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 rats from group 4, which were fed 25% hammour fish, showed granularity of epithelial lining glomerular tufts.

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Contents

1. Introduction .......................................................... 141
2. Aim of study ....................................................... 141
3. Materials and methods ........................................ 141
   3.1. Materials ...................................................... 141
   3.1.1. Preparation of hammour fish ......................... 141
   3.1.2. Experimental animals ................................. 141
   3.1.3. Alloxan .................................................. 141
3.2. Methods ........................................................ 141
   3.2.1. Biological experiment ............................... 141

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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 sequel 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 male mature 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 stainless 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% ham-mour fish
- Group 4: diabetic rats were fed basal diet containing 25% ham-mour 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 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:

\[
\text{BWG} \% = \frac{\text{Final weight} - \text{Initial weight}}{\text{Initial weight}} \times 100
\]

\[
\text{FER} = \frac{\text{Gain in body weight (g/day)}}{\text{Food Intake (g/day)}}
\]

Relative weight of organs = \(\frac{\text{Organs weight}}{\text{Animal body weight}}\) \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-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 lipoproteins) 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 α-Ketoglutarate resulting in the formation of pyruvate and L-Glutamate.

3.2.5.4.2 Determination of aspartate 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 placed in a crucible containing soft paraffin in an oven at 56 °C for 3–5 min according to Drury and Wallington (1967). Specimens 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).

| Ingredient                  | Amount |
|-----------------------------|--------|
| CaCO₃                       | 600 mg |
| K₂HPO₄                      | 645 mg |
| Ca HPO₄·2H₂O                | 150 mg |
| MgSO₄·2H₂O                  | 204 mg |
| NaCl                        | 334 mg |
| Fe (C₆H₅O₇)₂·2H₂O          | 55 mg  |
|KI                          | 1.6 mg |
|MnSO₄·4H₂O                   | 10 mg  |
|ZnCl₂                       | 0.5 mg |
|Cu SO₄·5H₂O                 | 0.06 mg|
|CaCO₃                       | 600 mg |

Source: Hegsted et al. (1941).

Table 3
The composition of vitamin mixture.

| Vitamin                | Amount |
|------------------------|--------|
| Vitamin E              | 10 μg  |
| Vitamin K              | 0.50 μg|
| Vitamin A              | 200 μg |
| Thiamin                | 0.50 mg|
| Pyridoxine             | 1.00 mg|
| Niacin                 | 4.00 mg|
| Calcium pantothenic acid| 0.40 mg|
| Vitamin D              | 100 μl |
| 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

| Parameters | Control (-) | Control (+) | 20% Hammour fish | 25% Hammour fish |
|------------|-------------|-------------|------------------|------------------|
| BWG (g)    | 81.75 ± 2.50| 55.00 ± 1.47| 86.00 ± 1.55     | 62.50 ± 1.91     |
| FI (g)     | 16.125 ± 0.81| 17.75 ± 0.45c| 21.4 ± 0.99     | 17.65 ± 0.55     |
| 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.

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 rat 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 hammer fish showed a decrease in the relative weight of the liver (3.44 ± 0.222, 4.463 ± 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 increase the weight of the liver compared 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 demonstrate significant increase compared to control (+ve) group.
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 kidney compared 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 ± 0.0085), (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 isoenetic 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 ± 2.83 |
| 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. |
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 (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 ± 1.70) mmol/L, respectively. As for ALP, the results showed that this value for all groups was significantly higher (P < 0.01) than that of the control negative.

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 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

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

| Parameters | Control (−) | Control (+) | 20% Hammour fish | 25% Hammour fish |
|------------|-------------|-------------|-------------------|------------------|
| Creatinine (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.
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.
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.

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