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Review

The effect of consuming different proportions of hummer fish on biochemical and histopathological changes of hyperglycemic rats

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ABSTRACT

Hammour fish (grouper fish) are known to be of great nutritional value for human consumption, as their protein has a high biological value and contains all the essential amino acids. Grouper fish are also a good source of minerals, vitamins, and fats that contain essential fatty acids. Thus, the current study aims to know the effect of different proportion of hummer fish on biochemical and histopathological changes of hyperglycemic rats. Twenty-four (24) Sprague Dawley-strain male albino rats, which weighed 150 ± 10 g, were divided into four groups. One group served as the negative control (normal), while the others were rendered diabetic using alloxan. One of the diabetic groups was considered the positive control and fed a standard diet, whereas the remaining two groups were fed with a 20% and 25% hammour fish diet for 28 days. At the end of the experiment, blood samples were taken from all the rats, and their organs were removed and subjected to biochemical analysis. The results indicated that the group fed with the 25% hammour fish diet exhibited significantly lower levels of liver, kidney, and heart damage, along with lower levels of serum glucose, total cholesterol, triglycerides, LDL, GOT, GPT and ALP, as compared to the positive control. The urea and creatinine levels were significantly higher for the rats that were fed the 20% hammour fish diet than for those in the positive control. The histopathological study of the heart showed a slight improvement of the heart tissues with the increase of hammour fish intake compare to the positive control, while kidney of rat from group 4, which were fed 25% hammour fish, showed granularity of epithelial lining glomerular tufts.

Contents

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1. Introduction

Hammour fish is known to have great nutritional value for human consumption, as its protein has a high biological value and contains all the required amino acids, Grouper fish are also a good source of minerals, vitamins, and fats, which contain the fatty acids that are essential for humans Bimal Prasanna et al. (2019). The pioneering observations in Greenland Eskimos suggest that high intakes of omega-3 fatty acids from fish and sea mammals prevent cardiovascular disease. This is in contrast with the high frequency of cardiovascular disease in Western populations, who have low fish intakes and high intakes of cholesterol and saturated fat. This is the presumed benefit of omega-3 fatty acids intake Kris-Etherton et al. (2002). Dietary grouper fish has shown to have a positive effect on glucose tolerance by increasing the insulin secretion capacity of the pancreatic beta cell and ameliorating insulin resistance Bjorn Liaset et al. (2019). However, it cannot be assumed that the effects of omega-3 fatty acids for patients suffering from diabetes mellitus are the same as those for non-diabetic individuals or patients with primary hyperlipidemia. The biosynthesis and composition of fatty acids are abnormal in diabetic patients. Many potential mechanisms implicated in the pathogenesis of atherosclerosis are present in diabetic patients, but the same is not necessarily true for non-diabetic individuals. The mechanisms of many of the risk factors in diabetic patients differ from the mechanisms of those abnormalities in non-diabetic subjects, reflecting the effect of insulin deficiency, hyperglycemia, and their sequels Api Chewcharat et al. (2020).

Diabetes is a disease in which the patient's blood sugar or blood sugar levels are excessively high. Glucose comes from the foods we eat, and insulin is a hormone that helps glucose reaches your cells to give them energy. With type 1 diabetes, the body does not produce insulin. With type 2 diabetes, which is the most common type, the body does not make or use insulin effectively. Without enough insulin, glucose stays in blood. Patient can also have prediabetes, which means that the blood sugar is higher than normal but not high enough to be diagnosed with diabetes. Having prediabetes increases the risk of developing type 2 diabetes. Cooke and Plotnick (2008). Ndisang and Vannacci (2017). National Diabetes Statistics Report (2017).

2. Aim of study

This work aimed to know the effect of consuming different proportion of Hummer fish on biochemical and histopathological changes of hyperglycemic rats.

3. Materials and methods

3.1. Materials

3.1.1. Preparation of hammour fish

First, hammour fish were purchased from the local market of Jeddah, KSA. Then, the fish were washed and cut into small slices before being dried in a drying oven at a temperature of 50° C for three days. Finally, these slices were crushed and milled into a fine powder.

3.1.2. Experimental animals

Twenty-four male albino rats (Sprague Dawley strain), weighing 150 ± 10 g, were used in the study.

3.1.3. Alloxan

Pure fine high-quality chemicals were purchased from Sigma, Cairo, Egypt.

3.2. Methods

3.2.1. Biological experiment

3.2.1.1 Basal diet composition of rats

3.2.2. Induced disease for rats

Diabetes mellitus was induced in normal healthy male albino rats via intraperitoneal injection of alloxan (150 mg/kg body weight) according to the method described by Desai and Bhide (1985). Six hours after the injection of alloxan, fasting blood samples were obtained using the *retro*-orbital method to estimate fasting serum glucose. The rats that presented a fasting serum glucose value of more than 185 mg/dl were considered diabetic N.D.D.G. (1994).

3.2.3. Experimental design and animal groups

Twenty-four mature male albino rats of the Sprague Dawley strain weighing 150 ± 10 g at the age of 14–16 weeks were included in this study. The animals were housed in plastic cages with metallic strainless covers and kept under strict hygienic conditions. Rats were fed the basal diet for seven days before the beginning of the experiment for adaptation purposes. The food was given to the rats in special non-scattering feeding cups to avoid the loss of food or contamination. Water was provided ad libitum via a narrow-mouthed bottle with a metallic tube tightly fixed at its mouth using a piece of rubber tube. The animals were subjected to a 12-hour light, 12-hour dark schedule and kept for seven days before the start of the experiment for acclimatization purposes, as aforementioned. The rats were divided into four groups, each containing six rats. The groups of rats were as follows:

- Group 1: rats were fed basal diet (control negative)
- Group 2: diabetic rats were fed basal diet (control positive)
- Group 3: diabetic rats were fed basal diet containing 20% hammour fish
- Group 4: diabetic rats were fed basal diet containing 25% hammour fish

3.2.4. Biological evaluation

During the experiment period (28 days), the consumed feed was recorded every day, and the body weight of each rat was recorded weekly. The body weight gain (B.W.G. %), food efficiency ratio (F.E.R), and organs weight were determined according to the method used by Chapman et al. (1959).

3.2.4.1. Blood sampling. At the end of the experiment period, the rats were euthanized using ether and anesthesia. Blood samples were obtained using the *retro*-orbital method and transferred to a clean, dry centrifuge tube. These were clotted by being left to stand at room temperature for 20 min and then centrifuged at 1500 rpm for 15 min. Serum samples were collected using a dry, clean syringe, poured into Wasserman tubes, and then kept frozen in a refrigerator at −10 °C till the biochemical analysis was conducted. The rats were thereafter dissected, and their liver, spleen, heart, lungs, and kidneys were removed and washed in a saline solution before being dried and weighed according to methods described by Drury and Wallington (1967).

3.2.4.2. Biological analysis. Food intake (consumption), body weight gain% (BWG %), feed efficiency ratio (FER) according to Chapman et al. (1959). Using the following equation:

$$BWG\% = \frac{Final\,weight - Initial\,weight}{Initial\,weight} \times 100$$

$$FER = \frac{Gain\,in\,body\,weight\,(g/day)}{Food\,Intake\,(g/day)}$$

$$\text{Relativeweightoforgans} = \frac{Organsweight}{Animalbodyweight} \times 100$$

3.2.5. Biochemical analysis

3.2.5.1 Food intake (consumption), body weight gain% (BWG %), feed efficiency ratio (FER) according to Chapman et al. (1959)

3.2.5.2 Determination of serum glucose: serum glucose was determined using chemical kits according to Trinder (1969).

3.2.5.3 Determination of serum lipids

3.2.5.3.1 Triglycerides: Enzymatic calorimetric determination of Triglycerides was carried out according to Fassati and Prencipe (1982).

3.2.5.3.2 Total cholesterol: The principle use of total cholesterol determination according to Allain (1974).

3.2.5.3.3 HDL-cholesterol: Phosphotungstic acid and magnesium ions selectivity precipitating all lipoproteins except the HDL fraction-cholesterol present in the supernatant can be determined by the same method used for total cholesterol, according to Lopez (1977).

3.2.5.3.4 V-LDL and LDL- cholesterol: The determination of VLDL (very low density lipoprotins) and LDL were carried out according to the method of Lee and Nieman (1996)

3.2.5.4 Determination of liver functions

3.2.5.4.1 Determination of alanine transferase (ALT): Determination of (ALT) was carried out according to the method of Tietz (1976). ALT catalyzes the transfer of the amino group from L-alanine to a-Ketoglutarate resulting in the formation of pyruvate and L-Glutamate.

3.2.5.4.2 Determination of asartate transferase (AST): Determination of (AST) was carried out according to the method of Henry (1974)

3.2.5.4.3 Determination of total protein: Total protein was measured according to the colorimetric method of Henry (1974).

3.2.5.5 Determination of e kidney functions

3.2.5.5.1 Determination of creatinine: Creatinine was determined according to kinetic method of Henry (1974).

3.2.5.5.2 Determination of urea: Urea was determined according to the enzymatic method of Patton and Crouch (1977).

3.2.6. Histopathological examination

Specimens from liver, kidney and heart were collected directly after euthanized of animals at the end of experimental period. Tissues were then fixed in 10% neutral formalin, washed overnight dehydrated in increased graded concentrations of ethyl alcohol, and cleared in xylene for 4–6 h. Thereafter, the samples were placed in a crucible containing soft paraffin in an oven at 56 °C for 8–12 h and embedded in paraffin wax. The embedded samples were then sectioned at five microns in thickness. To stain the sectioned samples with Hematoxylin and Eosin, Paraffin was firstly removed from the sections by two changes of Xylol five minutes in each, treated with absolute alcohol to remove Xylol, and then washed in tap water to remove the excess of alcohol. This followed by staining the sectioned samples with Hematoxylin and Eosin for 3–5 min according to Drury and Wallington (1967), washing with water, dehydrating in 4 changes with absolute alcohol, 1 min each), and clearing the sectiones with Xylol. Finally, the sections were mounted with Canda Balsam and covered with cover slides Carleton (1978)

3.2.7. Statistical analysis

Statistical analysis was calculated using one way classification. Analysis of variance (ANOVA), and least significant difference (LSD) according to Snedcor and Cochran (1967). (See Tables 1-3)

Table 1
Composition of basal diet.

Ingredients	Amounts*
Protein (casein)	10%
Corn oil	10%
Mineral mixture	4%
Vitamin mixture	1 %
Cellulose	5%
Choline chloride	0.2 %
Methionine	0.3 %
Corn starch	Up to 100%

Source: Reeves et al. (1993).

Table 2
The composition of salt mixture (g/100 g).

Ingredients	Amounts
$CaCO_3$	600 mg
K_2 HPO_4	645 mg
Ca $HPO_4{\cdot}2H_2O$	150 mg
$MgSO_4.2H_2O$	204 mg
Nacl	334 mg
Fe ($C_6H_5O_7$) $26H_2O$	55 mg
Kl	1.6 mg
$MnSO_4{\cdot}.4H_2O$	10 mg
$ZnCl_2$	0.5 mg
Cu $SO_4{\cdot}5H_2O$	0.06 mg
$CaCO_3$	600 mg

Source: Hegsted et al. (1941).

Table 3
The composition of vitamin mixture.

Vitamin	Amount
Vitamin E	10 Iu
Vitamin K	0.50 Iu
Vitamin A	200 Iu
Thiamin	0.50 mg
Pyridoxine	1.00 mg
Niacin	4.00 mg
Calcium panthothenic acid	0.40 mg
Vitamin D	100 Iu
Choline chloride	200 mg
Folic acid	0.02 mg
Inositol	24 mg
Para-amino – benzoic acid	0.02 mg
Vitamin B12	2.00 μg
Biotin	0.02 mg

Source: (Campbell. 1963).

4. Results and discussion

This work aimed to investigate the effect of different proportion of hummer fish on biochemical and histopathological changes of hyperglycemic rats.

4.1. Biological results

4.1.1. Effect of consuming different proportions of hammour fish (grouper fish) on on body weight gain (B. W. G.), food intake (F. I.), and food efficiency ratio (F. E. R.).for the diabetic rats

The data presented in Table 4 indicates the effect of consuming different proportions of hammour fish (grouper fish) on on body weight gain (B. W. G.), food Intake (F. I.), and food efficiency ratio (F. E. R.).for the diabetic rats

Body weight gain (B. W. G.):

It could be noticed that in control (-ve) normal rats body weight gain (B. W. G.) was 81. 75 ± 2. 50 g , while that in the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) hammour fish were (55. 00 ± 1. 47 ,86. 00 ± 1. 55, and 62. 50 ± 1. 91) g respectively. These results denote that there were significant increase in control (+ve) rats compared to control (-ve) group.

Food intake (F. I.):

It could be observed that for control (-ve) normal rats food intake (F. I.) was 16. 13 ± 0. 81 g.. while that in the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) hammour fish were (17. 7 5 ± 0. 45 , 21. 4 ± 0. 99, and 17. 65 ± 0. 55) g respectively. Diabetic rats fed on (20%, and 25%) hammour fish reflected significant increase compared to control (+ve) group.

Food efficiency ratio (F. E. R.):

It could be noticed that for control (-ve) normal rats food efficiency ratio (F. E. R.) was 0. 18 ± 0. 03, while that in the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) hammour fish were (55. 00 ± 1. 47, 0. 12 ± 0. 03, and 0. 13 ± 0. 02) g respectively. These result reflected significant decrease in control (+) compared to control (-ve) group. Diabetic rats fed on(20%, and 25%) hammour fish diet showed significant increase compared to control (+ve).

4.1.2. Effect of consuming different proportions of hammour fish (grouper fish) on the relative change in organ weights for the diabetic rats

The data presented in Table 5 indicates the effect of consuming different proportions of hammour fish on organ weight and the organ weight/body weight of both normal and alloxan-induced diabetic rats after four weeks of feeding.

The relative liver weight of the negative control rats group was (2.72 ± 0.01) gm/100gm. In contrast, the alloxan-induced diabetic rat groups (positive control, 20%, and 25% hammour fish) that were fed a diet containing different amounts of hammour fish showed a increase in the relative weight of the liver (3.44 ± 0.222 , 4.46.5 ± 0. 037, and 3.73 ± 0.036)gm/100gm, respectively. These results indicate that this parameter was significantly higher ($P < 0.01$) for all groups as compared to the control negative. Additionally, the results show that the alloxan-induced diabetic rats in groups (20%, and 25% hammour fish) gradually increas the weight of the liver compare to the positive control.

The relative kidney weight values in the normal rats group was (1.06 ± 0.026), while in the alloxan-induced diabetic rats groups (positive control, 20%, and 25% hammour fish) was (1.27 ± 0.012, 1.79 ± 0.0), and 1.29 ± 0.012), respectively. These results demon-

Table 4
Effect of consuming feeding different proportions of hammour fish (grouper fish) on body weight gain (B. W. G.), food Intake (F. I.), and food efficiency ratio (F. E. R.).for the diabetic rats.

Parameters	Control (−)	Control (+)	20% Hammour fish	25% Hammour fish
BWG (g)	81. 75 ± 2. 50b	55. 00 ± 1. 47f	86. 00 ± 1. 55 a	62. 50 ± 1. 91 e
FI (g)	16. 125 ± 0. 81 d	17. 7 5 ± 0. 45c	21. 4 ± 0. 99 a	17. 65 ± 0. 55c
FER	0. 18 ± 0. 03 a	0. 11 ± 0. 01f	0. 12 ± 0. 03 d	0. 13 ± 0. 02c

Values denote arithmetic means ± Standard error of the mean. One way ANOVA test, while those with similar letters are non-significant.

Table 5
Effect of feeding consuming different proportions of hammour fish (grouper fish) on relative organs weight of change on diabetic rats.

Parameters	Control (−)	Control (+)	20% Hammour fish	25% Hammour fish
Liver(g)	2.72 ± 0.01 a	3.44 ± 0.22b	4.46 ± 0.037c	3.73 ± 0.036b
Kidney(g)	1.06 ± 0.026 a	1.27 ± 0.012b	1.79 ± 0.02c	1.29 ± 0.017b
Heart(g)	0.37 ± 0.0085 a	0.55 ± 0.0085b	0.692 ± 0.0085c	0.69 ± 0.0085c

Values denote arithmetic means ± Standard error of the mean. One way ANOVA test, while those with similar letters are non-significant.

strate that the groups that had diets consisting of 20% and 25% hammour fish presented a significant increase ($P < 0.05$) in relative kidney weight when compared to the control negative. Additionally, the results show that the alloxan-induced diabetic rats in groups (20%, and 25% hammour fish) gradually increase the weight of the kindey compare to the positive control.

The relative heart weight value in the normal rats group was (0. 37 ± 0.0085) gm/100gm, while in diabetic rats groups that were fed different amounts of hammour fish (positive control, 20%, and 25%) presented corresponding values of (0.55 ± 0.00850.692 ± 0.0085, and 0.69 ± 0.0085), and (0.55 ± 0.0085) gm/100gm, respectively. These results showed that this value for all diabetic groups was significantly more ($P < 0.01$) than that of the control negative. Additionally, the results show that the alloxan-induced diabetic rats in groups (20%, and 25% hammour fish) gradually increase the weight of the heart compare to the positive control.

4.1.3. Effect of consuming different percentages of hammour fish (grouper fish) on blood glucose in diabetic rats

Table 6 illustrates the effect of different amounts of hammour fish on blood glucose in both normal and alloxan-induced diabetic rats after four weeks of feeding. The results proved that the blood glucose level was (84 ± 3.62) mg/dl for the negative control group rats, while that for the alloxan-induced diabetic rats groups was (215.7 ± 5.26, 180. 00 ± 2. 08 , and 140. 25 ± 1. 25) mg/dl for the positive control, 20% hammour fish-diet group, and 25% hammour fish-diet group, respectively, after four weeks of feeding. The same table indicates that the blood glucose showed a gradual decrease with the increase of hammour fish intake from 20% to 25%. The obtained results are in line with those of Ashraf et al. (2008). The result explained the beneficial effect of different types of fish on diabetic rats compared to diabetic rats treated with insulin. The result showed that the mean values of the serum glucose, cholesterol, triglycerides, LDL-c, HDL-c, VLDL-c, uric acid, urea nitrogen, aspartate amino transferase (AST) and alanine amino transferase (ALT) decreased in all treated groups, especially with the mackerel and sardine diet followed by bolti, as compared to the positive control groups (fed on a casein diet). Additionally, the levels of serum cholesterol and LDL-c increased in the groups fed on the herring diet. On the other hand, diabetic rats that were treated with low insulin dose and fed on the mackerel diet, showed non-significant differences in the levels of all parameters, as compared to non-diabetic rats.

4.1.4. Effect of consuming different proportions of hammour fish (grouper fish) on triglycerides and t-cholesterol

Table 7 presents the effect of consuming different amounts of hammour fish on triglycerides and t-cholesterol for both the normal and the alloxan-induced diabetic rats after four weeks of feeding.

The total cholesterol in the negative control rats group was (92 ± 1.29) mg/dl, while that in the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) was (189.5 ± 3.27, 176.5 ± 2.9, , and 175.25 ± 2.32) mg/dl, respectively.

The triglycerides values in the negative control rats group was (76 ± 1.08) mmol/L, while that in the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) was (126.5 ± 2.25), (140.7 ± 0.85), and 118.7 ± 2.83) mg/dl, respectively. The results showed that these values for all groups were significantly higher ($P < 0.01$) than those of the control negative. Additionally, the results show that the alloxan-induced diabetic rats in groups (20%, and 25% hammour fish) gradually reduce the level of triglycerides and t-cholesterol with the increase of hammour fish intake. Furthermore, the serum triglycerides value for rats fed with diets consisting of 20% and 25% hammour fish and the control positive was significantly higher ($P < 0.01$) than that of the control negative.

These results are in the line with those of Adriana and Piotr (2016), which suggests that the high-protein diet resulted in the lowered serum concentrations of triacylglycerol (by 19.2 protein, 5.6%; $P = 0.003$). Based on an analysis conducted with twenty hyperlipidemic men and women using the isoenergetic test (high protein) and control metabolic diets.

4.1.5. Effect of consuming different proportions of hammour fish (grouper fish) on LDL and HDL cholesterol

Table 8 presents the effect of consuming different amounts of hammour fish on LDL and HDL cholesterol in both normal and diabetic rats after four weeks of feeding.

The serum HDL levels in the negative control rats group was (49.5 ± 0.64) mg/dl, while those in the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) were (21.5 ± 1.7), (35.0 ± 0.7), and (37.3 ± 1.7) mg/dl, respectively. As for HDL, the

Table 6
Effect of different proportions of hammour fish (grouper fish) on serum glucose of diabetic albino rats.

Parameters	Control (−)	Control (+)	20% Hammour fish	25% Hammour fish
Serum glucose (mg/dl)	84 ± 3.62 a	215.7 ± 5.26 e	180. 00 ± 2. 08b	140. 25 ± 1. 25c

Values denote arithmetic means ± Standard error of the mean. One way ANOVA test, while those with similar letters are non-significant.

Table 7
Effect of consuming different proportion of Hammour fish (grouper fish) on triglyceride and total cholesterol in serum diabetic albino rats.

Parameters	Control (+)	Control (+)	20% Hammour fish	25% Hammour fish
T.G (mg/dl)	76 ± 1.08 a	126.5 ± 2.25c	140.7 ± 0.85 e	118.7 ± 2.83b
T.cholesterol (mg/dl)	92 ± 1.29 a	189.5 ± 3.27c	176.5 ± 2.9b	175.25 ± 2.32b

Values denote arithmetic means ± Standard error of the mean. One way ANOVA test, while those with similar letters are non-significant.

Table 8
Effect of different proportions of hammour fish (grouper fish) on serum of LDL and HDL serum diabetic albino rats.

Groups Parameters	Control (−)	Control (+)	20% Hammour fish	25% Hammour fish
LDL(mg/dl)	97.25 ± 1.37 a	113.25 ± 1.75 e	100.75 ± 2.1b	108.5 ± 2.10c
HDL(mg/dl)	49.5 ± 0.64 a	21.5 ± 1.7 e	35.0 ± 0.7c	37.3 ± 1.7b

Values denote arithmetic means ± Standard error of the mean. One way ANOVA test, while those with similar letters are non-significant.

results revealed that the corresponding values for alloxan-induced diabetic rats groups were significantly higher (P < 0.01) when compared to the control positive.

The serum LDL values in the negative control rats group was (97.25 ± 1.37) mg/dl, while those in the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) were (113.25 ± 1.75), (100.75 ± 2.17), and (108.5 ± 2.10) mmol/L, respectively. As for LDL, the results revealed that the corresponding values for all groups were significantly higher (P < 0.01) than those of the control negative. Our findings align with those of Gandham Bulliyya (2002), who showed that the fish-consuming population had a lower atherogenic risk than those of the non-fish-consuming population. The intake of fish may have substantial implications for public health and health economy by decreasing the risk of CVD. However, more studies are warranted to better define the mechanisms of cardioprotection by dietary fish and fish,.

4.1.6. Effect of consuming different proportions of hammour fish (grouper fish) on liver function (AST, ALT, and ALP)

Table 9 displays the effect of consuming different amounts of hammour fish on enzyme activity (AST, ALT, and ALP) in both the normal and alloxan-induced diabetic rat groups.

The AST level in the negative control rats group was (33.5 ± 1.7) u/l, while that in the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) were (41.5 ± 1.93 , 38.75 ± 1.49, and 36.5 ± 1.32 ,), u/l, respectively. The results showed that this parameter was significantly higher (P < 0.01) for the groups fed with diets that were 20% hammour fish and for the control positive as compared to the group with the diet of 25% hammour fish and the control negative. Further, the other groups showed non-significant differences when compared to the control negative.

The normal rats group presented ALT level of (19.75 ± 0.85) u/l, while the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) showed corresponding values of (28.25 ± 1.25 , 25 ± 1.29, and 22.75 ± 0.85) u/l, respectively. As for ALT, the results showed that this value for the group with diets that were 20% hammour fish and the control positive was significantly higher (P < 0.01) than that of the control negative.

With regard to Table 9 for ALP level the negative control rats group presented ALP levels of (85.5 ± 2.1) u/l, while the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) showed corresponding values of (124 ± 1.82,110.75 ± 2.17, and 104.5 ± 1.7) u/l. As for ALP, the results showed that the associated value for all groups was significantly higher (P < 0.01) than that of the control negative.

The obtained results indicated a gradual decrease in hepatic enzyme activity in both the normal and the alloxan-induced diabetic groups of rats with an increase in the amount of hammour fish included in their diet at different levels.

4.1.7. Effect of consuming different proportions of hammour fish (grouper fish) on kidney function (creatinine and urea values)

Table 10 presents the effect of different proportions of hammour fish intake on creatinine and urea values in both normal and diabetic rats.

The creatinine value recorded for the negative control rats group was (0.6 ± 0.04) mg/100 ml, while that for the alloxan-induced diabetic rats groups (positive control, 20%, and 25%) was (0.7 ± 0.057, 0.65 ± 0.0025, and 0.75 ± 0.028) mg/100 ml, respectively. The results revealed that this value for all groups was significantly higher (P < 0.01) than that of the control negative.

According to the same table, the negative control rats group recorded a serum urea level of (34.5 ± 1.04) mg/100 ml, while the groups fed with the hammour fish-supplemented diets (positive control, 20%, and 25%) presented corresponding values of (48.5 ± 1.93 ,45.25 ± 1.56, and 49.5 ± 1.84) mg/100 ml, respectively. The results indicated that this value for all groups was significantly higher (P < 0.01) than that of the control negative. The creatinine and urea levels were found to increase with a corresponding increase in the proportion of hammour fish included in the diet. Further, the obtained results showed a gradual increase in creatinine and urea in the alloxan-induced diabetic groups of rats as the hammour fish content was increased at different levels.

4.2. Histopathological results

4.2.1. Heart

Microscopically, the hearts of the rats from Group 1 (control negative) presented no histopathological changes (Fig. 1). On the other hand, the hearts of the rats from Group 2 (control positive) showed necrosis of some of the myocardial muscle fibers (Fig. 2). Further, the hearts of the rats from Group 3 (fed with a 20% hammour fish diet) showed no changes other than some mononuclear leucocytic cells infiltration (Fig. 3). No histopathological changes were observed in the hearts of the rats from Group 4 (fed with 25% hammour fish diet) (Fig. 4).

4.2.2. Kidneys

Microscopically, the kidneys of the rats from Group 1 (control negative) showed no histopathological changes (Fig. 5). On the other hand, the kidneys of the rats from Group 2 (control positive) presented necrobiotic changes in the endothelial lining of the glomerular tufts and distension in the Bowman's space (Fig. 6). Meanwhile, the kidneys of the rats from Group 3 (fed with a diet

Table 9
Effect of consuming different proportions of hammour fish (grouper fish) on some enzymes activity in serum diabetic albino rats.

Parameters	Control (−)	Control (+)	20% Hammour fish	25% Hammour fish
AST (u/l)	33.5 ± 1.7 a	41.5 ± 1.93c	38.75 ± 1.3b	36.5 ± 1.32 a
ALT (u/l)	19.75 ± 0.85 a	28.25 ± 1.25c	25 ± 1.29c	22.75 ± 0.85b
ALP (u/l)	85.5 ± 2.1 a	124 ± 1.82 d	110.75 ± 2.17c	104.5 ± 1.70b

Values denote arithmetic means ± Standard error of the mean. One way ANOVA test, while those with similar letters are non-significant.

Table 10
Effect of different proportion of hammour fish (grouper fish) on some parameter for kidney function of serum diabetic albino rats.

Groups Parameters	Control (−)	Control (+)	20% Hammour fish	25% Hammour fish
Criatinine (mg/100 ml)	0.6 ± 0.04 a	0.7 ± 0.057c	0.65 ± 0.0025b	0.75 ± 0.028 d
Urea (mg/100 ml)	34.5 ± 1.04 a	48.5 ± 1.93c	45.25 ± 1.56b	49.5 ± 1.84c

Values denote arithmetic means ± Standard error of the mean. One way ANOVA test, while those with similar letters are non-significant.

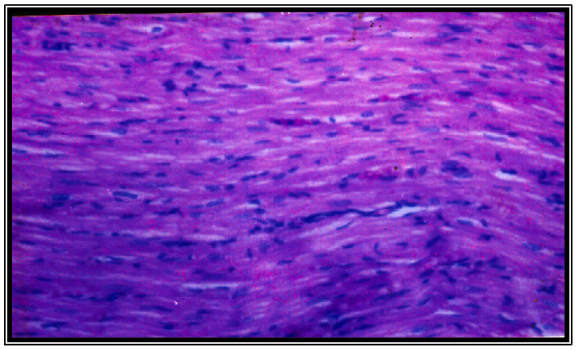

Fig. 1. Heart of control negative, untreated rat (group1) showed no histopathological changes (H and E × 200).

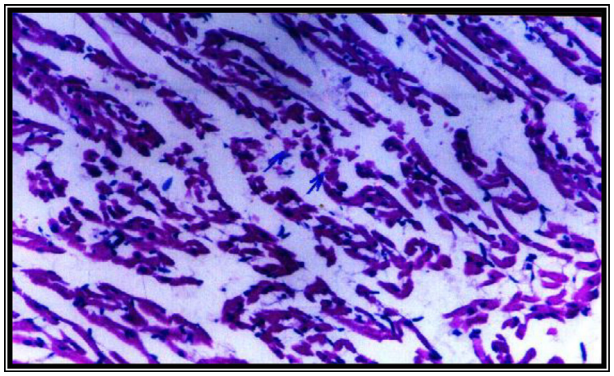

Fig. 2. Heart of rat from group 2 (control positive) showed revealed necrosis of some myocardial muscle fibers. (H and E × 200).

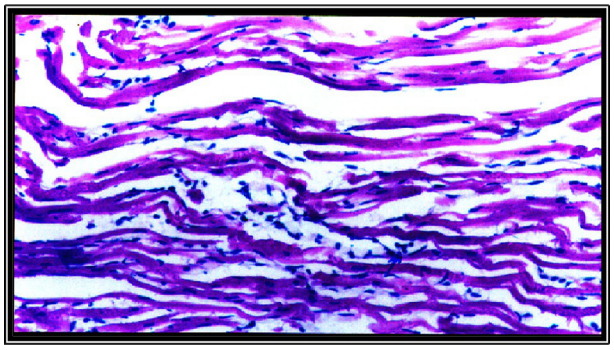

Fig. 3. Heart of rat from group 3 (fed 20% hammour fish) showed no changes except few mononuclear leucocytic cells (H and E × 200).

of 20% hammour fish) showed no changes other than in the granularity of the epithelial lining of certain renal tubules (Fig. 7). The kidneys of the rats from Group 4 (fed with a diet of 25% hammour fish) presented granularity in the epithelial lining of the glomerular tufts (Fig. 8).

5. Recommendations

1. It is suggested to use hammour fish powder for diabetic patients.
2. Different proportions of hammour fish powder may be suggested for lowering LDL and atherogenic index levels.

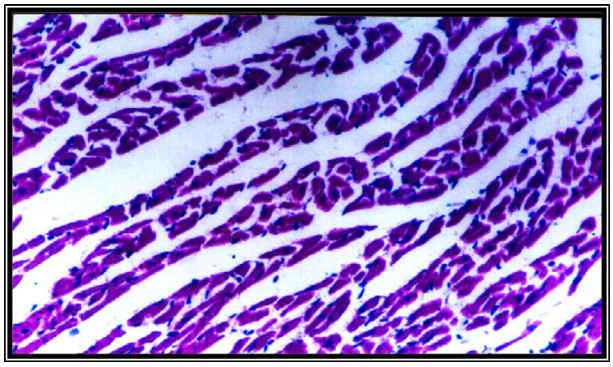

Fig. 4. Heart of rat from group 4 (fed 25% hammour fish) showing improvement of the heart tissues with the increase of hammour fish intake compare to the positive control (H and E × 200),

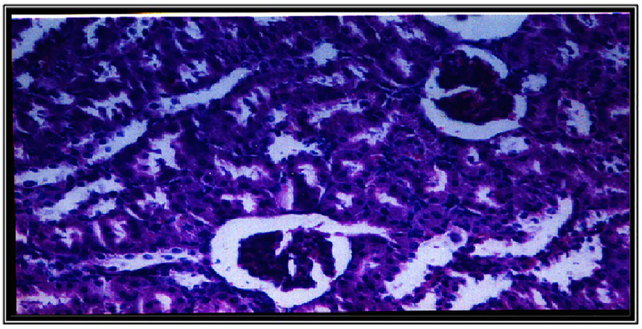

Fig 5. Kidney of rat from group 1 (control negative), untreated rat showed no histopathological changes were recorded in kidneys of rat (H and E × 200).

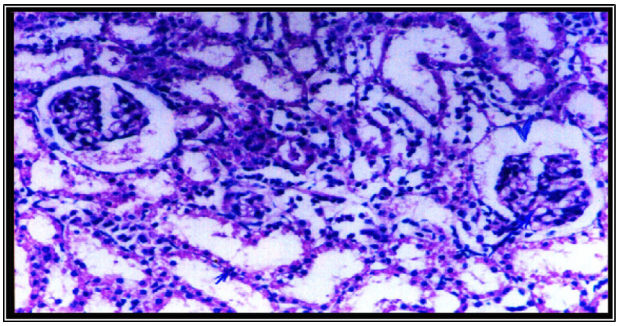

Fig. 6. Kidney of rat from group 2 (control positive) showed untreated rat revealed necrobiotic changes of endothelial lining glomerular tufts and distension of Bowman's space (H and E × 200).

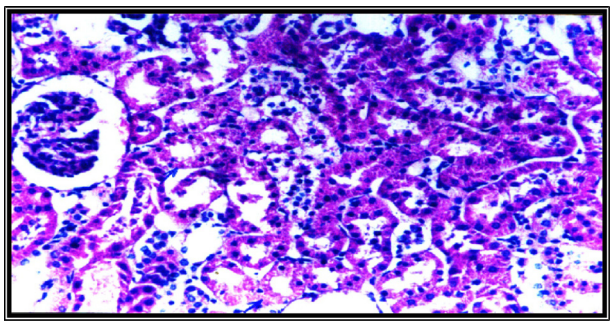

Fig. 7. Kidney of rat from group 3 (fed 20% hammour fish) showed no changes except granularity of epithelial lining some renal tubules (H and E 200).

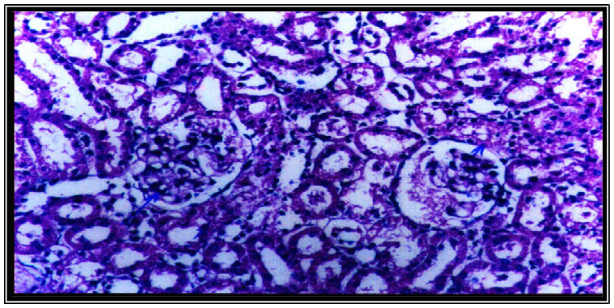

Fig. 8. Kidney of rat from group 4(fed 25% hammour fish) showed granularity of epithelial lining glomerular tufts (H and E × 200).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Adriana, M., Piotr, S., 2016. Polar and neutral lipid composition and fatty acids profile in selected fish meals depending on raw material and grade of products, February 2016. LWT- Food Sci. Technol. 70, 199–207.

Allain, C.C., 1974. Cholesterol enzymatic colorimetric method. J. Clin. Chem. 20, 470.

Ashraf Abdel-Megeid 1, A., Abd El-Rahman, M., Attia, Sonia S., Elmarasy, Abdou M. A., Ibrahim, 2008. Effect of different types of fish on rats suffering from diabetes. Nutr. Health 19 (4), 257–271.

Bulliyya, Gandham, 2002. Influence of fish consumption on the distribution of serum cholesterol in lipoprotein fractions: comparative study among fish-consuming and non-fish-consuming populations, Asia Pac J Clin Nutr. Asia Pac. J. Clin. Nutr.

Campbell, J. A.(1963): Methodology of Protein Evaluation. RAG Nutr., Document R.10, Led . 37. June Meeting, New york .

Carleton,H.(1978); Carleton Histopathological Technique, 4 the Ed .london, oxford university press. New York. Toronto.

Chapman, D.G., Castilla, R., Campbell, J.A., 1959. Evaluation of Protein in Food. I. A method for the deterinination of protein efficiency ration. Can. J. Biochem. Phosiol. 37, 679–686.

Api Chewcharat, Pol Chewcharat, Anawin Rutirapong, and Stefania Papatheodorou, (2020). The effects of omega-3 fatty acids on diabetic nephropathy: A meta-analysis of randomized controlled trials, Published: February 11, 2020 https://doi.org/10.1371/journal.pone.0228315.

Cooke, D.W., Plotnick, L., 2008. Type 1 diabetes mellitus in pediatrics. pediatr Rev 29 (11), 374–384.

Desai, A., Bhide, M., 1985. Hypoglycemic effect of Hanitonia suavecolens. Idian. J. Med. 81, 86–91.

Drury, R.A., Wallington, E.A., 1967. Carton's Histological Technique. Oxford univ.

Fassati, P., Prencipe, L., 1982. Triglyceride enzymatic colorimetric method. J. of Clin. Chem. 28, 2077.

Hegsted, D., Mills, R., Perkins, E., 1941. Salt mixture. J. Biol. Chem. 138, 459.

Henry, R. J. (1974). Clinical Chemist: Principles and Technics, 2nd Edition, Hagerstown (MD), Harcer, Row; 882.

Kris-Etherton, P.M., Harris, W.S., Appel, L.J., 2002. Fish consumption, (2002), fish oil, omega-3 fatty acids and cardiovascular disease. Circulation 106, 2747–2757.

Lee, R.D., Nieman, D.C., 1996. Nutritional Assessment. Mosby, Missoun, USA.

Bjorn Liaset, 1 Jannike Oyen, 1 Hélène Jacques, Karsten Kristiansen, and Lise Madsen1, (2019). Seafood intake and the development of obesity, insulin resistance and type 2 diabetes, Nutr Res Rev. 2019 Jun; 32(1): 146–167.

Lopez, M.F., 1977. HDL-cholesterol colorimetric method. J. Clin. Chem. 23, 882.

Bimal Prasanna Mohanty, Satabdi Ganguly, Arabinda Mahanty, Tandrima Mitra, Sinjini Patra, D. Karunakaran, Suseela Mathew, Kajal Chakraborty, B.N. Paul, D. Sarma, Syama Dayal, Shivadhar Singh and S. Ayyappan, (2019). Advances in Fish Research, Vol.-VII, Pages 189–218 Edited by: B.P. Mohanty Copyright © 2019, Narendra Publishing House, Delhi, India fish in human health and nutrition.

N.D.D.G, Nation Diabetes Data Group, 1994. Densification and diagnosis of diabetes mellitus and other categories of glucose intolerance. J. Diabetes 28, 1039–1057.

National Diabetes Statistics Report, (2017). Centers for Disease Control and Preventionwebsite. https://www.cdc.gov/diabetes/data/statistics/statistics-report.html External link. Updated July 17, 2017. Accessed October 19, 2017.

Ndisang, J. F. and Vannacci . (2017). Insulin resistance, type 1 and type 2 diabetes, and related complications 2017, Hindawi.

Patton, C.J., Crouch, S.R., 1977. Spectrophotometric and kinetics investigation of the Berthelot reaction for the determination of ammonia. Anal. Chem. 49, 464–469.

Reeves, P.G., Nielson, F.H., Fahmy, G.C., 1993. Reports of the American Institute of Nutrition, adhoc wiling committee on reformulation of the AIN 93. Rodent Diet. J. Nutri. 123, 1939–1951.

Tietz, N.W., 1976. Fundamental of Clinical Chemistry. Philadelphia th (2), W.B.53-56.

Trinder, P., 1969. Glucose enzymatic colorimetric method. J. Clin. Biochem. 6, 24.