-Sola J, Rotilio D, de Gaetano G, Rubin E, Urbano-Marquez A (2004). Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: A prospective randomized crossover trial effects of wine on inflammatory markers. Atherosclerosis, 175:117-123.

Frigo DE, Duong BN, Melnik LI, Sherif LS, Collins-Burow BM, Pace DK, McLanchlan JA, Burrow ME (2002). Flavonoid phytochemicals regulate activator proteins -1 signal transduction pathway in endometrial and kidney stable cell lines. J. Nutr., 132: 1848-1853.

Guilford JM, Pezzuto JM (2011). Wine and health: A Review. Am. J. Enol. Vitic., 62(4): 471-486.

Imhof A, Woodward M, Doering A, Helbecque N, Loewel H, Amouyel P, Lowe GD, Koenig W (2004). Overall alcohol intake, beer, wine, and systemic markers of inflammation in Western Europe: Results from three MONICA samples (Augsburg, Glasgow, Lille). Eur. Heart J., 25: 2092-2100.

Jacobs F (2001). Making wine from pineapple. Owerri: Ihem Davis Press.

Leitzman MF, Tsai C, Stampfer MJ, Rimm EB, Colditz GA, Willett WV, Giovannicci EL (2003). Alcohol consumption in relation to risk of cholestectomy in women. Am. J. Clin. Nutr., 78(2): 339-347.

Mingardi R, Avogaro A, Noventa F, Strazzabosco M, Stocchiero C, Tiengo A, Anderle G (1997). Alcohol intake is associated with lower prevalence of peripheral vascular disease in non-insulin dependent diabetic women. Nutr. Metab. Cardiovas., 7(4): 301-308.

Nakamura T, Fujiwara N, Sugaya T, Ueda Y, Koide H (2009). Effect of red wine on urinary protein, 8-hydroxydeoxyguanosine and liver- type fatty acid-binding protein excretion in patients with diabetic nephropathy. Metabolism, 58(8): 1185-1190.

Nakanishi N, Suzuki K, Tatara K (2003). Association between life style and white blood cell count: a study of Japanese male office workers. Occ. Med., 53: 135-137.

Nkondjock A, Ghadirian P, Johnson KC, Krewski D (2005). Dietary intake of lycopene is associated with reduced pancreatic cancer risk. J. Nutr., 135(3): 592-597.

Oduola T, Adeosun OG, Oduola TA, Agbaje NR, Raheem ZA (2005). Drinking patterns: biochemical and haematological findings in alcohol consumers in Ile-Ife, Nigeria. Afr. J. Biotechnol., 4(11): 1304-1308.

Olas B, Wachowicz B, Salukjuszczak J, Zielinski T (2002). Effect of resveratrol, a natutal polyphenolic compound, on platelet activation induced by endotoxin or thrombin. Thromb. Res., 107(3-4): 141-145.

Savage DG, Ogundipe A, Allen RH, Stabler SP, Lindenbaum J (2000). Etiology and diagnostic evaluation of macrocytosis. Am. J. Med. Sci., 319: 343-352.

Shaper AG, Pocock SJ, Ashby D, Walker M, Whitehead TP (1985). Biochemical and haematological response to alcohol intake. Ann. Clin. Biochem., 22: 50-61.

Tin MMY, Cho C, Chan K, James AE, Ko JKS (2007). Astragulus saponins induce growth inhibition and apoptosis in human colon cancer cells and tumor xenograft. Carcinogenesis, 28(6): 1347-1355.

Zimmermann M (1983). Ethical guidelines for investigation of experimental pain unconscious animals. Pain, 16: 109-110.