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Biological Evaluation of Okara in Rats Based on Plasma Lipid Profile and Histology

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Authors' contributions

This work was carried out in collaboration between all authors. Authors WHME and EMR designed the study, wrote the protocol and wrote the first draft of the manuscript. Authors WHME and MMM managed the literature analyses, searches, statistical analysis of the study, performed the biological and author AHM managed the experimental process and author EMR identified the species of okara and biological material. Author WHME managed the literature searches and addressed subsequent reviewer comments and suggestions for improvement. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study was conducted to determine the potentials effects of dietary fibre on plasma lipid profile and liver and heart histology of male albino rats fed in high-fat diet and dried okra by-product. Possible using okara is going to eliminate pollution and add economic value to this currently valueless. Okara was provided by Food Technology Institute (Agriculture Research Center, Giza, Egypt).
Place and Duration of Study: Experiment was started at Regional Center for Food and Feed during June 2012 to August 2012.
Methodology: Okara samples were dried and chemically analyzed. Then they were evaluated biologically on rats. The diets contained, the male albino rats grouped into five groups according diets as control group (-ve) received on diet contain no cholesterol (C-1) and free from okara, second one divided as (+ve) control group fed on 1% cholesterol and free okara (C-2), other remaining three (3-5) groups separately on composite diet contain 10%, 20% and 30% okara and 1% cholesterol for each group with remaining normal diet The plasma lipid profile was determined three times after fasting period. By the end of

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experiment rats organs (liver and heart) were kept to histological investigation.
Results: A significant variance ($p<0.05$) occurred between diet containing okara diet and negative control and positive control groups in the level of triglyceride throughout the whole experiment. By feeding groups on 20% okara (OK-20) had significantly lower ($p<0.05$) plasma LDL-C level than the 10 and 30% okara fed groups. The hematological analysis, in the exception of the platelets levels which does not varied significantly amongst diet groups. Focal area of hepatic necrosis associated with leucocytic cells infiltration in the liver of group fed on 10% okara with high fat diet. Examination of histology sections from group 30% okara with high fat diet showed reveal focal myolysis of cardiac myocytes associated with inflammatory cells infiltration.
Conclusion: Okara components might play an interesting role in the prevention of hyperlipidemia and could be used as a natural ingredient or supplement for functional food preparation.

Keywords: Cholesterol; dietary fiber; hematology; hyperlipidemia; histology; okara.

1. INTRODUCTION

Although the presence of fiber in food products is essential for an individual's health, it could alter food matrix characteristics and subsequent bioavailability of nutrients. Dietary fiber is known to increase bolus viscosity in the stomach and small intestine. It also binds bile acid and interacts with lipase, thereby reducing enzyme activity [1]. Moreover, fiber addition to the food formula may alter the time required to release oil from the digesta. However, the presence of fiber in food products is essential for an individual's health [2]. It is apparent that food matrix structure and characteristics could affect the rate of digestion and postprandial metabolic behaviors (e.g. amino acid absorption, blood plasma triglyceride and glucose concentrations, etc).

Intake of food rich in dietary fiber in the daily diet is important to reduce or regulate plasma cholesterol and triacylglycerol levels and to promote health [3]. Soy foods are associated with health claims for improved cardiovascular health. Several positive effects have been attributed to soybeans, including hypocholesterolemic effect [4]. Soy foods are associated with health claims for improved cardiovascular health. However, the processing of soybeans into soy milk and tofu requires large volumes of water that produces a highly moist by-product that is commonly used as animal feed.

Finding convenient ways to incorporate okara into food could eliminate a possible source of pollution and add economic value to this currently valueless product [5]. For each kilogram of soybean processed into soy milk an equal weight or more of okara is produced. Okara is a by-product of soy milk and tofu preparation, collected after filtration. About 1.1 kg of fresh OM is produced from every kilogram of soybean processed for soymilk [6]. Dried okara contains 25.4-28.4% protein, 9.3–10.9% oil, 40.2–43.6% insoluble fiber, 12.6–14.6% soluble fiber and 3.8– 5.3% soluble carbohydrates [7]. Therefore, okara is a rich source of dietary fiber. Epidemiological investigations have established that elevated levels of plasma total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) are major risk factors for the development of cardiovascular diseases, and a low ratio of TC to HDL-C are protective against them. Cholesterol abundance in the human body is governed by the levels of its dietary intake and internal production. The hypocholesterolemic effect of dietary fiber has been reported in different animal models (rat, mouse, hamster) [8] and in humans [9] colon cancer [10]. The physiological effects of dietary fiber *In vivo* can be predicted by *In vitro*

physicochemical properties, such as swelling, and water and oil retention capacity [11]. Villanueva et al. [12] suggest that the main components of okara, dietary fiber and protein, could be related with the total lipids and cholesterol decrease in the plasma and liver, as well as with the fecal output increase in high-fat fed hamsters. Okara might play an interesting role in the prevention of hyperlipidemia and could be used as a natural ingredient or supplement for functional food preparation. From a technological point of view, fibers are used as texturing and to increase volume, above all in manufacturing low-calorie foods, essentially due to their capacity to absorb water [13,14] suggest that okara, discarded as industrial waste, may be used in probiotic soy yoghurt, helping to increase the nutritional and functional properties without altering its acceptability.

Okara also contains high-quality protein [15]. It is used as an ingredient in many food products such as pickles, tempeh, salads, sauces, baked goods and desserts [16]. Extractions of proteins or soluble polysaccharides from okara have been reported [17]. However, the highly structure of fiber and high water-retention capacity of okara, the extraction of proteins and polysaccharides from okara has been investigated during recent years [15,18]. Soy soluble polysaccharide has a pectic-like structure containing a galacturonan backbone of homo galacturonan linked by α-1,4-glycosidic linkage, as well as rhamno galacturonan, which is a repeating unit comprised of α-1,2-rhamnose and α-1,4-galaturonic acid, then branched by β-1,4-galactan and α-1,3- or α-1,5- arabinan chains [19]. As a peptide glycan, soy soluble polysaccharide has been reported to have good emulsifying properties [20]. It can be used to stabilize acidified milk beverages [19]. The rate of protein digestion and oil release in the presence of okara were mainly due to the characteristics of polysaccharides in okara [21].

Aim of study was conducted to determine the potential effects of dietary fiber on the lipid profile in blood plasma, liver and heart histology of albino male rats fed on high-fat okara diet .

2. MATERIALS AND METHODS

Okara was kindly provided by a soy foods processor (Food Technology Institute, Agriculture Research Center and Giza. Egypt), and was kept at −20ºC prior to use. Okara has a high proportion of water. Fresh okara contains 80.9% moisture. It was dried at oven 55ºC/ 36 hr, and then dried okara was ground to a fine powder (pass through sieves 40 mesh) and vacuum packed to decrease lipid oxidation.

2.1 Nutritional Data

Moisture, protein, fat, total dietary fiber ash and calorie value in okara samples were determined by official methods of analysis [22]. Carbohydrate content was estimated by difference from total of chemical constituents of dried okara [23]. All data were expressed on a dry weight basis.

2.2 Mineral Contents

The mineral contents Fe, Na, P, Mn, K, Cu, Zn, Si, Ca and Mg in each composite dried okara and processed according to the method described in [22], digesting 0.5 g sample in concentrated HNO3 at a temperature of 85ºC and then in HClO4 at temperature of 180ºC until 1-2 ml of digested sample were left. The digested sample was then filtered and volume

was made up to 25 mL. These samples were then run through an Atomic Absorption Spectrophotometer (Varian, AA240 and Victoria, Australia) using air acetylene flame to determine the mineral content. Sodium and potassium were measured by atomic emission spectrometry with a detection limit of 0.1 micrograms per ml. Si was determined using Zeeman Atomic Absorption 4100 Perkin Elmer, U.S.A., according to [22].

2.3 Biological Evaluation

Before arrival of the rats, the animal house and stainless cages were properly cleaned and disinfected by detergent solution. The animal house was maintained at 22-24ºC with a relative humidity of 45-55%. A total 25 male weanling albino rats were used for experiment. Diets were prepared according to the composition of basal diet [24]. These 25 was obtained from National Center Farm, Cairo Egypt. The rats were allowed to acclimatize for 1 week. Then rats were divided randomly into five groups of five rats per group with average weigh 35 grams. Animals provided *ad-libtum* to rats for 60 days. All nutrients were added according to the recommended dietary allowance [RDA] for each rat and according to National Research Council method [25]. The diets were designed to provide similar amounts of proteins, lipids, carbohydrates, vitamins and minerals as the control diets. The composition of the controls and supplemented okara diets is given in Table 1. The diets were stored at 5ºC until used.

Table 1. Composition of the diets supplemented with / or without okara (g/kg dry weight)

Ingredient	C-1: control diet free okara g/kg*	C-2: control diet free okara g/kg**	10% okara (g/kg dry weight)	20% okara (g/kg dry weight)	30% okara (g/kg dry weight)
Sucrose	500	500	500	500	400
Casein	200	200	100	-	-
Corn starch	150	138	138	138	138
Corn oil	50	-	-	-	-
Cellulose	50	50.0	50.0	50.0	50.0
Mineral mix	35	35.0	35.0	35.0	35.0
Vitamin mix	10	10.0	10.0	10.0	10.0
DL-Methionine	3.0	3.0	3.0	3.0	3.0
Choline	2.0	2.0	2.0	2.0	2.0
Render Fat	-	50.0	50.0	50.0	50.0
Bile salt	-	2.0	2.0	2.0	2.0
Cholesterol	-	10.0	10.0	10.0	10.0
Dried okara	-	-	100	200	300
Total	**1000**	**1000**	**1000**	**1000**	**1000**

**C-1: -ve control diet free from render fat; okara and cholesterol (Basal diet)*
***C-2: +ve control diet with no okara and with render fat and cholesterol*
OK-10: diet high –Fat with 10% of okara
OK-20: diet high –Fat with 20% of okara
OK-30: diet high –Fat with 30% of okara

The animal grouped into five groups as control group (-ve) received on diet contain no cholesterol (C-1) and no okara, second one divided as (+ve) control group fed on 1% cholesterol and no-okara (C-2), other remaining three (3-5) groups separately on composite

diet contain 10%, 20% and 30% okara and 1% cholesterol for each group with remaining normal diet. High-fat diets (+ve control and supplemented diets) contained 5% render sheep fat + 1% cholesterol +0.2% Bile salt (add bile acid as a salt form into mineral mix)) and then homogenized by homogenizer.

Blood sample was collected after fasting overnight with not exceeds 16 hr., with 3 times at different period of experiment. The collected blood plasma samples are anaesthetized from carotid artery into tubes and plasma was obtained by centrifuging at 3000 rpm for 15 min as [26] for analyzing lipid profile. At the end of experiment, rats were fasted and collected blood, liver and hearts of rat groups rapidly removed and weighed then rinsed with 0.9% cold saline and preserve to histological examination.

2.3.1 Biochemical analysis

The plasma level of total cholesterol (TC), triglyceride (TG), high –density lipoprotein (HDL-C), low density lipoprotein (LDL-C), and very low density lipoprotein (VLDL-C) were determined by enzymatic colorimetric method using Microlab 200, Merck, Germany according to the methods outlined by [27,28]. Each determination was carried out in triplicate and the mean values are presented in the text.

2.3.2 Hematological analysis

Hematological analyses were performed on blood samples using an automated Complete Blood Count "CBC" by [29] where the Animal Blood Counter Veterinary was a fully automated (Microprocessor controlled) hematology analyzer used for the in vitro diagnostic testing of whole blood specimens. The recorded parameters were white blood cells, red blood cells, hemoglobin, hematocrit and platelets.

2.3.3 Histopathological examination

Histopathological analysis was carried out by using method according to [30]. Liver and heart specimens (n=4) of all groups were obtained and fixed in 10% neutral-buffered formaldehyde for 48 hr, embedded in paraffin and sliced at 5 μ m thickness. The sections were stained with haematoxylin and eosin (H and E) and examined by light microscopy (X200).

2.4 Statistical Analysis

All analyses were performed in triplicate and expressed as mean values standard deviation (SD). Statistical evaluation was performed by the two-way ANOVA method using a model with two main effects (animal group and type of diet). Interaction between times with animal groups using to consider lipid profile SPSS [31]. Values of $P<0.05$ was considered significant.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Okara

Okara is the residue left after soymilk and tofu production. Generally, okara is a raw material discarded or used as animal feed. The chemical composition of the okara by-product is shown in Table 2. The main components of the okara by-product are protein (40.0%), crude

fiber (16.7%) fat (17.3%), ash (3.7%) and carbohydrate (19.3%). This result is also agreed by [15,32]. The composition of okara will depend on the procedure followed to obtain it. Dietary fiber is the greatest fraction of okara [33]. Total dietary fiber of the okara is comprised 20.74% (Table 2) of which containing 40.13% soluble from of total dietary fiber (in either acid or alkaline solutions).

The dietary fiber content and fat content were higher than previously reported [15,34]. The insoluble dietary fiber of okara was hemicellulose, cellulose and lignin (Table 2). Table 2 is show the okara composition nearest to soy bean meal in their ratio of protein, ash and dietary fiber. The okara by- products have greater amount in acid dietary fiber 26.50%, and alkaline dietary fiber (13.63%). Lignin the most important fiber that reduces cholesterol in human lipid, the ratio was 7.31%.

Lignin is not a carbohydrate; rather, it is a polyphenolic compound with a complex three-dimensional structure in the soy seeds [35]. Reported that, lignin appears to prevent cholesterol gallstones in this model by improving cholesterol saturation of bile [36]. Indicated that, soluble dietary fibers may exert their.

Table 2. Proximate chemical composition of okara (OK)

Chemical composition	Okara composition % dry weight	Soy bean composition % dry weight **
Moisture %	3.0±0.48	8.20
Protein %	40.0±0.28	46.06
Fat %	17.3±0.21	20.03
Crude fiber %	16.7±0.45	
Fiber Fraction		
*Alkaline Dietary Fiber (ADL)**	13.63±0.6	
*Acid Dietary Fiber (ADF)**	26.50±0.14	
*Non Dietary Fiber (NDF)**	27.06±0.06	
Total Dietary Fiber	20.87±0.77	24.37
Hemicellulose	0.56±0.25	
Cellulose	12.87±0.48	
Lignin	7.31±0.95	
Ash %	3.7±0.12	4.47
Carbohydrate %	19.3±0.31	5.06
Calorie value (Kcal/100g)	392.9	

**This ratio from total dietary fiber*
*** Soy composition % of dry base according to [31].*
All values are means ± standard deviation of triplicate analyses

hypocholesterolemic effect by increasing excretion of fecal neutral sterols. In addition the prebiotic effect and other health-promoting properties of okara from soybean, a solid by-product composed mainly of dietary fiber, have recently been reported in rats [34]. Decreases plasma total and LDL cholesterol, such findings led the FDA to approve health claims like the following on labels of foods containing at least 0.75 g/serving of soluble fiber from whole oats: “Soluble fiber from foods such as oat bran, as part of a diet low in saturated fat and cholesterol, may reduce the risk of heart disease” [37].

The calorie values which obtained from 100g of okara were 392.9 Kcal (Table 2). Macroelements, mineral in the okara sample (Table 3). Potassium (801 µg/g), calcium (656 µg/g) and phosphorus (477.8 µg/g) were the most abundant macroelements in the okara (OK). Magnesium, sodium and copper are the lowest macroelements found in okara at 38.3, 26.1 and 1.4 µg/g, respectively. As it has been previously mentioned, okara by-product contains a significant amount of non-digestible polysaccharides and such ingredients can positively influence on calcium absorption [38]. Okara macroelements present similar values in this study to those given by [39] Furthermore, okara is a good source of potassium, it's recommended for people suffering from hypokalemia (low potassium level). Also showed that okara contained higher potassium and lower sodium, which was beneficial for lowering blood pressure. This is agreed by [40].

Table 3. Macroelements (µg/g dry matter) and microelements (µg/g dry matter) in dried okara

Mineral content	Dried Okara (OK)
Calcium µg/g	656.0±1.45
Copper µg/g	1.40±0.90
Iron µg/g	7.20±0.45
Potassium µg/g	801.00±0.28
Magnesium µg/g	38.30±1.02
Manganese µg/g	3.70±0.92
Sodium µg/g	26.10±0.84
Selenium µg/g	8.50±0.89
Zinc µg/g	3.20±1.13
Phosphorus µg/g	477.80±0.84

All values are means ± standard deviation of triplicate analyses

[41] evaluated the metabolism of calcium, magnesium and zinc in rats fed on a diet supplemented with okara and a significant enhancement on the absorption of calcium was appreciated. This fact seems to be caused by the increased solubility of calcium in the colon. Selenium and iron and are the predominant microelement major in okara at 8.50 and 7.20 µg/g. Manganese and zinc was at lower ratio of microelements level at about 3.5 µg/g. Generally legumes are containing appreciable amount of iron with low bioavailability. Meanwhile, Zinc bioavailability is relatively good in legumes, approximately a 25% from the zinc intake [42].

Table 4 shows the levels of plasma lipid profile of low-density lipoprotein and high –density lipoprotein of control rats, rats administered high – fat okara diet and cholesterol. The results (Table 4) show that, there were significantly difference ($p<0.05$) in the triglyceride in group 1 and 2 (C-1 control; C-2 control) and other fed groups on high-fat with okara diet rats throughout the whole experiment. There were a higher significant difference in triglyceride between group 2 (C-2 control) in fed on high fat diet no okara and other fed groups on high – fat okara at different levels. From, these obtained data, it could be found that, okara in different levels were maintained to reduce the triglyceride level although, the diet containing a high- fat and cholesterol. Generally okara has significant decreased role in the triglyceride in the high-fat okara diet similar to role of diet to maintain it as negative control effect by –ve control (C-1). These results were agreed by several studies when assess okara soybean by-products as potential hypolipidemic ingredients and for other health-promoting properties in animals [41,34].However, [43] concluded that the consumption of diets rich in soy protein (b-conglycinin) have an inhibitory effect on the development of atherosclerosis in mice.

3.2 Triglycerides and Lipoprotein Analysis in Plasma

Feeding albino rats with the high - fat okara diets at okara 10, 20 and 30% with high fat diets decrease significant ($P<0.05$) in plasma total cholesterol and LDL-C and compared to their control + ve control (C-2) (Table 4.). The group fed the diet supplemented with 20% okara (OK-20) had significantly lower ($p<0.05$) plasma LDL-C level than the 10 and 30% okara supplemented groups. There is significantly different could be occurred between groups with time interaction in the level of okara groups and high-fat diets of okara. By increase of okara levels from 10 up to 30% in the diets contain by high fat diet + cholesterol 1% is able to reduce VLDL-C comparing to +ve control (C-2) as shown in Table 4 Otherwise, there is non-significance difference ($p>0.05$) could be occurred in the level of VLDL-C corresponding to interaction between groups diet with time. Also, VLDL-C were similar ($P>0.05$) for okara groups at different levels of supplementation and the –ve control (C-1), while there exhibited the lowest VLDL-C concentration (Table 4) [44] showed that a diet containing 40% okara reduced the total and LDL cholesterol significantly in mice after 10 weeks. This significant decrease in level in LDL and VLDL according to okara concentration in diet, is due to soluble dietary fiber in okara meal. It's in agreement by [45] found that, the soluble dietary fiber residual from treated okara is significantly reduce the concentrations of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and triglyceride (TG) while elevating the concentration of high-density lipoprotein cholesterol (HDL-C) depend on *In vivo* experiment. The mean values of HDL-C is significantly higher in the all groups especially among group 4 which fed on diet containing 20% okara and high fat diet with 1% cholesterol. Higher HDL levels are considered beneficial since HDL picks up cholesterol from peripheral tissues and transports cholesterol in the blood back to the liver. The diets supplemented with 10, 20 and 30% okara (OK-10, OK-20 and OK-30) have significant decrease ($p<0.05$) the plasma cholesterols of rats fed a high-fat diet (render fat). These results indicated that not only fiber but other components of okara, such as protein, could be involved in the reduction of plasma lipids. Other studies have shown that soy protein consumption has beneficial effects on plasma lipid concentrations in animal models [12] suggested that the main components of okara, dietary fiber (lignin) and protein could be related with the total lipids and cholesterol decrease in the plasma and liver, as well as with the fecal output increase in high-fat fed hamsters. Okara might play an interesting role in the prevention of hyperlipidemia and could be used as a natural ingredient or supplement for functional food preparation. Since several components of okara (protein or dietary fiber) might be contribute to decrease blood lipid profile.

3.3 Hematological Analysis

The results of hematological analysis of rats whole blood samples fed different diets are presented in Table 5. In the exception of the platelets levels which does not varied significantly amongst diet groups, most of the hematological parameters varied significantly ($p<0.05$) compared to –ve control (C-1). In this respect rats fed okara at different level with high –fat diet group exhibited lower levels of white blood cell compared to –ve control (C-1), controversy , there where non-significant difference occurred between okara supplemented group than those fed on +ve control (C-2) as shown in Table 5. Meanwhile, this variation is lower than occur in group 2 which has a higher count of RBCs. The structural integrity of red blood cells and, hence, the cell phospholipids membrane are affected in hypercholesterolemic situation induced by a high fat and cholesterol diet. RBC's in such group 2 have increased red cell membrane. This membrane thickness of red blood cell was delayed cell death and also increases their count. This result is also indicating by [46,47]

concluded that, the high-fat diet feeding, erythrocyte membrane and leucocyte cholesterol and phospholipid contents were increased, cholesterol: Phospholipid molar ratio was elevated, and erythrocyte enzymes (glucose-6-phosphate dehydrogenase and 6 phosphate dehydrogenase) and leukocyte enzymes (cholesterol ester Hydrolase and cholesterol ester synthetase) were elevated. Erythrocyte membrane glycoprotein components showed marked increase, indicating possible alterations of membrane surfaces. Cellular changes indicate alterations in structure and function of blood cells due to High-fat diet feeding. Phagocytosis by peritoneal macrophages was significantly decreased in the animals fed high-fat diets, particular high saturated fat. Similarly, natural killer cell activity was markedly reduced in the mice with a high intake of saturated lipid, a finding which correlated with the in vitro production of interferon. These results indicate that diets high in fat influence immune responses and thus can affect the onset and severity of autoimmune disease.

3.4 Organs Weight

There were no significant differences ($P<0.05$) in heart weights (cardosomatic index) between the treated different diet groups and both controls rat, and the weights were within normal ranges in Table 6. However, there were a significant difference ($P<0.05$) in hepatosomatic index between –ve control and different groups of rats. Nevertheless, there were no differences $P<0.05$ between groups which received by okara supplemented and +ve control with high fat and free okara (Table 6).

3.5 Histological of Liver

Microscopically elucidate liver of rats from group 1 revealed histological structure of hepatic lobule (Fig. 1A). The microscopically examination of liver tissues revealed the presence of normal hepatic parenchyma, including normal hepatic cords, central veins and portal areas in group 1 (-ve control C-1 group). Meanwhile, liver of rat from group 2 showed liver tissues showed marked pathological changes such as wide extension at hepatocellular, vacuolar degeneration of hepatocytes, pyknosis of hepatocyticnuclui (Fig. 1B) and focal area of hepatic necrosis associated with leucocytic cells infiltration (Fig. 1C). Liver of rat from group 3 revealed vacuolar degeneration of hepatocytes (Fig. 1D). However, liver of rat from group 4 revealed vacuolar degeneration of hepatocytes (Fig. 1E, 1F and 1G), congestion of hepatic sinusoids (Fig. 1F) and leucocytic cells infiltration (Fig. 1G). Examined liver of rat from group 5 showed vacuolar degeneration of hepatocytes (Fig.1G and 1H). These changes in hepatocellular, it could be revealed to degenerative of high-fat diet okara. While, then diet containing okara may be impede the hepatic degenerative depend on isoflavones in okara constituent (isoflavones range 0.02-0.12% of okara as d.b [5]). It was agreed by [48].

3.6 Histological of Heart

Microscopically investigation, heart of rats from group 1 revealed normal cardiac myocytes (Fig. 2A).On the other hand, heart of rat from group 2 showed thickening and hyalinosis in the cell of cardiac blood vessels (Fig. 2B), as well as vacuolations in the tunica media of myocardial blood vessel (Fig. 2C). Heart of rats from group 3 showed no histopathological changes (Fig. 2D). Congestion of myocardial blood vessels was noticed in heart of rat from group 4 (Fig. 2E). Similar examined sections of group 5 showed sections revealed focal myolysis of cardiac myocytes associated with inflammatory cells infiltration (Fig. 2F). These fundamental changes in cardiac cell, it was considered to peroxidation of lipids in high –fat diet.

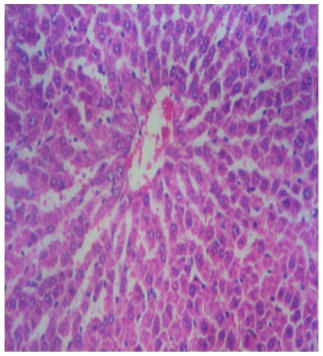

Fig. 1A: Rat liver from group 1 showing the normal histological structure of hepatic lobule (H and E × 200)

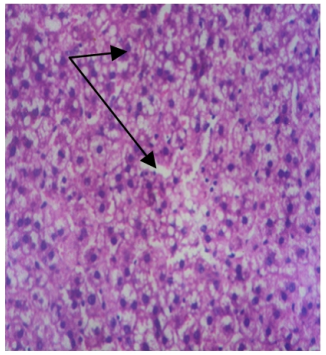

Fig. 1B: Rat liver from group 2 showing vacuolar degeneration of hepatocytes and pyknosis of hepatocytic nuclei (H and E × 200)

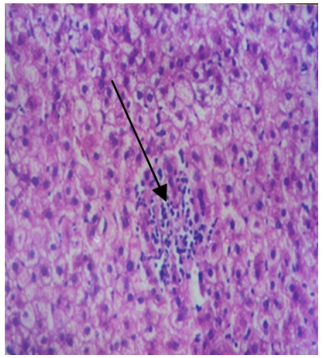

Fig. 1C: Rat liver from group 3 showing focal area of hepatic necrosis associated with leucocytic cells infiltration (H and E × 200)

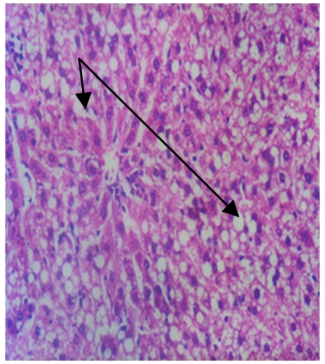

Fig. 1D: Rat liver from group 3 showing vacuolar degeneration fatty changes of hepatocytes (H and E × 200)

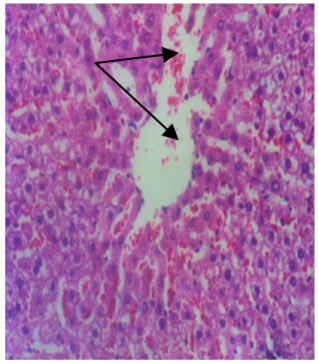

Fig. 1E: Rat liver from group 4 showing congestion of hepatic sinusoids and vacuolations of hepatocytes (H and E × 200)

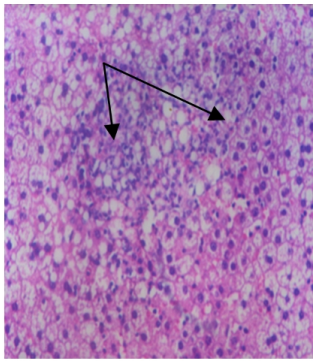

Fig. 1F: Rat liver from group 4 showing vacuolar degeneration of hepatocytes associated with leucocytic cells (H and E × 200)

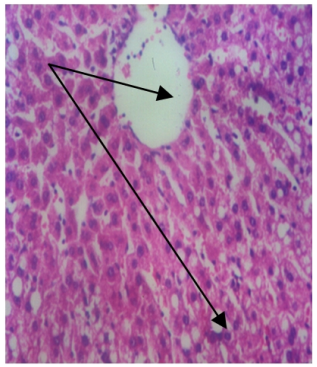

Fig. 1G: Rat liver from group 5 showing vacuolar degeneration of hepatocytes (H and E × 200)

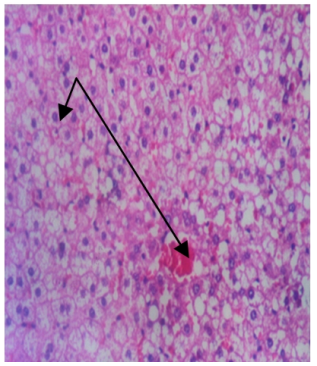

Fig. 1H: Rat liver from group 5 showing vacuolar degeneration of hepatocytes (H and E × 200)

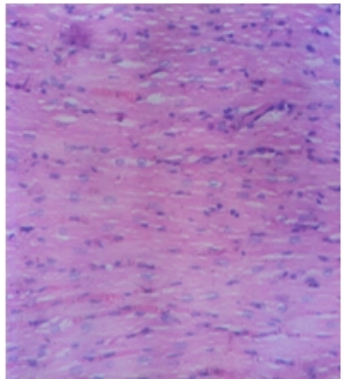

Fig. 2A: Rat heart from group 1 showing the normal cardiac myocytes (H and E × 200)

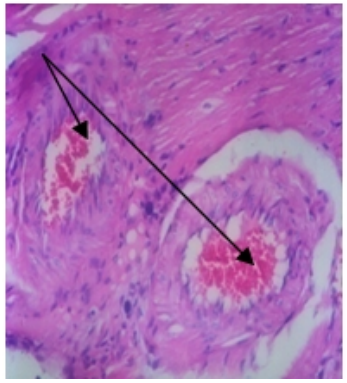

Fig. 2B: Rat heart from group 2 showing vacuolar thickening and hyalinosis in the wall of cardiac blood vessels (H and E × 200)

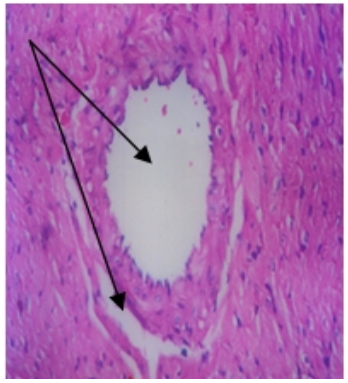

Fig. 2C: Rat heart from group 2 showing vacuolations in the tunica media of myocardial blood vessel (H and E × 200)

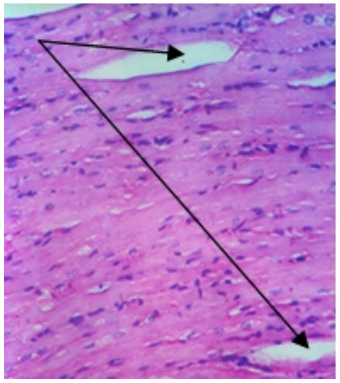

Fig. 2D: Rat heart from group 3 showing no histopathological changes (H and E × 200)

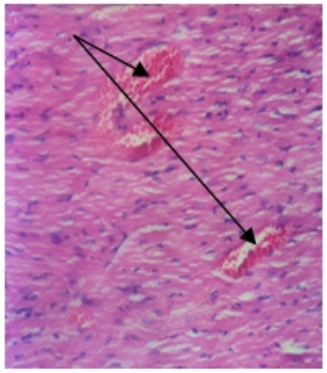

Fig. 2E: Rat heart from group 4 showing congestion of myocardial blood vessels (H and E × 200)

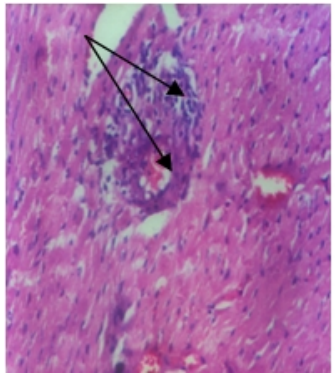

Fig. 2F: Rat heart from group 5 showing focal myolysis of cardiac myocytes associated with inflammatory cells infiltration (H and E × 200)

Table 4. Effect of the high-fat okara on plasma triglycerides, total cholesterol and lipoprotein concentrations (mg/dL) in fed groups rat

Plasma lipid profile	Control Free from okara C-1* (group1)	Control with high –Fat diet +10 % Cholesterol C-2 ** (group 2)	10% Okara OK-10 (group 3)	20% Okara OK-20 (group 4)	30% Okara OK-30 (group 5))	LSD at p<0.05	Interaction time × groups
Triglycerides (TG) mg/dL	64.54^{c} ±20.02	150.8^{a}±36.49	89.34^{b}±21.19	83.88^{b}±20.21	59.16^{c} ±18.22	8.761	15.17
Total cholesterol (TC) mg/dL	99.69^{c}±53.07	209.4^{a} ±40.06	153.8^{b}±3.40	135.0^{b} ±50.26	137.0^{b} ±2.08	22.35	38.72
VLDL-C mg/dL	17.47^{b} ±2.34	29.68^{a} ±6.60	17.83^{b}±5.68	16.73^{b}±8.09	11.79^{b}±10.25	6.042	NS
LDL-C mg/dL	43.91^{d}±3.55	150.0^{a} ±14.44	96.11^{b} ±8.59	67.31^{c} ±6.32	83.59^{b}±12.45	23.18	40.15
HDL-C mg/dL	42.42^{b}±3.83	29.64^{c}±7.56	39.85^{b}±7.78	50.82^{a}±6.76	41.33^{b}±9.32	3.015	5.222

Data are presented as Mean ± SD. Values in horizontal raw bearing same letters are significantly (p<0.05) not different
**C-1: -ve control diet free from render fat; okara and cholesterol (Basal diet)*
***C-2: +ve control diet with no okara and with render fat and cholesterol*
OK-10: diet high –Fat with 10% of okara
OK-20: diet high –Fat with 20% of okara
OK-30: diet high –Fat with 30% of okara

Table 5. Effect of the high-fat diets okara on hematological profile in fed groups rat

	Control Free from okara C-1* (group1)	Control with high –Fat diet +10 % Cholesterol C-2 ** (group 2)	10% Okara OK-10 (group 3)	20% Okara OK-20 (group 4)	30% Okara OK-30 (group 5)	LSD at p<0.05
WBC (10^3/mm^3)	31.07^{a}±4.22	21.40^{b}±3.12	20.13^{b}±3.05	18.10^{b}±5.04	21.42^{b}±2.18	6.64
RBC (10^3/mm^3)	7.352^{bc}±1.88	8.320^{a}±4.12	6.968^{c}±1.33	7.453^{bc}±0.98	7.918^{ab}±1.02	0.56
HGB g/dl	13.53^{a}±1.22	13.78^{a}±1.05	11.73^{c}±0.88	12.28^{bc}±0.07	13.13^{ab}±1.03	0.92
HCT %	42.12^{bc}±1.18	45.52^{a}±1.34	38.15^{d}±2.00	39.97^{cd}±1.62	43.17^{ab}±1.07	2.652
PLT (10^3/mm^3)	651.7^{a}±5.33	641.8^{ba}±12.35	744.2^{a}±20.13	727.5^{ab}±16.45	704.2^{a}±22.37	NS

Data are presented as Mean ± SD. Values in horizontal raw bearing same letters are significantly (p<0.05) not different
**C-1: -ve control diet free from render fat ; okara and cholesterol (Basal diet)*
***C-2: +ve control diet with no okara and with render fat and cholesterol*
OK-10: diet high –Fat with 10% of okara
OK-20: diet high –Fat with 20% of okara
OK-30: diet high –Fat with 30% of okara
Data are presented as Mean ± SD. Values in horizontal raw bearing same letters are significantly (p<0.05) not different

Table 6. Influence of high- fat diets with different okara percentage on liver and heart organs weight % of fed albino rats

Organ /body weight %	Experimental diet groups					
	Control Free from okara C-1* (group1)	Control with high –Fat diet +10% Cholesterol C-2 ** (group 2)	10% Okara OK-10 (group 3)	20% Okara OK-20 (group 4)	30% Okara OK-30 (group 5)	LSD at p<0.05
Hepatosomatic index (weight liver /body %)	2.85^{a}± 0.18	3.81^{b}±0.76	4.01^{b}±0.35	4.14^{b}±0.36	4.05^{b}±0.46	0.555
Cardiosomatic index (weight heart / body %)	0.32^{a}±0.04	0.30^{a}±0.05	0.31^{a}± 0.03	0.34^{a}±0.02	0.33^{a}±0.03	0.046

4. CONCLUSIONS

Okara is a rich source of nutrients that could be utilized in reducing hypercholesterolemia effects when albino rats were fed high-fat diets with different okara ratio (10, 20 and 30%) over 60 days. Results indicated that both dietary fiber and other okara constituent has significant role to decrease plasma lipids profile in high-fat fed rats. Whereas, the histopatholgical demonstrated that a high-fat ratio, it could effected in degenerative hepatocellular cell, whereas, okara is depressed this role. Okara constituents could improve the nutritional quality of other foods and is a promising functional ingredient that can be used in the development of fiber-rich foods.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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