ORIGINAL ARTICLE

INTERNATIONAL JOURNAL OF RESEARCH IN PHARMACEUTICAL SCIENCES

Published by JK Welfare & Pharmascope Foundation Journal Home Page: www.ijrps.com

The Effect of Hordeum Vulgare L. Extracts on Blood Cholesterol Level and Lipid Peroxidation Activity in a Hypercholesterolemia Rodent Model

Pang Jyh Chyang*, Mawaddah

Universiti Kuala Lumpur, Institute of Medical Science Technology, 43000 Kajang, Selangor, Malaysia

Article History:

Received on: 20 Apr 2020
Revised on: 20 May 2020
Accepted on: 29 May 2020

Keywords:

Hordeum vulgare L,
Antioxidant,
Cholesterol,
Melondialdhyde level

ABSTRACT

Hordeum vulgare L. (Barley) is an ancient and essential cereal grain crop with the claim that it has the potential to reduce cholesterol level and to lower oxidation activity in the liver.However, it hasn't been proven scientifically. Hence, this study was conducted to investigate the total phenolic content (TPC), total antioxidant activity (TAC) and liver peroxidation activity of barley aqueous and ethanol extractas well as assess the effect of ethanol extract on cholesterol level of high-fat diet rats. TAC of barley extract was determined by using ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) assay andDPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay. Meanwhile, Total Phenolic Content TPC was determined by Folin-Ciocalteu assay. A total of 15 Sprague Dawley rats were tested for the lowering cholesterol properties in barley and its association with lipid peroxidation product (Melondialdhyhe level) by adding the barley into their daily diet. The result indicated that TAC and TPC value of ethanol barley extract was high. Barley ethanol extracts effectively lowering cholesterol level in Sprague Dawley rats. Meanwhile, the malondialdehyde level in the liver tissue was a significant difference between the high-fat diet group of rats and the high-fat diet group of rats treated with ethanol barley extract. Conclusion, ethanol barley extract possess more phenolic content, antioxidant component and reducing cholesterol level of high-fat diet rats.

*Corresponding Author

Name: Pang Jyh Chyang
Phone: +6012-3580973
Email: jcpang@unikl.edu.my

ISSN: 0975-7538

DOI: https://doi.org/10.26452/ijrps.v11i4.3305

Production and Hosted by

IJRPS | www.ijrps.com

INTRODUCTION

Cholesterol is a crucial biological molecule that has roles in membrane structure as well as being the precursor for the synthesis of the steroid hormone and bile acids (Nayak, 2008). There is no recommended intake for cholesterol in daily diet as the body can produce enough cholesterol and people do not develop cholesterol deficiency disease if it is not consumed as diet (Isaacs *et al.*, 2011). However, the blood level tends to increase somewhat if the consumption of cholesterol is increased (Brown, 2013). An individual with a healthy lifestyle also has the possibilities in having high cholesterol level in blood.

According to health guidance that is released by the National Institute of Health, an individual needs to keep track of their cholesterol level in the blood to reduce the risk of developing coronary disease. Cholesterol-lowering agent or product can be found in the food containing the specific added ingredient (National Institute of Health, 2005). Lowering cholesterol by adopting healthy habits will help in weight loss and increase energy as well as helping

in preventing the cholesterol levels from becoming high. Weight reduction and exercise enhances the decrease the level of LDL-cholesterol that can also be achieved by reducing intake of saturated fats and cholesterol as well as contribute to the enhancement of energy level (Laclaustra *et al.*, 2018).

A study done by (Chen *et al.*, 2010) concluded that both the phytosterols and phytosterols diets significantly decrease the ratios of cholesterol in rat's plasma, red blood cells, liver, aorta and kidney. However, there is evidence that plant sterol and stanol help in reducing the cholesterol blood level, it is essential to remember that they are not a substitute for a healthy diet or a replacement for cholesterol-lowering medicines (Benelam, 2009; Trautwein *et al.*, 2018).

Phytosterols and phytostanols, also referred to as plant sterols and stanols, are common plant and vegetable constituent with structurally related to cholesterol, but differ in the structure of the side chain (Cantrill and Kawamura, 2008). Due to the similar structure to cholesterol, phytosterols and phytostanols can act in the intestine to lower cholesterol absorption by displacing cholesterol from intestinal micelles and due to their poorly absorbed characteristics, blood cholesterol levels will likely drop owing to increase excretion (Murray and Pizzorno, 2012).

Barley is an excellent source of dietary fibre, protein, and complex carbohydrates, and is a good source of specific vitamins and minerals (Balch, 2003). Barley is also a rich source of tocols, including to cophenols and to cotrienols, which are known to reduce serum low-density lipoprotein cholesterol through their antioxidant action (Madhusweta and Sumeet, 2016). According to Newman and Newman (2008), nutritional components of barley are generally reported as averages, when in reality, barley may differ significantly in chemical composition due to genotype, cultural practices, environmental growing conditions and might also be affected by the type of solvent to extract barley. Hence, the main objective for this research is to determine the phenolic content and antioxidant activity as well as the lowering cholesterol level effect of barley extract on rats fed with a high-fat diet.

MATERIALS AND METHODS

Preparation of Extract

Barley was purchased from a local wet market. Approximately 100g of barley was grounded and soaked in 1 Liter of aqueous and ethanol separately for seven days. The extractions were then filtered, driedusing Rota evaporatorand subsequently stored in the refrigerator before use. The working solution dilution of Barley extract was, according to Jaafar *et al.* (2014).

Experimental animal design

The animal ethic was obtained from an animal ethic committee of UniKL MESTECH. A total of fifteen male Sprague Dawley with body weighed between 300 – 400 g were used in this study.

The rats were kept acclimatized for five days under room temperature with 12 hour light and dark cycle and free access to water and standard basal diet.Hereafter, the rats were randomly divided into five groups with three rats in each groupand were treated for 20 days with a high-fat diet, except for the control negative group.

The high-fat diet formulation was adopted from the American Institute of Nutrition (Reeves *et al.*, 1993). The treatment group of ratswas given 500μg/μL barley extract daily (Jaafar *et al.*, 2014).

Determination antioxidant activity of aqueous and ethanol extract

Antioxidant activity of both extracts (aqueous and ethanol extract) was determined by using ABTS (2,2-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt) assay (Re *et al.*, 1999) and DPPH (2,2-diphenyl-1-picrylhydrazyl radical) assay (Brand-Williams *et al.*, 1995).

The test was done in triplicate. The percentage inhibition for both ABTS and DPPH assay was calculated by using the following formula

Percentage of inhibition = $[A_o - A_t] / A_o$ X 100%

where A_o is the absorbance value of radical cation solution and is absorbance value after radical cation solution treated with extract or antioxidant.

Total phenolic content

Total phenolic content of both extracts was determined by using Folin-Ciocalteu assay (Singleton *et al.*, 1999).

Gallic acid was used as a standard, and the total phenolics were expressed as mg/g gallic acid equivalents (GAE). The test was performed in triplicate.

Total cholesterol level

By applying diet planning; throughout 49 days experiment, blood was aspirated from the tail vein of each rat for the determination of cholesterol level.

Total cholesterol level was determined by using EasyTouch® Blood Cholesterol Test Strips with the EasyTouch® Cholesterol Meter.

The cholesterol level was performed in triplicate according to manufacturer protocol.

MDA assay

The lipid peroxidation activity of the treated group of rats was evaluated by using malondialdehyde (MDA) production, according to Ledwozyw *et al.* (1986) method. Total MDA concentration (nmol/mg) that represents lipid peroxidation activity was calculated by using the following formula

MDA = (MDA X dilution factor) / (Protein)

where dilution factor is 20.

Data Analysis

All statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS) statistical software version 17.0. Data were expressed in mean ± standard error of the mean (SEM) (Mean ± SD) and p-value less than 0.05 considered as significant. For statistical analysis, one-way analysis (ANOVA) followed by Tukey's test was applied to compare means among groups. Paired-T-test was used for the comparison between before and after treatment.

RESULTS AND DISCUSSION

Total Phenolic Content and Antioxidant Activity

Total phenols content in two different Hordeum vulgare L. extract was showed in Table 1. It was found that the TPC of ethanol extract of Hordeum vulgare L.is higher than the aqueous extract of Hordeum vulgare L. (Figure 1). Meanwhile, the TAC of Hordeum vulgare L. is also higher in ethanol extract compared with aqueous extract for both ABTS and DPPH radical. In this study, a higher level of TPC was found in ethanol extract may be attributable the complex formation of some phenolic compounds in the extract that is soluble in ethanol whereby these phenolic compounds may possess more phenol groups. Aof Hordeum vulgare L.antioxidant studies has been demonstrated in many studies (Anwar *et al.*, 2010; Zimmermann *et al.*, 2013; Oh *et al.*, 2014) where barley contains much more significant amounts of phenolic compound (0.2 – 0.4%) than other cereal grains which composed of polyphenols, phenolic acids, proanthocyanidins (PAs) and catechins (Quinde *et al.*, 2004).

Food Consume, Body Weight Gain and Feed Efficiency Ratio

In this study, the diet change (basal diet to high-fat diet) was slightly different for each group except rats from control negative group (basal diet) consistently

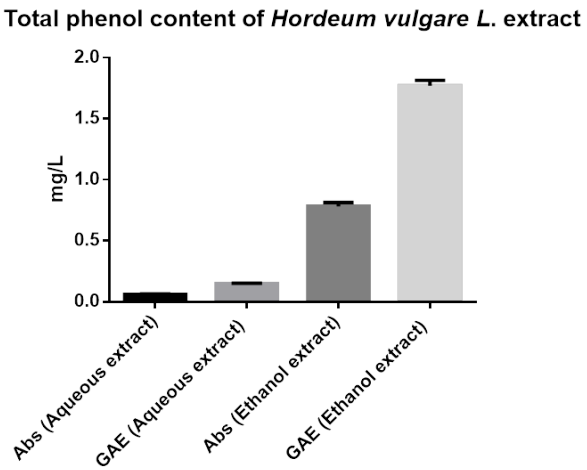

Figure 1: Total phenol content of Hordeum vulgare L. extract

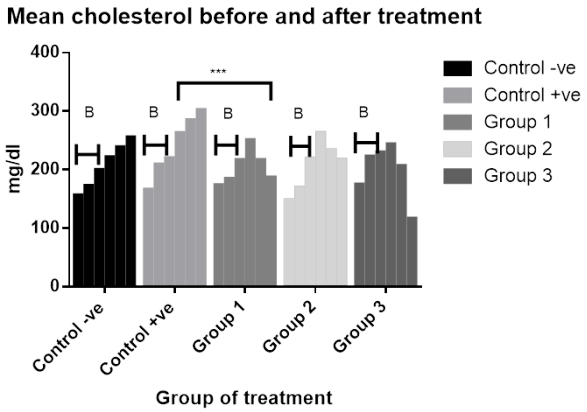

Figure 2: Mean ratcholesterol level before (B) and after treatment. Treatment group, group1 (high fat diet + barley ethanol extract), group 2 (basal diet + barley ethanolextract) and group 3 (high fat diet + Simvastatin) showed significantcholesterol decrease (p = 0.007).

ahead of other groups (data not shown). Meanwhile, rats from control positive group that received a high-fat diet consumed more than group 2 and group 3, which received a high-fat diet with barley and high-fat diet with simvastatin drug.

Initial weight and final weight of every rat (n=15) was taken for the calculation of body weight gain (BWG). There was no significant difference between the diet given and the rat's body weight gain in each group.

During the experimental period, the average food consumption (g) per week was measured. The food consumption before and after treatment were equally similar for each group. In Mahmoud (2013), a decrease of food consumed was observed when the diet changed, which is contradict with this study.

Table 1: Total Antioxidant Activity of Hordeum vulgare L. by ABTS and DPPH Scavenging method

Sample	ABTS			DPPH		
	Abs@ 734nm	% inhibition	TEAC value (μmol/ml)	Abs@ 734nm	% inhibition	TEAC value (μmol/ml)
Aqueous extract	0.630	12.011	39.831	0.707	-18.030	-1.565
Ethanol extract	0.588	17.877	130.07	0.547	8.681	-1.187

TEAC – Trolox Equivalent Antioxidant Capacity

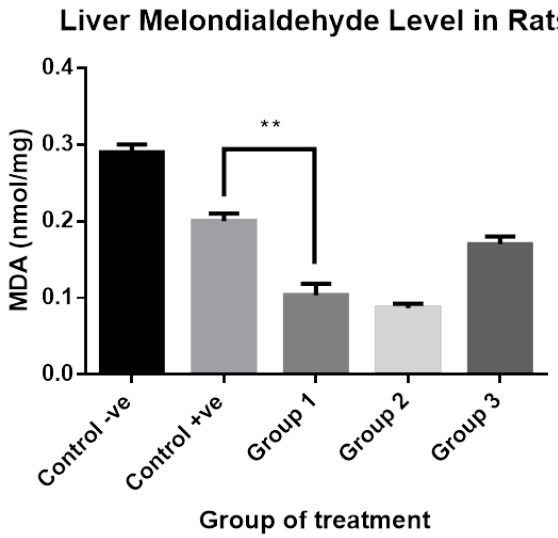

Figure 3: Malondialdehyde level in rats liver. It was found that MDA level in group 1 significantly lowerthan control lively group (p = 0.04)

This could be the addition of barley extract was well accepted, and it did not influence the acceptability of the diet by the rats.

Cholesterol Level of the Sprague Dawley

Mean cholesterol level after treatment for rats from control positive group that received high-fat diet was higher than rats from group 1 which receive high-fat diet with barley ethanol extract (p = 0.007) and group 3 which received a high-fat diet with simvastatin drug (Figure 2). When the mean cholesterol level after treatment was compared between group 1 and group 3, the cholesterol level in group 3 was slightly decreased than group 1 (p = 0.18).

Studies indicate that increase in high fat dietary intake in an animal could lead to hypercholesterolemia (Kishida *et al.*, 2002; Czerwiński *et al.*, 2004). Hypercholesterolemia is characterized by increased blood cholesterol levels above the normal range (Steinberg, 2011). In this study, before treatment started, all group of rats showed an average increase in cholesterol level after some time. Yet, after the treatment started, group 1, group 2, and group 3 exhibited a decrease in cholesterol levels. Group 1 and group 2 received an additional supplement of barley in their diet. By examine on a group that received barley extract in the daily diet, the similar results with Mahmoud (2013) study were obtained. Whereby a cholesterol level of rats was successfully lowered.

Barley was found contains β-glucan that lowering blood cholesterol level. The effectiveness of β-glucan in barley food products in lowering blood cholesterol has been reported in numerous publications (Pins, 2006). Besides that, barley is also rich of tocols, including tocopherols and tocotrienols, which are known for reducing serum LDL through their antioxidant action (Qureshi *et al.*, 1991). Hence, barley ethanol extract might contain a lot of β-glucan, tocopherols and tocotrienols.

Lipid Peroxidation

MDA level in the group supplemented with barley ethanol extract, group 1 and group 2, are significantly lower when compared to control positive (p = 0.04, Figure 3). A study by Yang *et al.* (2008) reported that hypercholesterolemia responsible for oxidative modification of LDL, protein glycation, glucose-autooxidation with excess production of free radicals and lipid peroxidation products which represent a significant risk factor for cellular damage where MDA level is increased. MDA is a protein that involves in hyperlipidemia which will provoke free radical attacks on membrane lipoproteins and polyunsaturated fatty acids. It is also the primary source of oxidative stress in rat liver (Ming *et al.*, 2009; Marczuk-Krynicka *et al.*, 2009).

CONCLUSIONS

When the barley ethanol extract was subjected to the rat's diet as a supplement, decreasing cholesterol level was observed. The presence of higher phenolic content in barley ethanol extract might be the cause of decreased MDA level in rat's liver. How-

ever, these need to be further study by using histology analysis and HPLC analysis.

ACKNOWLEDGEMENT

This project was supported by the Student's Final Year Project Grant.

Conflict of interest

The authors declare that they have no conflict of interest for this study.

Funding support

The authors declare that they have no funding support for this study.

REFERENCES

Anwar, F., Qayyum, H. M. A., Hussain, A. I., Iqbal, S. 2010. Antioxidant activity of 100% and 80% methanol extracts from barley seeds (Hordeum vulgare L.): stabilization of sunflower oil. *Grasas y Aceites*, 61(3):237–243.

Balch, P. A. 2003. Prescription for Dietary Wellness 2nd Ed. United States of America: Penguin group.

Benelam, B. 2009. Satiation, satiety and their effects on eating behaviour. *Nutrition Bulletin*, 34(2):126–173.

Brand-Williams, W., Cuvelier, M. E., Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Science and Technology*, 28(1):25–30.

Brown, J. E. 2013. Nutrition Now. 7th Ed. United States of America.

Cantrill, R., Kawamura, Y. 2008. Phytosterols, Phytostanols and Their Esters Chemical and Technical Assessment. 69th Join the FAO/WHO Expert Committee onFood Additives (JECFAI). *Phytosterols, phytostanols and their esters (CTA)* , pages 1–13.

Chen, Q., Gruber, H., Swist, E., Coville, K., Pakenham, C., Ratnayake, W. M., Scoggan, K. A. 2010. Dietary phytosterols and phytostanols decrease cholesterol levels but increase blood pressure in WKY inbred rats in the absence of salt-loading. *Nutrition & Metabolism*, 7(1):11.

Czerwiński, J., zbieta Bartnikowska, E., Leontowicz, H., Lange, E., Leontowicz, M., Katrich, E., Trakhtenberg, S., Gorinstein, S. 2004. Oat (Avena sativa L.) and amaranth (Amaranthus hypochondriacus) meals positively affect plasma lipid profile in rats fed cholesterol-containing diets. *The Journal of Nutritional Biochemistry*, 15(10):622–629.

Isaacs, J., Brown, J. E., Krinke, B., Lechtenberg, E., Murtaugh, M. 2011. NutritionThrough the Life Cycle. 4th Ed. United States of America.

Jaafar, N. J., Ambia, K., Md, Lian, H. H., Noah, R., Md 2014. The Effect of Mixture of Alpinia Galanga, Eurycoma Longifolio Jack and Syzygium Aromaticum Crude Extract on the Growth of Saccharomyces cerevisiae and Escherichia coli. *Journal of Life Sciences*, 8(5):399–403.

Kishida, T., Nogami, H., Ogawa, H., Ebihara, K. 2002. The Hypocholesterolemic Effect of High Amylose Cornstarch in Rats Is Mediated by an Enlarged Bile Acid Pool and Increased Fecal Bile Acid Excretion, Not by Cecal Fermented Products. *The Journal of Nutrition*, 132(9):2519–2524.

Laclaustra, M., Lopez-Garcia, E., Civeira, F., Garcia-Esquinas, E., Graciani, A., Guallar-Castillon, P., Banegas, J. R., Rodriguez-Artalejo, F. 2018. LDL Cholesterol Rises With BMI Only in Lean Individuals: Cross-sectional U.S. and Spanish Representative Data. *Diabetes Care*, 41(10):2195–2201.

Ledwozyw, A., Michalak, J., Stepien, A., Kadziolka, A. 1986. The Relationship Between Plasma Triglycerides, Cholesterol, Total Lipids and Lipid Peroxidation Products During Human Atherosclerosis. *Clinica Chimica Act*, 155(3):275–283.

Madhusweta, D., Sumeet, K. 2016. Status of Barley As A Dietary Component for Human. *Research and Review: Journal of Food and Dairy Technology*, 1:2347–2359.

Mahmoud, M. Y. 2013. Natural Antioxidants Effect of Mulberry Fruits (Morus nigra and Morus alba L.) On Lipids Profile and Oxidative Stress in Hypercholestrolemic Rats. *Pakistan Journal of Nutrition*, 12(7):665–672.

Marczuk-Krynicka, D., Hyryniewiecki, T., Paluszak, J., Krauss, H., Nowak, D. 2009. High-Fat Content in Diets and Oxidative Stress in the Liver of Non-Diabetic and Diabetic Rats. *Polish Journal of Environment Studies*, 18(2):249–253.

Ming, M., Guanhua, L., Zhanhai, Y., Guang, C., Xuan, Z. 2009. Effect of the Lycium barbarum polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo. *Food Chemistry*, 113(4):872–877.

Murray, M. T., Pizzorno, J. 2012. The Encyclopedia of Natural Medicine. United States of America. Simon and Schuster Publication.

National Institute of Health 2005. Your Guide to Lowering Your Cholesterol with TLC.

Nayak, B. S. 2008. Manipal Manual of Clinical Biochemistry (for Medical Laboratory and Msc Students). Jaypee.

Newman, R. K., Newman, C. W. 2008. Barley for Food and Health: Science,Technology, And Products. United States of America. John Wiley and

Sons Inc.

Oh, S., Yi, B., Ka, Hj, Song, J., Park, J., Jung, J., Kim, M. J., Park, K. W., Lee, J. H. 2014. Evaluation of In-vitro Antioxidant Properties of Roasted Hulled Barley (Hordeum vulgare L). *Food Science and Biotechnology*, 23(4):1073–1079.

Pins, J. J. 2006. A Review of the Effects of Barley beta-Glucan on Cardiovascular and Diabetic Risk. *Cereal Foods World*, 51(1):8–11.

Quinde, Z., Ullrich, S. E., Baik, B. K. 2004. Genotypic Variation in Color and Discoloration Potential of Barley-Based Food Products. *Cereal Chemistry Journal*, 81(6):752–758.

Qureshi, A. A., Chaudhary, V., Weber, F. E., Chicoye, E., Qureshi, N. 1991. Effects of brewer's grain and other cereals on lipid metabolism in chickens. *Nutrition Research*, 11(2-3):159–168.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9-10):1231–1237.

Reeves, P. G., Nielsen, F. H., Fahey, G. C. 1993. AIN-93 Purified Diets for Laboratory Rodents: Final Report of the American Institute of Nutrition Ad Hoc Writing Committee on the Reformulation of the AIN-76A Rodent Diet. *The Journal of Nutrition*, 123(11):1939–1951.

Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. 1999. Analysis of Total Phenols and Other Antioxidant Substrates and Antioxidant using Folin-Ciocalteu reagent. *Method Enzymol*, 299:152–178.

Steinberg, D. 2011. The Cholesterol Wars: The Skeptics vs The Preponderance of Evidence. USA. Academic Press.

Trautwein, E., Vermeer, M., Hiemstra, H., Ras, R. 2018. LDL-Cholesterol Lowering of Plant Sterols and Stanols—Which Factors Influence Their Efficacy? *Nutrients*, 10(9):1262.

Yang, R. L., Shi, Y. H., Hao, G., Li, W., Le, G. W. 2008. Increasing Oxidative Stress with Progressive Hyperlipidemia in Human: Relation between Malondialdehyde and Atherogenic Index. *Journal Clin. Biochem. Nutr*, 43(3):154–158.

Zimmermann, A. M., Rivero, A. C., Bezerra, A. S., Ruviaro, A. R., Novack, M. M. E., Nörnberg, J. L. 2013. Antioxidant potential of barley extract in rats subjected to a high-fat diet. *Food Science and Technology*, 33(1):167–171.