International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs **Coden: IJARQG(USA)** **Volume 6, Issue 3 - 2019**

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.03.014

Whole grain diet formulated with tiger nut and date! Its lipid lowering and anti-oxidative effect in high fat diet induced hyperlipidemic rat

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Abstract

High-fat diets resulting in adipose mass accumulation can be directly associated with hyperlipidemia and consequent oxidative stress. Whole grains, tiger nuts and date fruit are nutrient rich food with significant health benefits against imbalanced lipid levels and oxidative stress. This study, thus, determined the lipid lowering and anti-oxidative benefit of six blends of whole grain diet formulated with tiger nut and date (treatment diet).The five weeks study (n=5) compared the hematological parameters of the control and treatment diet groups; it examined the lipid profile and oxidative stress markers in the liver and heart of the experimental animals. The hematological parameters of rats fed the formulated diet showed no significant difference ($p>0.05$) with the control. High fat diet significantly ($p<0.05$) increased the weight and impaired the lipid profile which favored the progression of oxidative stress in both the liver and heart as compared with control. The treatment diet with no noticeable toxicity from the hematological parameters, enhanced a good lipid profile and antioxidant status with values comparable to control and was able to reverse the effects of high fat diet in group whose diet was changed from high fat to the treatment diet.

Keywords: Whole grain, Tiger nut, Date fruit, Hyperlipidemia, Anti-oxidative

Introduction

Fat accumulation is associated with increased lipotoxicity from the high levels of free fatty acids, free cholesterol and other lipid metabolites. As a consequence, mitochondrial dysfunction with oxidative stress, production of reactive oxygen species and endoplasmic reticulum (ER) stress-associated mechanisms are activated (Buzzeti, et al, 2016). High fat diet has been used for the induction of metabolic syndrome including obesity, insulin resistance, hyperlipidemia and fatty liver disease (Chun *et al.*, 2010; Kohli *et al.*, 2010) in experimental animals. Hyperlipidemia categorized by the increased blood levels of lipids and lipoproteins Viz a viz high concentrations of total cholesterol, low density lipoproteins (LDL), very low density lipoproteins (VLDL), triglycerides and reduced high density lipoproteins (HDL) concentrations, is a common risk factor for the development of fatty liver and cardiovascular diseases. It can be managed or prevented together with its associated complications by modulating nutritional habits with diets particularly rich in polyphenols, flavonoids and antioxidants having anti-atherogenic effects.

Whole grains foods rich in dietary fibers, vitamins, minerals, protein and phytochemicals (Jacobs et al., 1998)are evolving dietary constituent that delivers significant health benefits to its consumers and its consumption is associated with a reduction in the risk of developing many diseases. Whole grains with its richness in nutrients can improve lipid profile by decreasing total cholesterol and LDL-cholesterol concentrations and adequately increasing levels of HDL-cholesterol. Guinea corn, maize (white and yellow), millet, wheat and soya bean used in the diet formulation are nutritive and healthy whole foods. The phytochemicals contained in guinea corn have proven to be effective at naturally lowering the bad cholesterol and increasing the level of good cholesterol, preventing a variety of cardiovascular diseases. Maize and millet are low in cholesterol and fat content with wheat bran having a specific triacylglycerol-lowering effect. Soybean a flavonoid (isoflavone) rich food is a source of healthy, unsaturated fat and fiber, which helps lower total cholesterol.

Date, the fruit of date palm (*Phoenix dactylifera*), is considered an ideal food that provides a wide range of bioactive constituents (carotenoids, phytosterols, polyphenols) (Vayalil, 2012) with many potential health benefits. They are good source of fiber helpful in lowering LDL (Rahmani et al., 2014), contains many important vitamins and minerals, having also anti-oxidative properties (Allaith, 2008) contributed by the carotenoids and phenolic compounds present in them.

Tiger nut (*Cyperus esculentus*) is an edible perennial grass-like plant that produces sweet nut-like tubers, reported to have a positive effect on lowering cholesterol and possess a wide range of health promoting properties, including anti-inflammatory (Salem et al., 2005), cardiovascular health and anti-thrombotic properties (Chukwuma *et al*., 2010).

The individual benefits of each grains, tiger nuts and date fruits have been reported in several reviews and research articles. Thus, the lipid lowering and anti-oxidative effects of whole grain diet which is a blend of millet, guinea corn, maize (yellow and white), wheat and soy beans formulated with tiger nuts and date fruit was considered a prospective basis for study.

Materials and Methods

Sample collection and preparation

The grains (millet, white and yellow maize, guinea corn, wheat), soya beans, were obtained from a market in Ado-Ekiti, Ekiti State, Nigeria. They were cleaned, boiled, air dried and blended to a fine sample. The tiger nuts and date fruits were also blended and formulated into a diet with the grains.

Chemicals/Reagents

All chemicals and reagents used were obtained commercially and of analytical grade.

Diet formulation

The composition of the treatment diet and high fat diet is as follow:

Treatment Diet**:** 10% each of the grains (guinea corn, yellow maize, white maize, millet, wheat), 20% soy beans, 10% tiger nut, 6% date, 4% vitamin premix and 10% vegetable oil

High Fat Diet: 50% skimmed milk, 16% corn starch, 4% vitamin premix and 30% Lard.

Experimental design

Twenty (20) male Wistar rats weighing (100-150)g were obtained from the Animal House, College of Medicine, Ekiti State University. They were provided rat pellets and water, *ad libitium*, and subjected to standard environmental conditions such as temperature (26-30°c), relative humidity (45-55%) and 12hrs dark/light cycle. The animals were acclimatized for 7 days and randomly divided into four (4) groups: **group 1** (fed standard pelletized diet for 5weeks), **group 2** (fed treatment diet for 5weeks), **group 3** (fed high fat diet for 2weeks, replaced with treatment diet for 3weeks), and **group 4** (fed high fat diet for 5 weeks).

Biochemical analysis

At the end of five weeks, the animals were fasted overnight, sacrificed under chloroform anaesthesia, target organs (liver and heart) excised from each rat and blood collected into a plain sample bottle for serum analysis. Each organs were prepared in 0.25M sucrose solution using the Teflon homogenizer. The resulting homogenate was centrifuged at 10,000g for 10minutes in a cold centrifuge (4ºC) to obtain a clear supernatant for biochemical analysis. Storage was done between 0ºC to 4ºC to preserve enzyme activity and assays carried out using standard laboratory protocols.

Determination of hematology parameters

The packed cell volume (%) and hemoglobin concentration (g/dl) were determined according to the hematocrit method described by Alexander and Griffiths (1993); red blood cells ($\times 10^6 \mu l$) and white blood cells ($\times 10^6 \mu l$) were estimated by visual method using New Improved Neubauer counting chamber. The corpuscular constant were estimated as follows: Mean Corpuscular Volume (MCV in fl) = (PCV % x10)/RBC and Mean Corpuscular Hemoglobin (MCH in P_g) = (HB X 10)/RBC.

Lipid profile

Levels of total cholesterol (CHOL), triglyceride (TRIG) and HDL-cholesterol was assayed using diagnostic kit obtained from Randox Laboratories U.K. LDL-cholesterol was calculated using the formula of Friedwald et al., (1972) as given below:

LDL cholesterol =

$$\text{Total cholesterol} - \frac{\text{Triglycerides}}{5} - \text{HDL-cholesterol}$$

Liver biomarkers

Serum AST and ALT activity was estimated following procedures in the diagnostic kit obtained from Randox Laboratories, U.K. The activity (u/l) was obtained by matching absorbance reading against the standard values provided in the kit.

Antioxidant assays

The Biuret method described by Weichselbaum (1995) was employed in the determination of total protein in the samples using commercially available kits (Randox laboratories, UK). The activity of superoxide dismutase was determined using the method proposed by Misra and Fridovich (1972), reduced glutathione was determined according to Beutler et al., (1963), glutathione peroxidase activity (GPx) as described by Rotruck et al., (1973) and catalase (CAT) activity was determined according to the method of Sinha (1972). Extent of lipid peroxidation was assessed by measuring the level of free malondialdehyde (MDA) generated according to methods of Varshney and Kale (1990).

Statistical analysis

Data are presented as mean ± standard deviation, analyzed using student's t-test to compare the difference between the control and test groups. *P*-values less than 0.05 ($p < 0.05$) was considered as indicative of significance. Graph Pad Prism 5.00 was also used for statistical calculations.

Results

Table 1: Effect of whole grain diet formulated with tiger nut and date on hematology parameters

	Groups	
Parameters	**Control**	**TD**
PCV (%)	47 ± 0.28	46 ± 0.14
Hb (g/dl)	15.6 ± 0.28	15.3 ± 0.15
RBC ($\times 10^6 \mu l$)	5.15 ± 0.04	4.47 ± 0.07
WBC ($\times 10^6 \mu l$)	76 ± 2.83	60 ± 1.41
MCV (fl)	92.2 ± 0.14	92 ± 0.85
MCH (P_g)	30 ± 1.27	34 ± 0.42

Values are expressed as **Mean ± S.D**; statistically not significant **$p > 0.05$**
TD→ Treatment Diet
The hematological parameters in table 1 showed no significant difference ($p>0.05$)

Table 2: Effect of whole grain diet formulated with tiger nut and date on percentage change in body weight in high fat diet induced hyperlipidemic rats

Group	Initial weight (g)	Final weight (g)	weight-gain (g)	%increase
Control	160.0 ± 13.7	208.3 ± 14.7	48.38	23.2
TD	150.3 ± 7.73	188 ± 19.2	37.65	20.0
HFD+TD	141.6 ± 2.20	194.6 ± 17.4	52.96	27.2
HFD	120.1 ± 9.03	187.6 ± 16.9	67.53^{a}	36.0

Values are expressed as **Mean ± S.D;** significant difference **$p < 0.05^{a}$** → compared to control
TD → Treatment Diet; HFD → High Fat Diet
The changes in body weight is presented in table 2with no significant difference (p > 0.05) in percentage change in body weight across all groups except for high fat diet group

Table 3: Effect of whole grain diet formulated with tiger nut and date on liver biomarkers (u/l) in high fat diet induced hyperlipidemic rats

	Groups			
Parameters	**Control**	**TD**	**HFD+TD**	**HFD**
ALT	23.7 ± 2.31	17.0 ± 0.00^{a}	26.3 ± 2.31^{bc}	50.7 ± 2.31^{ab}
AST	13.7 ± 2.08	15.0 ± 1.73	43.0 ± 3.46^{bc}	84.7 ± 7.51^{ab}

Values are expressed as **Mean ± S.D**
Significant difference **$p < 0.05^{a}$** → compared to control, ^{b}→ compared to TD group, ^{c} → compared to High Fat Diet group
TD→ Treatment Diet; HFD → High Fat Diet
The activity of serum liver biomarkers are presented in table 3: the serum concentrations of ALT and AST in the TD group were not statistically significant with the control but the concentrations in the HFD group increased significantly (p<0.05) compared to control and TD groups.

Table 4: Effect of whole grain diet formulated with tiger nut and date on serum lipid profile (mmol/l) of high fat diet induced hyperlipidemic rats

	Groups			
Parameters	**Control**	**TD**	**HFD+TD**	**HFD**
HDL	0.90±0.03	1.06 ± 0.04^{a}	0.74 ± 0.03^{abc}	0.60 ± 0.03^{ab}
LDL	0.91±0.31	1.67 ± 0.30^{a}	1.82 ± 0.41^{a}	2.30 ± 0.06^{ab}
CHOL	2.05±0.35	2.90 ± 0.25^{a}	2.77 ± 0.50^{a}	3.09 ± 0.06^{a}
TRIG	1.20±0.02	0.89 ± 0.01^{a}	1.08 ± 0.33	0.98±0.15

Values are expressed as **Mean ± S.D**
Significant difference **$p < 0.05^{a}$** → compared to control, ^{b}→ compared to TD group, ^{c} → compared to High Fat Diet group
TD→ Treatment Diet; HFD → High Fat Diet
The serum lipid profile in HFD group were significantly different compared to control and TD group.

Table 5: Effect of whole grain diet formulated with tiger nut and date on liver lipid profile and antioxidant status of high fat diet induced hyperlipidemic rats

Groups Parameters	Control	TD	HFD+TD	HFD
HDL	1.06±0.06	1.16±0.02[a]	0.81±0.04[ab]	0.73±0.01[ab]
LDL	0.62±0.51	1.26±0.47	1.20±0.42[a]	1.95±0.84[a]
CHOL	1.86±0.60	2.59±0.44	2.14±0.44	2.87±0.79[a]
TRIG	0.90±0.17	0.85±0.05	0.63±0.05	0.94±0.19
SOD	0.50±0.04	0.95±0.02[a]	0.44±0.02[bc]	0.34±0.05[b]
GPx	24.7±2.93	33.6 ±4.04	22.3 ±3.56[b]	16.7 ±0.67[b]
GSH	0.020±0.004	0.033±0.003[a]	0.012±0.004[b]	0.007±0.001[ab]
CAT	0.55 ±0.00	1.19 ±0.19[a]	0.24 ±0.02[abc]	0.17 ±0.05[ab]
LPO	0.088±0.02	0.091±0.02	0.091±0.02[c]	0.118±0.01[b]

Values are expressed as **Mean ± S.D**
Significant difference **$p < 0.05$[a]** → compared to control, **[b]**→ compared to TD group, **[c]** → compared to High Fat Diet group
TD→ Treatment Diet; HFD → High Fat Diet

Table 5 presents the lipid profile (mmol/l) and antioxidant status of the liver: the lipid profile of the TD group was not significant ($p>0.05$) with the control except for HDL which was significantly higher. The lipid profile of the HFD group was statistically significant ($p<0.05$) with the control. The GSH level and catalase activity of the TD group was significantly higher than control while the overall antioxidant status (SOD, GPx, GSH, CAT, LPO) of the HFD group was statistically significant with control and TD group.

Table 6: Effect of whole grain diet formulated with tiger nut and date on heart lipid profile and antioxidant status of high fat diet induced hyperlipidemic rats

Groups Parameters	Control	TD	HFD+TD	HFD
HDL	0.70±0.02	0.78± 0.05	0.58±0.01[abc]	0.52±0.01[ab]
LDL	1.63±0.07	1.28±0.21	0.86±0.33[ac]	2.67±0.21[ab]
CHOL	2.52±0.06	2.14±0.25	1.55±0.28[ac]	3.34±0.25[ab]
TRIG	1.00±0.12	0.39±0.05[a]	0.53±0.17[ac]	0.75±0.18[a]
SOD	0.59±0.03	0.72±0.04[a]	0.38±0.04[abc]	0.29±0.02[ab]
GPx	57.0±11.6	43.9±2.31	46.6±5.20[c]	34.8±2.90[ab]
GSH	0.02±0.006	0.02±0.001	0.01±0.001[b]	0.008±0.000[b]
CAT	0.46±0.04	0.62±0.03	0.32±0.03[abc]	0.226±0.04[ab]
LPO	0.30±0.09	0.26±0.05	0.30±0.04[b]	0.31 ±0.04[b]

Values are Expressed as **Mean ± S.D**
Significant difference **$p < 0.05$[a]** → compared to control, **[b]**→ compared to TD group, **[c]** → compared to High Fat Diet group
TD → Treatment Diet; HFD → High Fat Diet

Table 6 presents the lipid profile (mmol/l) and antioxidant status of the heart: the lipid profile and antioxidant status of the TD group was not statistically significant ($p>0.05$) with the control except for triglyceride level which was significantly lower and SOD activity which was significantly higher. The lipid profile and antioxidant status of the HFD group was statistically significant ($p<0.05$) with control and TD groups.

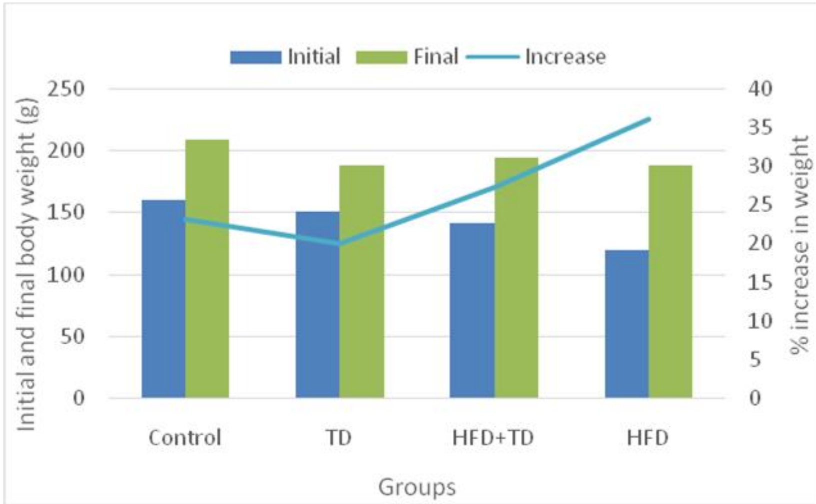

Figure 1: Effect of whole grain diet formulated with tiger nut and date on percentage change in body weight of high fat diet induced hyperlipidemic rats

TD → Treatment Diet; HFD → High Fat Diet

Discussion

High-fat diets (HFD) are known to lead to a positive fat balance and consequently to adipose mass accumulation which can be directly associated with hyperlipidemia and oxidative stress (Arango et al., 2009; Messier et al., 2007). Abnormally high body weight gain as a result of increased fat intake can lead to obesity and other related metabolic diseases but according to Slavin et al., (2013), weight control can be made easier by eating more whole grains instead of higher-calorie foods. The HFD used in the study enhanced a significant increase in weight of the rats when compared to other groups.

A normal liver is oxidative stress-resistant while fatty liver is vulnerable to oxidative stress as consumption of a calorie-rich diet results in lipid accumulation, excess production of inflammatory cytokines, and macrophage infiltration that favours the progression of liver disease (Mastroianni et al., 2014; Wei et al., 2007). Thus, the significant difference in ALT and AST (liver function biomarkers) activity in groups fed high fat diet with similar significance observed for lipid profile and antioxidant status in the liver compared with control infers that there was an insufficiency of liver function. This correlates with Uthandi and Ramasamy (2011) who reported that feeding on HFD can lead to a significant elevation in liver aspartate aminotransferase (AST), and alanine aminotransferase (ALT).

Hypercholesterolemia characterized by elevated cholesterol particularly LDL-cholesterol (causing deposition of cholesterol in the arteries and aorta), is a risk factor for cardiovascular diseases. High fat diet increases blood cholesterol levels and heart attack (Hession et al, 2009; Suliman, 2008) which justifies the significant difference ($p<0.05$) in heart lipid profile of the HFD group especially decreased high density lipoprotein (HDL) and increased low density lipoprotein (LDL) levels when compared to control. Increase LDL level causes deposition of cholesterol in the arteries and aorta and hence a step to coronary heart disease. The impairment of lipid profile in the liver and heart resulted in depleted antioxidant status as revealed by the activities of SOD, GPx, and Catalase with levels of GSH and LPO since fat accumulation is associated with increased lipotoxicity and a consequent mitochondrial dysfunction, oxidative stress and production of reactive oxygen species (Buzzeti, et al., 2016).

Dietary manipulation plays a vital role in the management of hyperlipidemia and consequent oxidative stress that works through lowering of raised cholesterol levels. The hematology parameters observed by feeding with the whole grain, tiger nut and date diet had no significant difference with the control and together with the absence of mortality throughout the study suggests the safety of the diet. The whole grain diet had adequate nutritional composition (Adeleke, 2019) and its formulation with tiger nut and date significantly ensured a good lipid profile and antioxidant status when compared with control group suggesting the healthy benefits of the diet.

Subsequent change in diet from high fat to the formulated whole grain, tiger nut and date diet in treated group (group 3) resulted in significant difference in parameters (weight gain, liver biomarkers, lipid profile and antioxidant activity) assessed when compared to high fat diet group (group 4).The treatment diet enhanced increased HDL levels, decreased LDL, cholesterol and triglyceride levels with high antioxidant activity and low MDA levels observed in the heart and liver compared to group fed high fat diet all through. These findings are in accordance with those of Galaly et al. (2014) who stated that male rats fed with a high fat diet had significantly higher levels of MDA and lower levels of GSH and SOD.

The high content of antioxidants present in whole grains justifies the beneficial effects on the liver and cardiovascular system as phenolics in grains may inhibit LDL cholesterol oxidation (Kris-Etherton, 2002). More so, dates are a good source of fiber, and contain many important vitamins and minerals helpful in lowering of LDL (Rahmani et al., 2014). Its extract also reduces oxidative stress by decreasing hepatic levels of malondialdehyde (MDA) and increasing hepatic glutathione levels (Mohammed et al., 2008). Tiger nut also have high content of unsaturated fatty acids especially oleic acid with positive effect on losing weight (Imam *et al.,* 2013) and lowering cholesterol level (Mohamed, 2016).

Conclusively whole grain diet formulated with tiger nut and date enhanced a good lipid profile and Antioxidant status and was able to reverse the detrimental effects of high fat diet when replaced with the whole grain diet.

Acknowledgments

The authors acknowledge the contribution of Dr Oseni, O.A, College of medicine and the useful insight of Miss Oguntuase, M.A, Department of Science Laboratory Technology both of Ekiti State University, Ado-Ekiti, Nigeria.

Conflict of interest

The authors declares no conflict of interest.

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Quick Response Code	Subject: Biochemistry
DOI:10.22192/ijarbs.2019.06.03.014	

How to cite this article:
Adeleke, O.V., Agomoh, J.N., Olajide, O.F. and Adanikin, T.O. (2019). Whole grain diet formulated with tiger nut and date! Its lipid lowering and anti-oxidative effect in high fat diet induced hyperlipidemic rat. Int. J. Adv. Res. Biol. Sci. 6(3): 232-239.
DOI: http://dx.doi.org/10.22192/ijarbs.2019.06.03.014