

The ameliorative effect of dietary barley and malted barley on hyperlipidemic rats

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Abstract

The present study was carried out to investigate the efficiency of barley or malted barley in ameliorating lipid profile and oxidative stress alteration caused by high fat, high cholesterol diet (HFHC). Adult male albino rats were divided into six groups; each group consisted of 12 rats. Group (1) Served as control, fed on balanced diet (without treatment); groups (2) and (3), rats were fed on balanced diet containing 10 % barley and malted barley respectively, group (4), rats were fed on HFHC (50 g lard+10 g cholesterol/kg diet) to induce hypercholesterolemia. Groups (5) and (6), rats were fed on HFHC contains 10% barley and malted barley respectively.

Results reflected a significant depletion in all parameters of lipids profile and the percentage of change reached 22.04 % 24.87%, 25.19 %, 40.34 % , 34.11 % , 54.37 % , 54.89 % for Total lipid (TL), Total cholesterol (TC) , Triacylglycerols (TAG) , Low density lipoprotein cholesterol (LDL-C) , Very low density lipoprotein cholesterol (VLDL-C) , Atherogenic index (AI) and Risk factor ratio (RF) respectively when compared with HFHC group treated with barley with an exception of high density lipoprotein cholesterol (HDL-C) showed a significant increase when compared with HFHC group. On the other hand the results showed a significant increment in reduced glutathione level (GSH) in blood and liver. The percentage of changes in GSH in liver showed 13.82 % and 21.47 % as well as, super oxide dismutase (SOD) activity by 33.05 % and 42.39 % . while a significant decrement in malondialdehyde (MDA) in serum and liver, the percentage of decrement in liver by 38.47% and 46.36% in groups fed on HFHC diet and treated with barley or malted barley respectively when compared with group fed on HFHC ($p < 0.05$). It can be concluded that barley or malted barley can reduce lipids profile and oxidative stress but malted barley is more effective than barley.

Key Words: Barley –Malted barley –Lipids profile–High fathigh cholesterol diet -
Oxidative stress

1. Introduction

Hyperlipidemia is a predominant risk for cardiovascular diseases (CVD) which remain as one of the leading causes of death (**Lim et al., 2012 and Alokbi et al., 2014**).Hypercholesterolemia is the presence of high levels of cholesterol in the blood (**Durrington 2008**).Hyperlipidemia and thereby atherosclerosis is the leading cause of cardiac illness and deaths (**Tiwari et al., 2006**). About 70% of total cholesterol in the human is synthesized *de novo* and the remaining is supplied by absorption from diet (0.3-0.5 g/day) (**Kumar et al., 2012**). In 1984, it was demonstrated for the first time that there exists a link between serum cholesterol levels and risk to coronary heart disease (CHD). So 1% drop in serum cholesterol reduces the risk for CHD by 2%. The primary cause of CHD is atherosclerosis, a chronic disease, characterized by the accumulation of lipids and fibrous connective tissue on the arterial wall, resulting in a narrowing of the vessel lumen and ultimately hardening of the vascular system, which may lead to ischemic heart disease, myocardial infarction, and stroke (**Alaa et al., 2011**).

Barley (*Hordeumvulgare.L*) contains many nutrients, including dietary fiber, antioxidants (*p*-coumaric acid),phenolic compounds, vitamins, minerals such as (calcium, potassium, phosphorous, magnesium) in addition of sphingolipids and unsaturated fatty acids. This diverse composition allows barley product to have myriad of benefits and appealing characteristics. It is also used as nutraceutical ingredient because it contains high content of soluble fiber, especially as a rich source of beta glucan. Because of its nutritional and chemical properties in particular a high dietary fiber component, barley is considered the as most suitable grain in human diet. (**Oscarsson et al, 2014**).

Barley's fiber can prevent or help with a number of different conditions. For example, when barley's fiber binds to and removes cholesterol-containing bile, this can be very beneficial for people struggling with heart disease since it forces the body to make more bile by breaking down cholesterol, thus lowering cholesterol levels (**Cleland et al., 2006**).

Barley has gained popularity due to the functional properties of its bioactive compounds in barley-based different healthy food products. Germination is a process in which physical modification of endosperm is carried out to increase the bioactive compounds (**Madhujith and Shahidi 2007**). Worldwide, the greatest use of barley is for malting purposes, most specifically for the brewing industry. However, in recent years, there has been a growing interest in incorporating barley into the human diet because it is wholesome, readily available, and relatively inexpensive (**Keenan et al., 2007**).

Those phenolic compounds in malting barley include polyphenols (benzoic and cinnamic acid derivatives), flavonoids, proanthocyanidins, tannins, and amino phenolic compounds (**Bonoli et al., 2004**), all of which are known to inhibit nonenzymatic lipid peroxidation and widely recognized as having important antioxidant and antiradical properties. Therefore, the presence of the Natural antioxidants in malting barley and screening of malting barley variety with the highest) Level of radical scavengers seems very important to produce malt with high levels of antioxidant activity (**Maillart et al., 2010**).

Barley and malted barley have been considered as ingredients for production of functional foods due to their concentration of antioxidant compounds furthermore malt should be considered as a new source of natural antioxidant for dietary needs (**Qingming et al., 2010**).

2. Materials and Methods

2-1 Materials

2.1.1 Barley

A- Barley was obtained from local markets in summer2014.

B-Malted barley was obtained by three steps: Steeping, Germination and Drying to ensure product stability.

2.1.2 Chemicals

Cholesterol was obtained from El- Gomhouria Company for trading chemicals, Cairo Egypt. Chemical used in the present study were analytical pure grade.

2.1.3 Animals

The healthy experimental animals used throughout this work were 72 adult male albino rats weighing (140 ± 5.6) gram, were supplied from the Animal House of National Cancer Institute, Egypt.

2. 1.4 Diet

The experimental diet used in the present study was the balanced diet prepared according to **AIN-1993M** adjusted *by Reeves et al. (1993)*.

2.Methods:

2.2.1 Experimental design

The biological experiment divided into six groups; each group consisted of 12 rats. Group (1) Served as control ,fed on balanced diet (without treatment);groups (2) and (3), rats were fed on balanced diet containing 10 %barley and malted barley respectively, group (4), rats were fed on HFHC (50 g lard+10 g cholesterol /kg diet) to induce hypercholesterolemia. Groups (5) and (6), rats were fed on HFHC diet contains 10%barley and malted barley. All rats were offered the balanced diet with drinking water *ad libitum*.

2.2.2 Biochemical analysis

At the end of the experimental period (6 weeks), the animals were fasted for 12 hr, and then anesthetized under diethyl ether anesthesia and whole blood samples were taken from hepatic portal vein in two centrifuge tubes. The first tube contained heparin as anti coagulant for immediate determination of enzymatic antioxidant such as reduced glutathione (GSH) .The second tube for separating serum by allowing blood samples left for 15 minutes at temperature of 25 °C then centrifuged at 4000 rpm for 20 minute by using EBA8 centrifuge, diameter = 9.8 cm (made in china),serum was removed and frozen in plastic vials and kept at -25 °C until biochemical analysis as Serum lipid profile such as total lipid (TL), total cholesterol (TC), triacylglycerols (TAG) High density lipoprotein (HDL-c),low density lipoprotein (LDL-c),atherogenic index (AI),risk factor ratio (RF),very low density

lipoproteins (VLDL-c), lipid peroxidation product measured as malondialdehyde (MDA) and finally determined reduced glutathione, malondialdehyde levels and superoxide dismutase activity (SOD) in liver tissue by perfusing liver with a PBS (phosphate buffered saline) solution, PH7.4 containing 0.16 mg/ml heparin to remove any red blood cells and clots. Homogenizing 0.25 of liver in 5 -10 ml cold buffer (50mM potassium phosphate PH 7.5.1 mM EDTA) per gram liver, using tissue homogenizer, Centrifuge at 4.000 rpm for 15 min and Remove the supernatant for assay.

2.2.3 Statistical analysis

Data were statistically analyzed by statistical package for social science (SPSS) version 16.0 statistical packages. Values were presented as mean \pm standard deviation (S.D).Statistical differences between groups were performed using one way ANOVA,The mean difference was significant at the ($p<0.05$)level according to (Levesque2007).

3. Results and Discussion

The results presented in table (1 and 2) revealed that the rats fed on a diet containing HFHC caused a highly significant increase in all studied lipids parameters except that HDL-C. The increment reached 27.22 %, 40.18%, 49.74%, 61.21%, 49.75%, 72.28% and 72.97%for TL, TC, TG, VLDL-C, AI and risk factor respectively in HFHCgroup as compared to control group. On the other hand, HDL-C was significantly ($P<0.05$) decreased in atherogenic rats it recorded 36.25 ± 2.91 as compared to control rats. After treatment with barley and malted barley reflected a significant depletion in all parameters of lipids profile and the decrement reached 22.04 % 24.87%, 25.19 %, 40.34 % , 34.11 % , 54.37 % , 54.89 % for Tl, TC, TG, LDL-C, VLDL-C, AI and risk factor for HFHC fed rats group co-treated with barley when compared with HFHC group with an exception of good cholesterol (HDL-C) showed a significant increase in the mean value to reach (47.62 ± 2.44) mg/dl, (57 ± 5.70) mg/dlfor HFHC groups co-treated with barley and malted barley respectively when compared with HFHC group (36.25 ± 2.91) mg/dl.

Table (1): Serum lipids profile(TL, TC, and TAG) mg/dlin healthy and hyper-cholesterolemic rats fed on barley and malted barley

Parameters Groups	TL (mg/dl)	TC (mg/dl)	TAG (mg/dl)
Control(without treatment)	373.83±5.36 ^d	119.35±5.47 ^d	55.76±2.13 ^d
Control+barley	350.21±76.55 ^c	115.86±6.14 ^c	50.65±7.16 ^b
Control+ malted barley	340.10±10.49 ^c	110.12±28.91 ^c	44.21±2.32 ^b
High fat high cholesterol	513.67±4.66 ^a	199.52±17.13 ^a	110.96±11.62 ^a
High fat high cholesterol +barley	400.45±9.69 ^{cd}	149.89±6.70 ^{dc}	73.12±4.43 ^b
High fat high cholesterol+malted barley	390.61±5.38 ^c	127.59±2.68 ^c	60.68±4.05 ^c
LSD	30.32	13.91	4.09

Values are represented (Mean ±SD) for 12 rats for each group.

There was no significant difference between means have the same letter in the same column (p<0.05).

Table (2): Serum lipids profile (HDL-C, LDL-C, VLDL-C) mg/dl, atherogenicindex and risk factor ratio in healthy and hypercholesterolemic rats fed on barley and malted barley

Parameters Groups	HDL-C (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)	Atherogenic index	Risk factor ratio
Control (without treatment)	51.96±2.06 ^b	56.99±7.70 ^e	11.15±0.43 ^d	1.30±0.17 ^d	1.10±0.18 ^d
Control+ barley	65±3.54 ^a	49.88±4.57 ^d	10.13±1.43 ^b	1.06±0.11 ^d	0.89±0.14 ^d
Control+ malted barley	64.62±5.68 ^a	36.66±14.07 ^e	8.84±0.46 ^b	0.70±0.27 ^d	0.56±0.29 ^d
High fat high cholesterol	36.25±2.91 ^e	146.93±7.44 ^a	22.19±0.32 ^a	4.69±0.49 ^a	4.07±0.44 ^a
High fat high cholesterol +barley	47.62±5.70 ^a	87.65±8.82 ^e	14.62±0.89 ^b	2.14±0.30 ^b	1.84±0.23 ^d
High fat high cholesterol+malted barley	57±2.44 ^c	58.42±3.63 ^c	12.13±0.81 ^c	1.60±0.19 ^c	1.55±0.16 ^c
LSD	3.93	8.73	0.83	0.29	0.29

Values are represented (Mean ±SD) for 12 rats for each group.

There was no significant difference between means have the same letter in the same column (p<0.05).

Kishida et al., (2002) found that rats fed on cholesterol-enriched diet resulted in a significant increase in total lipids, total cholesterol, triacylglycerols, LDL-C, VLDL-C, and LDL/HDL ratio accompanied with decrease in HDL-C, thus providing a model for dietary hyperlipidemia. The physiological effects of barley beta -glucan is related to its ability to form a hydrated viscous mass in gastrointestinal tract (GIT) .The increased viscosity of GIT contents leads to trapping and reduced absorption of bile acids, which reduces their plasma levels and enhances excretion in the feces. Reduced absorption of cholesterol also induces higher rate of cholesterol synthesis by the liver because of the need to produce more bile acids. Apart from the physical effects, fermentation of beta-glucan in the colon produces large amounts of propionate, short chain fatty acid that inhibits cholesterol synthesis. The action of propionate is thought to be mediated through inhibition of activity of hepatic HMG-COA reductase, a key hepatic enzyme involved in cholesterol synthesis **Zaher and Ann (2015)**.

Sindhu and Khetarpaul (2003) found that the rats group fed on 10 % the probiotic fermented barley diet significantly decreased the serum cholesterol and triacylglycerols, whereas, HDL cholesterol increased compared to control group diets. **Xiao et al., (2003)** showed that probiotics including *Bifidobacterium* had a hypocholesterolemic effects in both rat and human. The mechanisms involved may be as follows (1): Fermentation products of lactic acid bacteria inhibit cholesterol synthesis enzymes and thus reduce cholesterol production; (2): The bacteria facilitate the elimination of cholesterol in feces; (3): The bacteria inhibit the absorption of cholesterol back into the body by binding with cholesterol; (4): The bacteria interfere with the recycling of bile salt (a metabolic product of cholesterol) and facilitate its elimination, which raises the demand for bile salt made from cholesterol and thus results in body cholesterol consumption;(5): The assimilation of lactic acid (**Beena and Prasad 2011**).

Yamamoto et al., (2010) stated that serum cholesterol level of rats fed 1% and 5% fermented barley diets was significantly lower than that of the high fat high cholesterol group.

Tong et al., (2015) reported that the hypocholesterolemic effects of dietary hull-less barley beta-glucan (HBG) on cholesterol metabolism are reducing the concentration of plasma LDL cholesterol by promoting the excretion of fecal lipids and regulating

the activities on hydroxyl methylglutaryl-CoA-reductase (HMG-CoA) reductase and cholesterol 7 α hydroxylase activity (CYP7A1) in hypercholesterolemic hamsters.

Table (3): Blood reduced glutathione (mg %) and malondialdehyde(μ mol/l) levels in healthy and hypercholesterolemic rats fed on barley and malted barley

Parameters Groups	(GSH) (mg %)	(MDA)(μmol/l)
Control(without treatment)	25.77\pm3.65^a	3.89\pm0.334^{cd}
Control +barley	27.19\pm2.27^{ab}	3.22\pm0.17^d
Control+ malted barley	29.65\pm2.91^c	2.85\pm1.42^{cb}
High fat high cholesterol	16.86\pm2.52^d	5.87\pm1.13^b
High fat high cholesterol+ Barley	22.87\pm1.51^e	4.09\pm0.63^a
High fat high cholesterol+ malted Barley	24.14\pm4.04^a	3.54\pm2.30^{bc}
LSD	3.03	1.23

Values are represented (Mean \pm SD) for 12 rats for each group.

There was no significant difference between means have the same letter in the same column ($p < 0.05$).

Table (4): The livers tissues (GSH) mg/g,(MDA) μ mol/g levels and superoxide dismutase activity (SOD) U/g in livers of healthy and hypercholesterolemic rats fed on barley and malted barley

Parameters Groups	(GSH) mg/g In liver tissue	(MDA) μmol/g In liver tissue	(SOD) U/g In liver tissue
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Control(without treatment)	19.35±2.56^a	4.5±0.58^{bc}	307.05±3.24^e
Control+barley	21.44±1.66^d	3.95±0.57^c	314.83±2.89^d
Control+malted barley	23.25±3.16^f	3.04±1.75^c	340.55±2.43^a
High fat high cholesterol	14.66±4.22^b	1 1.15±0.64^a	162.39±3.53^b
High fat high cholesterol+ Barley	17.01±2.24^c	6.86±0.46^d	242.57±1.76^g
High fat high cholesterol+ malted barley	18.67±1.74^g	5.98±0.34^c	281.90±5.41^f
LSD	3.03	0.88	5.83

Values are represented (Mean ±SD) for 12 rats for each group.

There was no significant difference between means have the same letter in the same column (p<0.05).

From the results showed in table (3) revealed that the level of reduced glutathione in blood recorded 25.77± 3.65 mg% this value decreased significantly to reach 16.86 ± 2.52 mg% in HFHC group. While, the rats co-treated with barley and malted barley showed a significant increase in the level of reduced glutathione that reach 22.87±1.51mg% and 24.14±4.04 mg% respectively when compared with HFHC group (P< 0.05). The level of serum MDA level in control group recorded 3.89±0.334 µmol/l this value increased significantly to reach 5.87±1.13 µmol/l in HFHC group while, the rats treated with barley and malted barley caused a significant decrease in the serum level of MDA which recorded 4.09±0.63µmol/l and 3.54±2.30 µmol/l for HFHC co-treated with barley and malted barley respectively when compared with HFHC group.

The results in table (4) demonstrated that the rats fed on HFHC diet showed a significant decrease in the level of reduced glutathione and superoxide dismutase activity in liver tissue which recorded 14.66±4.22 mg/g tissue and 162.39±5.53 mg/g tissue respectively when compared with control groups. On the other side the rats treated with barley and malted barley cause a significant increase in the level GSH and SOD activity in liver so the percentage of changes in GSH in liver are 13.82 %

and 21.47 %.on the same manner the percentage of changes in SOD level are 33.05 % and 42.39 % in groups of rats treated with barley and malted barley respectively when compared with HFHC group. On the other hand the level of MDA in liver is $4.5\pm 0.58\mu\text{mol/g}$ tissue, this value increase significantly to reach $11.15\pm 0.64\mu\text{mol/g}$ tissue by the percentage of increment is 59.61 %in HFHCgroup. while the rats treated with barley and malted barley caused a significantly decrease in the level of MDA that reach $6.86\pm 0.46\mu\text{mol/g}$ tissue, $5.98\pm 0.34 \mu\text{mol/g}$ tissueby the percentage of decrement 38.47% and 46.36% respectively when compared with HFHC group.

Holasovaa, et al., (2002) showed that the antioxidant activity of barley beverage(BB) and barley beverage fermented (BBF) may be due to presence of phenolic compounds and proanthocyanidins in the barley. Antioxidant activity was related to the total phenolic contents. Phenolic compounds such as ferulic, vanillic and p-coumaric acids are the major functional antioxidants in the barley. Moreover theextracts from barley were possessed antioxidant activity, including reducing power, radical scavenging activity and the lipid peroxidation inhibition (**Qing and Huiyuan2007**).

Mateos (2005)and Fardet et al., (2008) investigated that the supplementation with barley bran to the hyperlipidemic rats are greatly ameliorated the lipid peroxide and antioxidant levels this is due to barley bran phenolic acids such as ferulic acid may scavenge free-radical oxygen species both *in vitro and vivo*.

Pan et al.,(2009) Said that the malt extract from barley exhibited high antioxidant activities both *in vitro* and *vivo*, evidenced by its ability to scavenge hydroxyl- and superoxide-radicals, high reducing power, and protection against biological macromolecular oxidative damage. Furthermore, malt extract prevented the decrease of antioxidant enzyme activities, decreased liver malondialdehydelevelsand carbonyl content, and improved total antioxidant in treated mice. So it demonstrated potential antioxidant activities and anti aging effect of malt, providing scientific support for the empirical use of malt as an antioxidant for diseases caused by reactive oxygen species.

Giriwono et al., (2011) investigated that Fermented barley extract (FBE) supplementation sustained liver anti-oxidative enzymes, reduced glutathione and

superoxide dismutase, thus suppressing oxidative stress markers such as MDA by 42%.

Aly(2013) and Balkan et al., (2013) suggested that feeding hypercholesterolemic rats (fed 1% cholesterol in the diet) on diets containing 10% barley bran showed a significant decrease in the level of MDA and increase in the level of antioxidant GSH compared with those received only 1% cholesterol.

4. Conclusion

It can be concluded that barley and malted barley can reduce lipids profile and oxidative stress but malted barley is more effective than barley.

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الملخص باللغة العربية

التأثير المحسن للمتناول الغذائي للشعير و الشعير المتخمّر علي الجرذان المصابة بارتفاع دهون الدم

فاطمة عبد الحميد خليل- سحر موسى جلال- مديحة عبد العظيم حفني

قسم الكيمياء الحيوية والتغذية-كلية البنات للأداب والعلوم والتربية جامعة عين شمس

يهدف هذا البحث الي التحقق من مدي فاعلية وكفاءة الشعير و الشعير المتخمّر علي مستوي الدهون في الجرذان المتناولة وجبات عالية الدهون والكوليسترول . ومن ثم تم تقسيم الجرذان الي 6 مجموعات تحتوي كل مجموعة علي 12 جرذ. المجموعة (1):مجموعة الكنترول تتناول غذاء متوازن بدون اي معاملة. المجموعة (2) و(3) :غذاء متوازن بالإضافة الي 10% من الشعير ، 10 % من الشعير المتخمّر علي التوالي. المجموعة (4) تتناول وجبات عالية الدهون والكوليسترول (50 جرام دهون صلبة + 10 جرام كوليستيرول لكل كيلو جرام للوجبة) لإحداث إرتفاع في مستوي الكوليستيرول. المجموعة (5) و(6) :تتناول وجبات عالية الدهون والكوليستيرول (50 جرام دهون صلبة + 10 جرام كوليستيرول لكل كيلو جرام للوجبة) بالإضافة الي 10 % من الشعير ، 10 % من الشعير المتخمّر علي التوالي. وبعد انتهاء فترة التجربة (6 أسابيع) أظهرت النتائج كالتالي :حدث انخفاض في مستوي الدهون الكلية ، الكوليستيرول ، الجليسيريدات الثلاثية ، الليبوبروتينات المنخفضة الكثافة ، الليبوبروتينات المنخفضة الكثافة جدا، معامل التصلب و معامل الخطورة. و علي النحو الأخر هناك إرتفاع في مستوي نشاط الجلوتاثايون المختزل و سوبر اوكسيد ديسميوتيز و إنخفاض في مستوي المالنوندايفي الكبد والسيرم في كلا من المجموعات التي تمت معالجتها بالشعير و الشعير المتخمّر مقارنة بالمجموعة التي تناولت وجبة عالية من الدهون والكوليستيرول بدون معالجة. وخلص هذا البحث الي ان الشعير و الشعير المتخمّر لهما تأثير خافض لمستوي الدهون و الكوليستيرول ورافع لمستوي مضادات الاكسدة ويعتبر الشعير و الشعير المتخمّر من الاغذية الهامة التي لها دور علاجي للحد من أمراض القلب و الشرايين.

