Pakistan J. Zool., vol. 49(5), pp 1631-1637, 2017. **DOI**: http://dx.doi.org/10.17582/journal.pjz/2017.49.5.1631.1637

Effect of Wheat and Corn Bran and Barley and Sorghum ß-Glucan Extracts on the Plasma Cholesterol Level of Dietary-Induced Hypercholesterolemic Rats

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Article Information
Received 11 May 2016
Revised 10 September 2016
Accepted 26 September 2016
Available online 22 August 2017

Authors' Contribution
AH conceived and designed the study, executed the experimental trials, analyzed the data and wrote the article. MI and SK helped in article writing.

Key words
Hypercholesterolemia, Cardiovascular disease, Wheat bran feed, Maize bran feed, Sorghum β glucan feed, Barley β glucan feed, Triglycerides, Lipid profile, Total cholesterol.

ABSTRACT

Hypercholestrolemia leading to cardiovascular diseases are very common problem in Pakistan and in most cases it is caused due to insufficient intake of dietary fiber. A study was conducted to evaluate the role of dietary fiber and ß-glucan from major food grains used locally on plasma cholesterol level of dietary-induced hypercholesterolemic rats. Insoluble dietary fiber (lignin and cellulose) was extracted from wheat and maize, while ß glucan was extracted from barley and sorghum. Male rats (n = 32), weighing 200-220 g were induced with hypercholesterolemia and were equally divided into 5 groups to study the effect of purified diet-AIN-93 G, wheat bran feed (WBF) , maize bran feed (MBF), sorghum ß glucan feed (SGF) and barley ß glucan feed (BGF) on plasma total cholesterol (TPC), very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride level. Group I comprised control group of induced hypercholesterolemic rats fed on purified diet AIN-93 G. Group II comprised induced hypercholesterolemic rats fed on WBF. Group III comprised induced hypercholesterolemic rats fed on MBF, Group IV comprised hypercholesterolemic rats reared on SGF and Group V comprised hypercholesterolemic rats fed on BGF. Both BGF and SGF significantly lowered the TPC in experimental animals compared with purified diet AIN-93, WBF and MBF. The VLDL, LDL and triglycerides levels were also decreased accordingly while a higher ratio of HDL was recorded in rats fed on BGF and SGF compared to the other feeds. The results suggest that BGF and SGF containing the soluble ß-glucan extract can reduce TPC, VLDL, LDL, and triglycerides and higher HDL.

INTRODUCTION

More than half of the total dietary energy is obtained through cereals grains due to their high percentage of carbohydrate content throughout the world. Wheat (*Triticum aestivum* L.) is the most widely used cereal grain and its nutritional properties make it a staple food around the world. The wheat grain can be ground and treated to produce various products ranging from whole grain wheat, flour, refined flour, semolina, *etc*. Wheat is of a great benefit and makes it a nutritionally-balanced staple crop that saves millions from deficiency diseases (Rao *et al*., 1989). Similarly maize (*Zea mays* L.) has a tremendous market in the rural reigns of Pakistan as it is eaten in the form of thick bread with vegetable curry (Venkatesh *et al*., 2003; Shobha *et al*., 2010). Barley (*Hordeum vulgare* L.) is also consumed by people around the world and its straw is used as animal fodder and can be ranked as the fourth most widely cultivated crop in the World (Akar *et al*., 2004; Pourkheirandish and Komatsuda, 2008). Likewise sorghum (*Sorghum bicolor* L.) is one of the oldest cultivated cereals and is widely cultivated in the Pakistan. Both barley and sorghum are used as fodder and as cereal grain for human consumption. The flour from both the cereals can be coarsely ground to be used as porridges and side dishes in dry tracts in Pakistan, Africa, Central America, and South Asia (Iqbal and Iqbal, 2015). Sorghum flour can also be mixed with other cereals and used as chapptti (flat bread) (Imran *et al*., 2016) .

Epidemiologic studies showed that the intake of dietary fiber was negatively correlated to the hypercholesterolemia (Kushi *et al*., 1985; Kromhout *et al*., 1982; Khaw and Barrett-Connor, 1987). Whole wheat and wheat fiber has been known to reduce the cholesterol level in studies carried out on human subjects as well as on experimental animals (Erkkila *et al*., 2005; Swain *et*

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0030-9923/2017/0005-1631 $ 9.00/0

al., 1990). Studies have supported the fact that due to the presence of insoluble dietary fibers and soluble dietary fiber such as ß glucans, barley can lower the cholesterol and low-density-lipoproteins to as significant level (McIntosh *et al*., 1999; Oakenfull and Fenwick, 1978; Fastnaught, 2001). Likewise sorghum ß-d-glucan extract has also cholesterol-lowering effect and has been shown to regulate hepatic cholesterol metabolism in experimental mice (Kim *et al*., 2015).

The ß-d-glucan fraction present in various cereals is known to have a hypocholesterolemic effect (Bourdon *et al*., 1999). It is also known to enhance the phagocytosis which in turn decreases LDL-cholesterol in blood (Kerckhoffs *et al*., 2003) which in turn may reduce the risk of coronary heart disease and ischemic heart disease (Keogh *et al*., 2003).

The present study was aimed (i) to compare the nutritional composition of wheat, corn, barley and sorghum produced in Pakistan and (ii) to determine the hypocholesterolemic effect of crude dietary fiber extracted from wheat and maize and ß-glucan extracted from sorghum and barley on the plasma lipid profile of the hypercholesterolemic rats.

MATERIALS AND METHODS

Animals

Forty male *Sprague Dawley*, albino rats 4 to 8 months old, weighing 200-220 g, were housed in the Animal House of PCSIR Laboratories at 23°C to 26°C. The rats were acclimatized for one week.

The rats were divided into four groups, each of 8 rats. Each rat was kept individually in wire-bottom stainless steel cages, with food and tap water provided *ad labitum* for a period of four weeks. The average feed intake was 12-16g/day for an individual rat. The weight of the animals was also recorded at the end of each week.

Induction of hypercholesterolemia

The rats were induced with hypercholesterolemia by addition of cholesterol powder, bile salt and dried animal fat to the standard diet in percentage of 1%, 0.25% and 4%, respectively (Schurr *et al*., 1972; Minhajuddin *et al*., 2005; Pengzhan *et al*., 2003). Cholesterol powder was purchased from Simga-Aldrich and bile salts from Becton, Dickinson (BD) U.S.A. The animal fat was obtained from the local meat market. This preparation was fed to the rats for two weeks. The condition of hypercholesterolemia was confirmed on 15th day by using diagnostic kit.

Chemical evaluation of feed additives

Whole grain seeds of wheat, maize and barley were purchased from The National Seed Council. The nutritional composition of the raw ground samples of indigenously produced varieties of Wheat Inqlab-91, Maize MMRI Yellow, Sorghum F-9917 and Barley Nal-03 was determined according to AOAC (2005). The moisture, fat, protein and ash was analyzed by AOAC (2005), crude dietary fiber by Weende Method (Van Soest and McQueen, 1973), cellulose by Kurschner and Hank (1930) method, lignin by Calixto *et al*. (1983) and the ß-glucan content was analyzed by method mentioned by McCleary and Codd (1991).

For the extraction of bran from wheat and maize, the seeds were ground in grinders, after grinding the hull and aleurone layers were separated. The ground whole grain mixture was then sieved using a sieve of pore size 100 µm to separate the bran from the rest of the seeds components. The process of sieving was repeated several times to obtain the maximum percentage of dietary fiber from the samples (Anonymous, 1989; Bohm *et al*., 2011).

The barley and sorghum grains were milled and passed through 0.5 mm sieve. The level of ß-d-glucan was estimated using McCleary and Codd (1991).

Formulation of the basic purified diet and high fiber diet

The experimental diets were prepared after modification in the basic purified diet AIN-76 A. Crude dietary fiber and ß-d-glucan fractions were obtained by a specific three step procedure of grinding and sieving (Knuckles *et al*., 1992). After final sieving the coarse material had the highest ß–glucan content. For the wheat and maize coarsely ground cereals were sifted using sieve of 200-300 mesh size (Anonymous, 1989).

The feed for four experimental rats was prepared with the addition of crude dietary fiber of wheat and maize and soluble ß-d-glucan fraction obtained from barley and sorghum in the purified diet AIN-93G diet for rats and rodents recommended by Reeves (1997) (Table II).

Experimental plan

Four groups of rats, each of eight, were fed on wheat bran feed (WBF), maize bran feed (MBF), barley ß glucan extract feed (BGF), and sorghum ß glucan extract feed (SGF) for 28 days. Fifth group of 8 rats, which was control, was fed on AIN-93G.

At the end of 28 days the animals were anaesthetized with chloroform. The blood samples were drawn from the heart ventricle using 19 to 25G needle with 1 to 5 ml syringe (Parasuraman *et al*., 2010) in plastic tubes containing ethylenediamine tetraacetic acid (EDTA), dipotassium salt, 0.8mg/ml of blood. The blood samples were centrifuged at 1500x g at 4°C for 30 min to get plasma from the blood samples. The plasma samples were then stored at -70°C. The cholesterol level in the plasma

Table I.- Percent composition of whole wheat, whole maize, whole barley, whole sorghum, wheat bran, maize bran barley β-glucan extract and sorghum β-glucan extract g % (Mean ±SD).

	Moisture	Fat	Protein	Ash	β-Glucan	Cellulose	Lignin	Total CDF
Wheat Inqlab-91 (whole)	11.73±0.40	3.92±0.27	13.50±0.34	1.50±0.5	1.12±0.99	1.23±1.45	1.50±0.99	2.21±0.67
Maize MMRI Yellow(whole)	12.15±1.90	5.62±1.54	12.22±0.56	1.36±0.91	1.87±0.43	2.33±0.90	1.56±0.56	3.70±0.93
Barley Nal-3(whole)	10.58±0.54	5.22±1.03	10.83±0.34	2.03±0.25	6.32±1.33	3.54±0.34	2.48±0.34	5.66±0.71
Sorghum F-9917(whole)	9.57±0.59	6.98±0.65	12.22±0.56	1.69±0.59	3.46±0.88	2.12±0.56	1.96±0.59	4.10±0.89
Wheat bran	9.45±0.45	1.43±0.39	7.93±0.50	2.50±0.72	0.98±0.53	10.56±0.98	8.45±0.45	20.22±0.67
Maize bran	7.99±1.02	3.72±0.67	8.11±0.20	3.71±0.87	1.01±0.81	9.89±1.32	4.65±0.23	18.55±0.98
Barley β-glucan extract	8.99±0.98	4.22±0.85	10.83±0.34	2.90±0.72	19.21±0.65	2.44±0.33	2.44±0.44	5.77±0.33
Sorghum β-glucan extract	9.66±1.22	5.21±0.51	10.83±0.34	2.08±0.98	12.91 ±0.76	4.77±1.90	3.22±0.79	8.34±0.54

Table II.- Composition (% g/kg) wheat bran feed (WBF), maize brain feed (MBF), barley β-glucan extract feed (BGF), sorghum β-glucan extract feed (SBF)..

	Cornstarch	Casein (85% protein)	Dextrinized corn starch	Sucrose	corn oil	Crude fiber/ bran	β glucan Extracts	Mineral mix	Vitamin mix	L-Cystine	Choline bitartrate	Tert-butyl-hydroquinoneone
Purified Diet AIN-93G	397.486	200.000	132.000	100.00	70.00	50.000	…	35.000	10.000	3.00	2.500	0.014
Wheat BF	197.486	200.000	132.000	100.00	70.00	250.00	…	35.000	10.000	3.00	2.500	0.014
Maize BF	197.486	200.000	132.000	100.00	70.00	250.00	…	35.000	10.000	3.00	2.500	0.014
Barley β-GEF	197.486	200.000	132.000	100.00	70.00	…	250.00	35.000	10.000	3.00	2.500	0.014
Sorghum β-GEF	197.486	200.000	132.000	100.00	70.00	…	250.00	35.000	10.000	3.00	2.500	0.014

samples was analyzed through enzymatic colorimetric procedures using Sigma diagnostic kit 352 and triglyceride estimated through Gilford diagnostic kit 23422.

For estimation of lipoproteins, 0.5ml plasma of each group were pooled, to which protease inhibitor, epsilon-amino caproic acid (1.3/mg/ml of plasma) and anti-microbial agent (garmicine 50mg/ml, 10 µl/ml of plasma) were added as preservatives. A sample of 1 ml was taken from pooled plasma of each group and was fractionated by density-gradiant ultracentrifugation for the estimation of lipoproteins (Havel *et al*., 1955). Later background density of the sample was adjusted to 1.019 with NaCl. Plasma was centrifuged at 40,000 rpm for 18 h at 17°C in a Beckman 50.3 rotor (Beckman, Palo Alto CA,). The top 0.34 ml layer, containing the VLDL fraction, was removed with a Pasteur pipette. The next 0.34 ml layer was removed as background; the *subnactant* density was adjusted to 1.063, and centrifuged at 40,000 rpm for 24 h at 70°C. The top 0.34 ml was removed as the LDL fraction; another 0.34 ml layer was removed as background. The subnatant contained HDL. Lipoprotein fractions were analyzed for cholesterol using the procedure described for plasma. The triglycerides were determined using the colorimetric method of Fossati and Prencipe (1982).

Statistical analysis

Descriptive statistics was used for all the parameters studies (percentage, mean and standard deviations). For further detailed statistical analysis, analysis of variance (ANOVA), and paired comparison post-hoc LSD test were done. A value was $p < 0.05$ considered significant.

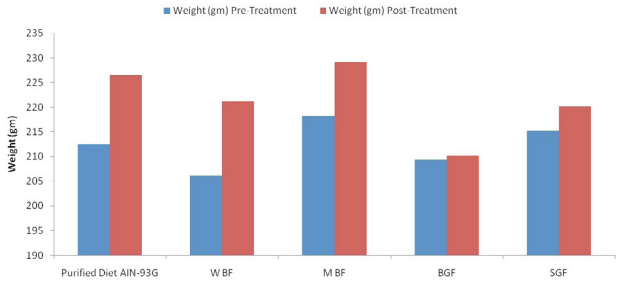

Fig. 1. Pre-treatment vs post comparison of change in weight (g) of different groups on purified diet AIN-93G W.

RESULTS AND DISCUSSION

Weight

Figure 1 shows weights of rats before and after they were fed on specific diets. The most significant weight gain was recorded in the hypercholesterolemic group of rats fed on purified diet AIN-93, while the hypercholesterolemic rats fed on MBF also had a significantly higher post-treatment weight gain as compared to the rest of the groups, whereas the hypercholesterolemic rats given BGF showed the least gain in post-treatment weight.

Total plasma cholesterol (TPC)

Table III shows effect of different feed formulations on TPC, VLDL, LDL, HDL and triglyceride of normal and diet induced hypercholesterolemic rats. The TPC of the group fed on purified diet AIN-93G was significantly ($p<0.05$) higher than the rest of the groups. No significant difference was recorded in the mean TPC of the rats fed on WBF and MBF, while both of these groups had significantly lower TPC than the control group fed on purified diet AIN-93G. The hypercholesterolemic rats fed on BGF had significantly ($p<0.05$) lower TPC compared to the other hypercholesterolemic groups. Similar results were observed by Bryan *et al*. (2003) who observed that both oats and barley ß-glucan had cholesterol lowering effect on hamsters. Similarly, Lupton *et al*. (1994) and Behall *et al*. (2004) showed that diets containing barley beta-glucan reduced lipids in mildly hypercholesterolemic men and women.

Very low density lipoproteins (VLDL)

No significant difference was observed in the VLDL of the hypercholesterolemic rats of all the groups. The hypercholesterolemic group fed on purified diet AIN-93G had highest VLDL level yet there was no significant difference between the mean VLDL of hypercholesterolemic rats fed on MBF. The mean VLDL of the hypercholesterolemic rats fed on BGF and SGF were significantly ($p<0.05$) lower than the rest of the groups (Table III). Queenan *et al*. (2007) recorded a significant ($p<0.05$) decrease in the total cholesterol, VLDL, LDL, and HDL in mildly hyper-cholestermic adult male and female participants who consumed 6g of barley ß-glucan enriched diet. Similar results were also reported by Keogh *et al*. (2003) that showed soluble-fiber ß-glucan derived from barley can reduce CVD risk through reductions in total and LDL cholesterol serum cholesterol. However it was observed that a prolonged use was necessary for a significant effect.

Low density lipoproteins (LDL)

SGF fed hypercholesterolemic rat had a significantly low LDL (Table III). These findings are similar to those of Cho and Ha (2003) who studied on the effect of prosomillet and sorghum on cholesterol metabolism. The hypercholesterolemic rats fed on BGF also had significantly ($p<0.05$) lower LDL level as compared to the hypercholesterolemic rats fed on purified diet AIN-93G, WBF and MBF.

Table III.- Effect of various diets on (TPC), (VLDL), (LDL), (HDL) and triglycerides of dietary induced hypercholesterolemic rats on pre-treatment and post-treatment.

	TPC mg/dl (Mean ±SD)		VLDL mg/dl(Mean ±SD)		LDL mg/dl(Mean ±SD)		HDL mg/dl(Mean ±SD)		Triglycerides mg/dl (Mean ±SD)	
	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T	Pre-T	Post-T
Purified Diet AIN-93G,	297.45±3.77a	320.45±3.27a	57.54±2.77a	54.54±5.27a	76.14±0.88a	72.94±0.88a	142.15±±1.56a	136.15±1.56a	300.33±2.55a	320.71±7.81a
W BF	299.12±9.44a	270.12±2.26b	60.43±0.99b	46.23±0.55b	76.54±1.78a	65.25±1.32b	138.12±2.54a	132.57±2.89b	321.15±3.21b	256.32±5.22b
M BF	286.2±8.44b	268.21±1.09bd	57.32±1.12a	50.97±1.29a	75.21±2.24a	64.16±1.51b	134.32±1.76a	135.22±2.60a	320.56±2.57b	247.12±2.66b
BGF	287.33±3.44b	249.23±3.78c	58.43±1.77a	40.34±2.24c	74.21±1.14a	58.41±0.98c	132.93±2.61b	144.16±1.55b	320.25±3.78b	200.78±2.50c
SGF	289.23±4.33b	252.13±3.51cd	56.21±0.87a	41.43±0.77c	76.67±0.56a	56.70±1.20c	130.27±3.55b	142.09±3.12b	289.24±2.34a	210.41±3.59c

Mean values followed by different letters within a column are significantly different ($p<0.05$). Purified diet AIN-93, Wheat Bran Feed (WBF), Maize Brain Feed (MBF), Barley β-glucan Extract Feed (BGF), Sorghum β-glucan Extract Feed (SGF) on Total Plasma Cholesterol (TPC), Very Low Density Lipoproteins (VLDL), Low Density Lipoproteins (LDL), High Density Lipoproteins (HDL) and Triglycerides of Hypercholesterolemic Rats Pre- Treatment (Pre-T) and Post- Treatment (Post-T)

High density lipoproteins (HDL)

The hypercholesterolemic group fed on purified diet AIN-93 and MBF had significantly ($p<0.05$) higher HDL level than the rest of the groups. Brites *et al*. (2011) observed that bread made from maize resistant starch/dietary fiber was responsible for higher body weight gain and cholesterol in experimental rats. There was no significant difference in the Post-T HDL of the hypercholesterolemic groups fed on WBF, SGF and BGF.

Triglycerides

The hypercholesterolemic group fed on purified diet AIN-93 had the highest level of triglycerides among all the groups. The hypercholesterolemic groups fed on WBF and significantly higher triglyceride. Borel *et al*. (1990) and Swain *et al*. (1990) observed that wheat bran did not affect the absorption of and uptake of dietary cholesterol, triglycerides and free fatty acids hence have no significant effect on lowering of cholesterol and triglycerides. The hypercholesterolemic groups of rats fed on BGF and SGF had significantly lower triglyceride levels as compared to the rest of the groups. The results of the present study indicate that ß glucan had a pronounced effect in reduction of total lipids, cholesterol, LDL, HDL, VLDL, and triglycerides in blood in hypercholesterolemic rats.

CONCLUSION

STC level of hypercholesterolemic rats is significantly reduced after feeding on BGF as well as SGF for six weeks. Likewise a significant decrease in VLDL and LDL was also recorded in this group, while the HDL post treatment was increased significantly as compared to the pre-treatment reading. In conclusion under the conditions of this study the β-glucan extract from barley and sorghum can help in reducing the TPC, VLDL, LDL and triglyceride and increasing HDL level in the induced hypercholesterolemic rats. However, further in-depth study is required on the human subjects before any recommendations to patients of hypercholesterolemia.

Statement of conflict of interest

Authors have declared no conflict of interest.

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