

Hepatotoxicity of High Protein Diet in Diabetic Rats: An Indication for Necessary Dietary Precaution

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Abstract

Chronic consumption of high protein diets has been reportedly linked to range of functional and morphological changes in organ physiology and histoarchitecture. However, only few studies reported the impact of such chronic intake on diabetic liver structures and enzymatic profile. This experimentally-controlled designed nutritional study aimed to determine the effect of high dietary protein intake on hepatic enzymes profile and histoarchitecture, total body and organ weights in alloxan-induced male diabetic rats. Thirty-two male Wistar rats each weighing ≥ 200 g were randomly categorized into four experimental groups ($n = 8$, each): Normal control (NC) fed with standard rat feed; Diabetic control (DC) fed with standard rat feed; Diabetic on high protein diet (DP) and Normal on high protein diet (NP). Diabetes was induced with freshly prepared alloxan monohydrate solution (150 mg/dL, intraperitoneally). Rats were fed for a period of eight weeks according to the experimental design with water *ad-libitum* while their weights were measured twice weekly and recorded. At the end of eight weeks, blood samples were taken from each rat to measure the serum hepatic enzymes (ALT, AST and GGT) concentrations while the animals were sacrificed to extract the liver for gross analysis and tissue histology. Graphpad-Prism version 6 and SPSS version 22 were used to analyze the data. P values < 0.05 were considered significant. A significant ($p < 0.05$) increase in mean liver weight, serum ALT, AST and GGT concentrations and significant reduction ($p = 0.002$) in mean body weight gain were observed in high protein-fed rats compared with their respective controls. Histological analysis of liver sections revealed histopathologic lesions of mild to moderate hepatocytic vacuolar degeneration with periportal fibroplasia and cellular infiltration in high protein-fed rats. In conclusion, high protein diet consumption induced hepatotoxicity in diabetic rats via distortion of liver ultrastructures and enzymatic profile.

Keywords: Hepatotoxicity; Diabetic Rats; Protein Diet

Introduction

Dietary proteins of plant or animal origin are large macronutrient biomolecules consisting of one or more long chains of amino acid residues (polypeptides) essential for life support including catalysis of metabolic reactions, DNA replication, response to stimuli and transportation of molecules [1]. Hepatic cells are important targets for lesions in the presence of excessive dietary components since they are absorbed through the intestinal mucosa and quickly reach the liver through the portal vein [2]. It is essentially important to under-

stand the potential hepatic lesions that can result from dietary modifications since the primary metabolic reactions involving macronutrients occur in the liver. Despite numerous medical publications related to high protein diet, the results from a majority of such studies are inconclusive and have failed to demonstrate benefits and effects, especially in hepatic metabolism [3]. Inadequate or excess dietary consumption of proteins is detrimental to health thus, optimal amount if not contraindicated, is expected to be consumed daily. Chronic intake of high dietary protein has been reportedly linked to range of functional and morphological changes in body organ physiology and histoarchitecture [2,4]. However, only few studies reported the impact of such chronic intake on diabetic liver structures and enzymatic profile. Thus, additional enzymatic and histopathological studies of livers of diabetic animals fed a high-protein diet are required since the results to date have been conflicting [5,6]. In order to assess the potentiality, safety and suitability of such diet for human consumption, this experimentally-controlled designed nutritional study aimed to determine the effect of high dietary protein intake on hepatic enzymes profile, hepatic histoarchitecture, and total body and organ (liver) weights in alloxan-induced male diabetic rats.

Materials and Methods

Experimental Animals and Design

Thirty-two adult male Wistar rats (*Rattus norvegicus*) weighing $\geq 200g$ were purchased from the disease-free stock of Olu Animal Research Farm, Sango 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 mm² 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 8 rats each: Normal control (NC) fed with standard rat feed; Diabetic control (DC) fed with standard rat feed; Diabetic on high protein diet (DP) and Normal on high protein diet (NP). Each group had a close entry value of mean body weight (Table 2) 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 [7] with the approval of the Animal Care and Use Review Committee of the Institution.

Test Diets, Composition and Feeding

The composition of the diets in this study was based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The control (normal ration) and the test (high protein ration) diets were prepared from ingredients purchased from a commercial market in Ibadan metropolis, Oyo State, Nigeria according to the compositions (expressed in percentage) shown in Table 1. The animals were fed according to the experimental design for 8 weeks with water *ad-libitum*. Body weight and total food intake of each group of rats were measured and recorded weekly while the food conversion ratio (food intake/weight gain) was calculated.

Ingredients	Normal ration (%) - Control	High protein ration (%) - Test
Maize	30	24
Corn bran	15.5	10
Wheat	15	15
Palm kernel cake	20	20
Groundnut cake	12	12
Soya bean meal	10.5	15.5
Fish meal 72%	3	14.5
Oyster shell	1	1
Bone meal	3	3
Salt	0.25	0.25
Growth premix	0.25	0.25
Total additives	100	100
Lysine	0.1	0.1
Methionine	0.1	0.1
Metabolizable energy (kcal/kg)	2313.55	2330.25
Crude protein (%)	18.29	25.09

Table 1: Composition of Control and Test Diets.

Induction of Diabetes

After 15 hour, overnight fast following acclimatization, rats in DC and DP groups 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 [8]. 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 eight week study period.

Blood Collections and Biochemical Assays

The blood samples were collected from the tail veins of the rats and transferred into the k3 EDTA (Ethylene Diamine Tetraacetic Acid) sample bottles. Samples were centrifuged at 3000 revolutions to obtain the plasma fractions which was kept in a refrigerator (at -700C) until used. The sera obtained were used for the biochemical assay. Serum Aspartate Aminotransferase (AST), Gamma GlutamylTransferase (GGT) and Alanine Aminotransferase (ALT) levels were measured using detection techniques of colorimetric analysis and spectrophotometric measurements with commercial kits from Sigma Aldrich Corporation (United Kingdom).

Liver Extraction, Measurement and Histological Analysis

At the end of the study, animals in all groups were anesthetized via an intraperitoneal injection of a solution containing 10 mg/100 g body mass of ketamine and 1mg/100 g body mass of xylazine and then dissected to extract the livers which were rinsed in NaCl 0.9% and weighed. Liver tissues were histologically processed using standard laboratory histotechniques. Extracted livers were fixed in Bouin's solutions. All samples were then dehydrated in graded ethanol series, cleared in toluene and embedded in paraffin wax; 5 - 6 μ m 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 was computed, analyzed and summarized using appropriate statistical methods and program of Graphpad-Prism version 6 and SPSS version 22. Results (all mean values) are expressed using descriptive statistics such as arithmetic mean and standard error of mean (mean \pm SEM). Comparisons between groups were made using Student's t-test and one way analysis of variance (ANOVA). P-values < 0.05 were considered statistically significant.

Results

Effect of High Dietary Protein Intake on Body and Organ weights

Body Weight and Weight Gain

The effects of high dietary protein intake on mean body and organ (liver) weights are presented in Tables 2 and 3 respectively. Overall percentage weight gain after 8 weeks was significantly reduced ($P = 0.002$) in high protein diet-fed rats (DP- 3.62%; NP- 1.51%) compared with their respective controls control (NC - 24.28%; DC - 24.5%) as suggested by standard ANOVA. High protein diets have similar effects on weight gain in both diabetic and non-diabetic rats but much more in diabetic rats. No significant difference observed in total food intake ($P > 0.05$) in High protein-fed rats compared with the control rats. Repeated measures ANCOVA using the total food intake for each animal as a covariable, revealed that there was a significant effect of diet on weight gain while there was no interaction of diet and time over the 8-week period. Mean weights gain at 8 weeks were significantly ($p < 0.05$) lower in DP and NP rats compared with their control rats while a significant ($p < 0.05$) difference was observed in the food conversion ratio (food intake/weight gain) between high protein-fed rats and the control rats.

Parameters	Experimental Animal Categories			
	Non-diabetic		Diabetic	
	NC	NP	DC	DP
Initial Weight (g)	201.83 ± 2.37	200.01 ± 4.47	200.10 ± 4.47	201.67 ± 6.54
Final Weight (g)	250.83 ± 5.38	198.50 ± 4.88	247.83 ± 5.07	194.38 ± 5.40
Weight change (%)	24.28	1.51**	23.85	3.62*

Table 2: Effect of High Protein Diet on Body Weight (n = 8).

Values are expressed in mean ± SEM, *Significant (p < 0.05) when compared with diabetic control - DC.

**Significant when compared with normal control - NC.

Organ (Liver) Weight

Table 3 shows the effect of high protein diet intake on liver weight during the eight week study period. High protein diet caused a significant (p < 0.05) increase in the mean weight of the livers of DP (8.97 ± 1.0%) and NP (5.51 ± 0.2%) rats compared with their respective controls. The difference in the values for NP and DP rats was comparably significant (p < 0.05).

Parameter	Experimental Animal Categories			
	Non-diabetic		Diabetic	
	NC	NP	DC	DP
Liver weight (g)	5.45 ± 0.01	5.75 ± 0.02*	5.46 ± 0.00	5.95 ± 0.02**

Table 3: Effect of High Protein Diet on Liver Weight (n = 8).

Values are expressed in mean ± SEM, ** Significant (p < 0.05) when compared with DC.

*Significant (p > 0.05) when compared with NC.

Effect of High Protein Diet on Hepatic Enzymes Concentrations

Effect of high dietary protein on serum hepatic enzymes concentrations is expressed in Table 4. A significant (p < 0.05) increase in serum GGT, ALT and AST levels was observed in DP and NP rats compared with their respective control. The difference in the values obtained between NP and DP rats was comparably significant (p < 0.05).

Parameters	Experimental Animal Categories			
	Non-diabetic		Diabetic	
	NC	NP	DC	DP
ALT (U/L)	31.82 ± 3.61	52.10 ± 1.15	32.4 ± 3,25	60.68 ± 3.54
AST (U/L)	160.55 ± 10.12	202.55 ± 13.53	158.25 ± 13.04	208.55 ± 15.16
GGT (U/L)	3.38 ± 10.12	4.06 ± 12.10	3.58 ± 10.12	4.64 ± 10.42

Table 4: Effect of High Protein Diet on Liver Enzymes (n = 8).

Values are expressed in mean ± SEM

Effects of High Protein Diet on Liver Histoarchitecture

Under high power magnification (x 400) light microscopic examination, the photomicrographs (H and E stained) of the liver sections were closely examined. Photomicrographs of the NC and DC rats showed normohistoarchitecture of the livers while that of NP and DP rats respectively displayed mild and moderate hepatocytic vacuolar degeneration with periportal fibroplasia and cellular infiltration respectively as shown in Figure 1 to 3 below.

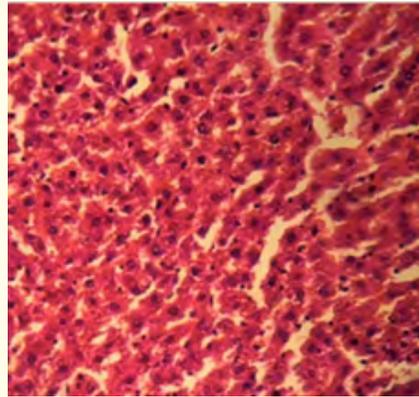


Figure 1: Liver photomicrograph of NC and DC rats showing normal histoarchitecture.

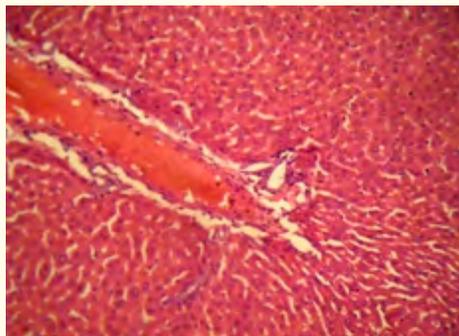


Figure 2: Liver photomicrograph of NP rats showing mild hepatocytic vacuolar degeneration with periportal fibroplasia and cellular infiltration.

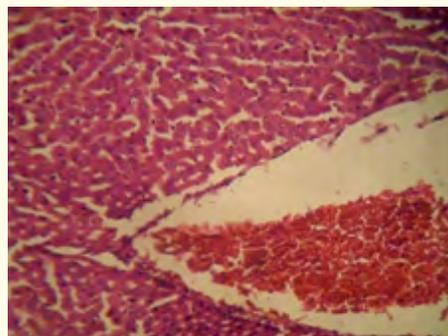


Figure 3: Liver photomicrograph of DP rats showing moderate hepatocytic vacuolar degeneration with periportal fibroplasia and cellular infiltration.

Discussion

The effects of high protein diet consumption on liver weight, ultra structure and enzymatic profile in diabetic and non-diabetic healthy rats was assessed in this experimentally-controlled nutritional study to determine its potentiality, safety and suitability for human consumption. Findings obtained revealed that high protein diet significantly reduced weight gain, increased liver weight, hepatic enzymes activities and serum concentrations and pathologically altered the histoarchitecture of the liver thus, expressing its hepatotoxicity effect in diabetic and non-diabetic healthy rats – an indication for necessary dietary precaution.

The composition of the diets used in this study was based upon the standard diet formulas used to assess weight gain in rodents during commercial feeding studies. The effect of high protein diet on body and liver weights in healthy and diabetic rats was examined. A significant reduction in mean body weight and an increase in liver size and weight were observed in DP and NP rats compared with their respective controls. Hepatomegally observed in this study agrees with the result of a recent study [9] that noticed an enlargement of the liver in rodents fed with 50% protein diet. The amount of protein in diet may have specific effects. From broader dietary perspective, the choice of protein will inevitably influence other dietary components and may be a critical determinant for the health outcome. In this study, both plant and animal proteins were combined in proportion to formulate test diets used to assess the hepatic impacts. The alteration in liver size and weight observed in this study calls for valid concern and precaution as it may be since studies [10,11] have shown that organ weight measurement is important to assess general toxicity because any change in organ weight is a sensitive indicator of toxicity. In theory, organ weight will be affected by the suppression of body weight as described by Michael [12] and observed in this study also. Dietary approach to weight reduction by consumption of high protein diet has long been employed [13]. However, non-persistent outcome resulting from such intake has cast doubt on the use of such diet in chronic reduction of body weight without adverse effect on overall health. Recently, diabetics who have restrictions to consumptions of carbohydrates and fats with resolution to increasing their protein intake, have been reported to have increased risks of organ and metabolic problems of public health importance with associated all-cause and cause-specific mortality [4,14].

Effect of high protein diet on hepatic enzymes (GGT, ALT and AST) activities was evaluated in this study which revealed a significant increase in the serum concentrations of liver enzymes in NP and DP rats. The values obtained for diabetic rats were significantly higher than those of the non-diabetic healthy rats. ALT and AST measurements are considered precise liver function tests since high levels of such transaminases confirms presence of liver lesions or damage involving hepatocyte destruction with plasmatic membrane disruption [15,16]. ALT is a specific liver enzyme located in the hepatocyte cytoplasm while AST can also be expressed by muscles and kidney. Views of different researchers with regards to effect of high protein diet on hepatic enzymes vary. However, research findings of different investigations vary with the percentage of macronutrients used in the experimental diet. While a study [2] noticed an increase in levels of ALT and AST in the same order or degree of increment in the amount of protein content of the diet, a recent study [9] however, observed no alteration in the levels of the enzymes but noticed hepatic enlargement when 50% of protein is used in the diet. Jean., *et al.* [17] in their study observed increased ALT level without alteration in AST level. In our own study, the levels of ALT, AST and GGT increased significantly in high protein-fed rats when compared with their controls. As a result of these conflicting and inconsistent research findings, additional enzymatic and histopathological studies of livers of rodents fed a high-protein diet are required to establish the potentiality, safety and suitability of such diet. Meanwhile, based on the findings of many studies with elevated levels of hepatic enzymes in addition to altered ultra-structure of the liver indicating hepatotoxicity, a necessary dietary precaution must be indicated when recommending protein diet for any purpose or treatment.

The photomicrographs of the liver sections highlighted further the effect of high protein diet at hepatic tissue level. 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 [18]. The liver sections under high power magnification (x 400) light microscope were closely examined. Photomicrographs

of the NC and DC rats showed normohistoarchitecture of the livers while that of NP and DP rats displayed mild and moderate hepatocytic vacuolar degeneration with periportal fibroplasia and cellular infiltration respectively. This finding contrasts with the result of the study of Lacroix, *et al.* [5] who noticed no lesions in the liver histoarchitecture. However, presence of hepatotoxicity as indicated by hepatomegally, liver histoarchitecture distortion and elevated levels of transaminases in this study summons attention for dietary precaution in both healthy and diabetic individuals to prevent associated risks of morbidity and mortality.

Conclusion

Findings from this nutritional study demonstrated the hepatotoxicity of high protein diet consumed in diabetic and healthy non-diabetic rats. Thus, taking necessary precautions with optimal dietary guidelines in protein diets recommendation, associated risks of morbidity and mortality would be averted or prevented while its beneficial potentiality, safety and suitability ensured.

Conflict of Interest

No conflict of interest exists.

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