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Effects of adding whole barley flour to bread and its impact on anti-obesity action of female rats fed a high-fat diet

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ABSTRACT

The present investigation was focused on the effect of whole barley on balady bread properties and its nutritional and biological evaluation through different sets of rats. Chemical, sensorial and microbial properties were determined. The anti-obesity action of high levels of whole barley 60, 80% supplemented bread through 30 female rats evaluated. The increasing level of whole barley flour (WBF) incorporated into bread significantly increased crude fiber and ash contents. High levels from WBF led to decrease the microbial load in the bread. Concerning biological evaluation, body weight gain of groups treated with barley had a significant decrease. Nearly all fatty cells from group 5 treated with bread (80% WBF) are small like negative group as demonstrated by histopathology of fatty tissue. Barley bread stabilized blood glucose levels, ameliorated lipid profile, liver enzymes and urea level but creatinine level was in similar level and improved the histological structure of liver tissues. In conclusion, this study demonstrates that incorporating high levels of whole barley into balady bread not only enhances its nutritional and microbial properties but also exerts anti-obesity effects and promotes overall metabolic health in rats. These findings highlight the potential of barley-supplemented bread as a functional food product with various health benefits. These findings highlight the potential of barley-supplemented bread as a functional food product with various health benefits.

1. Introduction

In recent years, obesity has become a common epidemic in developing countries and the most prevalent metabolic disease. It is correlated with several of chronic diseases, including diabetes, hyperlipidemia, certain cancers and hypertension coronary artery disease (Flegal et al., 2010). The problem of obesity has been close to an epidemic level in Egypt. About 48 % of men and 70 % of adult Egyptian women are obese or overweight according to 1998 statistics. Several studies in Egypt referred to the main reason of this problem may be attributed to economic prosperity, bad eating habits and absence of the health-conscious culture (Khan et al., 1996 & Galal et al., 2002). The over ingestion of fat is closely correlated with the development of obesity. The long-term feeding of rats on high fat diet can cause obesity with hyperglycemia, hyperphagia, insulin resistance and hyperlipidemia. Furthermore, it has been revealed that high-fat diet may cause a raise in brown and white adipocytes growth (Viuda et al., 2010). With

the increasing of obesity prevalence, the need for therapeutic and preventive strategies became urgent (Mubarak et al., 2013).

Increasing research on barley in human diet is attributed to the several health benefits related with its dietary fiber, which lowers plasma cholesterol (Cavallero et al., 2002 & Hanhineva et al., 2010). Barley has not only a high content of dietary fiber, but also contains other beneficial compounds for instance, phenolic compounds which is mostly referred to as antioxidants (Holtekjølen et al., 2006).

There are many cultivars of hull-less barley contain low or high (beta-glucan), variation degree of extract viscosity, and normal or waxy starch are found. β -glucan is a main constituent of soluble fiber which can reduce the risk of hypoglycemia, hypercholesterolemia and lower of cancer colon in experimental animals (Bhatty, 1999). Barley and oats possessed the highest contents of β -glucan (2 %-16 %) relative to other grains (Wood et al., 1984). Through the fermentation of these soluble fiber in the large intestine, the short chain fatty acids is produced which play an important role in lowering low density lipoprotein (LDL) or bad

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Fig. 1. Hull-less barley processing and milling.

cholesterol in the serum, control blood glucose levels and raise the production of immune cells (Behall et al., 2004 & Keenan et al., 2007). FDA indicated that the regular consumption of three grams of barley beta-glucan per day can reduce serum cholesterol and low-density lipoprotein and thus decrease the risk of heart disease and atherosclerosis (FDA, 2005, 2006). Wholegrain barley foods are also related to weight loss and lowering the appetite (Baik et al., 2008). However, the clear correlation between weight loss and soluble fibers effects on subjective measures of satiation are not apparent. Pectin, β -glucan, guar gum and psyllium are more widely related to decrease appetite and satiation perceptions than no / low fiber food (Dikeman et al., 2006). Diets included fiber have many health benefits as slowing gastrointestinal transit time, adding bulk, prolonging nutrient absorption and promoting satiation as well (Delgado et al., 2004). Epidemiological studies reveal that consuming of foods contained whole grain are inversely associated with weight gain in adulthood (Koh-Banerjee et al., 2004 & Bazzano et al., 2005) and body mass index (BMI) (Jacobs et al., 1998, McKeown et al., 2002, Newby et al., 2003). Kaul et al., (1993) performed experiments on obese patients (20 % or more high than the ideal body weight). These patients consumed a meal exchange for eight months. This meal exchange contained a high-fiber level (14.68 g) from whole grains, nonfat milk, lactase and nuts. The resultant data showed that the ingestion of fiber resulted in controlling blood cholesterol levels and weight loss. Many studies refer to the incorporation of barley flour or beta-glucan in wheat flour cause a reduction in the quality of breads, especially loaf volume. As a consequence of these quality deficits, it could be a stumbling block in the production of a viable white bread containing barley. However, if these quality parameters can be conquered, there would appear to be a good potentiality for production nutritious bread product containing barley (Sullivan et al., 2013). Balady bread represents the product as major edible ingredient for poor and rich Egyptian consumers. But, there is a substantial cavity between wheat grain consumption and its production, where the total production of wheat grains covers only approximately 55 % of the daily needs (Yaseen et al., 2010). Thereby, several studies investigated the potentiality of substituting wheat flour with other cheaper replacements (Khalil et al., 2000, McWatters et al., 2004, Edema et al., 2005, Olaoye et al., 2006). Consequently, this work was undertaken to incorporate whole barley flour into balady bread to add nutritional value. Also, through different sets of experimental rats, the effect of whole barley bread on serum parameters and the potentiality of using whole barley as anti-obesity agent were assessed.

2. Materials & methods

2.1. Materials

2.1.1. Hull-less barley grains

(*Hordeum vulgare*) were obtained from Crops research department, field research institute, A.R.C., Giza, Egypt. Wheat flour 72 % extraction rate was pursued from the local market in Cairo, Egypt.

Table 1

Formula used for balady bread preparation.

Ingredients	Samples		
	Control	Barley bread 60 %	Barley bread 80 %
Refined Flour	100 g	40 g	20 g
Whole barley flour	Zero	60 g	80 g
Yeast	0.5 g	0.5 g	0.5 g
Salt	1.5 g	1.5 g	1.5 g
Water	75–80 mL tap water	75–80 mL tap water	75–80 mL tap water

2.1.2. Chemicals

Chemicals used in this study obtained from Elgomhoria Company, Cairo, Egypt.

2.1.3. Sheep fat

Sheep fat used was obtained from the local market in Cairo, Egypt.

2.1.4. Female albino rats

(30, weighed 180 ± 10 g) were about in the same age and provided from Experimental Animal House of Food Technology Institute, Agric. Research. Center, Giza, Egypt.

2.2. Methods

2.2.1. Technological methods

2.2.1.1. Milling process. Hull-less barley grains were moistened to 14% moisture for 24 h. and then ground by hammer mill to whole meal barley flour as shown in Fig. 1.

2.2.1.2. Preparation of balady bread. Balady bread was prepared according to the method mentioned by Hussein et al., (2013) from wheat flour (72 %) after mixing with different levels of barley flour (60 and 80 %). Table 1 is indicating a different mixture blended as a formula. The dough was hand-kneaded approximately 6 min till homogeneity and it was fermented for about (1 h. /30 °C and then divided to pieces (125 g), a fine layer of bran was sprinkled on a wooden board and the pieces were arranged on it to ferment again up to 45 min in warm place. The fermented pieces were formed to produce a loaf approximately 20 cm in rounds. The formed loaves were left at 30–35 °C for 15 min then the loaves were baked at 400–500 °C for about 1–2 min., they were left to cool for approximately 1 hour before different analysis as shown in Fig. 2.

2.2.2. Analytical method

Determination of Moisture, protein, crude lipids, crude fiber and ash as the method described by AOAC (2000) % Carbohydrates = $100 - (\% \text{Protein} + \% \text{Moisture} + \% \text{Ash} + \% \text{Lipids} + \% \text{Fiber})$.

2.2.3. Sensory evaluation of balady bread

Sensory properties of prepared bread were quantified by ten



Fig. 2. Balady bread prepared by using Whole barley flour as a partial substitute of wheat flour.

Table 2
Experimental diet.

Ingredients	g/100 diet
Corn starch	67.6
Casein	11.6
Corn oil	10
Vitamin mixtures	1
Salt mixture	4
Cellulose	5
D.L methionine	0.3
Choline chloride	0.2

panelists from our laboratory and the scoring card of sensory attributes included the following parameters: appearance, odor, texture, color and taste on ten points for each parameter according to the method mentioned by Aly (2019) and Patel et al., (2019).

2.2.4. Microbiological methods

Total bacterial load & Mold and yeast were determined according to the method described by Marshall (1992).

2.2.5. Biological evaluation

2.2.5.1. Experimental diet. Experimental diets (g/100gm) were blended and prepared as mentioned by AIN (1993) and indicated in Table 2.



Fig. 3. Rats abdomen were carefully opened to remove the organs.



Fig. 4. Blood drawing from orbital venous.

Table 3
Chemical composition of balady bread supplemented with whole barley flour.

Breads	Moisture	Protein	Fat	Ash	Crude fiber	Carbohydrates
Control	31.3 ± 1.1 ^c	9.1 ± 0.3 ^b	1.59 ± 0.22 ^b	1.2 ± 0.3 ^c	0.9 ± 0.1 ^c	56.2 ± 1.9 ^a
40 %WF + 60 %WBF	36.4 ± 0.7 ^b	10.5 ± 0.5 ^a	2.27 ± 0.38 ^a	2.2 ± 0.3 ^b	2.7 ± 0.1 ^b	46 ± 0.6 ^b
20 %WF + 80 %WBF	38.6 ± 1.2 ^a	11.1 ± 0.2 ^a	2.46 ± 0.42 ^a	2.9 ± 0.2 ^a	3.8 ± 0.07 ^a	40.9 ± 0.9 ^c
LSD* _{0.05}	2	0.7	0.69	0.52	0.2	2.9

WBF: Whole barley flour WF: wheat flour. Different letters (a, b) within column show significant differences between values ($p < 0.05$). Data are indicated as: average for three replicates ± standard deviation (SD); % on dry weight basis.

2.2.5.2. Experiment design. Female rats were housed in polycarbonate cages measuring 40 × 24 × 20 Cm (each cage contained 6 rats), kept in a room at 25 °C ± 2 °C and maintained under normal healthy conditions. After feeding on Basel diet for ten days, they were dispensed to two essential groups: The first group (6 rats) continued consuming Basel diet as a negative control group (healthy rats). The second group (24 rats) fed on high fat diet (20 % fat sheep + 0.2 % bile salt) for 1 month. After induction of obesity, the rats divided into five groups and fed different diets for 12 weeks. Group1: Rats continued feeding with Basel diet as a negative control (healthy rats). Group2: Rats treated with high fat diet (20 % sheep fat + 0.2 % bile salt) as a positive control (obese rats). Group3: Rats treated with high fat diet + control balady bread which added in a ratio of 50 %. Group4: Rats treated with high fat diet + balady bread contained 60 % WBF in a ratio of 50 %. Group5: Rats treated with high fat diet + balady bread contained 80 % WBF in a ratio of 50 %.

2.2.5.3. Determination of food intake and body weight. Body weight for each rat recorded every week and food intake were measured every two days during the 12 weeks of the experimental period. Food intake was determined by calculating the rested amount in the feed bin subtracted from weight of food content placed one day before according to [Ennouri et al., \(2006\)](#).

2.2.5.4. Organs weight. Rats abdomen were carefully opened ([Fig. 3](#)) to remove the organs including liver, kidney and heart from each rat and then washed with saline solution, dehydrated by filter paper and weighted. The organs kept at -20 °C for further analysis ([Chapman et al., 1959](#)). The relative organ's weight was recorded according to the following formula:

$$\text{Relative organ weight} = \frac{\text{Organ weight}}{\text{Animal body weight}} \times 100$$

2.2.5.5. Biological test. At the start of the trial before treatment (after induction of obesity) and then at the end of the experimental period (12 weeks) rats were fasted for 12 h., blood was drawn from orbital venous after anesthetizing rats with diethyl ether as shown in [Fig. 4](#). Thereafter the blood samples were taken in a clean dry centrifuge vial to get and separate the serum. Blood samples without anticoagulant were centrifuging at 3500 R.P.M. for 10 min ([Schermer, 1967](#)). The serum preserved at frozen at -20 °C for further analysis. All following parameters were determined: "S Glucose according to [Trinder \(1969\)](#), lipid profile tests " Total Cholesterol as described by [Allain et al., \(1974\)](#), Triglycerides according to [Fassati et al.,\(1982\)](#), Low density Lipoprotein (LDL-C) according to [Friedwald et al., \(1972\)](#) and High density lipoprotein (HDL-C) according to [Lopes-Virella et al., \(1977\)](#)," liver enzymes "glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT)" as described by [Bergmeyer et al.,\(1986\)](#) and kidney function parameters "creatinine as described by [Bartels et al., \(1971\)](#) and urea as described by [Tabacco et al., \(1979\)](#).

2.2.5.6. Histopathological investigation. Tissue samples of the livers were taken. Specimens were immersed in 10 % neutral buffered formalin solution and left for about 24 h, then washed in water for the histopathological investigation according to the method described by

Table 4
Sensory properties of balady bread supplemented with whole barley flour.

Samples Characteristics	Control	40 %WF + 60 %WBF	20 %WF + 80 %WBF	LSD* _{0.05}
Appearance (10)	9.5 ± 0.4 ^a	8.00 ± 0.8 ^b	7.4 ± 0.5 ^c	0.6
Color (10)	9.55 ± 0.44 ^a	7.7 ± 0.42 ^b	7.5 ± 0.47 ^b	0.4
Texture (10)	9.4 ± 0.46 ^a	7.7 ± 0.5 ^b	7.4 ± 0.47 ^b	0.4
Taste 10	8.9 ± 0.3 ^c	9.2 ± 0.3 ^a	9.6 ± 0.4 ^a	0.3
Odor (10)	8.9 ± 0.52 ^c	9.25 ± 0.26 ^b	9.55 ± 0.16 ^a	0.3
Overall acceptability (10)	9.54 ± 0.37 ^a	8.15 ± 0.47 ^b	8.15 ± 0.47 ^b	0.5

WBF: Whole barley flour WF: wheat flour. Different letters (a, b) within column show significant differences between values ($p < 0.05$). Data are indicated as: average (for ten scores) ± standard deviation (SD).

[Banchroft et al., \(1996\)](#).

2.2.5.7. Peritoneal fat pad. After induction of obesity before treatment in the first of trial and then in the end of the trial, peritoneal perinephric fat pad was collected from the rats after dissection, then kept at -20 °C for histopathology examination of fatty tissues as mentioned by [Azain et al., \(2000\)](#).

2.2.6. Statistical analysis

The statistical data was expressed as arithmetic mean ± standard deviation (SD). The analysis of variance performed by Excel (ANOVA) method and the level of statistical significance was $P \leq 0.05$ as mentioned by [Aly et al., \(2020\)](#).

3. Results and dissection

3.1. Results

3.1.1. Chemical composition of bread

[Table 3](#) shows the chemical composition of balady bread samples which were prepared by blending 60 % and 80 % WBF with WF. The resultant data illustrated that the higher level of WBF incorporated in bread recorded the highest content of moisture (38.6 %) as compared with the lower level (36.4 %) and control bread (31.3 %). Supplementation caused a significant increase in protein contents which ranged from (9.1 to 11.1 %) in samples. The higher level of WBF incorporated into bread had the highest ash and crude fiber contents (2.9 and 3.8 %) while the control bread showed the least for these values (1.2 and 0.9 %).

3.1.2. Sensory attributes of balady bread

The results in [Table 4](#) illustrate the sensory scores of prepared balady bread supplemented with 60 % and 80 % whole barley flour (WBF). It could be noticed that bread prepared from 20 %WF + 80 %WBF had the highest score for taste and odor followed by bread prepared from 40 % WF + 60 %WBF compared to control bread and this preference due to barley flour's gummy taste. Control sample had the highest score for

Table 5

Microbial load of produced balady bread during storage period at room temperature (18 ± 2 °C) for 8 days.

Microbial parameter (cfu/gm)	Storage Period	Microbial counts of balady bread supplemented with WBF		
		Control	40WF + 60 %WBF bread	20WF + 80 %WBF bread
Total count Bacteria	Zero Time	NC	NC	NC
	2 days	$(4.2 \times 10^3 \pm 2.6)^a$	$(2.8 \times 10^3 \pm 4.2)^b$	$(2.9 \times 10^3 \pm 5.00)^b$
	LSD* _{0.05} 0.8			
	4 days	$(7.5 \times 10^3 \pm 4.5)^a$	$(5.2 \times 10^3 \pm 4.9)^b$	$(4.3 \times 10^3 \pm 2.1)^c$
	LSD* _{0.05} 0.8			
	6 days	R	$(7.2 \times 10^3 \pm 2.0)^a$	$(5.9 \times 10^3 \pm 5.6)^b$
LSD* _{0.05} 0.9				
Mold & yeast	8 days	R	R	R
	Zero Time	NC	NC	NC
	2 days	$(9.3 \times 10^2 \pm 2.5)^a$	$(4.3 \times 10^2 \pm 2.1)^b$	$(3 \times 10^2 \pm 1.0)^b$
	LSD* _{0.05} 3.9			
	4 days	$(41.3 \times 10^2 \pm 3.2)^a$	$(30 \times 10^2 \pm 2.6)^b$	$(26.3 \times 10^2 \pm 3.5)^b$
	LSD* _{0.05} 6.2			
	6 days	R	$(41.7 \times 10^2 \pm 7.02)^{NS}$	$(38.3 \times 10^2 \pm 7.5)^{NS}$
	LSD* _{0.05} 16.4			
8 days	R	R	R	

Cfu.: colony forming units; WBF: Whole barley flour; WF: wheat flour; NC: No colonies were noticed; R: Rejected; NS: non-significant. Different letters (a, b) within column show significant differences between values ($p < 0.05$).

appearance, color and texture. A significant decrease in appearance, crust color and texture were recorded in all supplemented breads. These data are consistent with Erefej et al., (2006) who showed that when barley flour level increase over 45 % in balady bread, lead to darker in color, harder and non-uniformly-shaped, as the fiber gives its own brownish color to bread and it can be attributed to the lack of gluten.

3.1.3. Microbial evaluation (total count bacterial & mold and yeast) of produced balady breads during storage period (8 days) at room temperature (18 ± 2 °C)

Total bacteria count is used for indicating of bacteria load in a sample. The loaves of bread were stored for 8 days at (18 ± 2 °C). The data in Table 5 shows that balady bread samples have no detected of growth bacteria or growth mold and yeast at zero time these data are in

Table 6

Effect of balady bread supplemented with different levels of WBF on body weight gain (BWG) and food intake (FI) in obese rats.

Parameters Groups	Initial Weight(g)	Final Weight (g)	B.W.G	% B.W.G	Food Intake (g/day/rat)
Control negative(G1)	187.75 ± 6.5^b	220.3 ± 11.0^c	32.5 ± 8.9^b	15.4 ± 4.7^b	17.58 ± 1.03^b
Control positive(G2)	225.75 ± 12.42^a	276 ± 4.2^a	50.3 ± 11.3^a	22.5 ± 6.4^a	18.12 ± 0.66^a
Control bread(G3)	209.75 ± 8.04^a	255 ± 6.8^b	46.0 ± 2.1^a	$22.1 \pm 1.7^{a,b}$	16.95 ± 0.73^c
40WF + 60 %WBF(G4) bread	216.75 ± 12.01^a	250.7 ± 16.2^b	29.5 ± 4.4^b	13.6 ± 2.1^b	15.09 ± 1.11^d
20WF + 80 %WBF (G5) bread	213 ± 9.1^a	241.3 ± 10.7^b	28.3 ± 5.3^b	13.3 ± 2.5^b	14.92 ± 0.94^d
LSD* _{0.05}	14.9	15.1	10.7	5.9	0.5

WBF: Whole barley flour; WF: wheat flour; B.W.G: Body weight gain. Different letters (a, b) within column show significant differences between values ($p < 0.05$). Data are indicated as: average \pm standard deviation (SD).

Table 7

Effect of balady bread supplemented with different 8 levels of WBF on organs weight and relative organs weight in obese rats.

Rat Groups	Liver		Kidney		Heart	
	Weight (g)	Relative Organ weight	Weight (g)	Relative Organ weight	Weight (g)	Relative Organ weight
Control negative	9.1 ± 0.14^d	4.1 ± 0.14^c	2.1 ± 0.3^b	0.96 ± 0.1^{NS}	0.8 ± 0.04^d	0.4 ± 0.03^c
Control positive	11.9 ± 0.1^a	4.3 ± 0.1^a	2.7 ± 0.2^a	0.99 ± 0.1^{NS}	1.6 ± 0.1^a	0.6 ± 0.1^a
Control bread	11.0 ± 0.2^b	4.3 ± 0.1^a	2.6 ± 0.1^a	1 ± 0.01^{NS}	1.36 ± 0.21^b	0.5 ± 0.1^b
40WF + 60 %WBF bread	10.1 ± 0.5^c	4.02 ± 0.1^d	2.4 ± 0.2^a	0.99 ± 0.1^{NS}	1.1 ± 0.1^c	0.4 ± 0.03^c
20WF + 80 %WBF bread	9.9 ± 0.4^c	4.2 ± 0.1^b	2.4 ± 0.3^a	0.99 ± 0.1^{NS}	0.97 ± 0.1^c	0.4 ± 0.04^c
LSD* _{0.05}	0.47	0.1	0.32	0.13	0.21	0.1

WBF: Whole barley flour; WF: wheat flour. Different letters (a, b) within column show significant differences between values ($p < 0.05$). Data are indicated as: average \pm SD; NS means non-significant.

agreement with Ravimannan et al., (2016) who revealed that all the fresh bread samples (control and experimental) had no detected total count bacteria before storage. Control balady bread recorded the highest count during the storage period as compared with WBF-wheat bread. It was obviously visible to the naked eye that control bread was spoiled and rejected on the 6th day. It reached to the maximum count in the fourth day (7.5×10^3 cfu/gm) while balady bread prepared with 60,80 % WBF were rejected on 8th day and reached to the maximum count in the sixth day (7.2×10^3 cfu/gm, 5.9×10^3 cfu/gm), respectively. Mold and yeast are one of the major causes of food spoilage besides bacteria load. Balady bread control contained higher counts of mold and yeast count (9.3×10^2 , 41.3×10^2 cfu/gm) after 2 and 4 days of storage period, respectively than balady bread supplemented with 60 % WBF which were 4.3×10^2 , 30×10^2 , 41.7×10^2 cfu/gm after 2,4,6 days of storage, and balady bread supplemented with 80 % WBF had 3×10^2 , 26.3×10^2 , 38.3×10^2 cfu/gm after 2, 4 and 6 days of storage.

3.1.4. Biological evaluation

3.1.4.1. Effect of balady bread supplemented with different levels of whole barley flour on body weight gain (BWG) of obese rats. Data in Table 6 shows the changes in body weight gain of normal rats and obese rats. Obese groups indicated a significant increase in body weight gain as compared with the normal rats at the end of experiment period. All treated groups with whole barley bread decreased the mean value of BWG as compared to group treated with control bread and the positive control group. It can be attributed to decrements in the consumption of total calories and carbohydrates other than those in the experimental diets. In this respect, Burton et al., (2000) mentioned that all of mechanisms explained the satiating properties of soluble dietary fibers are associated with appetite regulation including absorption taste, a fermentable substrate and gastric emptying. There are not significant variances between whole barley bread treatments (G4, G5). These results are in agreement with Kalra et al., (2000) who demonstrated the effects of three barley cultivars containing 6-23, 4-60, 2-18 % b-glucan and showed that final body weight and weight gain for the control treatment were the highest after 40 days of feeding in comparison to test groups. In addition, Aoe et al., (2017) studied the effects of high b-glucan barley cultivar on obesity and the barley was incorporated into rice as a replacement for the ordinary pearled rice. During the

Table 8

Effect of balady bread supplemented with different levels of WBF on serum glucose in obese rats.

Groups of rats	Glucose mg/dl		
	Zero time	Week 12	% Var.
Control negative	115.4 ± 3.5 ^b	118.4 ± 1.1 ^c	2.6 ↑
Control positive	126.9 ± 1.8 ^a	145.9 ± 5.8 ^a	15↑
Control bread	132.1 ± 1.8 ^a	142.6 ± 2.9 ^a	7.9↑
40WF + 60 %WBF bread	130.7 ± 4.1 ^a	131.9 ± 4.2 ^b	0.9↑
20WF + 80 %WBF bread	132.03 ± 1.00 ^a	131.3 ± 1.8 ^b	0.6 ↓
LSD* _{0.05}	5.7	6.8	

WBF: Whole barley flour; WF: wheat flour; %Var.: Variation Different letters (a, b) within column show significant differences between values ($p < 0.05$). Data is indicated as average (or three replicates ± standard deviation (SD)).

experiment, body weight and BMI significantly lowered in the experimental groups. From the resultant data, it could be concluded that the long term consumption of diet containing whole barley flour can help in weight control.

3.1.4.2. Food intake (FI). As indicated in Table 6, results of FI recorded a significant increase in positive control group as compared with negative rats (18.12 ± 0.66 and 17.58 ± 1.03 g/day, respectively). All obese groups treated with barley bread recorded a significant decrease in FI (15.09 ± 1.11 and 14.92 ± 0.94 g/day) as compared with both control negative and control positive group. These results are in agreement with those obtained by Darwiche et al., (2003) who mentioned that dietary fibers including soluble fibers, an ingredient of whole grains, can act as a satiating agent. Some cereal such as barley and oats contain viscous dietary fibers causing delay stomach emptying and gastric distention as well as form viscous gel. It is worth mentioning that, diet contained antioxidants could play a prophylactic role against the side and toxic effects of high fat diet in rats (Shehata et al., 2012).

3.1.4.3. Effect of experimental diets on relative organ weights of liver, kidney and heart in obese rats. The differences in organs weight listed in Table 7. It is clearly to seen that the weights of liver, kidney and heart in obese rats increased significantly in comparison to healthy negative group and these data are consistent with Athesh et al., (2012) who mentioned that the liver, spleen and kidney absolute weights of high fat diet groups had a significantly higher values than animals group fed on negative diet. The mean value of liver and heart relative weight were reduced in response to the 60 % and 80 % WBF breads in comparison with other obese groups and recorded the best results. The absolute weights of heart inclined to be lower in rats-fed on WBF 80 % than 60 % WBF group, but these differences were not statistical significance. **Biological analyses.**

3.1.4.4. Effect of balady bread supplemented with different levels of whole barley flour on serum glucose of obese rats. The serum glucose values of rats fed on different diets under investigation during feeding period presented in Table 8. Concerning positive control group, the resultant

Table 9

Effect of balady bread supplemented with different levels of WBF on serum cholesterol and triglycerides in obese rats.

Groups of rats	S. Cholesterol mg/dl			Triglycerides mg/dl		
	Zero time	Week 12	% Var.	Zero time	Week 12	% Var.
Control negative	84.67 ± 4.51 ^b	89.3 ± 2.08 ^c	5.5↑	115.7 ± 5.9 ^c	124.83 ± 7.18 ^d	7.9↑
Control positive	115.3 ± 9.1 ^a	133.87 ± 5.00 ^a	16.1↑	169.53 ± 9.04 ^b	220.9 ± 11.7 ^a	30.3↑
G3	109.7 ± 11.5 ^a	123.77 ± 8.46 ^a	12.8↑	180 ± 3.00 ^a	208.97 ± 9.49 ^a	16.1↑
G4	107.9 ± 9.97 ^a	104.8 ± 5.57 ^b	2.9↓	188.63 ± 4.40 ^a	181.20 ± 7.02 ^b	3.9↓
G5	112.2 ± 13.5 ^a	95.93 ± 4.38 ^{b,c}	14.5↓	171.27 ± 5.61 ^b	161.95 ± 5.67 ^c	5.4↓
LSD* _{0.05}	18.5	10.5		10.79	16.51	

G: Group; S, serum; %Var.: Variation; Different letters (a, b) within column show significant differences between values ($p < 0.05$). Data are indicated as: average for three replicates ± standard deviation (SD).

Table 10

Effect of balady bread supplemented with different levels of WBF on serum lipoprotein in obese rats.

Groups of rats	HDL-C mg/dl			LDL-C mg/dl		
	Zero time	Week 12	% Var.	Zero time	Week 12	% Var.
Control	43.3 ±	45.7 ±	5.5↑	18.2 ±	18.7 ±	2.7↑
Negative	1.53 ^a	4.2 ^a		4.2 ^b	7.4 ^c	
Control	33.7 ±	24 ± 3 ^d	28.8↓	47.7 ±	65.7 ±	37.7↑
Positive	1.2 ^b			7.2 ^a	3.8 ^a	
G3	32.1 ±	25.2 ±	21.5↓	41.6 ±	56.8 ±	36.5↑
	1.00 ^b	0.47 ^d		12.7 ^a	8.4 ^a	
G4	33 ±	30.8 ±	6.7↓	37.2 ±	37.1 ±	0.3↓
	2.46 ^b	1.25 ^c		13.5 ^a	6.5 ^b	
G5	33.3 ±	35.4 ±	6.3↑	44.6 ±	27.8 ±	37.7↓
	1.5 ^b	1.48 ^b		12.4 ^a	4.2 ^{b,c}	

G: Group; C: Cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; %Var.: Variation Different letters (a, b) within column show significant differences between values ($p < 0.05$). Data are indicated as: average for three replicates ± standard deviation (SD).

data shows that the mean value of serum glucose in positive control group had a significant increase compared to control negative group (145.9 ± 5.8 , 118.4 ± 1.1 mg/dl). All treated groups with barley bread causing a significant decrease in serum glucose compared with positive control group (G2) and control bread (G3). The mean values of serum glucose in rats fed on lower level of barley in G4 caused slight increase at the end of feeding period. There were non-significant variances between the two groups G4, G5 treated with barley bread 131.9 ± 4.2 , 131.3 ± 1.8 mg/dl, respectively.

3.1.4.5. Effect of balady bread supplemented with different levels of whole barley flour on lipid profile of obese rats. The effects of treatment with barley balady bread on lipid profile of obese rats during feeding period presented in Table 9. High fat diet had significantly increased serum total cholesterol, low density lipoproteins and triglycerides, and decreased serum high density lipoproteins. All groups treated with barley showed a significant reduction in serum cholesterol compared with positive control group (G2) and bread control group (G3). In G3, cholesterol level was raised from 109.7 ± 11.5 to 123.77 ± 8.46 by 12.8 % in the end of experiment. The higher level of barley in G5 (80 %) ameliorated S. cholesterol more than the lower one in G4 (60 %). In G5, cholesterol level was reduced from 112.2 ± 13.5 at zero time to 95.93 ± 4.38 by 14.5 % at week 12. Likewise, Rats fed on diet formulated with 80 % WBF bread (G5) had the lowest level of triglycerides 161.95 mg/dl followed by (G4) which fed on 60 % WBF bread 181.20 ± 7.02 mg/dl compared with control positive and the group treated with control bread 208.97 mg/dl.

Data indicated in Table 10 shows that barley bread in G4 which treated with (60 %WBF bread) caused a slight decrease in HDL level at the end of experimental period. While in G3 which treated with control bread, these value decreased by 21.5 %. The higher level of whole barley flour had a more effect for the mean value of HDL-c. and recorded the

Table 11
Effects of balady bread supplemented with different levels of WBF on liver functions in obese rats.

Groups of rats	GPT (U/L)			GOT(U/L)		
	Zero time	Week 12	% Var.	Zero Time	Week 12	% Var.
Control negative	21.73 ± 1.92 ^b	23.91 ± 1.47 ^c	10.03↑	28.67 ± 4.73 ^b	27.03 ± 3.00 ^c	5.7↓
Control positive	31.77 ± 2.84 ^a	36.7 ± 2.1 ^a	15.5↑	37.67 ± 4.38 ^a	46.33 ± 2.78 ^a	22.9↑
G3	31.33 ± 1.29 ^a	36.22 ± 1.19 ^a	15.6↑	38.07 ± 2.25 ^a	41.95 ± 2.74 ^a	10.2↑
G4	30.37 ± 0.87 ^a	31.44 ± 2.08 ^b	3.5↑	39.57 ± 1.10 ^a	37.22 ± 0.84 ^b	5.9↓
G5	32.11 ± 2.22 ^a	32.04 ± 2.2 ^b	0.22↑	37.76 ± 0.96 ^a	33.81 ± 2.73 ^b	10.5↓
LSD* _{0.05}	3.6	3.3		5.68	4.6	

G: Group; GPT: Glutamic pyruvic transaminase and GOT: Glutamic oxaloacetic transaminase. %Var.: Variation; Different letters (a, b) within column show significant differences between values ($p < 0.05$). Data are indicated as: average for three replicates ± standard deviation (SD).

Table 12
Effect of balady bread supplemented with different levels of WBF on kidney function in obese rats.

Groups of rats	Urea nitrogen Mg/dl			Creatinine Mg/dl		
	Zero Time	Week 12	% Var.	Zero Time	Week 12	% Var.
Control negative	22.8 ± 1.1 ^b	24.3 ± 1.53 ^c	6.6↑	0.62 ± 0.15 ^{NS}	0.68 ± 0.04 ^{NS}	9.8↑
Control positive	30.4 ± 1.4 ^a	36.57 ± 2.71 ^a	20.3↑	0.63 ± 0.04 ^{NS}	0.72 ± 0.1 ^{NS}	14.3↑
G3	31.5 ± 3.40 ^a	35.97 ± 1.10 ^a	14.2↑	0.65 ± 0.15 ^{NS}	0.74 ± 0.04 ^{NS}	13.8↑
G4	30.7 ± 2.49 ^a	32.33 ± 1.53 ^b	5.3↑	0.60 ± 0.1 ^{NS}	0.65 ± 0.1 ^{NS}	8.3↑
G5	30.4 ± 2.08 ^a	30.13 ± 1.72 ^b	0.89↑	0.66 ± 0.19 ^{NS}	0.75 ± 0.09 ^{NS}	13.6↑
LSD* _{0.05}	4.3	3.27		0.26	0.13	

G: Group; %Var.: Variation; Different letters (a, b) within column show significant differences between values ($p < 0.05$). Data are indicated as: average for three replicates ± standard deviation (SD).

highest 35.4 ± 1.48 mg/dl, followed by the lower level in G4 30.8 ± 1.25 mg/dl as compared to positive control group (G2) 24 ± 3 mg/dl or the control bread group (G3) 25.2 ± 0.47 . These data are confirmed by [Shaban et al., \(2006\)](#) who showed that the least value for HDL-c was recorded for bread control group. While the maximum value was recorded for the bread contained a high level of barley flour. The obese rats treated with balady bread supplemented with barley (G4 and G5) decreased the mean value of LDL as compared to control bread. Concerning G4, the mean value of serum LDL was 37.1 ± 6.5 mg/dl at the end of the experimental period. While G5, serum LDL was reduced from 44.6 ± 12.4 mg/dl to 27.8 ± 4.2 mg/dl by 37.7 %.

3.1.4.6. Effects of balady bread supplemented with different levels of whole barley flour on liver functions of obese rats. The effects of treatment with barley balady bread on the level of glutamic pyruvic transaminase (GPT) and glutamic oxaloacetic transaminase (GOT) enzymes of obese rats during feeding period (12 weeks) presented in [Table 11](#). Feeding obese rats as positive group on high fat diet led to a significant increase in serum (GPT) enzyme as compared to negative group at the end of experiment. Barley bread groups (G4, G5) resulting a slight increase in the activity of GPT enzyme at the end of experiment. Groups treated with barley bread recorded a significant decrease in serum GPT and GOT enzymes compared with positive control and control bread groups. On the other hand, the higher level of whole barley incorporated into bread in G5 reduced the activity of S.GOT enzyme by 10.5 % more than the lower one in G4 5.9 %.

3.1.4.7. Effect of balady bread supplemented with different levels of WBF on kidney function of obese rats. The obtained data presented in [Table 12](#) illustrates that feeding obese rats as positive group on high fat diet led to a significant elevation in serum urea compared with negative group 36.57 ± 2.71 vs. 24.3 ± 1.5 mg/dl, respectively. Treating obese rats with diet containing barley either G4 or G5 caused a slight increase in urea level at the end of experiment. The mean values of urea in G4 or G5 were significantly lower 32.33 ± 1.53 , 30.13 ± 1.72 , as compared to positive group 36.57 ± 2.71 and control bread group 35.97 ± 1.10 mg/dl. The results of [El Rabey et al., \(2013\)](#) support our findings, when they reported that the mean values of urea in oat bran and barley bran groups

were lower than the positive control in hypercholesterolemia rats. Regarding creatinine level, the level of creatinine in positive control group was not significantly higher than that in the negative control group 0.72 ± 0.1 and 0.68 ± 0.04 mg/dl. The treatment with WBF bread in G4 and G5 had non-significant difference on serum creatinine level.

3.1.5. Histopathological examination of liver

As shown in [Fig. 5](#), microscopically liver of rats from group 1 showed the normal histological structure of hepatic lobule from central vein and hepatocytes ([Fig. 5.1.](#) & [Fig. 5.2.](#)). On contrary, liver of rats from group 2 showed severe histopathological changes as hepatocellular vacuolar degeneration ([Fig. 5.3.](#)), focal hepatocellular necrosis associated with inflammatory cells infiltration ([Fig. 5.4.](#)) and sinusoidal leukocytosis ([Fig. 5.5.](#)). Meanwhile, liver of rats from group 3 revealed no histopathological alterations ([Fig. 5.6.](#)) except hepatocellular vacuolar degeneration of some hepatocytes ([Fig. 5.7.](#)) Moreover, liver of rats from group 4 showed improved picture, most examined sections revealed no histopathological alterations ([Fig. 5.8.](#)) and some sections showed small focal hepatocellular necrosis associated with inflammatory cells infiltration ([Fig. 5.9.](#)). Also, some sections from group 5 showed no histopathological alterations and approximately restored normal histological structure ([Fig. 5.10.](#)) another sections cleared slight kupffer cells activation ([Fig. 5.11.](#)).

3.1.6. Histopathology of fatty tissue

At the first of the trial (after the induction of obesity), Photomicrography of perinephric fat was evaluated and the resultant data illustrated that as shown in [Fig. 6.](#), feeding on high fat diet caused an observed expansion of perinephric fatty cells size in positive control group. More accumulation of adipocytes resulted in marked enlargement of fatty cells size ([Fig. 6.1.](#)) as compared with those in negative control group which were small ([Fig. 6.2.](#)). At the end of trial period, Photomicrography of perinephric fat was also determined ([Fig. 7.](#)) and the obtained data revealed that a difference in adipocytes size between groups. Negative group showed small sized fatty cells ([Fig. 7.1.](#)). On contrary, positive group showed an enlargement in fatty cells ([Fig. 7.2.](#)). Rats from group 3 which treated with control bread had variable sized fat cells most of them are large ([Fig. 7.3.](#)). While obese rats which treated with bread (60 % WBF) showed variable sized fat cells most of them are

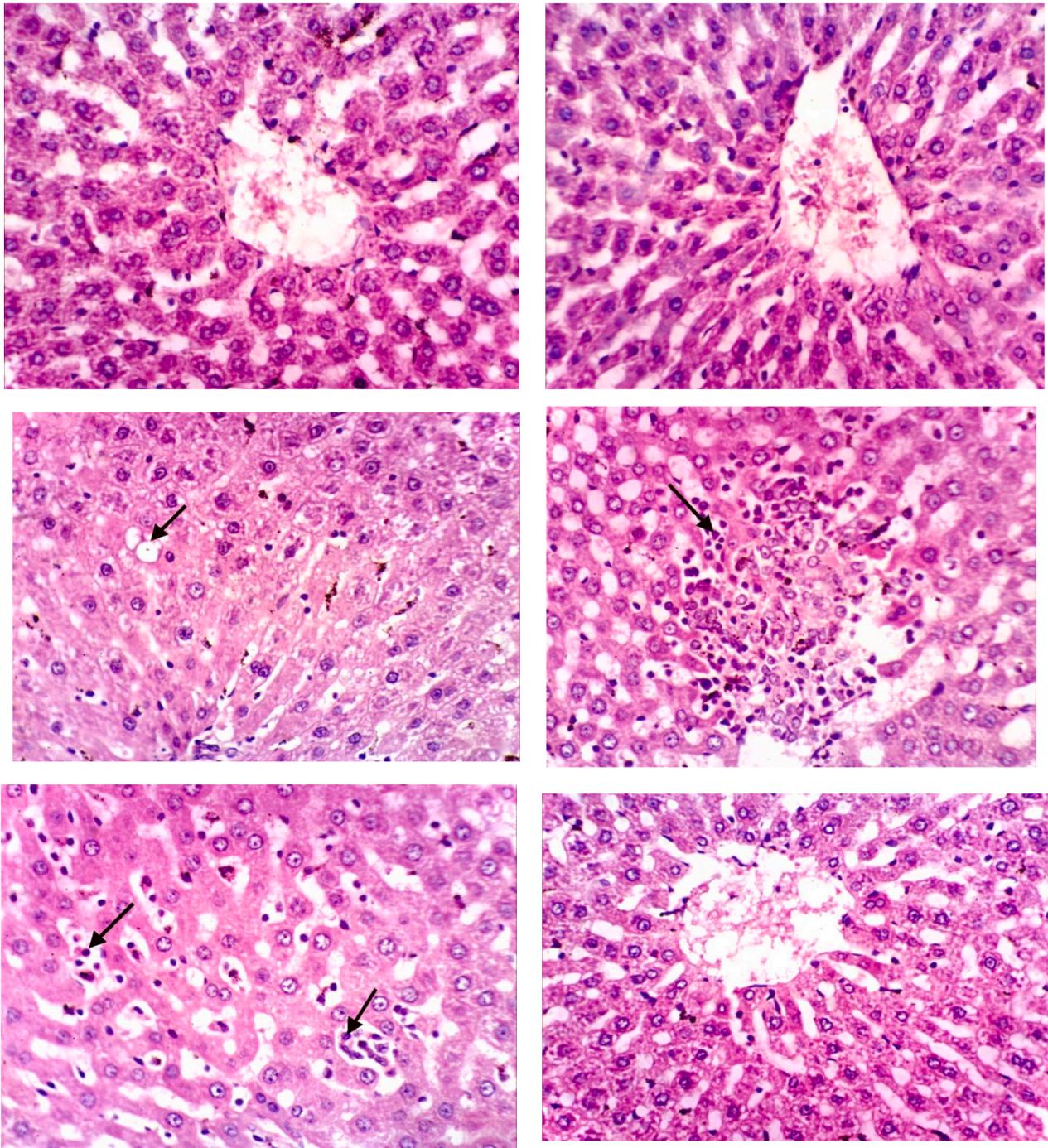


Fig. 5. Histopathological examination of liver tissue of different groups treated with breads supplemented with different levels of whole barley. (Fig. 5.1.) Liver of rat from group 1 showing the normal histological structure of hepatic lobule. Normal central vein and hepatocytes (H & E X 400). (Fig. 5.2.) Liver of rat from group 1 showing the normal histological structure of hepatic lobule. Normal central vein and hepatocytes (H & E X 400). (Fig. 5.3.) Liver of rat from group 2 showing hepatocellular vacuolar degeneration (H & E X 400). (Fig. 5.4.) Liver of rat from group 2 showing focal hepatocellular necrosis associated with inflammatory cells infiltration (H & E X 400). (Fig. 5.5.) Liver of rat from group 2 showing sinusoidal leukocytosis (H & E X 400). (Fig. 5.6.) Liver of rat from group 3 showing no histopathological alterations (H & E X 400). (Fig. 5.7.) Liver of rat from group 3 showing hepatocellular vacuolar degeneration of some hepatocytes (H & E X 400). (Fig. 5.8.) Liver of rat from group 4 showing no histopathological alterations (H & E X 400). (Fig. 5.9.) Liver of rat from group 4 showing small focal hepatocellular necrosis associated with inflammatory cells infiltration (H & E X 400). (Fig. 5.10.) Liver of rat from group 5 showing no histopathological alterations (H & E X 400). (Fig. 5.11.) Liver of rat from group 5 showing slight Kupffer cells activation (H & E X 400).

small (Fig. 7.4.) and nearly all fatty cells from group 5 treated with bread (80 % WBF) are small like negative group (Fig. 7.5.).

3.2. Dissection

The increasing in fiber content causes an elevation in water absorption (Sudha et al., 2007). Supplementation caused a decrease in carbohydrate content. These data is confirmed by Hussein et al., (2013)

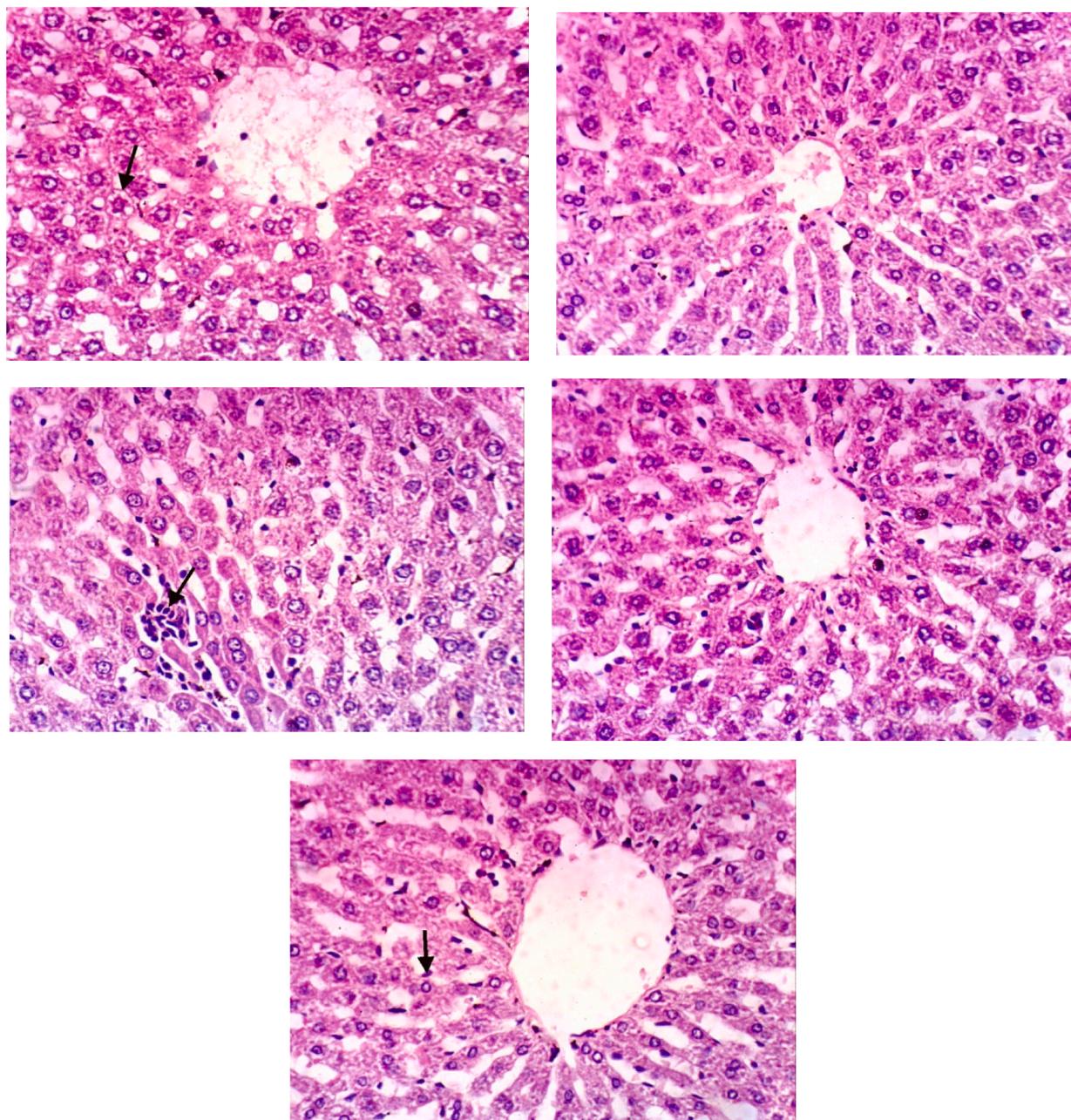


Fig. 5. (continued).

who revealed that WBF-wheat bread was higher in fiber, fat, protein, ash and lower in carbohydrate content than WB. Also, some ingredients, such as β -glucan in barley flour can tightly bound appreciable amounts of water in the dough when added to wheat flour during bread making. Thus β -glucan can affect the texture and volume so dough and availability of water is necessary for the development of the gluten network. An underdeveloped gluten network can cause lower loaf volume and raise bread firmness. Moreover, B-glucan can suppress in the amount of steam generated causing in greater firmness crumb texture and decreased loaf volume (Gill et al., 2002). The results of microbial evaluation of balady breads are confirmed by Liu, (2004) who stated that barley has a several spectrum of phenolic components which considered as a source of antioxidant metabolites that are synthesized in the colon or act as antioxidants themselves. Phenolic compounds play a prominent role in growth and reproduction which can create a defense system against parasites and pathogens (Lattanzio et al., 2006; Mithofer et al., 2012). In conclusion, the incorporation of WBF in balady bread with

different levels (60 and 80 %) can increase the shelf life of stored balady bread at room temperature due to its antioxidant properties. the results of effect of balady bread supplemented with different levels of whole barley flour on body weight gain (BWG) of obese rats are in agreement with Kalra et al., (2000) who demonstrated the effects of three barley cultivars containing 6.23, 4.60, 2.18 % b-glucan and showed that final body weight and weight gain for the control treatment were the highest after 40 days of feeding in comparison to test groups. In addition, Aoe et al., (2017) studied the effects of high b-glucan barley cultivar on obesity and the barley was incorporated into rice as a replacement for the ordinary pearled rice. During the experiment, body weight and BMI significantly lowered in the experimental groups. From the resultant data, it could be concluded that the long term consumption of diet containing whole barley flour can help in weight control. There were not significant differences in kidney relative weights between all groups. These results are on the same line with Choi et al., (2010) who mentioned that the treatment with barley containing 2 and 4 % b-glucan

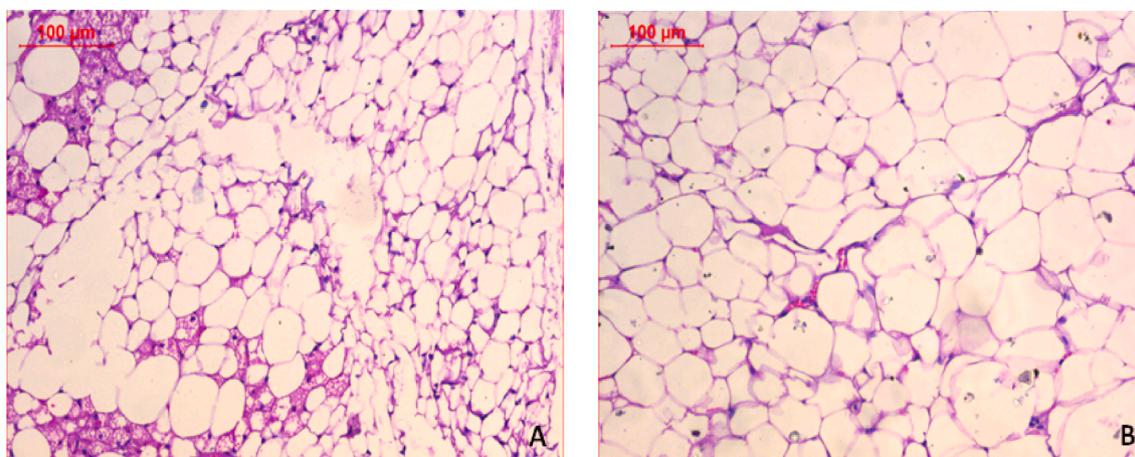


Fig. 6. Photomicrography of perinephric fat (H&E,x200) of negative and positive group after induction of obesity at the first of experimental period. (Fig. 6.1.) Fat cell size of rats from negative group is small. (Fig. 6.2.) Fat cell size of rat from positive group is large.

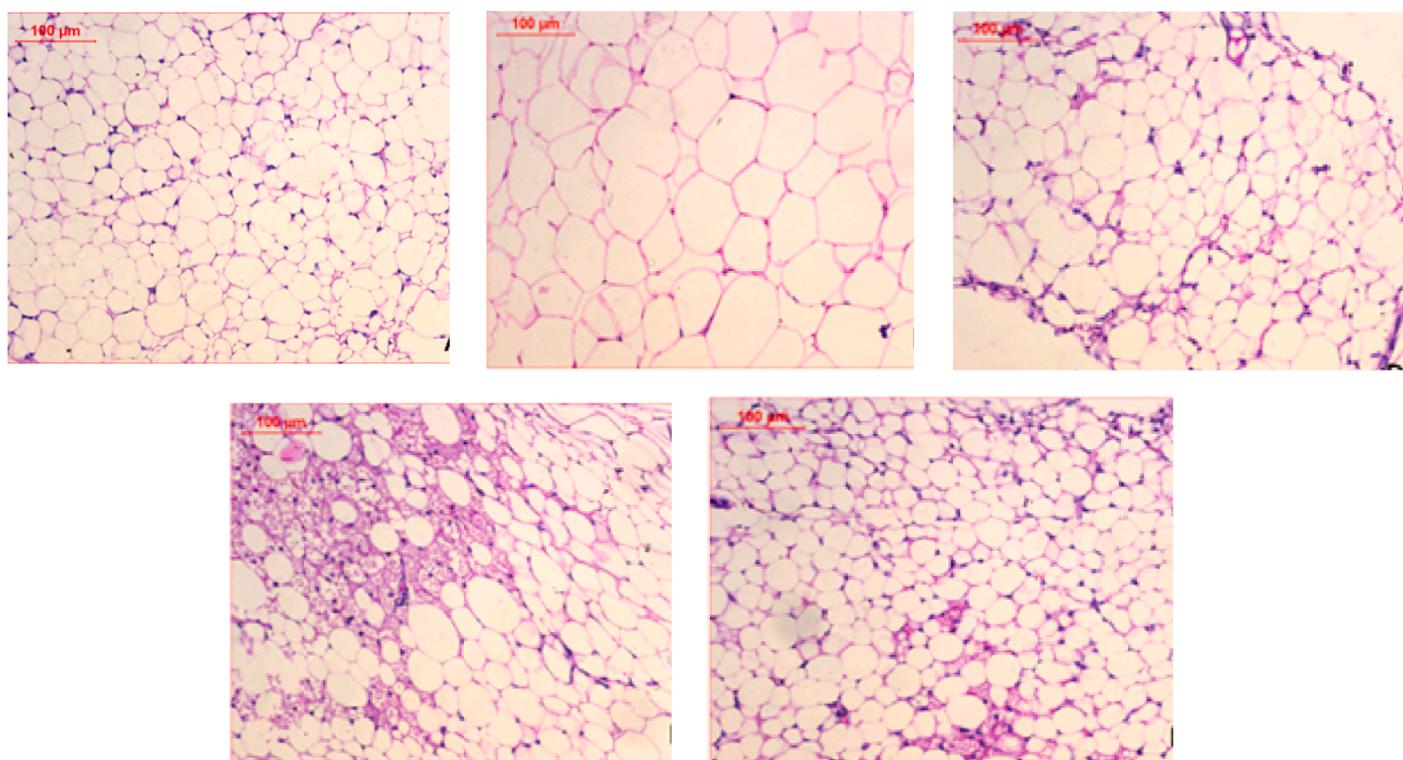


Fig. 7. Photomicrography of perinephric fat showing difference in fat cell size between different groups treated with breads supplemented with different levels of whole barley at the end of experimental period (H&E, x200). (Fig. 7.1.) Small sized fat cells in negative group. (Fig. 7.2.) Large sized fat cells in Positive group. (Fig. 7.3.) Variable sized fat cells, most of them is large figure in group 3. (Fig. 7.4.) Group 4 variable sized fat cells, most of them is small in group 4. (Fig. 7.5.) Nearly all fat cells are small like negative group in group 5.

(BG) for 12 week led to reducing in weights of liver by 19 and 18.6 % in b- glucan diets 2 and 4 %, respectively and there were no significant reductions in other organ weights after BG treatment. Also, Xia et al., (2018) mentioned that after 8 weeks of feeding high level of hull-less barley, it significantly lowered the organ indexes of liver and abdominal fat in high fat diet rats. In this respect, the liver is considered a prominent agent in keeping lipid homeostasis (Ogata et al., 2009). In addition, Puskas et al., (2004) showed that an accumulation of intracellular lipid in heart was related to cholesterol and triglycerides elevation in diet. The data of balady bread supplemented with different levels of whole barley flour on serum glucose of obese rats are in agreement with Choi et al., (2010) who revealed that the high levels of

β -glucan in barley led to reduce insulin-response index and stabilize glucose levels in the blood. Also, Hajifaraji et al., (2012) showed the effect of barley and oat breads on serum glucose in dyslipidemia and type 2 diabetic rats and demonstrated that significant variances were observed in fasting plasma glucose between groups, and these differences were more apparent in the oat bread group at the end of the study. Along with that Brennan et al., (2007) stated the effects of adding Glucagel (a beta-glucan isolate) at ratios 2.5 % and 5 % into breads and the resultant data showed that however, the supplementation led to a reduction in the quality of the end bread, breads supplemented with 5 % Glucagel caused a significant decrement in sugar release after a 300 min of digestion and the authors recommended that we can use beta-glucans

against hyper-insulinaemia and hyperglycemia and thus decrease the risk of diabetes and the development of similar chronic diseases. On contrary, Li et al., (2003) studied the effects of barley intake on fasting plasma glucose and found that there were not significant differences between experimental and standard diet after 4 weeks and the authors suggested that, short term consumption of barley had no effect on fasting plasma glucose. In this respect, Wood et al., (1994) reported that β -glucan (from both barley and oat cereals) is considered an important factor in stabilizing of serum glucose and insulin. Soluble dietary fibers can causing many mechanisms process such as stabilize postprandial serum glucose level via delay the release of reducing sugars from food moreover lowered amylolysis (Colonna et al.,1990) and lowering the stomach emptying at the gastrointestinal level (Cherbut, 1995). Similar findings for lipid profile of obese rats fed balady bread supplemented with different levels of whole barley flour were previously mentioned by Ranhotra et al., (1998) who mentioned that serum TG levels showed a dose dependent (decreasing) response to barley in the diet. Compared to diet A, serum TG levels were 9.2 % lower in animals consuming diet B1 (25 % barley diet), 16.8 % lower with diet C1 (50 % barley diet), and 31.1 % lower in animals consuming diet D1 (75 % barley diet). In this respect, Aman (2006) reported that high cholesterol concentration in the blood represents an independent diet-associated risk factor for atherosclerosis. It is known that certain types of dietary fiber such as psyllium fiber and partly soluble β -glucan, help to decrease the cholesterol concentration. Previous investigations have established that dietary fiber can reduce the up-take of lipids and dietary cholesterol through interfering with the micellar solubility of these lipids or likely modify the site and ratio of up-take (Schweizer et al., 1991 & Marlett et al., 1994). The data liver functions of obese rats fed balady bread supplemented with different levels of whole barley flour are on the same line with Maraia et al., (2017) who reported that the daily treatment of whole barley 200 mg/ kg.b.w and 100, 200 mg/kg.b.w. barley bran in hyperlipidemia rats ameliorated liver enzymes in comparison with positive control group at the end of experimental period. Along with that, Abulnaja et al., (2015) reported that the daily dose with 5 %,10 % barley bran significantly lowered all liver enzymes bran in hypercholesterolemia male rats and found that the higher level of barley bran decreased the liver enzymes activity more than the lower one. Liver function tests are usually used in clinical practice to examine liver disease and monitor the development of known disease. The most common liver function tests are the serum aminotransferases (ALT& AST), alkaline phosphatase, bilirubin, albumin and prothrombin (Fleming, 2001). The data of kidney function of obese rats are in agreement with Abulnaja et al., (2015) who studied the effects of barley bran 5, 10 % on kidney function of the hypercholesterolemia rats and found that the concurrent barley bran supplementation had non-significant decrease on creatinine value. Creatinine is the main chemical waste product that's procreated by muscle metabolism. In the healthy kidney, creatinine is leached by the glomerulus and efficiently secreted by the tubules. Furthermore, test creatinine shows of how well the kidneys filter (Foley et al., 2005 & Stevenes et al., 2006).

4. Conclusion

We can fully exploit WBF in bakery products for increasing daily fiber intake as revealed by chemical analyses. But, the incorporating high levels of WBF into leavened bakery products lead to a decrease in the sensorial quality of these products. Thereby, we need more research to expand the utilization of barley to add healthy properties to wheat-based products. The high levels of whole barley flour improved serum lipids parameters, ameliorated liver enzymes and improved histological structure of liver. The higher level of whole barley incorporated into bread improved those parameters than the lower one. The long term consumption of WBF-wheat bread with high β -glucan levels can help weight management and this can likely due to the decrement of total calories intake and promoting satiation. The outer bran of whole barley

which possessed the majority of soluble fibers and β -glucan could possibly have preferential effects than any other whole barley fractions.

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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