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Research Article Dietary Inclusion of Locally Made Sweet Potato Wine Protects Against Biochemical Alterations in High Cholesterol Fed Rats

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Abstract

Background and Objective: Sweet potato (*Ipomoea batatas*) is the 6th most important food crop and possesses anticancer, antidiabetic and anti-inflammatory properties. This study investigated the effect of sweet potato wine in altered biochemical indices in cholesterol fed rats. **Materials and Methods:** Wister albino rats weighing between 90-120 g were randomized into 8 groups of 10 rats each fed with fresh sweet potato and sweet potato extract. Group 1 (control) were fed normal rat feed and were given sterile placebo (distilled water). Group 2 (negative control) were fed normal rat feed and 2.5% cholesterol. Group 3 (positive control) were fed normal diet, 2.5% cholesterol and standard red wine (400 mL/70 g). Group 4-7 were the treatment groups as follows: TI (2.5% cholesterol+400 mL/70 g potato wine), T2 (2.5% cholesterol+800 mL/70 g potato wine), T3 (400 mL/70 g potato wine) and T4 (800 mL/70 g potato wine). Group 8 (standard control) were fed normal diet and standard red wine (400 mg/70 g). The wines and the cholesterol were daily administered at 9 am and 4 pm, respectively for 5 weeks and the animal were sacrificed 24 h after the last administration. **Results:** The sweet potato wine and extract: restores abnormalities in lipid profiles in the cholesterol fed rats, does not have a significant effect on the serum renal function biomarkers and the liver function biomarkers were significantly increased. **Conclusion:** The study suggests that administration of sweet potato wine would be beneficial in restoring lipid profiles while eliciting liver damage which might not have a significant effect on kidney function.

Key words: Potato wine, sweet potato, Soxhlet apparatus, cholesterol, lipid profiles

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The sweet potato (*Ipomoea batatas* L.), an herbaceous dicotyledonous plant that is large, starchy, sweet-tasting and tuberous roots which are a root vegetable and is widely cultivated throughout the world¹. Being a favorite staple of many cultures, it is a favorite ingredient in many ethnic cuisines². Besides simple starches, raw sweet potatoes are rich in complex carbohydrates, dietary fiber and beta-carotene (a provitamin A), with moderate contents of other micronutrients, including vitamin B5, vitamin B6 and manganese³. Proteins and fats are contained in sweet potatoes in small quantities⁴. When cooked by baking, small variable changes in micro-nutrient density occur to include a higher content of vitamin C at 24% of the daily value per 100 g serving⁵.

The nutritional value of sweet potatoes is ranked as highest among several other foods⁶. However, new varieties now produce more edible energy per day than any other major food crop and 30% more starch/unit area⁷. Sweet potato tubers are used as an energy crop: The tubers can be fermented to produce alcohol and the plant grows in areas when maize does not. Other sweet potato products are suitable for livestock⁸.

Sweet potato leaves were classified as a physiologically functional food which offers protection from various diseases and health promotion by reducing oxidative stress^{8,9}. Their role is mediated by the ability to promote more favorable antioxidant status, free radical scavenging capacities and prevent processes involved in disease pathogenesis⁹. Active constituents were grouped into two types of polyphenols as anthocyanins and phenolic acids. The anthocyanins were acylated cyanidin and peonidin type while the phenolic acids were composed of caffeic acid and five kinds of caffeoylquinic acid derivatives⁸. Physiological functions related to these polyphenols as radial scavenging, antimutagenic, anticancer, antidiabetic, antibacterial, anti-inflammatory, cardioprotective and chemopreventive activities have been demonstrated *in vitro* and *in vivo*^{8,9}.

Wine making and cultivation of grape spread throughout the world. It is an alcoholic beverage made from fermented grapes. Yeast consumes the sugar in the grapes and converts it to ethanol, carbondioxide and heat¹⁰. Varieties of grapes and strains of yeasts produce different style of wine. This variation results from the complex interactions between biochemical development of the grape, the reactions involved in fermentation, the terroir and the production process. Fruits such as; apple, berries, blackcurrants, orange and water melon are sometimes fermented for wine production¹¹.

Purple Sweet Potato (PSP) has been successfully used for preparation of wine^{12,13}. High anthocyanins with peonidin and cyanidin as the major components in purple and red fleshed sweet potatoes have been reported by several investigators^{14,15}. Also, wide variation is observed in the total anthocyanins content among the PSP cultivars and breeding lines. Teow et al.15 reported that the total anthocyanins contents of PSP varied in the range of 17-531 mg kg¹ roots among 19 genotypes. Many researchers reported that PSP anthocyanins (antioxidant) could scavenge free radicals, attenuate liver dysfunction, enhance memory function, decrease blood sugar, lower insulin resistance and could also inhibit cancer cell growth and other functions^{14,16,17}. Anthocyanins have been reported to exert cancer chemopreventive activity¹⁸. Furthermore, PSPs contain more types of anthocyanins and acylated anthocyanins compared with those in other fruits and vegetables, they were found to have a comparatively higher degree of stability in heat and liaht¹⁶.

Humans are exposed to a large number of chemical, biological and physical agents, as well as environmental determinants that can impinge on health. The ability of humans to fight against these factors is important for maintenance of their health and productivity¹⁹. Oxygen free radicals and other reactive oxygen species are produced in the human body as by-products through numerous physiological and biochemical processes. As a result of aerobic metabolism, oxygen related free radicals (superoxide and hydroxyl radicals) and reactive species (hydrogen peroxide, nitric oxide, peroxynitrite and hypochlorous acid) are produced in the body²⁰. An increase in oxidative stress is the result of an imbalance between the rates of free radical production and elimination via endogenous antioxidant mechanisms such as; glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT)²¹. In humans, free radicals have been associated with over 100 diseases which include arthritis, hemorrhagic shock, atherosclerosis, early aging, ischemia and reperfusion injury of many organs. Alzheimer's and Parkinson's disease, gastrointestinal dysfunctions, tumor promotion and carcinogenesis and more²². Consequently, endogenous and exogenous antioxidants are believed to be important for preventing such diseases. The exogenous antioxidants that are widely available in fruits, vegetables, nuts and seeds have a broad spectrum of biological, pharmacological and therapeutic activities against free radicals and oxidative stress. Although, the bioavailability and therapeutic efficacy of antioxidants differ vastly, the antioxidants have been demonstrated to improve human health²³. This study was aimed at determining the anti-oxidant properties and phenolic compounds of locally made wine by using different *in vitro* techniques like DPPH assay, FRAP, total phenolic contents, total anthocyanins content and evaluation of its possible effect on some biochemical parameter on cholesterol fed rat.

MATERIALS AND METHODS

Chemical and reagents: The research was conducted at the Department of Medical Biochemistry and Pharmacology, Kwara State University, Malete, Nigeria from December, 2018-June, 2019. Fresh sweet potato wine was obtained from the Department of Biosciences and Biotechnology, Kwara State University, Malete. Eden classics red grape drink was a product of Nigeria Distillery Limited, Sango Ota, Nigeria. The entire study was carried out in 2 months between January-March, 2019. Cholesterol, methanol, Folin reagents, potassium ferricyanide, chloroform, sucrose, sodium sulphate (Na₂SO₄), copper sulphate (CuSO₄), sulphuric acid (H₂SO₄), sodium hydroxide (NaOH) and hydrochloric acid (HCI) were products of BDH Chemicals Limited, London, UK. All other chemicals used were obtained from the local supplier and were of analytical grade.

Sweet potato extraction: More than 20 tubers of sweet potato were cut into tiny slice, air dried for 2 weeks and grinded into fine powder. The powder was kept in an air-tight container in dark until used. About 121 g of the powder was extracted for 10 h in 750 L methanol using Soxhlet apparatus. The resulting extract was concentrated with rotary evaporator to about 15 mL and kept at -4° C until use.

Proximate analysis: The proximate analysis were carried out by using standard methods, reducing sugar^{24,25}, carbohydrates^{24,26}, total protein^{24,27}, moisture content²⁴ and vitamin C²⁸.

In vitro antioxidant activity and total phenolic content: DPPH radical scavenging capacity of the samples were estimated according to the method of Brand-Williams *et al.*²⁹ with slight modification. Ferric Reducing Antioxidant Power (FRAP) of the samples were determined by the method of Benzie and Strain³⁰. Anthocyanins levels were measured by the pH differential method described by Giusti and Wrolstad³¹. Total Phenolics (TP) content was estimated spectrophotometrically by Folin-Ciocalteu method described by Makkar³².

Experimental animals: Subsequent to obtaining Department of Medical Biochemistry and Pharmacology, Kwara state University, Malete Ethical clearance for the study, a total of 80 Wister rats weighing between 90-120 g were collected from the Animal Facility Center of the department. The rats were housed in clean metallic cages and were acclimatized for 7 days at a temperature of 28-30°C, 12 h light (12 h dark cycle). They all had *ad libitum* access to drinking water and rat feeds. The whole treatment was in accordance with the protocol of National Research Council Guide for the Care and Use of Laboratory Animals³³.

Animal grouping, treatment and management: The rats were completely randomized into 8 groups of 10 rats each for this study. Rats in group 1 were fed normal rat feed and were given sterile placebo (distilled water) and designated as control (CG). Group 2 comprised rats designated as negative control (NG) and was fed normal rat feed and 2.5% cholesterol. Group 3 rats was designated as positive control (PC) and were fed normal diet, 2.5% cholesterol and standard red wine (400 mL/70 g). Group 4-7 were the treatment groups designated TI-T4 and were placed on normal rat feed. In addition, rats in the treatment groups were treated as follow: TI (2.5% cholesterol+400 mL/70 g potato wine), T2 (2.5% cholesterol+800 mL/70 g potato wine), T3 (400 mL/70 g potato wine) and T4 (800 mL/70 g potato wine). Group 8 was the Standard Control (SC) and were fed normal diet and standard red wine (400 mg/70 g).

The locally made wine and standard red wine were administered at 9 am daily while the cholesterol was administered at 4 pm daily. All administrations were carried out for 5 weeks and the animal were sacrificed 24 h after the last administration.

Serum and plasma preparation: Serum and plasma were prepare as described by Tuck *et al.*³⁴, 24 h after the last administration and after 12 h fasting, the rats were humanely sacrificed by halothane anaesthetization and blood was collected into heparinized and plain sample bottles by cutting jugular vein. The blood was then centrifuged at 3000 rpm for 10 min to obtain the plasma and serum, respectively. The plasma and serum were collected into clean sample bottles and kept frozen until analysis. The rats were also quickly dissected and the liver and kidney isolated, blotted with clean

tissue paper, cleaned of fat and weighed. The organs were then homogenized with a dilution factor of 5 in an ice cold 0.25 M sucrose solution.

Biochemical assays

Lipid profile assay: TG, TC and HDL-C were analyzed by enzymatic colorimetric assay by using Hitachi 704 Analyzer (Roche Diagnostics, Indianapolis, USA). LDL-C was calculated from measured values of TG, TC and HDL-C in mg dL⁻¹ according to the equation of Friedewald *et al.*³⁵. The Atherogenic Index of Plasma (AIP) was determined as described by Dobiasova and Frohlich³⁶ and Castelli's Risk Index (CRI-I and CRI-II) were determined by the method of Castelli *et al.*³⁷.

Kidney function test: Serum creatinine concentration was determined by using the method of Bartels *et al.*³⁸, serum urea concentration was estimated by using the method of Veniamin and Vakirtzi-Lemonias ³⁹ and serum uric acid was determined according to the method described by Tietz⁴⁰. Serum Na, Ca, Mg, K, Cl and HCO₃ ions were determined by atomic absorption spectrometric method of Shokrollahi *et al.*⁴¹.

Liver function test: Serum AST and ALT activities were determined as described by Reitman and Frankel⁴², serum LDH activity was determined by following the principle of Wroblewski and Ladue⁴³, serum ALP activity was determined as described by the method of Recommendation of German Society of Clinical Chemistry⁴⁴, serum albumin was determined by the method of Doumas *et al.*⁴⁵ and serum bilirubin level was determined by the method of Jendrassik and Grof⁴⁶.

Statistical analysis of data: Data were analyzed by SPSS (version 20) and Mean±SEM were determined for all

parameters. Significant differences among the groups were determined by one-way Analysis of Variance (ANOVA) and levels of significance were evaluated by using Duncan's Multiple Range Test (DMRT) at p<0.05.

RESULTS

Percentage DPPH inhibitory activities of sweet potato wine:

The percentage inhibition of sweet potato wine at 20 mg mL^{-1} was significantly higher than that of red wine and significantly lower than that of the sweet potato extract while at 40, 60, 80 and 100 mg mL⁻¹, the percentage inhibition of sweet potato wine was significantly higher than that of red wine and sweet potato extract (Table 1).

FRAP, anthocyanins and TP contents of sweet potato wine: There was no significant difference in the FRAP content of the red wine, sweet potato wine and sweet potato extract. The anthocyanins content was significantly higher in sweet potato wine as compared to the red wine and sweet potato extract. While the total phenol content was significant higher in sweet potato extract when compared with red wine and sweet potato wine (Table 2).

Lipid profile: The serum total cholesterol level in the rats showed significant reduction in T2, T3, T4 and T5 groups when compared with CG and NC groups. Though there was no significant difference in the serum total cholesterol level in the T1, CG and NC groups (Fig. 1a). The serum triglyceride level showed significant reduction in PC, T1, T2 and T3 groups when compared to CG group but no significant difference in PC, T1, T2 and T3 groups when compared to NC group. There was no significant difference in the triglyceride level between T1, CG and NC groups (Fig. 1b). The serum HDL cholesterol level showed significant reduction in PC, T2, T3, T4 and SC groups

Samples	Inhibition (%)					
	 20 (mg mL ⁻¹)	40 (mg mL ⁻¹)	60 (mg mL ⁻¹)	80 (mg mL ⁻¹)	100 (mg mL ⁻¹)	
Red wine	73.91±0.56ª	80.24±0.37 ^b	81.31±0.47 ^b	81.17±0.09 ^b	81.92±0.19 ^b	
Sweet potato wine	76.93±0.51 ^b	46.78±1.40 ^a	45.99±1.40ª	48.04±0.98ª	48.04±0.98ª	
Sweet potato extract	80.62±0.19°	82.22±0.93°	83.22±0.09°	84.06±0.37 ^b	84.06±0.37 ^b	
Butylated hydroxy toluene	79.73±0.71°	81.10±0.76 ^b	85.72±0.34 ^d	91.68±0.18 ^d	100.83±0.57 ^d	

*Data were expressed as Mean±Standard deviation (SD) of 3 replicates *a, b, c and d represents the statistical level of significant difference such that a<b<c<d.

Table 2: FRAP, anthocyanins and TP contents of samples

Samples	FRAP (mg/100 mL)	Anthocyanins (mg/100 mL)	TP (mg/100 mL)
Red wine	223.54±26.00ª	10.11±0.01ª	790.16±1.98ª
Sweet potato wine	199.19±11.18ª	20.92±0.21 ^b	660.22±0.99ª
Sweet potato extract	223.71±9.12ª	00.77±0.91ª	105.18±7.71 ^b
Gallic acid	235.34±6.61ª	52.79±2.56°	199.16±4.75 ^d

*Data were expressed as Mean ± Standard Deviation (SD) of 3 replicates, *a, b, c and d represents the statistical level of significant difference such that a < b < c < d

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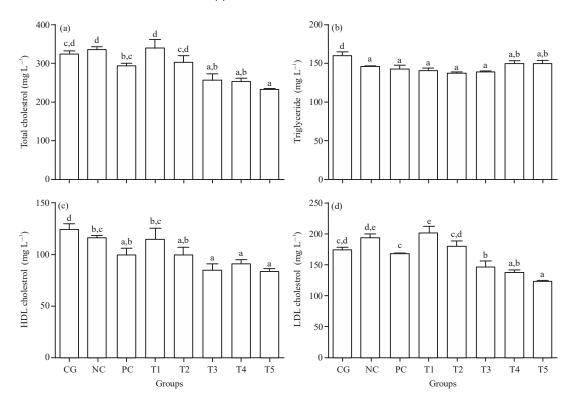


Fig. 1(a-d): Effect of potato wine of lipid profile levels in (a) Total cholesterol, (b) Triglyceride, (c) HDL cholesterol and (d) LDL cholesterol

Bars with different letters indicate significant difference at p<0.05, *a, b, c and d represents the statistical level of significant difference such that a < b < c < d

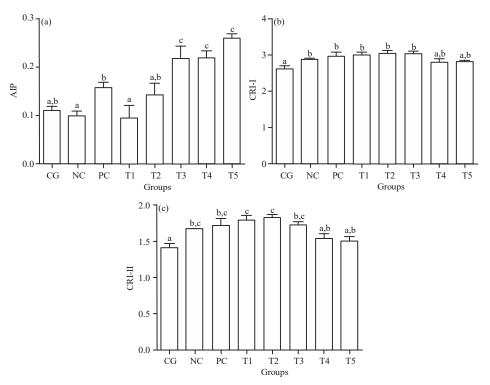


Fig. 2(a-c): Effect of potato wine of (a) AIP, (b) CRI-I and (c) CRI-II Bars with different letters indicate significant difference at p<0.05, *a, b and c represents the statistical level of significant difference such that a<b<c

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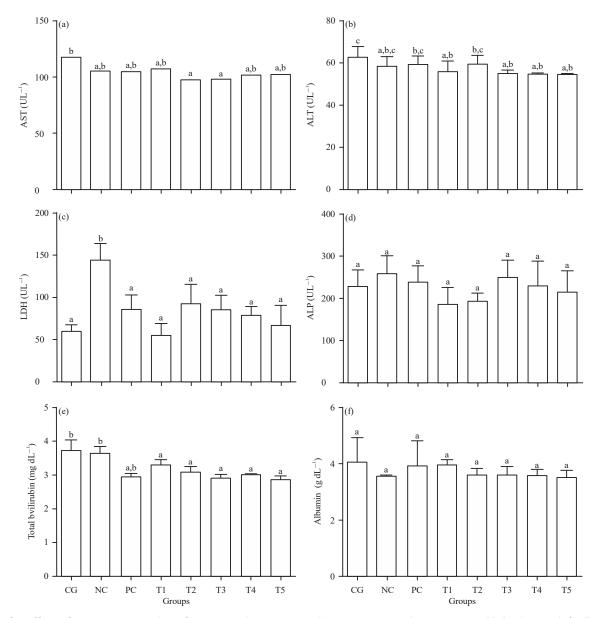


Fig. 3(a-f): Effect of potato wine on liver function indices (a) AST, (b) ALT, (c) LDH, (d) ALP, (e) Total bilirubin and (f) albumin Bars with different letters indicate significant difference at p<0.05, *a, b and c represents the statistical level of significant difference such that a<b<c

when compared to CG and NC groups, the level in T1 group is same with NC group but significantly less than CG group (Fig. 1c). The serum LDL cholesterol level showed significant reduction in PC, T3, T4 and SC groups when compared to NC group, the level is significantly higher in T1 group when compared with CG and PC groups and there was no significant difference between T2 group and PC, CG and NC groups (Fig. 1d).

Atherogenic ratios: The result showed that AIP of PC group was significantly greater than that of NC and T1 groups. There was no significant difference in AIP among CG, NC, PC, T1 and T2 groups, while AIP of T3, T4 and T5 groups was significantly

higher than that of all other groups (Fig. 2a). The result showed CRI-1 of NC, PC, T1, T2 and T3 groups significantly greater that of CG group and no significantly difference among the CRI-1 of CG, NC, T4 and SC groups (Fig. 2b). The result also showed CRI-II of NC, PC, T1, T2 and T3 groups significantly greater than that of CG group while, there was no significant difference in the CRI-II among CG, NS, T4 and T5 groups (Fig. 2c).

Liver function indices: The result showed that AST activity in T2 and T3 groups was significantly greater than that of the CG group, while no significant difference exists in the activity of AST among NC, PC, T1, T4 and SC groups (Fig. 3a). The result showed that the activity of ALT in T4 and T5 groups was

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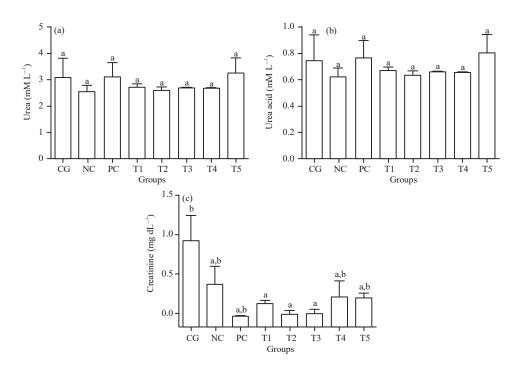


Fig. 4(a-c): Effect of potato wine on kidney function indices (a) Urea, (b) Uric acid and (c) Creatinine Bars with different letters indicate significant different at p<0.05, *a and b represents the statistical level of significant difference such that a
b

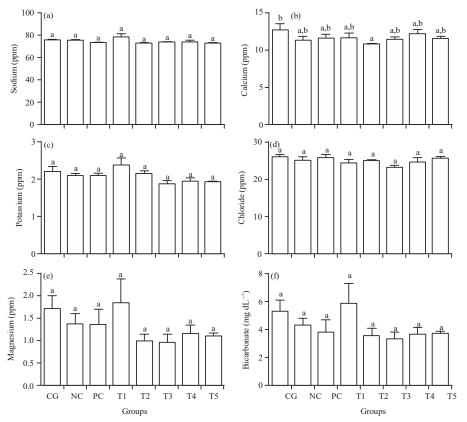


Fig. 5(a-f): Effect of potato wine on serum ion levels (a) Sodium, (b) Calcium, (c) Potassium, (d) Chloride, (e) Magnesium and (f) Bicarbonate

Bars with different letters indicate significant difference at p<0.05, *a and b represents the statistical level of significant difference such that a
b

significantly greater than CG group whereas, no significant difference was observed in the activity of ALT among NC, PC, T1, T2 and T3 groups when compared with the CG group (Fig. 3b). The result showed a significant reduction in the activity of LDH in the CG, PC, T1, T2, T3, T4 and T5 groups when compared with NC group (Fig. 3c). The result also showed no significant difference in the activity of ALP in all the treatment and control groups (Fig. 3d). The result showed a significant reduction in the level of total bilirubin in the treatment groups (T1-T5) when compared to CG and NC groups and no significant difference in the level of total bilirubin treatment in groups (T1-T4) when compared with PC group (Fig. 3e). The result showed no significant difference in the level of albumin within the treatment groups (T1-T4) and when compared with the compared with the control groups (Fig. 3f).

Kidney function indices: The result showed no significant difference in the level of urea and uric acid within the treatment groups (T1-T4) and when compared with the control groups (Fig. 4a and b). The result showed a significant reduction in the level of creatinine in the T1, T2 and T3 groups, when compared to CG group but no significant difference in the level of creatinine in the T1, T2 and T3 groups when compared with NC, PC, T4 and T5 groups (Fig. 4c).

Serum ion levels: The serum ion level's result has been presented in Fig. 5a-f. The result showed no significant difference in the level of sodium, potassium, chloride, magnesium and bicarbonate ions within the treatment groups (T1-T4) and when compared with the control groups (Fig. 5a, c-f). On the other hand, the result showed a significant reduction in the level of calcium ion in the T2 group when compared to CG group, but no significant difference in the level of calcium ion among the NC, PC, T1, T3, T4 and SC groups (Fig. 5b).

DISCUSSION

In this study, sweet potato wine was shown to have lower antioxidant activity and phenolic content than the red wine. Previous study has reported that red wine contains an antioxidant known as resveratrol that mop up free radical and help to protect lining of blood vessels of the human heart⁴⁷⁻⁴⁹. Anthocyanins and total phenol levels in the wines and the extract were similar to that obtained in blueberry and in some of the 18 non-traditional Brazilian tropical fruit⁵⁰. The trend of DPPH inhibitory activities (%) in the 3 samples was similar to that obtained in cashew apple wine which is volume-dependent scavenging of DPPH free radical within the tested amount range, which may be attributable to the hydrogen-donating ability of the present phenolics^{51,52}. The DPPH inhibitory activities (%) depends majorly on different structural features which include O-H bond dissociation energy, resonance delocalization of the phenol radical and steric hindrance derived from bulky groups substituting hydrogen in the aromatic ring^{52,53}. The FRAP content was higher than those reported by Ljevar *et al.*⁵⁴ in commercially available fruit wine.

Deposition of total cholesterol, LDL-cholesterol and triglyceride on the arterial walls have been previously reported to cause plaques leading to cardiovascular diseases and atherosclerosis⁵⁵. The lower level of total cholesterol and LDL-cholesterol and higher level of HDL-cholesterol in the groups of experimental rats that were administered with sweet potato wine is evidence that the wine may possibly reduce the risk of developing atherosclerosis and then heart diseases. Consumption of cashew by people with high LDL-cholesterol or who are at risk of high LDL-cholesterol has been known to results in decreases in total cholesterol, LDL-cholesterol and non-HDL-cholesterol and this consistent with the effects of other tree nuts^{56,57}. Cashew nut consumption has been reported to increases HDL-cholesterol in type 2 diabetes⁵⁸. Apple juice has also been reported to reduce in LDL-cholesterol and increase HDL-cholesterol in rabbit⁵⁹.

The marker enzymes of the liver were assayed and are specifically located in hepatic cells, however, they can leak into the serum or other parts as a result of injury to the liver where they are located^{60,61}. Low level of AST is normally found in the blood, however, when the liver or heart is damaged additional AST is released into the blood stream. The ALT is produced within the cells of the liver and is the most sensitive marker for liver cell damage^{61,62}. Any form of hepatic cell damage can result in an elevation in the ALT, as the cells are damage, the ALT leaks into the blood stream leading to a rise in the serum levels. Therefore, the high AST, ALT and ALP levels in the serum of rats to which the wines (red wine and potato wine) were administered are indications of leakage into the blood stream due to liver damage⁶³. The LDH is an enzyme found in the cells of many body tissues, including the heart and liver⁶⁴. Owning to its widespread distribution in the tissues, elevation of the total LDH in the serum is generally of little value in diagnosis. Serum LDH is usually within the normal range in chronic renal diseases associated with urea, high activities usually occur in infective hepatitis. Mononucleosis and toxic jaundice in which hepatocellular damage occurs. The result of the enzyme activities clearly demonstrated that regardless of the quantity of potato wine administered, a significant effect

is shown. Osna et al.65 reported that alcohol consumption affects the liver structure or cells and that tissue enzyme assay detect liver damage before structural damages are detected by conventional histological technique. Awe and Olayinka⁶⁶ observation also showed that cashew wine at 7.5 and 10% alcohol content induced marked liver failure characterized by a significant increase in serum AST, ALT, LDH and Gamma Glutamyl Transpeptidase (GGT) activities, but at 5% alcohol content of the cashew wine showed no apparent disruptions of the normal liver structure by histological and enzyme activities assessment. Awe et al.67 also reported the preservation of the liver architecture in the liver of rats to which 5.0% alcohol content cashew wine was administered while there was alteration in the architecture of the liver of rats to which 7.5 and 10% alcohol content cashew wine was administered.

To determine the kidney function in this study, the amount of certain metabolite present in the kidney during certain synthetic and degradation processes was estimated. Result from our study showed that consumption of sweet potato wine brought about an increase in the level of creatinine in the serum in the positive and negative controls which was reduced in the treatment groups. However, administration of sweet potato wine shows no significant difference in the Na⁺, K⁺, Mg⁺, Cl⁻, Ca²⁺, bicarbonate, uric acid and urea levels.

CONCLUSION

The report in this study suggest that sweet potato contains bioactive components which might be responsible for the observed medicinal activities of the wine, however, the study revealed that regular consumption of potato wine for may have the potential of interfering with some liver and kidney functions such as; the level of creatinine and atherogenic ratios.

SIGNIFICANCE STATEMENT

This study may help researchers to note that intake of the wine may help to reduce the risk of heart diseases due to the presence of antioxidants and phenolics compounds. While, moderate consumption of low alcoholic content sweet potato wine is recommended.

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