

Full Length Research Paper

Study on the biochemical effects of barley fiber on the hypercholesterolaemic rats

Sakhawat Ali¹, Saima Nazir¹, Shumaila Usman¹, Zahida Nasreen^{1*}, Ume Kalsoom² and Tanzeela Inam²

¹Biotechnology and Food Research Centre, PCSIR Labs Complex, Lahore 54600, Pakistan.

²Institute of Molecular Biology and Biotechnology, the University of Lahore, Pakistan.

Received 18 May, 2011; Accepted 27 February, 2014

Chemical compositions and nutritive value of Barley (*Hordeum vulgare*) samples (barley grain and barley hull) were determined by proximate analysis. The percentage value of moisture, ash, protein, fat, fiber, cellulose, lignin and carbohydrate in barley whole grain and hull is deliberated. Moreover, evaluation of functional properties of barley, anti-hypercholesterolaemic effect of barley hull high fiber diet is studied. The in house prepared barley hull high fiber diet proved effective as it lowered the cholesterol levels significantly in hypercholesterolaemic rat.

Key words: Nutritive value, barley and anti hyper cholesterol.

INTRODUCTION

Barley is a cereal grain derived from the annual grass *Hordeum vulgare*. It serves as a major animal feed crop, with smaller amounts used for malting (mostly for beer and certain distilled beverages) and in health food. Barley is an important cereal food all over the world. It is abundantly used in Africa, Asia and semi-arid tropics and is also cultivated in Europe, America and Australia (Keogh et al., 2003; Erkan et al., 2006).

Barley is the second most important winter cereal in Pakistan. It grows on an area of 107.7 thousand hectares with production of 99.6 thousand tones. The average yield is 925 kg per hectare (Government of Pakistan, 2008). About two third of the area devoted to barley in Pakistan is rained and one third is irrigated.

Barley contains many nutrients, including dietary fiber, antioxidants, vitamins, minerals (calcium, magnesium, potassium, phosphorus) sphingolipids and unsaturated fatty acids. This diverse composition allows barley products to have myriad of benefits and appealing characteristics (Oscarsson et al., 1996). Barley is a nutritious cereal grain that offers consumers many bio-active compounds that can help improve their health. The barley is also used as neutraceutical ingredient because it contains high content of soluble fiber, especially as a rich source of β -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.

*Corresponding author. E-mail: zahidan nasreen_pcsr@yahoo.com. Tel: 920429230688.

Many agencies also encourage an increase fiber intake. Barley grain is an excellent source of both soluble and insoluble dietary fiber (DF) with clinically proven health benefits. Beta Glucan (BG) the major fiber constituents in barley have been shown to lower the plasma cholesterol, reduce glycemic index and reduce the risk of colon cancer (Erkan et al., 2006). Other more general beneficial physiological effects of consumption of whole grains include reduced transit time for foods, which may reduce risk of colon cancer (Bruce et al., 2000), and reduced absorption of nutrients which may reduce glucose and insulin responses and risk of obesity (Wisker et al., 1994, 1992).

The purpose of the present work was to investigate the biochemical composition of barley and to estimate hypocholesterolaemic effect of high fiber diet on hypercholesterolaemic rats. We also aimed to prove hypothesis that it reduces cardiovascular disease risk comparatively with other sources of soluble fiber.

MATERIALS AND METHODS

Procurement and cleaning of raw material

Barley whole grain used in this study was purchased from local market. Barley grain and hull was cleaned manually. After removal of foreign material, weeds and non-grain matters, samples were stored in clean polythene bags until used.

Estimation of Moisture, Ash Fat and Fiber

The moisture, Ash and fat contents were determined by using method of (AOAC, 2012). 3 to 5 g of dried sample was in use in each assessment.

Estimation of protein

Protein content of barley and hull sample was estimated by using Kjeldahl method as reported by AOAC, 2012

Estimation of lignin

Lignin content was estimated by the method of A.S.T.M (1961). 1 g of defatted sample was used for reflux with 70 mL 1.25% H₂SO₄ for 2 h. Reflux sample washed with hot water and then with chloroform. Washed sample treated with 72% H₂SO₄ for four hours with constant stirring. Sample was filtered (add 10 -12 mL distilled water) with Whatman filter paper No-1 and ignited it at 550°C for 4 h.

Estimation of cellulose

Cellulose estimated by using the method of Kurschner and Hanak (1930). Missing) 1 g of defatted sample was used for reflux with 15 mL of 80% acetic acid and 1.5 mL conc. HNO₃ acid for 2 h. Reflux sample washed with hot water. Washed sample was treated with 72% H₂SO₄ for four hours with constant stirring. Sample was filtered (add 10 to 12 mL distilled water) with Whatman filter paper No. 1

washed with distilled water until it become neutral and finally it washed with alcohol and ignited it at 550°C for 6 h.

Estimation of carbohydrates

The carbohydrates were determined by applying following equation. Moisture + Ash + Fat + Fiber + Protein - 100 = CHO.

1) Development of high fiber diet

The complete dehulling of barley hull from barley groats carried out. Barley hull was used as fiber source in diet. After grinding of barley hull, fiber was extracted by hydrolysis. Acid hydrolysis of extract was carried out with dilute sulphuric acid. After hydrolysis, fiber was neutralized by NaOH. Sample was washed with the distilled water for the complete removal of NaOH.

2) Composition of diet

Twenty five (25 g/kg) barley bran high fiber diet contained 175 g/kg casein protein to fulfill the protein requirements in animal body. After that 12 g/kg corn oil was added in diet to provide essential fatty acids. Corn starch and sucrose was added in diet as sweetness and carbohydrates source. DL- Methionine added in diet for amino acid source. Vitamin mixture and mineral mixer was added 10 and 35 g/kg diet, respectively. Barley bran was used as a fiber source and 257 g barley bran was added in per kg diet (Remi et al., 1992). After manually mixing, diet was pelleted with water and dried in oven and store in air tight bags.

Feed intake

Feed intake was determined by the weighing the feed hoppers. Consumption, expressed as grams per rat per day, was obtained by dividing food intake by the number of rats per cage (Jackson et al., 1994). The average feed in take was 13 g/day/rat.

Functional properties of barley bran high fiber diet

High fiber barley hull diet was used for the determination of functional properties of barley by the determination of antihypercholesterolemic effect of high fiber barley hull diet on hypercholesterolaemic rat.

Experimental design and induction of hypercholesterolemia

Thirty (30) albino rats of either sex weighing between 200 to 300 g were selected for the experiment. The rat was divided into 3 groups each group consist of 10 rats.

Induction of Hypercholesterolemia

The rat was made Hypercholesterolaemic by oral administration of 1% of cholesterol powder (1 g/kg) for 10 days as prescribed by (Reeves et al., 1993). Then, the hypercholesterolaemic condition was confirmed by using respective diagnostic kit at the 11th day of experiment. After confirmation of hypercholesterolaemic condition of rats, the day at which rats were treated with 25% barley bran high fiber diet was considered as first day of experiment.

Table 1. Proximate analysis of whole grain barley and hull of barley.

Parameter	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	Fiber (%)	Cellulose (%)	Lignin (%)	Carbohydrate (%)
Barley whole grain	11.5 ± 0.38	2.5 ± 0.30	12.5 ± 0.26	1.8 ± 0.15	4.4 ± 0.21	6.6 ± 0.26	0.6 ± 0.21	67.30
Barley hull	6.43 ± 0.25	4.13 ± 0.15	9.2 ± 0.15	1.23 ± 0.20	26.3 ± 0.85	37.5 ± 0.3	2.67 ± 0.25	52.71

The mean value was calculated by applying standard deviation.

Blood collection and analysis

One (1) ml blood was collected from coccygeal vein of albino rats. Collected blood was centrifuged at 3000 rpm for 10 min and serum was separated. The enzymatic kit was used to assess serum levels using spectrophotometer.

Examination of serum cholesterol

The cholesterol level was determined by using method of (Richmond, 1973).

Estimation of cholesterol by enzymatic kit

Twelve (12) test tube were used, two of them labeled as blank/standard. Remaining 10 tubes were labeled as 1, 2, 3.....10 for each sample of rat's serum from each hypercholesterolemic group. 1000 µl reagent was taken in all the tubes by micro pipette. 10 µl of standard solution from kit was added to the tube labeled as standard and 10 µl of serum sample was taken in tubes labeled as 1, 2, 3.....12. Contents of all the tubes were mixed well and then incubated at 37°C for 10 min. Absorbance of standard and sample was measured against the blank at wavelength of 546 nm. Similar procedure was used the rest of the samples.

Statistical analysis

The data were subjected to analysis of variance (ANOVA) using Co Stat-2003 software following the method as described by (Steel and Torrie, 1982). The Duncan multiple range (DMR) was used to determine the level of significance between samples.

RESULTS

The values of moisture, fat, ash, protein, fiber, carbohydrates, lignin and cellulose in whole grain barley and hull of barley are given in Table 1. The moisture content (11.5±0.38) and fat content (1.8±0.15) of whole grain barley was higher than the moisture content (6.43±0.25) and fat content (1.23±0.2%) of hull of barley. The whole grain barley had lower ash contents (2.5±0.30%) as compared to hull of barley (4.13±0.1%). The increased value of crude protein in whole grain barley was 12.5 ± 0.26%. In addition to it was observed that the percentage fiber content of whole grain barley was lower than the fiber content of hull of barley.

Contrary, carbohydrate value in whole grain barley was higher than the hull of barley (67.3 and 52.71%)

respectively. Proximate composition of the whole grain barley and hull of barley showed that the crude lignin of whole grain barley was lower than the hull of barley. Also the cellulose contents in whole grain barley were lower as compared to hull of barley.

Cholesterol level (mg/dl)

In group A (control) the mean value of cholesterol level at day 0, 1, 14 and 28 of the experiment were 157 mg/dl, 159 mg/dl, 158 mg/dl and 159 mg/dl respectively. Slight variation in cholesterol level from day 1st to 28 was observed which was insignificant.

DISCUSSION

Most research studies have shown the use of barley and barley product as a source of soluble fiber (Dongowski et al., 2002). Barley provides a potential source of low cost protein with good nutritional assessment. The use of Barley in human diet has also been motivated by the quality of its fiber components which act as hypoglycemic and hypocholesterolemic agents. Barley was selected as cereal source because it is rich in important fiber components. The barley contains substantially higher amounts of functional ingredient β-glucan. The use of β-glucan extracted from barley as human food due to its positive role in human health has received a growing attention (Carpita, 1996).

Our results of proximate analysis of barley sample (barley whole grain and barley hull) and hypocholesterolaemic effect of barley high fiber diet are given in Table 2, which, correlated the earlier findings for barley varieties by (Keogh et al., 2003).

The values of moisture, fat, ash, protein, fiber, carbohydrates, lignin and cellulose in whole grain barley and hull of barley are given in Table 3. The moisture content (11.5±0.38) and fat content (1.8±0.15) of whole grain barley was higher than the moisture content (6.43±0.25) and fat content (1.23±0.2%) of hull of barley (Anderson et al., 1984). The whole grain barley had lower ash contents (2.5±0.30%) as compared to hull of barley (4.13±0.1%), these results are in line with earlier findings of Bridges et al 1992, who have also observed that same combination in case of oat and barley. Increase value of

Table 2. Cholesterol level (mg/dl).

Group	Cholesterol level 0 Day (before Hypocholesterolaemia) (Mean ± S.D)	Cholesterol level 1 Day (after Hypocholesterolaemia) (Mean ± S.D)	Cholesterol level 14 Day (after Treatment) (Mean ± S.D)	Cholesterol level 28 Day (after Treatment) (Mean ± S.D)
Group A: Control	157 ± 4.42	159 ±4.31	158 ± 4.13	159 ± 4.85
Group B: hypocholesterolaemic rats + chick starter diet	162 ± 6.89	220 ± 3.70	198 ± 1.19	174± 1.58
Group C: hypocholesterolaemic rats + high fiber diet	211± 5.10	223 ± 5.73	179 ± 7.30	167 ± 6.70

Table 3. Multiple comparisons of cholesterol level (mg/dl) among different groups.

Dependant variable	Group	Group	Mean difference	Std. Error	Significant
Cholesterol level day 1	Group A Control	Group B: hypocholesterolaemic + chick starter diet	-61.6000	2.08487	0.000*
		Group C: hypocholesterolaemic + high fiber diet	-64.6000	2.08487	0.000*
	Group B hypocholesterolaemic + chick starter diet	Group A: Control	61.6000	2.08487	0.000*
		Group C: hypocholesterolaemic +diet	-3.0000	2.08487	0.162**
	Group C hypocholesterolaemic +diet	Group A: Control	64.6000	2.08487	.000 *
		Group B: hypocholesterolaemic + chick starter diet	3.0000	2.08487	.162**
Cholesterol level day 14	Group A Control	Group B: hypocholesterolaemic + chick starter diet	-67.1000	2.18920	.000*
		Group C: hypocholesterolaemic +diet	-21.3000	2.18920	.000*
	Group B hypocholesterolaemic + chick starter diet	Group A: Control	67.1000	2.18920	.000*
		Group C: hypocholesterolaemic +diet	45.8000	2.18920	.000*
	Group C hypocholesterolaemic +diet	Group A: Control	21.3000	2.18920	.000*
		Group B: hypocholesterolaemic + chick starter diet	-45.8000	2.18920	.000*
Cholesterol level at day 28	Group A Control	Group B: hypocholesterolaemic + chick starter diet	-59.2000	2.17647	.000*

Table 3. Contd.

	Group C: hypocholesterolaemic +diet	-8.6000	2.17647	0.07**
Group hypocholesterolaemic + chick starter diet	Group A: Control	59.2000	2.17647	0.000*
	Group C: hypocholesterolaemic + high fiber diet	50.6000	2.17647	0.000*
Group C hypocholesterolaemic +diet	Group A: Control	8.6000	2.17647	0.001*
	Group B: hypocholesterolaemic + chick starter diet	-50.6000	2.17647	0.000*

crude protein in whole grain barley was $12.5 \pm 0.26\%$, similar findings of protein content were estimated by Jackson (1994) in same cereals. In addition to it was observed that the percentage fiber content of whole grain barley was lower than the fiber content of hull of barley. Significant increase in fiber content in hull of barley was reported by Gorinstein et al. (2002). Barley grain is excellent source of soluble and insoluble dietary fiber.

The cellulose content of whole grain barley and hull of barley is given in Table 1. The cellulose and lignin content of whole grain barley was than hull of barley. These results are comparable with those reported by Jacobs et al. (1998).

The second part of this research was aimed to estimate the cholesterol lowering effect of high fiber diet. It was inferred from the outcomes of the present study that the barley high fiber significantly lowers the cholesterol level in hypercholesterolaemic rats.

In this experiment blood analysis was conducted to estimate the cholesterol lowering effect of high fiber diet of barley on hypercholesterolaemic rat. Significant reduction in cholesterol was observed in hypercholesterolaemic group C hypercholesterolaemic high fiber diet as compared to hypercholesterolaemic control.

Significant reduction in cholesterol was observed in hypercholesterolaemic group C hypercholesterolaemic high fiber diet) as compared to hypercholesterolaemic control group.

Barley dietary fiber is high in β -glucan, which helps to lower cholesterol by binding to bile acids and removing them from the body via the feces. Bile acids are compounds used to digest fat that are produced by the liver from cholesterol (Behall et al., 2004).

When they are excreted along with barley fiber, the liver must produce new bile acids and uses up more cholesterol, thus lowering the amount of cholesterol in circulation. Soluble fiber may also minimize the amount of cholesterol prepared in liver (Bazzano et al., 2003).

Conflict of Interests

The author(s) have not declared any conflict of interests.

Conclusion

It can be concluded from the present study that the highest soluble fiber intake had the greatest effect on total cholesterol. The outcomes of present study indicate that the addition of barley to a healthy diet can reduce risk of cardiovascular disease by reducing cholesterol level in hypercholesterolaemic conditions.

REFERENCES

- AOAC (Association of Official Analytical Chemist) 2012. Official method of analysis 19th Ed. Washington D.C.
- Anderson JW, Storey L, Sieling B (1984). Hypercholesterolemic effects of oat bran or bean intake for Hypercholesterolemic men. *J. Am. Clin. Nutr.* 40:1146.
- ASTM (1961). American Society for Testing Materials and standard method of lignin in wood. 1106-56T 848:71-79.
- Bazzano LA, Ogden LG, Loria CM, Whelton PK (2003). Dietary fiber intake and reduced risk of coronary heart disease in US men and women. *Arch. Intern. Med.* 163(16):1897-1904.
- Behall KM, Scholfield DJ, Hallfrisch JG (2004). Lipids significantly reduced by diets containing barley in moderately hypercholesterolemic men. *J. Am. Coll. Nutr.* 23:55-62.
- Bridges SR, Anderson JW, Deakins DA, Dillon DW, Wood CL (1992). Oat bran increases serum acetate of hypercholesterolemic men. *Am. J. Clin. Nutr.* 56:455-459.
- Bruce B, Spiller, Klevay LM, Gallagher SK (2000). A diet high in whole and unrefined foods favorably alters lipids, antioxidant defenses, and colon function. *J. Am. Coll. Nutr.* 19:61-67.
- Carpita NC (1996). Structure and biogenesis of cell walls of grasses. *Annl. Rev. Plant Physiol. Plant Mol. Biol.* 47:445-476.
- Dongowski G, Huth M, Gebhardt E, Flamme W (2002). Dietary fiber-rich barley products beneficially affect the intestinal tract of rats. *J. Nutr.* 32:3704-3714.
- Erkan H, Celik S, Bilgi B, Koksel H (2006). A new approach for the utilization of barley in food products: Barley tarhana. *Food Chemistry*, 97:12-18.

- Gorinstein S, Pawelzik E, Delgado-Licon E, Haruenkit R, Weisz M, Rakhtenberg S. (2002). Characterization of pseudo cereals and cereals proteins by protein and amino acid analyses. *J. Sci. Food Agric.* 82:886-891.
- Government of Pakistan (2008). Government of Pakistan, Finance Division, Economic Adviser Wing Islamabad, Pakistan.
- Jackson KA, Suter DAI, Torring DL (1994). Oat bran, barley and malted barley lower plasma cholesterol relative to wheat bran but differ in their effect on cholesterol in rat feed diet with and without cholesterol. *J. Nutr.* pp.1678-1684.
- Jacobs DR, Marquart L, Slavin J, Kushi LH (1998). Whole grain intake and cancer: An expanded review and meta-analysis. *Nutr. Cancer* 30:85-96.
- Keogh GF, Cooper GJS, Mulvey TB, McArdle BH, Coles GD, Monro JA, Poppitt SD (2003). Randomized controlled crossover study of the effect of a highly beta-glucan-enriched barley on cardiovascular disease risk factors in mildly hypercholesterolemic men. *Am. J. Clin. Nutr.* 78:711-718.
- Oscarsson M, Andersson R, Salomonsson AC, Aman P (1996). Chemical composition of barley samples focusing on dietary fiber composition. *J. Cereal Sci.* 24:161-170.
- Remi DS, Dirk F, Annick V (1992). Cholesterol lowering effects and utilization of protein lipid, fiber and energy in rats fed unprocessed and baked oat bran. *J. Nutr.* 22:1318-1324.
- Richmond W (1973). Cholesterol enzymatic, lipid, colourimetric test-CHOD/PAP method *Clinical Chemistry*, 19:1350.
- Steel RGD, Torrie JH (1982). Principles and procedures of statistics 2nd edition, McGraw, Hill Book, New York (USA).
- Wisker E, Godau A, Daniel M, Peschutter G, Feldheim W (1992). Contribution of barley fiber to the metabolizable energy of human diets. *Nutr. Res.* 12:1315-1323.