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Moderate Consumption of Red Wine Impacts Beneficial Anti-Obesity Potential and Histo-Protective Effect on Target Organs in Diabetic Rats

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Abstract

Therapeutic use of red wine in diabetes mellitus as well as oxidative stress-induced diseases has been recently reported. However, adequate reports on its impact on the histoarchitecture of major target organs are still lacking. This experimentally-controlled designed nutritional study aimed to determine the effects of moderate red wine consumption on cardiac, hepatic, renal and pancreas histoarchitecture and body mass index (BMI) in diabetic rats fed on high carbohydrate diet. Twenty-eight male Wistar rats each weighing ≥ 200g were randomly categorized into four experimental groups (n = 7, each): Normal control (NC) fed with standard rat feed; Diabetic control (DC) fed with high carbohydrate diet; Diabetic on high carbohydrate diet treated with 4 ml/kg red wine (DT4) and Diabetic on high carbohydrate diet treated with 6 ml/kg red wine (DT6). Diabetes was inducted with freshly prepared alloxan monohydrate solution (150 mg/dL, intraperitoneally). Anthropometrical parameters such as weight (g), length (cm) and waist circumference (cm) were measured weekly by standard methods to determine the BMI over a study period of 5 weeks while the red wine was orally administered via oral cannula. Animals were sacrificed after five weeks to extract organs (Heart, Kidney, Pancreas and Liver) for tissue histology. Graph Pad Prism and statistical program SPSS version 20 were used to analyze the data. P values < 0.05 were considered significant. Significant ($p < 0.05$) decrease in percentage mean weight gain - WG (DT6 - 9.2%; DT4 - 10.8%) and % BMI change (DT6 - 6.3%; DT4 - 8.5%) was observed in red wine-treated diabetic rats compared with DC rats (WG - 17.4%; BMI - 11.3%). Histological photomicrographs of the extracted organs revealed a dose-dependent improved histoarchitecture in red wine-treated diabetic rats over that of the diabetic control. In conclusion, moderate consumption of red wine impacts beneficial anti-obesity potential and histo-protective effect on target organs in diabetic rats fed on high carbohydrate diets.

Keywords: *Body Mass Index; Carbohydrate Diet; Diabetic Rats; Histoarchitecture; Red Wine; Target Organs*

Introduction

Recommendation of red wine as part of diabetic diets has been fraught with controversy due to its constituent alcohol which has been implicated in a number of health disorders and problems. Recently, studies have shown that moderate consumption of red wine improved glycemic profile and reduced weight gain [1] with reduction in the risk of developing Type 2 diabetes mellitus [2] and delayed progression of diabetic complications [3]. Effect of red wine in human studies showed that moderate wine intakes do not worsen postprandial glucose homeostasis in patients with diabetes [4,5]. While the potential health benefit of red wine has been attributed to its constituent compounds which were reported to possess orgarno-protective and anti-obesity properties, obesity still remain a leading public health

problem associated with an increased risk of multiple chronic conditions including hypertension, type 2 diabetes mellitus, cardiovascular disease, certain cancers (e.g. breast), depression, lower quality of life, disability as well as premature mortality [6-8]. Despite various reports on the positive biochemical and metabolic effects of red wine on weight and blood glucose lowering, very few studies reported the short and long term impacts of moderate red wine consumption on the histoarchitecture of major target organs. This experimentally-controlled designed nutritional study using diabetic rats therefore, aimed to examine the histological effect of red wine on the heart, kidney, liver and pancreas (which are target organs of most ingested products) and determine its impact on the body mass index through standard measurement of anthropometric parameters.

Materials and Methods

Experimental Animals and Design

Twenty-eight adult male Wistar rats (*Rattus norvegicus*) weighing ≥ 200 g were purchased from the disease-free stock of Anthony Olu farm, Ibadan, Oyo state, Nigeria. They were fed initially with standard rat chow and water ad libitum for the 2 weeks acclimatization in raised stainless steel cages with 6 mm^2 mesh floor (to maintain same physical activity) kept in a well-ventilated animal house (at 23˚C and a 12h light and dark cycle). Replaceable numbered blotters papers were placed under each cage to catch the spilled diet that was measured to make up for the daily serving ration. After acclimatization, the rats were randomly divided into four groups of 7 rats each: Normal Control (NC) group; Diabetic group (DT4) treated with 4 mls/kg red wine; Diabetic group (DT6) treated with 6 mls/kg red wine and Diabetic Control (DC) group. Each group had a close entry value of mean body weight (Table 1) and coefficient of variation. All animal weights were measured twice weekly and recorded. This study using experimental animals was conducted in accordance with the internationally accepted principles for laboratory animal use and care [9] with the approval of the Animal Care and Use Review Committee of the Institution.

Induction of Diabetes

After 15 hour overnight fast following acclimatization, all rats except those in NC group were injected by single intraperitoneal injection of 150 mg/kg body weight of freshly prepared 2% Alloxan monohydrate (Sigma chemicals, USA) dissolved in sterile 0.9% normal saline in a standard volumetric flask strapped with foil to prevent alloxan instability. Diabetes was confirmed 4-7 days later by use of glucometer (On Call Plus Blood Glucose Monitoring System, ACON Laboratories, Inc. San Diego, USA.) and compatible strips. Rats with Fasting Blood Glucose (FBG) level > 150 mg/dl were considered diabetic and used for this study since the level of serum glucose considered to be normal in *rattus norvegicus* ranges from 50-135 mg/dL [10]. Diabetes was allowed to stabilize for 5 days before exposure to experimental diets. Fasting blood glucose level of all rats in each experimental group was measured on weekly basis for the five week study period.

Animal feeding and Red Wine Administration

The animals were fed according to the experimental design for the period of study with water ad-libitum. During acclimatization period, all animals were fed on standard rat chow and thereafter, following grouping, were exposed to high carbohydrate diet formulated based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The red wine (Merlot varietal) containing 13% alcohol purchased from a reputable store was administered to the rats in DT4 and DT6 groups via oropharyngeal cannula at 4 mls/kg and 6 mls/kg respectively.

Measurement of Body Anthropometrical parameters

Anthropometrical parameters (body weight, length and waist circumference) of all the rats were measured twice a week according to standard methods over a period of five weeks Weight were measured to the nearest gram using automated weighing balance while the length and waist circumference expressed in centimeters were measured by using a measuring tape beginning from the nose to the anus

and around the waist at hip region above the iliac crest respectively. Values obtained were used to calculate the body mass index (BMI) expressed as weight (g)/length square (cm^2).

Extraction and histology of the Kidney, Liver, Pancreas and Heart

At the end of the study, animals in all groups were anesthetized using Ethyl Ether in a glass dome and then dissected to extract the kidney, liver, pancreas and heart organs for histological studies. Tissues were histologically processed using standard laboratory histo-techniques. Extracted organs were placed in 10% formalin solution for a day. All samples were then dehydrated in graded ethanol series, cleared in toluene and embedded in paraffin wax; 5-6 µm sections were routinely stained with Harris hematoxylin and eosins stains (Sigma-Aldrich) and were assessed under light microscope (Nikon Eclipse E400).

Statistical Analysis

The data obtained were computed, analyzed and summarized using Graph Pad Prisim and SPSS program version 20. Results (all mean values) are expressed as mean ± SEM. Comparisons between groups were made using Students' t-test and one way analysis of variance (ANOVA). P - values < 0.05 were considered statistically significant.

Results

Effect of Red Wine on Body Anthropometric Parameters

Body Weight

The effect of red wine on anthropometric parameters is presented in Tables 1 and 2. A significant ($p < 0.05$) decrease in mean body weight gain expressed in percentage was observed in red wine-treated rats (DT4 - 10.82%; DT6 - 9.20%) compared with diabetic control (DC – 17.40%). Impact of red wine on body weight gain was dose-dependent (Table 1).

	Experimental Animal Categories			
Parameters	**Non-diabetic**	**Diabetic**		
Mean Body Weight (g)	**NC (Normal Control)**	**DT4 (4 ml/kg RW treated)**	**DT6 (6 ml/kg RW treated)**	**DC (Diabetic Control)**
Initial	205.40 ± 1.11	200.60 ± 6.54	202.00 ± 8.28	204.00 ± 6.43
Final	237.00 ± 3.47	222.30 ± 3.65	220.30 ± 3.59	239.50 ± 6.28
Overall weight gain (%)	15.39	10.82*	9.20*a	17.40

Table 1: *Effect of Red Wine on Body Weight (g) Gain in Experimental Rats (n = 7 each/group).*

*Values are expressed in mean ± SEM *= significant ($p < 0.05$) when compared with diabetic control. [a] = not significant ($p > 0.05$) when compared with DR4.*

Length and Waist Circumferences

A significant ($p < 0.05$) decrease in waist circumference without significant alteration in body length was observed in wine-treated rats compared with the diabetic control (Table 2). Difference in the waist circumference values of DT6 and DT4 rats was comparably insignificant.

Effect of Red Wine on Body Mass Index (BMI)

Table 3 depicts the impact of red wine on BMI. A significant dose-dependent decrease ($p < 0.05$) in mean BMI was observed in DT4 and DT6 rats compared with the diabetic control.

	Experimental Animal Categories			
Parameters	**Non-diabetic**	**Diabetic**		
	NC (Normal Control)	**DT4 (4 ml/kg RW treated)**	**DT6 (6 ml/kg RW treated)**	**DC (Diabetic Control)**
Mean Body Length (cm)				
Initial	19.20 ± 0.37	18.40 ± 0.24	17.80 ± 0.37	18.20 ± 0.20
Final	19.60 ± 1.14	18.60 ± 0.84	18.00 ± 0.71	18.60 ± 0.54
% length change	2.08	1.09	1.12	2.19
Mean Waist Circumference (cm)				
Initial	11.94 ± 0.25	11.40 ± 0.18	11.20 ± 0.19	11.50 ± 0.22
Final	15.50 ± 1.14	9.20 ± 0.38	8.80 ± 0.38	14.80 ± 0.38
% waist circumference change	29.82	19.30*	21.43*a	28.70

Table 2: *Effect of Red Wine on Length (cm) and Waist Circumference (cm) in Experimental Rats (n=7 each/group).*

*Values are expressed in mean ± SEM. *= significant ($p < 0.05$) when compared with diabetic control. [a] = not significant ($p > 0.05$) when compared with DR4.*

	Experimental Animal Categories			
Parameters	**Non-diabetic**	**Diabetic**		
Mean BMI (g/cm^2)	**NC (Normal Control)**	**DT4 (4 ml/kg RW treated)**	**DT6 (6 ml/kg RW treated)**	**DC (Diabetic Control)**
Initial	0.57 ± 0.01	0.59 ± 0.01	0.64 ± 0.03	0.62 ± 0.01
Final	0.63 ± 0.04	0.64 ± 0.20	0.68 ± 0.01	0.69 ± 0.02
% BMI Change	10.52	8.48*	6.25*a	11.29

Table 3: *Effect of Red Wine on BMI (g/cm2) in Experimental Rats (n = 7 each/group).*

*Values are expressed in mean ± SEM. *= significant ($p < 0.05$) when compared with diabetic control. a = not significant ($p > 0.05$) when compared with DT4.*

Effect of Red Wine on Target Organ Histoarchitecture

Effect on Cardiac Tissue Histoarchitecture

The photomicrographs of the hearts of all grouped rats under x400 magnification (H & E stained) showed no apparent disruption or visible lesion of cardiac histoarchitecture as depicted in Plate 1(a-d) below.

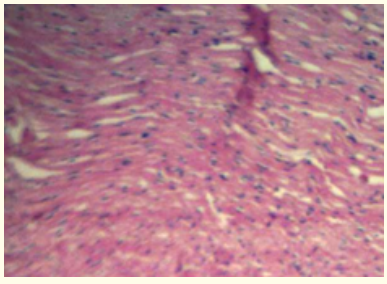

PLATE 1(a): *Heart photomicrograph of DT6 rats showing preserved histoarchitecture.*

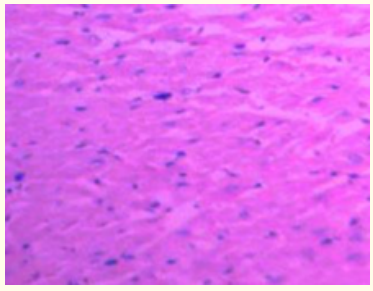

PLATE 1(b): *Heart Photomicrograph of DC rats showing no visible lesion.*

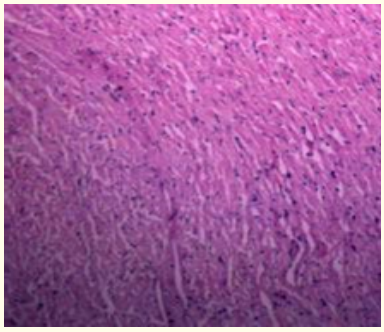

PLATE 1(c): *Heart photomicrograph of NC rats showing normal histoarchitecture.*

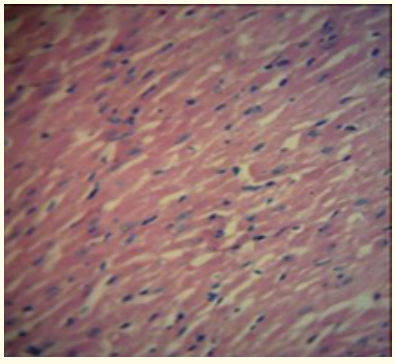

PLATE 1(d): *Heart photomicrograph of DT4 rats showing preserved histoarchitecture.*

Effect on Renal Tissue Histoarchitecture

Plate 2 (a-d) under x400 magnification (H and E stained) depicts the renal tissue histoarchitecture in grouped rats. Moderate consumption of red wine impacted an improved, dose-dependent, histoprotective effect on the kidneys of wine-treated rats (Plate 2c and 2d).

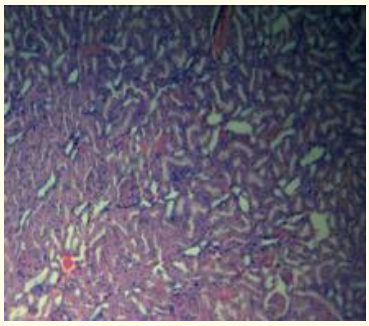

PLATE 2(a): *Renal photomicrograph of NC rats showing normal histoarchitecture.*

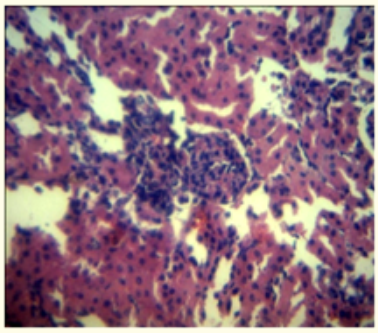

PLATE 2(b): *Renal photomicrograph of DC rats showing mild periglomerular congestion and numerous tubular lumen protein casts.*

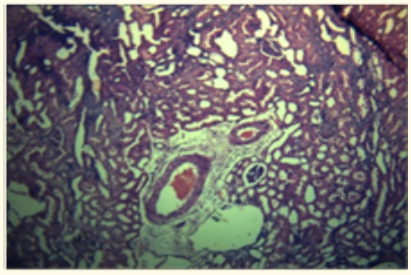

PLATE 2(c): *Renal photomicrograph of DT6 rats showing mild renal cortical congestion with few intratubular protein casts.*

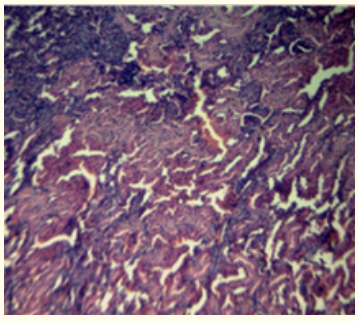

PLATE 2(d): *Renal photomicrograph of DT4 rats showing mild renal cortical congestion with scanty intratubular protein casts.*

Effect on Hepatic Tissue Histoarchitecture

Plate 3 (a-d) shows the photomicrographs of the liver of grouped experimental rats (x400 magnification, H and E stained). Moderate red wine consumption improved hepatic histoarchitecture in DT4 and DT6 rats by minimizing histopathological changes observed in DC rats (Plate 3c and 3d).

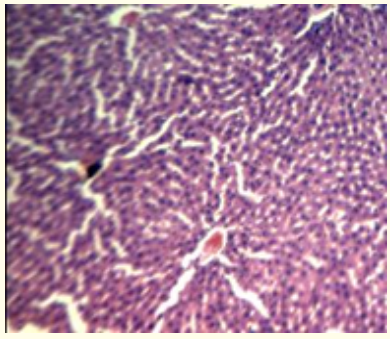

PLATE 3(a): *Liver photomicrograph of NC rats showing normal histoarchitecture.*

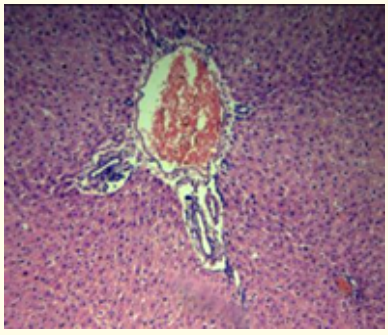

PLATE 3(b): *Liver photomicrograph of the DC rats showing a diffuse, mild vacuolar degeneration of the hepatocytes with severe portal congestion and mild periportal cellular infiltration by mononuclear cells.*

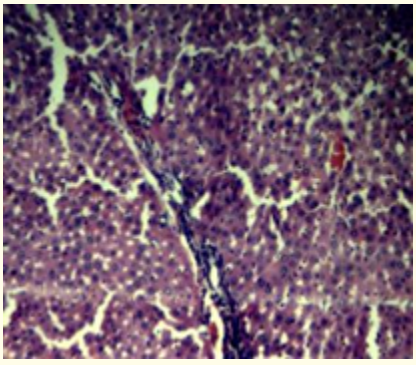

PLATE 3(c): *Liver photomicrograph of DT4 rats showing mild to moderate portal congestion and periportal cellular mononuclear cells.*

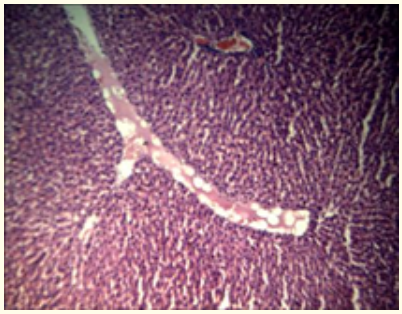

PLATE 3(d): *Liver photomicrograph of DT6 rats showing moderate portal and central congestion with mild periportal cellular infiltration by mononuclear cells.*

Effect on Pancreatic Tissue Histoarchitecture

Plate 4 (a-d) shows the photomicrographs of the pancreas of grouped experimental rats (x400 magnification, H and E stained). Moderate consumption of red wine minimized interstitial congestion of the islets in red-wine treated rats (Plate 4b and 4c) than the diabetic control rats (Plate 4d).

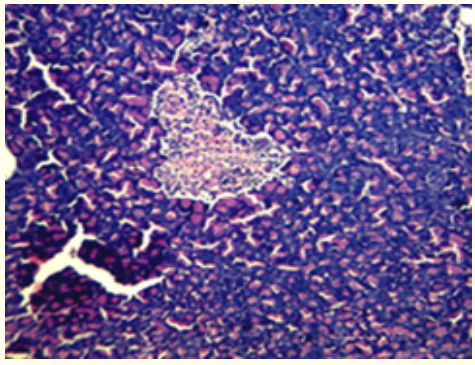

PLATE 4(a): *Pancreas photomicrograph of NC rats showing normal islets histoarchitecture.*

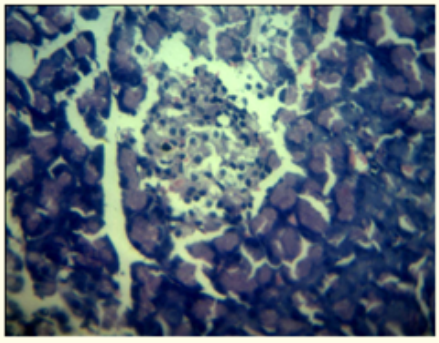

PLATE 4(b): *Pancreas photomicrograph of DT6 rats showing mild islets interstitial congestion.*

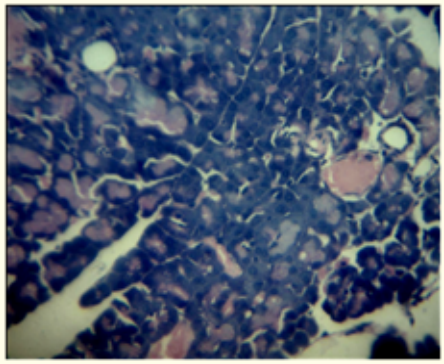

PLATE 4(c): *Pancreas photomicrograph of DT4 rats showing moderate islets interstitial congestion.*

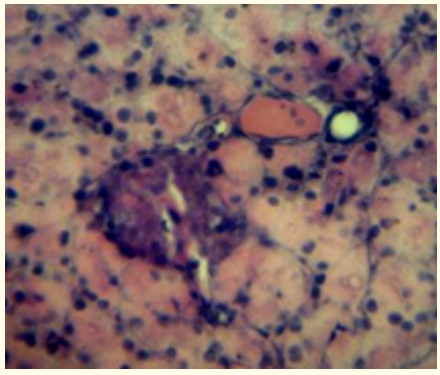

PLATE 4(d): *Pancreas photomicrograph of DC rats showing severe islets interstitial congestion.*

Discussion

This experimentally-controlled nutritional study determined the impact of moderate consumption of measured portions of red wine on body mass index and target organs histoarchitecture in diabetic rats fed on high carbohydrate diet. The findings obtained showed that moderate consumption of red wine significantly reduced BMI and protected target organs ultrastructures from further pathologic changes in diabetic rats.

Effects of red wine consumption on body anthropometrical parameters such as length, weight and waist circumference in experimental rats were determined in this study while the BMI was calculated from their values using the universal standard formula. The observed decrease in the percentage mean BMI change at the end of study was a reflection of the significant impact of red wine intake on weight gain and waist circumference. Length of the body of the rats was not significantly changed. Waist circumference has been reported as an indicative additional metric that can be more useful in determining visceral adiposity [10] which poses more risk than fat elsewhere. According to the United State National Institute of Health (NIH), waist circumference in excess of 102 centimetres (40 in) for men and 88 centimetres (35 in) for (non-pregnant) women is considered to be high risk for type 2 diabetes, dyslipidemia, hypertension, and cardiovascular disease. In populations, such as those of Asian descent, waist circumference is a better indicator of disease risk than BMI. It is also of greater value for estimating risk for obesity-related disease in older people. Weight gain however, as well as unfavorable changes in body composition, was reported to be associated with increased risk and decreased survival in some medical disorders [11]. The World

Health Organization (WHO) used BMI as the standard for recording obesity statistics since the early 1980s. This general correlation is particularly useful for consensus data regarding obesity or various other conditions. While obesity may be easily estimated from the BMI in rats, studies [12] have shown that alterations in BMI values were associated with dyslipidemic profile and oxidative stress in serum of rats which may predict the adverse consequences of obesity. Elevated BMI is associated with increased cardiometabolic risks [13,14]. Weight management should be the primary nutritional strategy in managing glucose control in type 2 diabetes for people who are overweight or obese [15]. The significant reductions in weight gain and waist circumference observed in this study potentiate the beneficial anti-obesity effect of red wine in this regard and likewise suggest its recommendation at meal for diabetic individual except an absolute contraindication exists.

Pathological changes at tissue level may result from acute or chronic damage to organ from any etiological cause. Similarly, histological analysis of target organs monitoring for pathological changes enhances screening assessment of quality of product prior to recommendation for human consumption. Opportunity of such quality assessment is enhanced by use of animal models as employed in this study which highlighted the histological outcome of red wine on some target organs. The observed histological findings support moderate recommendation of red wine for preventive and curative health unless absolutely contraindicated. The kidney, liver, heart and pancreas of the grouped experimental rats fed on high carbohydrate diet in this study were histologically examined after 5 weeks of oral administration of red wine. Minimal apparent disruption of the histoarchitecture of the organs of treated rats was noted in the organs photomicrographs (Plate 1-4) compared with the diabetic control. No visible histopathological change observed in the cardiac photomicrograph of DT4 and DT6 rats compared with the normal and diabetic controls. The fact that excessive consumption of alcohol predisposes the heart to damage leading to development of cardiac disorders such as cardiomyopathy, stroke, hypertension and varying degrees of arrhythmias [16] solicits for moderation in alcohol consumption. The histopathologic periglomerular congestion and the numerous intratubular protein casts shown on the renal photomicrograph of the DC rats were improved in the wine-treated rats in a dose-dependent manner thus depicting renoprotective effect of red wine. This beneficial effect in conjunction with its anti-diabetic property [1], may help in reducing the polyuria and subsequent overall dehydration associated with diabetes. Photomicrographs of the pancreas closely examined under high power magnification showed that red wine improved the pancreatic islet cells architecture by minimizing islets cells interstitial congestion. Studies have shown that polyphenols (one of the major constituents of red wine) in general, can stimulate β-cell growth and subsequently improve islet β-cell proliferation, survival and mass [17,18]. This protective effect of red wine on pancreas may slow down the progress of diabetic complications and also reduce incidence of type 2 diabetes among moderate drinkers [2]. The liver photomicrographs of the DC rats showed a diffuse, mild vacuolar degeneration of the hepatocytes with severe portal congestion and mild periportal cellular infiltration by mononuclear cells. These histopathological changes noted in DC rats were improved in DT4 and DT6 rats' photomicrographs. Susceptibility of the liver to damage by excess alcoholic drinking has been reported by several studies. Thus, the observed hepatoprotective effect of red wine on the liver structure calls for moderate drinking in order to derive the beneficial protective effect of red wine on the liver and other target organs.

Conclusion

This nutritional study demonstrated the effect of moderate consumption of red wine on anthropometrical parameters and its beneficial impact on BMI. It also provided the evidence-based histoprotective effects of red wine on target organs in diabetic rats fed on high carbohydrate diet. In the absence of absolute contraindication, moderate recommendation of red wine after or during meals should be suggested or considered to abate tendency of weight gain and its associated risks and co-morbidities. In conclusion, moderate consumption of red wine confers beneficial anti-obesity and histo-protective effects on target organs in diabetic rats.

Conflict of Interest

No conflict of interest exists.

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