

**PHYSIOLOGICAL AND HISTOPATHOLOGICAL STUDY FOR THE EFFECT OF BARLEY (HORDEUN VULGARE) FLOUR ON INDUCED DIABETIC**

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**ABSTRACT**

The aim of this study is to evaluate the effect of barley consumption on sugar reduction in diabetic patients in male and female rats. The mice were divided into six groups with 10 mice per group. The mice injected the substance with 200 mg / kg body weight to induce diabetes. The study included 60 mice of both sexes, injected with aloxane and a dose of 200 mg / kg body weight to induce diabetes. The first group was represented by the control group as its members took standard food from the experimental animal food. The second group, which represents the induced control group as its members took the general measure of food, but injected the substance of the aloxane for comparison. While the third and fourth groups were fed an equal mixture of wheat flour and barley in the form of dry paste for both females and males for mice, respectively. The fifth and sixth groups were fed barley flour only for both sexes. Measure both body weight and blood sugar at the beginning and end of the two weeks of the experiment, where it was fed daily with various food treatments mentioned previously. Different blood parameters were measured, The physiological and histological changes of liver, kidney and brain were also studied. The results showed that there was a significant decrease in the percentage of blood clots in groups with diabetes, and showed groups treated with barley decreased cholesterol and triglyceride with the emergence of changes in the tissue in the liver and kidneys, while the brain appeared in its natural form. Barley has a protective effect against diabetes.

Key Words: diabetic mice, blood glucose, hematological analysis, blood parameters.

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دراسة فسلجية ونسجية لتأثير حبوب الشعير في الفئران المستحثة بالسكري

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المستخلص

تهدف الدراسة الى تقييم تأثير تناول الشعير في خفض السكر لمرضى السكري في ذكور وإناث الفئران. قسمت الفئران الى ستة مجاميع وبواقع عشرة فئران لكل مجموعة. وحقت الفئران بمادة الاكسان بجرعة 200ملغم/كغم من وزن الجسم لحث مرض السكري لديها. شملت الدراسة 60 فأرة من كلا الجنسين وحقت بمادة الالوكسان وجرعة 200ملغم/كغم من وزن جسم الحيوان وذلك لغرض حث داء السكري لديها. مثلت المجموعة الاولى بمجموعة السيطرة إذ تناولت أفرادها الغذاء القياسي العام من غذاء حيوانات التجربة. المجموعة الثانية، والتي تمثل مجموعة السيطرة المستحثة إذ تناولت أفرادها الغذاء القياسي العام ولكن حقت بمادة الالوكسان للمقارنة. بينما المجموعتين الثالثة والرابعة فقد غذيت على خليط متساوي من طحين الحنطة والشعير على شكل عجينة جافة لكل من الإناث والذكور للفئران على التوالي. اما المجموعتين الخامسة والسادسة غذيت على طحين الشعير فقط لكلا الجنسين. قيس كل من وزن الجسم وسكر الدم في بداية ونهاية الاسبوعين للتجربة حيث غذيت يوميا بمختلف المعاملات الغذائية المذكورة مسبقاً. وكذلك تم قياس بيانات الدم المختلفة، كما تم دراسة التغيرات المظهرية والنسجية لكل من الكبد، الكلية والدماغ. بينت النتائج أن هناك انخفاضاً كبيراً في نسب الكلوكوز بالدم في المجاميع المصابة بالسكري، كذلك أظهرت المجاميع المعاملة بالشعير انخفاضاً في نسبة الكوليسترول والدهون الثلاثية مع ظهور تغيرات نسيجية في الكبد والكليتين، في حين أن الدماغ ظهر بشكله الطبيعي. يعتبر الشعير ذا تأثير وقائي ضد الإصابة بداء السكري.

الكلمات المفتاحية: الفئران المستحثة، كلوكوز الدم، بيانات الدم، التغيرات النسيجية

## INTRODUCTION

Barley plant is the fourth most produced cereal in the world after wheat, maize and rice, scientifically known as *Hordeum vulgare L.* and an ancient grain. "Barley is a very good source of molybdenum, manganese, dietary fiber, and selenium (13)". Also, (23) presented that barley supplies a good source of the copper, chromium, phosphorus, magnesium, niacin and vitamin A. Barley is supplying a high nutritional values of  $\beta$ -glucan as a soluble and insoluble fibers which act to reduce blood cholesterol, lowered post-prandial blood glucose and insulin levels and risk factor of heart disease (18; 23). Barley content of  $\beta$ -glucan was varying between 2 and 9% (10; 27). Instead of that barley is very important for supplying protein (7), vitamins (vit. E) and many essential minerals such as iron and zinc (4; 20). The presence of soluble fiber in the bowel leads to delayed gastrointestinal passage and is suppressed in the large intestine. Also, there is a sufficient time to produce short chain, low-molecular fatty acids that are thought to be beneficial and closely linked to lowering blood cholesterol (14). Now days, there is a renewed interest in supplementing barley for many bakery products and meals for human food. Barley products are highly content of fiber, low fat that help to promote satiety score (12; 22) and that can be useful tools for weight loss and weight maintenance (29). Also, many studies have been founded that barley  $\beta$ -glucans effectively lower blood glucose levels, which is important in the prevention and management of type 2 diabetes (11; 17) and also increase satiety, which supported weight management (8; 28). Furthermore, barley's nutritional advantages, barley also has an excellent taste. So, barley through its functional and sensory properties, is considered a promising future for the production of various healthy foods and pastries (25). Thus, the current study was aimed to determine the effect of consumption of barley flour in reducing blood sugar and other blood parameter in laboratory diabetes white mice after eating barley paste for two weeks. Also it was conducted to determine the effect of barley flour consumption on histopathical changes in liver, kidney and brain tissues of the mice.

## MATERIALS AND METHODS

### Preparation of barley flour

Five kilo grams of white barley seeds were taken and washed in tap water to remove the suspended soil, gravel, straw and exotic impurities, and then left for half an hour to get rid of excess water. Then, It was published in trays of stainless steel under the sun until dry, and then removed from anything strange, then grind well in a laboratory mill then, sieved with sieve at diameter 250 $\mu$ m to fine powder in sealed nylon bags and kept in dry placed till used.

**Preparation of dried barley and wheat flour paste:** One hundred gms of barley flour and 100 gms. were weighed Of wheat flour and mixed well with lukewarm water until got a stiff dough then cut into small pieces and left to dry in sun for several days until it used to mice daily.

### Preparation of dry barley paste alone

One kilo gm of barley flour was weighed, mixed well with amount of lukewarm water until got a stiff dough, then cut into small pieces and left to dry for several days until it used to mice daily.

### Laboratory animals

Sixty fifty adult albino mice (male and female) age 8-10 weeks weighing between 20-25gm. used for experiment that obtained from animal house of Al-Razi center. All the experiments were done by the veterinary staff lab. of veterinary drug Research Center which had approved all the experimental protocols for ethical reason. The mice were given a normal standard food while providing the drink water from the tap for a whole week to get well adapted. Then after passing the week, glucose level was measured after pulling blood out of the tail vein. Mice were divided into six groups (10 mice) for each one (table 1). Animals were fasted for 12 hr., then five groups were induced for diabetes by intraperitoneal injection of a single dose of 200 mg /kg of alloxan (Alexis Biochemical, Lot-L24553) as (DC), while one group was assigned to be non-diabetic control (N-DC). After given an alloxan, the blood glucose level of all animals was analyzed every day from the tail vein during one week. The animals that showed stable hyperglycemia > 200 mg/dL were used for experiments as diabetics and the

others were eliminated. Blood glucose level was tested by using the test strips for the Glucose (Accua chek, Germany). Then all groups except control group (N-DC) were fed barley flour only or combined flour of barley and wheat. All mice groups were individually housed in stainless steel cages in a room at  $21\pm 3^{\circ}\text{C}$  for 12hr dark cycle and 60–65% humidity. Each mice had free access to its respective diet and drinking water during the experimental period and weighing daily. At the end of the 14<sup>th</sup> day of the experiment, mice were given a lethal overdose of isoflurane by inhalation. Then instantly the blood samples were taken from the heart by syringe and laid on in box full of ice, then blood of all samples were immediately centrifuged at 3000 g for 5 min at  $4^{\circ}\text{C}$ , after that serum was separated, and it was kept at  $-80^{\circ}\text{C}$  until analyzed.

**Table 1. Design of the experiment**

Groups	Receiving diet
1-NDC	Commercial standard diet (CSD)
2-DC	Diabetic control group (DC) fed CSD
3-FBW	Female fed barley & wheat flour
4-MBW	Male fed barley & wheat flour
5-FB	Female fed barley flour only
6-MB	Male fed barley flour only

### Experimental design

Mice in group 1 was [non-diabetic control group (N-DC), and in group 2 diabetic control group (DC) were fed on commercial standard diet, CSD) included: brown rice, oats, wheat, soy, and fish meal, Calcium Carbonate, Yeast Culture Dehydrated, Flax Seed Meal (Linseed), Inulin, Monocalcium Phosphate, Soy Oil and needed vitamins and minerals" as shown in (Table 2). Female and male mice in groups 3 & 4 were fed barley and wheat flour 50:50% denoted with (FBW & MBW) respectively. Also, female and male mice in groups 5 & 6 were fed on barley flour only, denoted with (FB & MB) respectively. The rats in all groups continued to eat their usual diet and distilled water (Table 1).

**Table 2. Nutrient Contents analysis in mice commercial standard diet**

Constituents	%
Crude carbohydrate	59.3
Protein	16.0
Fat	4.0
Crude fiber	8.0
Moisture	10.5
Calcium	1.3
Phosphorous	0.9

### Commercial standard diet (CSD): Brown Rice, Oats and Wheat, Soy and Fish Meal.

#### Chemical composition of barley flour

Moisture (44-16 A), crude ash (08-07) were determined by incineration in a muffle furnace at  $580^{\circ}\text{C}$  for 8hr.; fat was estimated by using Soxhlet extraction (30-10) according to AACC-approved methods (1). The kjeldahl method was used for determination the crude protein level, its expressed by conventional factor  $N \times 6.25$  where the multiplying total content of nitrogenous compound were corresponding to the total nitrogen content. Dietary fiber was estimated according to (Asp, et al. 1983) method; Dry matter evaluation by drying in an electric oven with ventilation (JR AD/ Italy, www.jradco.org) at  $105^{\circ}\text{C}$  until reaching constant weight (2); total carbohydrate was estimated by difference.=

#### Determination of blood glucose level

Blood glucose was estimated by drawing blood from the tail vein by using the sugar test strips (Accua chek, Germany) and the examination was done daily after the administration also recorded the changes in the mice weights treated and untreated daily.

#### Blood analysis & lipid profile

At the end of the experiment, animals were slaughtered and blood was withdrawn directly from the heart. Blood hematology parameters (WBC, RBC, HGB, HCT, MCV, MCh, MCHC and PLT) were determined, also serum glucose, triglycerides (TGs) and total cholesterol were estimated by using enzymatic method at Al-Maghreb Lab, Baghdad

#### Histopathological changes

After slaughtering all mice, the important organs were taken to evaluate the histopathology changes in kidney, liver and brain of the mice that induced with alloxan. Kidney, liver and brain biopsies were taken on the 14<sup>th</sup> day of the study. The samples were dipped and dehydrated by 50:50% of formaldehyde and alcohol properly. Then the dipped samples were poured and embedded by paraffin, then were cut in 3-micron thickness and stained (H and E staining) for examining by light microscopy in the central lab / Ibn al-Haytham College.

### RESULT AND DISCUSSION

**Chemical composition of barley flour:** It was found the highest constituents in barley flour

were dry matter, followed with total carbohydrates, dietary fiber, crude protein, moisture, fat and then ash were 89.6, 52.2%, 20.2, 12.5, 10.4, 2.5 and 2.2% respectively as was shown in (Table 3). Also, barley flour contain relatively high dietary fiber ratios that are important to human health. This finding were accordance with finding by (4). Also, (7) found almost approximately accordance percentages of chemical content.

**Table 3. Chemical composition of barley flour.**

Parameters	%
Moisture	10.4
Dry matter	89.6
Ash	2.2
Crude protein	12.5
Fat	2.5
Dietary fiber	20.2
Total carbohydrate $\bar{Y}$	52.2

= $\bar{Y}$ =estimated by differences

**Effect of barley flour in weight of mices:**After Alloxan injection, fasting blood sugar FBS, elivate in the animals over 250.0 mg/dl which expressed as diabetes animals. It was shown clearly in (Table 4), there were an increase in females weight group, which were given barley and wheat flour paste, and the

males group was not significant differences compared with control group. While, groups 5 & 6 there were no significant differences by consumption barley flour only Table 4. The role of barley in an increasing the weight may be due the nature of the functional ingredients contained which lead to obesity as presented by (9). The result was shown that the barley works as appetizer and increase the intake of food and its play an important role in the incidence of obesity in both sex and regulates the body weight through different mechanisms and reduced energy spending as pointed by (21). Also, it was presented that barley has the ability to increase fat synthesis through stimulating cholesterol synthesis in liver, decreasing bile acid secretion and reducing the activity of the acyl-transferase enzyme to increase the absorption of fat droplet in GIT (18). In this study, it was notice in all mice groups that given barley were appeared tendency to stable condition without any signs of nervous or stress during the period of experiment that was considered the positive effects of barley and as actual effect in reducing disorders of the central nervous system (19).

**Table 4. Bodies weight of animals during two weeks of applying different diet**

1 (N-DC) Control	2 (DC) Diebete control	Body wt. (gm) in mices groups				Days
		3 Female (FBW)	4 (Male (MBW)	5 Female (FB)	6 Male (MB)	
30.5	33.6	35.0	28.1	29.0	29.0	1
29.5	31.5	35.2	28.3	29.0	30.0	2
29.6	31.1	35.5	28.5	32.0	30.3	3
29.6	31.0	35.8	28.9	33.1	30.3	4
30.0	34.6	36.0	32.0	33.0	30.5	5
30.5	35.0	36.7	33.0	34.0	31.0	6
30.5	33.1	36.9	33.3	34.1	31.5	7
31.0	33.8	37.1	34.0	34.5	31.6	8
31.0	33.1	37.1	34.0	34.6	32.0	9
32.2	33.4	37.0	34.4	35.1	32.1	10
32.5	34.0	37.2	35.0	35.3	32.1	11
33.0	33.6	37.3	35.5	35.3	33.0	12
33.0	31.5	37.5	35.2	36.0	33.0	13
33.1	31.1	37.5	34.5	36.1	32.5	14
$\Sigma$ 436	$\Sigma$ =460.4	$\Sigma$ 512.0	$\Sigma$ 454.7	$\Sigma$ 47.1	$\Sigma$ 405.9	-
M=31.1 b	M=32.9	M=36.6 a	M=32.5 b	M=33.7 ab	M=28.9 b	-
4.073 *						LSD value

**Determination the blood glucose level**

The results in Table 5 shows that it was found after 14 days of barley flour feeding along period of the experiment, a significant reduction in FBS in group 5 (DMB) comparing with control and diebete control group. ( $p < 0.05$ ), as well as in group 6 (FB) ( $p$

= .03) was seen in comparison with group 1 (N-DC) & 1a (DC). Also, there were a significant differences in group 4 (FB). Thus, it was concluded the directly effect of barley on blood glucose level. Whereas, a slight significant decrease in the groups that given the barley and flour compared with control

group. This confirms the ability of barley flour to decrease blood glucose when taken appropriately (9; 16). The results were proved the ability of barley to decrease the painful symptom of diabetes in fat mice, and may act as a therapeutic supplement to regulate blood glucose levels due to fiber content in barley and also help to prevent blood sugar levels

from rising too high in people with diabetes (15). "In addition, the barley and other whole grains are rich sources of magnesium, a mineral that acts as a co-factor for more than 300 enzymes, including enzymes involved in the body's use of glucose and insulin secretion (16; 21)".

**Table 5. The effect of barley on blood glucose in male and female mice after two weeks.**

LSD value	Blood glucose (mg/dl) in mices groups						Days
	1 (Control) St. diet	1a Diabets rats fed (CSD flour)	2 Diebete Female fed (B&W)	3 Diebete Male fed (B&W)	4 Diebete Female fed (B only)	5 Diebete Male fed (B only)	
41.07 *	120.5	250.0	250.3	255.5	252.5	255.0	1
38.66 *	120.5	250.0	250.0	245.0	251.0	253.0	2
38.71 *	121.6	251.5	249.5	244.2	250.2	251.0	3
39.52 *	120.6	252.4	249.6	244.0	248.0	245.5	4
44.93 *	119.0	251.2	248.0	244.0	248.1	244.5	5
38.32 *	119.5	250.0	248.0	242.0	245.5	244.0	6
36.79 *	118.8	251.3	242.5	232.5	240.0	230.5	7
39.25 *	118.0	253.0	240.8	232.0	235.0	230.0	8
42.81 *	119.0	253.0	240.5	222.0	232.1	223.9	9
38.73 *	120.2	252.5	230.2	211.5	230.0	120.0	10
37.27 *	120.5	252.0	225.0	211.2	200.5	119.5	11
41.72 *	121.0	252.5	200.0	200.4	189.2	119.0	12
37.92 *	120.0	253.5	180.8	198.5	180.0	115.0	13
40.09 *	120.1	253.0	175.5	190.0	170.0	114.0	14
---	1679.3 $\Sigma$	$\Sigma=3524.4$	$\Sigma=3230.7$	$\Sigma=2917.3$	$\Sigma=2921.1$	$\Sigma=2764.9$	-
---	119.95 M	M=251.7	M=230.7	M=208.4	M=208.7	M=197.5	-

\* (P<0.05).

#### Blood analysis and lipid profile

Table 6, was noted that the barley has an effect on red blood cells and hemoglobin in groups given barley or (barley & wheat flour) paste, due to the presence of proteins in sufficient amounts of barley that helps the blood system to produce important components (9). Also the results were shown decreasing in a number of platelets after given the groups both paste (barley only or barley-wheat) paste, that may be due to the defect in bone marrow or deficiency resulting from break in the platelets after leaving the bone marrow and pass through the blood vessels to spleen and sticking together with active molecules of barley thus make it a strange body for the immune system that led to attack and the hemolysis (21). Barley also rich in beta-

glucan, which plays a role in lowering cholesterol, especially LDL cholesterol and maintaining the health of the blood system. Result of this study showed a high significant differences in cholesterol and triglyceride content in all mices that fed barley flour only, also there were a significant differences in mices groups that fed a mixed equally amount of barley and wheat flour of both sex of mices. This finding was comparable with others who stated that barley fiber reduces glucose, cholesterol and triglycerides through the increase the activity of GIT (18). The low cholesterol level have been founded may be due to the ability of barley to effect on protein receptors that in turn effect on the liver and led to reduce the production of triglycerides (5; 6; 24). "In addition, the soluble barley's dietary

fiber available in barley is an appropriate environment for friendly bacteria in the large intestines. When these helpful bacteria ferment soluble barley's fiber, they produce a short-chain fatty acid as *butyric acid*, which serves as the primary fuel for the cells of the large intestine and helps maintain a healthy colon. Also, these helpful bacteria create two other short-chain fatty acids, *propionic* and *acetic acid*, which are used as fuel by the cells of the liver and muscles (26). As well as barley's

dietary fiber contain a high *beta glucan*, which assisted to lower the cholesterol by binding with bile acids and removing them from the body through feces. Bile acids have been shown to be useful for cholesterol-producing liver enzymes, so when bile acids are secreted into the gut, they mix with the dietary fiber. The liver builds up these new bile acids using a higher amount of cholesterol to manufacture these acids and thus reduces any cholesterol in the blood (13)".

**Table 6. Changes in blood parameter in mice feeding barley and wheat or barley flour after two weeks**

Hematology parameter	Different treatments of barley paste in mice groups						LSD value
	1 (Control) St. diet	1a (Diabets rats)	2 Female (B&W)	3 (Male (B&W)	4 Female (B only)	5 Male (B only)	
WBC ( $\times 10^3/\mu\text{l}$ )	3.67	2.50	3.14	2.98	3.20	3.07	0.562 *
RBC ( $\times 10^6/\mu\text{l}$ )	3.3	4.7	4.77	5.24	4.20	4.43	0.931 *
HGB (g/dl)	9.88	8.60	8.33	9.84	9.50	10.27	1.89 NS
HCT (%)	40.6	55.6	36.0	35.4	39.00	45.7	7.59 *
MCV (fl)	85.8	70.5	80.5	84.9	85.5	84.3	9.41 *
MCH (pg)	17.6	16.2	17.4	16.6	17.5	16.8	2.58 NS
MCHC (g/dl)	34.7	29.2	30.05	31.24	34.56	35.4	4.94 *
PLT ( $\times 10^3/\mu\text{l}$ )	172 <sup>3</sup>	150 <sup>3</sup>	129 <sup>3</sup>	125.7 <sup>3</sup>	126 <sup>3</sup>	130 <sup>3</sup>	18.73 *
Serum Cholesterol (mg/dl)	205.0	218.5	176.1	150.5	115.5	110.6	32.92 *
Triglyceride (mg/dl)	206.0	224.0	150.0	113.4*	180.2	122.5	51.63 *

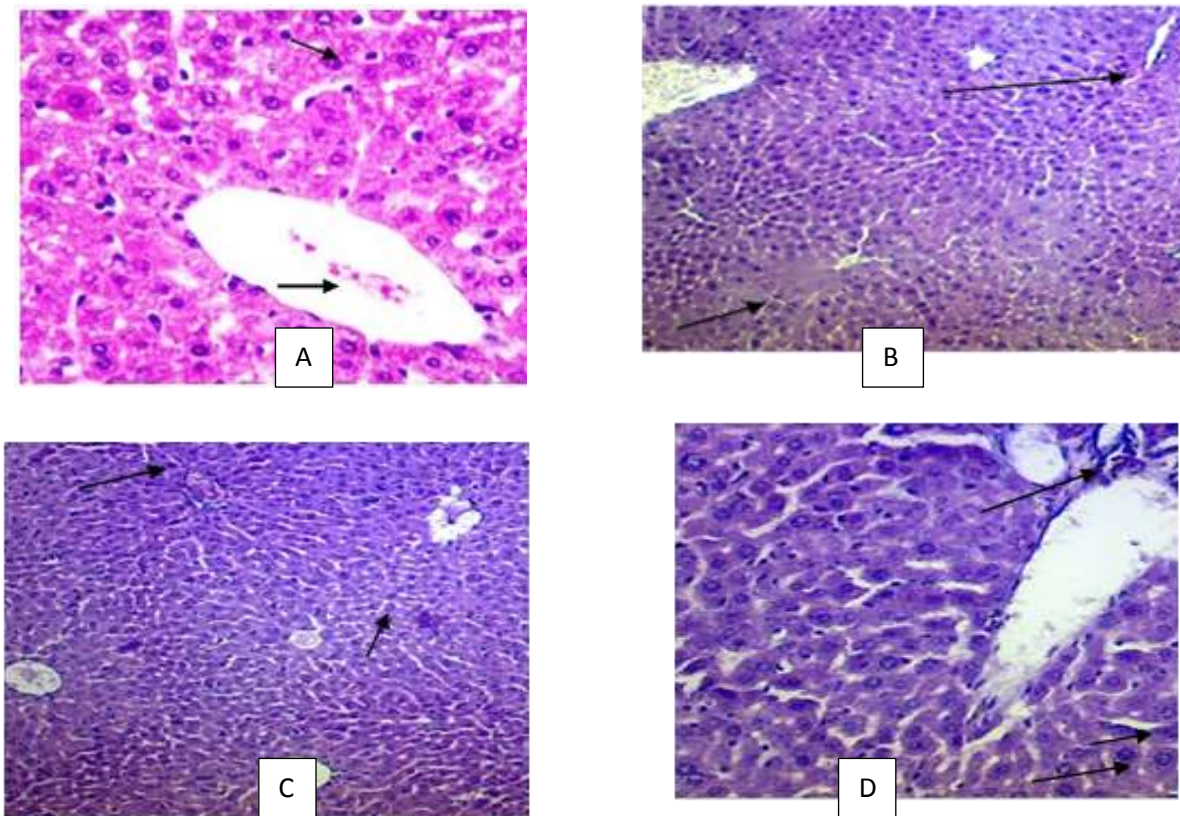
\* ( $P < 0.05$ ).

#### Histopathological changes in liver

The liver section as shows in Fig. 1, section A, the control group showed the section reveal radial arrangement of hepatocyte, sinusoid originates at lobules margin toward the hepatic vein (c.v. ) (H&E stain X). While, section B, diabetic mice that were feeding standard food had congestion and necrosis due to the high level of glucose which effect badly the liver of mice. section C group 3 &4, that mice were feeding barley and wheat flour also showed

heavy congestion with infiltration of inflammatory cell, dilation of number of hepatocyte cells with apoptosis (H&E stain X40). In section D, group 5 &6, mice were feeding barley flour only, were also shown still a congestion in liver vessels with infiltration of inflammatory cells and enlargement of hepatic cells in the liver with the emergence of kupffer cells. This may be need more time, until barley flour effects positively to heal the kidney tissues.



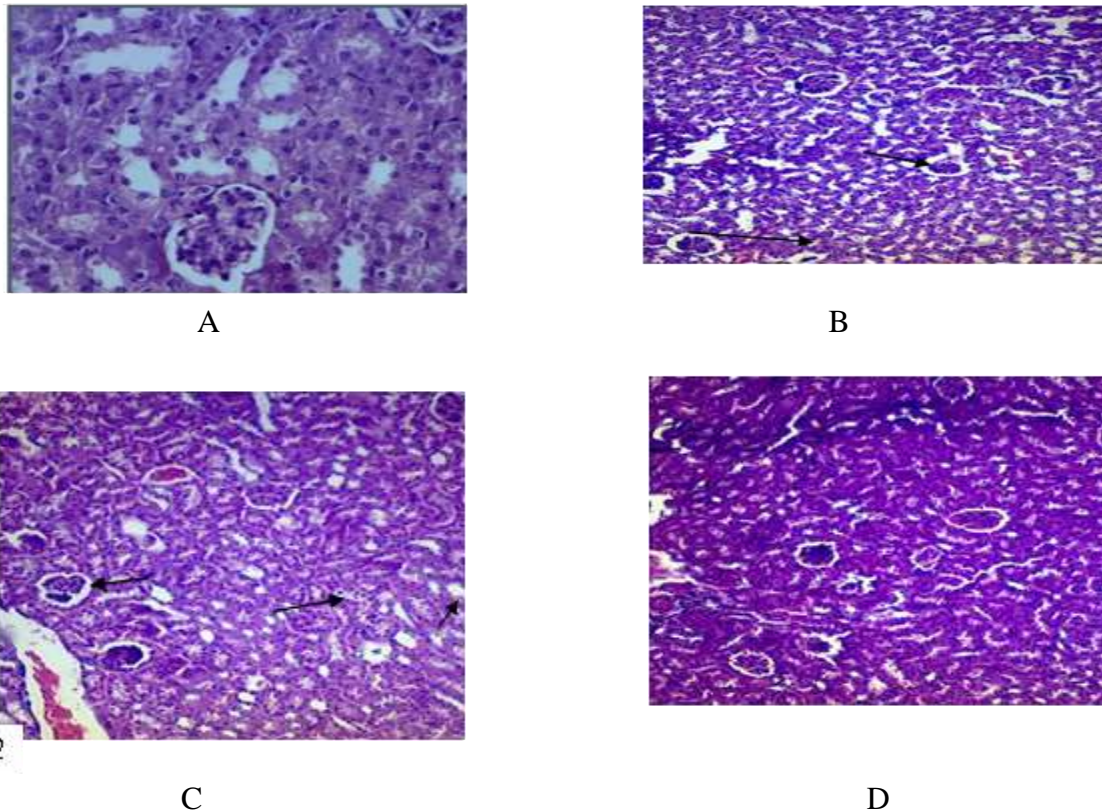


**Fig. 1. Histopathological shapes of mice liver in different treatments: A=NDC, noted on non-diabetic control group; B=noted on diabetic control group; C=FBW & MBW, noted on female & male fed barley and wheat flour; D= FB & MB, noted on female & male fed barley only**

#### **Histopathological changes in kidney**

The kidney section indicate in Fig. 2, section. A, the control group showed the appearance of renal tissue which consist glomeruli and renal tubules ( Proximal and distal convoluted tubules ). While, section B, diabetic mice that were feeding standard food kidney was showed hemorrhage in its tissue. dialation in glumeruli and convoluted tubules, presence of vacuoles with degenerative changes ( H&E X40). Section C group 3 &4, that mice were feeding barley and wheat flour also were

showed hemorrhage in kidney tissue, dialation in glumeruli and convoluted tubules, presence of vacuoles with diffusion of large number of inflammatory cells ( H&E X40). In section D, group 5 &6, mice were feeding barle flour only, were also shown still a showing hemorrhage in kidney tissue, dialation in glumeruli and convoluted tubules, presence of vacuoles with diffusion of large number of inflammatory cells. This may be need more time, until barley flour effects positively to heal the kidney tissues ( H&E X40).

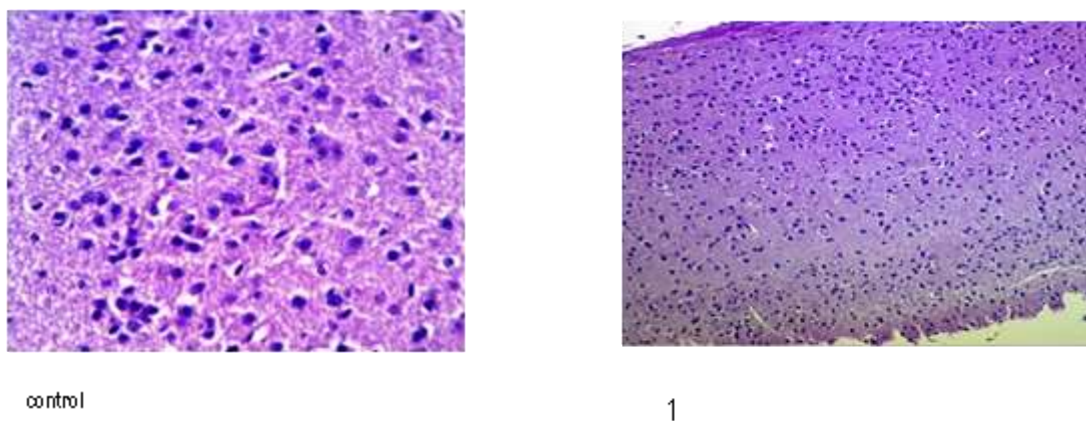


**Fig. 2. Histopathological shapes of mice kidney in different treatments: A=NDC, noted on non-diabetes control group; B=noted on diabetes control group; C=FBW & MBW, noted on female & male fed barley and wheat flour; D= FB & MB, noted on female & male fed barley only**

**Histopathological changes in brain**

It was found that barley consumption had not any effect on brain tissues as shown normal

glial cells and other cells section 1 in Fig. 3, compared with normal group as clear in Fig. 3.



**Fig. 3. Histopathological shapes of mice brain in different treatments: A=NDC, noted on non-diabetes control group; B=noted on diabetes control group; C=FBW & MBW, noted on female & male fed barley and wheat flour; D= FB & MB, noted on female & male fed barley only**

The study show the efficiency of barley in the reduction of blood glucose in male and female. And the ability of barley paste to reduce triglyceride and cholesterol in all groups

without exception, as well the histological change in liver and kidney may probably due to giving a paste in continuous period, while there were no strange change in brain tissues



of any. So it is necessary to urge to provide barley flour and to educate the owners of factories for the manufacture of cereals for the production barley's pastas, noodles, bread, roller-milled barley bran and flour, panacakes, muffins, doughnuts, biscuits, waffles...etc. of such products because of the lack of Iraqi markets for such products and their importance to consumer health, which is looking for the provision of these products because there is currently awareness and nutrition awareness of barley and its importance For public health and for diabetics, especially through private satellite channels, health and food.=

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