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Effect of antioxidant rich nutraceuticals on histological changes due to high fat diet induced obesity in rats

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ABSTRACT

Objective: Obesity is an important, multifaceted chronic disorder which has become one of the leading health problems globally as a result of its morbidity and mortality. The aim of this research was to induce obesity using high fat diet feeding and to assess the effect of treatment with antioxidant rich nutraceuticals in the pathology of the liver, kidney and heart.

Methods: Nutraceuticals were formulated using locally available foodstuffs (onions, garlic, tomatoes, lemon, palm oil and crayfish in the ratio of 5:5:2:4:2:2) based on recommended daily allowances (RDA). Obesity was induced using high fat diet feeding for ten weeks. Albino rats of both sexes weighing 150-220 g were used for the study. The animals were grouped into 4 group of 8 rats each: group I: normal, control; group II: high fat diet, untreated; group III: high fat diet treated with 250 mg of nutraceutical daily; group IV: high fat diet treated with 500 mg of nutraceutical daily for ten weeks. Sections of liver, kidney and heart were taken for histopathological study.

Results: The data obtained showed that high fat diet feeding significantly increased body mass and body mass index and was associated with an abnormal lipid distribution. Histopathological examination showed that high fat diet feeding was associated with hepatic, myocardial and renal necrosis, degeneration and congestion of associated blood vessels. However, treatment with antioxidant rich nutraceutical reverses the damage to near normal.

Conclusion: High fat diet induced obesity is associated with pathological changes in the heart, kidney and liver. Antioxidant rich nutraceuticals could provide a protective effect against pathological changes in obesity.

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KEYWORDS

Body mass index; high fat diet; obesity; nutraceuticals

Introduction

Obesity is a metabolic disorder caused by imbalance in energy intake and energy expenditure and is one of the leading causes of death globally [1]. In 2010, estimates show that around 3.4 million people died due to overweight or obesity [2]. Over the years, the prevalence of obesity has been shown to be increased worldwide [3]. Obesity is associated with a range of metabolic disorders such as hypertension, atherosclerosis, hyperlipidemia, type 2 diabetes, and fatty liver [4]. Non alcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disorder [5] and obesity is an important risk factor for NAFLD and cirrhosis-associated death [6].

Oxidative stress is associated with a variety of inflammatory and metabolic disorders, including obesity [7]. Oxidative stress induce damage to the body due to free radicals that are inadequately neutralized by the body antioxidants defence system [8]. More so, oxidative stress has also been regarded as one of the major causative factors for the development of insulin resistance which is present in obesity [9].

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About 2000 years ago, Hippocrates correctly emphasized "Let food be your medicine and medicine be your food". The term 'nutraceutical' was coined by combining the terms 'nutrition' and 'pharmaceutical' in 1989 [10]. Hence a nutraceutical is any substance that may be considered a food or part of a food that provides medical or health benefits, including prevention and treatment of diseases. Several nutraceuticals are available with documented health benefits [11].

Pharmacological interventions for weight loss are available for treating obesity. For example fat absorption suppressants, appetite-suppressants and thermogenic drugs are used in treating obesity [12]. Orlistat is a popular anti-obesity drug which reduces dietary fat absorption by inhibiting pancreatic lipase [13]. Marketed anti-obesity drugs are associated with several negative outcomes which include stomach pain, headache and steatorrhea [14]. These negative outcomes and possible high cost make search for cheaper and safer anti-obesity agents an active area of research.

The liver, heart and kidney are vital organs of the body that are affected in obesity. It is therefore the objective of the present study to investigate the effect of supplementation with antioxidant rich nutraceuticals on the histopathology of the liver, kidney and the heart of high fat died induced obese rats.

Materials and Methods

Chemicals and reagents

All chemicals and reagents used for this study were of analytical grade, products of either Randox Laboratory or Cayman Chemical Company (USA).

Experimental animals

This study was conducted in accordance with the standards set for the use and care of laboratory animals and the protocol approved by the Ethical committee of the Faculty of Science, Usmanu Danfodiyo University Sokoto-Nigeria. Wistar albino rats of both sexes weighing 150-220 g were used for this study. The animals were purchased from the Department of Biochemistry, University of Ilorin, Nigeria. They were allowed to acclimatize for 7 days before the commencement of the experiment. They were fed with pelletized grower's feed (Vital Feed, Jos, Nigeria) and were allowed access to water *ad libitum* before and during the experimental period.

Formulation of high fat diet (HFD)

HFD was formulated in accordance with Kim *et al* [15] with some modifications. The formulation consists of fat (46%), carbohydrate (24%), protein (20.3%), fibre (5%), salt mixture (3.7%) and vitamins mixture (1%). Obesity was induced through feeding with the formulated diet for 10 weeks.

Nutraceutical formulations

Antioxidants rich nutraceutical were prepared from locally available foodstuffs (onions, garlic, tomatoes, lemon, palm oil and crayfish in the ratio of 5:5:2:4:2:2) based on recommended daily allowances (RDA) [16]. The foodstuffs were purchased from Sokoto central market. Taxonomic identification was done by comparing the plant materials in the preserved identified vouched materials in the herbarium of the Department of Biological Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria. Briefly, 25g of onions, 25 g of garlic, 10 g of tomatoes were mixed and blended in 100 ml of distilled water. Ten grams of crayfish was then added and blend again. Twenty grams of lemon juice and 10 g of palm oil were added. The formulation was mixed and stored at 4^0C until required.

Grouping of animals and treatment

The animals were divided into four groups:

- Group I: normal control received normal pelletized diet.
- Group II: high fat diet, untreated.
- Group III: high fat diet treated with 250 mg/kg of nutraceuticals.
- Group IV: high fat diet treated with 500 mg/kg of nutraceuticals.

The appropriate dosages of the nutraceutical were administered to the animals orally once daily by intubation using intravenous cannula tube for 10 weeks, weight changes of the rats were monitored throughout the experimental period.

Determination of body mass index (BMI)

Body mass index was measured on weekly basis for 10 weeks of the experimental period. Rats were weighed in grams. Naso-anal length was measured in centimeter. BMI was estimated as an index of obesity as body weight (g)/length^2 (cm^2) [17].

Histopathological study

Liver, kidney and heart tissues were fixed using the routine formalin/paraffin embedded technique. Sectioned at 4 µm and stained with hematoxylin and eosin as described by Luna [18]. Each section was scored on the severity of fat accumulation. Scoring was done as follows: (0) no visible lesion; (1) mild lesion, 1-10% cells affected; (2) moderate, 11-30% cells affected; (3) severe, more than 30%

cells affected. These were used to ascertain the degree of congestion, degeneration and necrosis in all three tissues.

Data analysis

Data were expressed as mean ± standard error of mean (SEM). Using GraphPad InStat software (version 5; San Diego, CA, USA), parameters were analyzed by one-way analysis of variance (ANOVA) in which lesion scoring was subjected to Kruskal Wallis test. Results were considered significant at $P < 0.05$.

Results

The effect of antioxidant rich nutraceutical on BMI is presented on Table 1. Administration of HFD to rats for 10 weeks resulted in significant increase for BMI ($P < 0.05$, HFD untreated group). On the other hand, no significant difference was observed between the control group and HFD-induced obesity and nutraceuticals-treated group ($P > 0.05$).

With regard to the heart tissues, Table 2 shows that the mean rank for congestion of the normal group has the lowest score compared with both HFD-treated group and HFD untreated group while the mean score of degeneration was similar among all groups. The mean rank of necrosis score of untreated group has the highest score compared to control and treated groups.

For the liver tissues, Table 3 presents that the mean score for congestion of the normal group and group treated with 250 and 500 mg/kg nutraceutical were lower compared to untreated group. The mean rank for degeneration showed similar scores for both normal and treated groups, but differ significantly ($P < 0.05$) compared to untreated group. The mean score for necrosis of untreated group was higher compared to control and treated groups.

Kidney tissue evaluation is presented in Table 4, which demonstrates that the mean value for congestion, degeneration and necrosis scores differ significantly among all groups. However, the degeneration score of control group remained similar compared to the group treated with 500 mg HFD.

Representative histological photomicrographs for heart, liver and kidney tissues of normal and HFD-fed rats are given in Figures 1 and 2, respectively.

Table 1. Effect of high fat diet (HFD) feeding on body mass index (BMI).

Groups	Treatment	Initial weight (g)	Final weight (g)	BMI (g/cm^2)
I	Normal, control	85.43 ± 3.39^a	160 ± 7.66^a	0.49 ± 0^a
II	HFD untreated	92.29 ± 1.97^b	187.67 ± 5.8^b	0.7 ± 0.03^b
III	HFD treated with nutraceutical (250 mg)	95.63 ± 3.32^b	169.5 ± 10.63^c	0.53 ± 0^a

Mean values having different superscripts are significantly different ($P < 0.05$).

Table 2. Effect of antioxidant nutraceuticals supplementation on heart tissue of high fat diet (HFD)-induced obesity rats.

Groups	Treatment	Congestion	Degeneration	Necrosis
I	Normal, control	8.5^a	11.5^a	10.5^a
II	HFD untreated	14.25^b	11.5^a	14.17^b
III	HFD treated with nutraceutical (250 mg)	11.63^c	11.5^a	10.5^a
IV	HFD treated with nutraceutical (500 mg)	11.67^c	11.5^a	10.5^a

Numeric values are standing for the histopathologic evaluation scores. Mean ranks having different superscript letters in the same column are significantly different (Kruskal Wallis Test, $P < 0.05$).

Table 3. Effect of antioxidant nutraceuticals supplementation on liver tissue of high fat diet (HFD)-induced obesity rats.

Groups	Treatment	Congestion	Degeneration	Necrosis
I	Normal, control	8.5^a	10.5^a	10^a
II	HFD untreated	16^b	15.5^b	15.5^b
III	HFD treated with nutraceutical (250 mg)	13.75^c	10^c	10^a
IV	HFD treated with nutraceutical (500 mg)	8.5^a	10^c	10^a

Numeric values are standing for the histopathologic evaluation scores. Mean ranks having different superscript letters in the same column are significantly different (Kruskal Wallis Test, $P < 0.05$).

Table 4. Effect of antioxidant nutraceuticals supplementation on kidney tissue of high fat diet (HFD)-induced obesity rats.

Groups	Treatment	Congestion	Degeneration	Necrosis
I	Normal, control	7.5[a]	9.5[a]	5[a]
II	HFD untreated	19[b]	15.08[b]	19[b]
III	HFD treated with nutraceutical (250 mg)	10.38[c]	12.13[c]	12.5[c]
IV	HFD treated with nutraceutical (500 mg)	8.75[d]	9.5[a]	9.83[d]

Numeric values are standing for the histopathologic evaluation scores. Mean ranks having different superscript letters in the same column are significantly different (Kruskal Wallis Test, $P < 0.05$).

Discussion

Rodents are often used as animal models for studying obesity and its associated health effects. Diet modification play important role in the incidence of obesity and its related disorders [19,20]. However, the major contributing factor for obesity is excessive caloric intake, availability of energy-dense meals, urbanization and sedentary life style [21]. Not just being overweight is characteristic of obesity, but also characterized as metabolic disorder due to the accumulation of excess dietary calories into visceral fat and the release of high concentrations of free fatty acids (FFA) into various organs [22]. The liver, kidney and heart are important organs of the body that play important role in energy metabolism. These organs are being affected due to accumulation of excess triglycerides and FFA [23]. In the present work, rats fed with high fat diet significantly increased final body mass index (BMI) (Table 1) and resulted with accumulation of fat in liver, heart and kidney than those fed normal diet (Figures 1 and 2).

The histological study of the heart (Table 2) indicated that the mean score for degeneration, congestion and necrosis of the untreated group fed with high fat diet has high value as when compared with the group supplemented with antioxidant nutraceuticals. This might be due to lipid accumulation in blood vessels as reported by Pinar *et al* [24]. The structural changes in the heart observed in this study could be related with functional changes that may be detrimental to the health status of the rats.

The histological study of the liver (Table 3) indicated that the mean score for degeneration, congestion and necrosis of the untreated group fed with high fat diet have higher values as when compared with the group supplemented with antioxidant nutraceuticals. In many of obese people increase of hepatic triglyceride levels causes hepatic steatosis. In this study, it could be suggested that high fat diet is the main cause of hepatocellular necrosis formation in liver tissue (due to free radicals or oxidative damage).

The histological study of the kidney (Table 4) indicated that the mean score for degeneration, congestion and necrosis of the untreated group fed with high fat diet have higher values as compared with the group supplemented with antioxidant nutraceuticals (Figure 2).

In this study, the therapeutic effects of antioxidant nutraceutical in managing obesity were determined through BMI and histopathological examination of liver, heart and kidney tissues of rats fed with HFD. As result, antioxidant nutraceuticals treatment ameliorates the HFD-induced obesity and lipid accumulation-induced damage in the liver, kidney and heart. Further studies are required to elucidate the safety as well as the mechanism of action of the nutraceuticals.

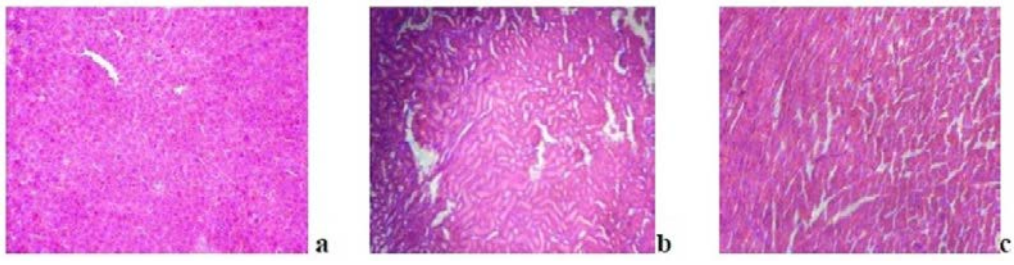

Figure 1. Representative pictures for tissues of rats fed with normal grower's mesh: (a) liver; (b) kidney; and (c) heart. (HE ×200).

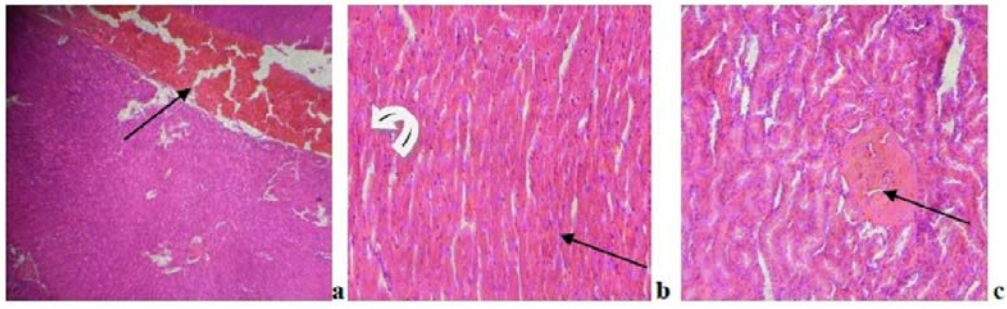

Figure 2. Representative pictures for tissues of rats fed with high fat diet: **(a) liver**, note vascular congestion (arrow); **(b) heart**, congestion (black arrow) and hemorrhage (curved arrow); **(c) kidney**, severe glomerular degeneration (arrow). (HE ×200).

Conflict of Interest

Nil.

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