

Research Article

Effect of β -glucan extracts on low-density Lipoprotein (LDL) and high-density Lipoprotein (HDL) levels of hypercholesterolemic rats

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Abstract

Cardiovascular diseases are more prevalent in Asian countries as compared to Western countries. The insufficient intake of dietary fibre is the main cause of hypercholesterolemia leading to cardiovascular diseases. This study was designed to extract Beta Glucans (BG) from various sources including oat, barley and yeast cells and evaluate their influence on total cholesterol, low-density lipoproteins (LDL), high-density lipoproteins (HDL), very low-density lipoproteins (VLDL) levels as well as on triglycerides in induced hypercholesterolemic rats. It was observed that BG obtained from barley reduced the levels of total cholesterol to 125 mg/dl from 180 mg/dl, levels of LDL from 30 mg/dl to 27 mg/dl while it did not show any effect on HDL levels. Yeast extracted BG resulted in the reduction of total cholesterol from 180 mg/dl to 40 mg/dl, decreasing the value LDL from 30 mg/dl to 8 mg/dl, though, it also did not influence HDL levels. BG extracted from oat lowered the total cholesterol and LDL from 180 mg/dl to 171 and from 30 mg/dl to 10 mg/dl respectively. Though, plant extracted BG increased the level of HDL from 40 mg/dl to 125 mg/dl. These results indicated that yeast extracted glucan was more efficient in lowering of serum lipid components in rats and they can be used as a supplement in order to decrease the cardiovascular disease.

Keywords: Animal models; Cardiovascular diseases; Cholesterol levels; Dietary fibers; Metabolism disorders

Introduction

Dietary fibers are consisting of unique bioactive components like vitamins, minerals, antioxidants and phytochemicals. In the last several decades, considerable attention towards the potential health benefits of these bioactive components has been increased. Though, beta-glucans are an active component of cereal fibers for

the lowering of cholesterol [1]. Beta-glucans are water-soluble fibers and are widely distributed in natural sources like barley, oat and yeast. All beta-glucans are polysaccharides regardless of their source, and they are consisting of glucose molecules. The glucose units of beta-glucans are connected by various linkage for instance β -1,4/ β -1,6 or β -1,3/ β -1,6

[2]. The molecular weight of beta-glucans ranges from 200,000 g/mol to about 687,000 g/mol which is quite lofty [3]. Beta-glucans are found in both soluble as well as in insoluble form [4]. Feeding trials of oats and barley have shown different levels of hypocholesterolaemic effects of supplementation. The difference in cholesterol-lowering effects can be attributed to certain factors such as solubility, viscosity and molecular weight (MW) of beta-glucans [5]. According to previous studies, cardiovascular diseases are more prevalent in developing countries as compared to developed countries and beta-glucans have many health benefits like they play a crucial role in lowering the cholesterol level [6]. They are also helpful in the control of many cardiovascular diseases and lower bowel transit interval [5], they also help in prevention in constipation and produce short-chain fatty acids [7]. Beta-glucans also boosts the growth of useful bacteria in the colon and enhance digestion [8]. Several studies have shown that beta-glucans reduce the level of LDL in humans with a high concentration of cholesterol and triglycerides [9, 10]. Although, the exact mechanism is unknown how beta-glucans lower the cholesterol in the blood. But the most possible explanation is that they reduce the reabsorption of the bile acid. Furthermore, the hepatic LDL cholesterol receptors become upregulated, which leads to a decrease in serum LDL cholesterol concentration [11]. During this study, the effect of beta-glucans extracted from different sources on the lipid profile of rats was determined and compared.

Materials and Methods

Barley (*Hordeum vulgare*) and Oat (*Avena sativa*) were purchased from Shah Alam market, Lahore (31.5764° N, 74.3174° E) and stored at room temperature until further analysis. Baker's yeast (*Saccharomyces cerevisiae*) was purchased from AB mauri Pvt Ltd, Lahore

Extraction of BG from Barley and Oats and Yeast

Barley and oat (each 40 gm) was ground into powder form, inactivation of endogenous glucanases was attained by refluxing with 90% ethanol and defatting them with 2-propanol /petroleum ether (2:3). The defatted product (oat & barley) was added to an aqueous solution of NaOH (0.25 N) and mixed for 8 hours at 25°C. *Saccharomyces cerevisiae* was obtained from AB mauri Pvt Ltd, Lahore. *Saccharomyces cerevisiae* was grown in Sabouraud dextrose agar (SDA) having chloramphenicol (0.005%) at 30°C for 3 days. The yeast suspension was made in 500 mL of YPG broth (yeast extract 1%, peptone 2%, and glucose 2%) and incubated at 30°C in shaking incubator (150 rpm) for 48 hours. The resultant slurry of each was then centrifuged at 10000rpm for 20 min at 4°C. After centrifugation, the supernatant was parted from the residue by pouring. The pH of the supernatant was then adjusted to 7 and then kept at -20°C overnight. Flasks were thawed to room temperature. A jelly-like material (BG) settled down and was separated by decanting the supernatant. It was dried in a water bath at 70°C [12].

Estimation of BG

Estimation of extracted BG from each source was done by dinitrosalicylic acid (DNS) method [13]. A standard curve of glucose was prepared using different dilutions of glucose standard solution (Fig. 1). Extracted BG was subjected to acid hydrolysis and released reducing equivalents were quantified by the DNS method and compared with the standard curve to obtain the concentration of BG.

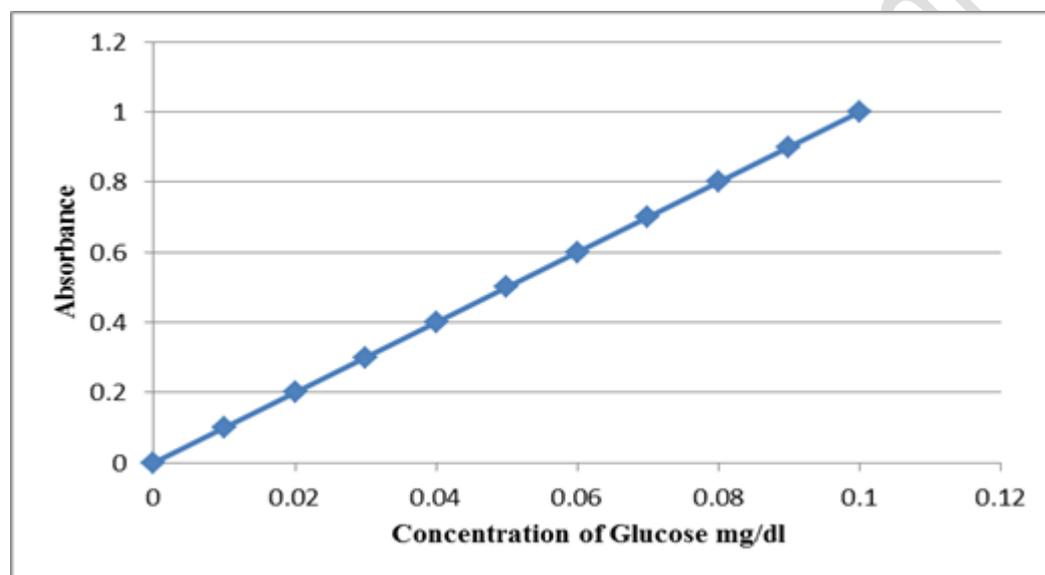
Experimental model

Twenty four albino rats of weight 100-150 g were attained from the Animal House of the University of Veterinary and Animal Sciences, Lahore. All the experiments dealing with animals were performed according to the guidelines for the care and use of laboratory animals and approved by the ethical committee for laboratory

animals. Rats were fed on a basal diet and water ad libitum. They were kept in cages in an air-conditioned room at 28°C and 55% humidity for four weeks. Rats were fed with corn starch (23%), skimmed milk (35%), sucrose (15%), dalda ghee (25%), potato starch (5%) / wheat straw (5%), vitamin and mineral mixture (2%) to induce the hypercholesterolemia. After 30 days, the serum lipid profile was determined by Crescent Diagnostic Kit [14]. Hypercholesterolemic rats were randomly distributed into eight units each having three rats. Four diets (A, B, C and

D) were constructed and served in each group (Table 1).

The diets were given randomly to rats such that there were 2 replicates on each ration and the feeding trial was prolonged for one month. The rats were weighed initially and thereafter weekly. After one month all rats were taken from each group, weighed and their blood was collected by puncturing directly their heart after anaesthetizing the rats and blood was collected in EDTA containing tubes and was preserved at 4°C till further use [15].



Standard curve of glucose

Figure 1. Standard curve of glucose

Table 1. Summary of diet served to four groups of hypercholesterolemic rats

S. #	Groups	Diet plan
1	Control G1	Diet D: Basal diet only
2	Group 2: G2	Diet A: Basal diet + Barley extracted BG (10%)
3	Group 3: G3	Diet B: Basal diet + Oat extracted BG (10%)
4	Group 4: G4	Diet C: Basal diet + Yeast extracted BG (10%)

Lipid profile analysis

On the 30th day, the rats were sacrificed and blood samples were obtained through cardiac puncture using 5 ml syringe. The blood was collected into properly labelled sample bottles pre-supplied with ethylene diamine tetra acetic acid (EDTA) to act as anti-coagulant, and centrifuged at 1500xg

for 5 min. The supernatants were decanted and stored at -20°C for examining biochemical parameters. Concentrations of total cholesterol, LDL-cholesterol, HDL-cholesterol, VLDL-cholesterol and triglycerides were examined in the serum samples of rats by Crescent Diagnostic Lipid Profile Kit according to

manufacturer's instruction (Cat NO: CS.603).

Statistical analysis

The data was subjected to one-way examination of variance (ANOVA) and the p-value < 0.05 was considered as significant.

Results and Discussion

The effect of BG extracted from barley, oat and yeast was examined on the lipid component of serum of albino rats. The control group was fed with diet without BG while others were fed a basal diet supplemented with BG extracted from various sources including oat, barley and yeast cells.

Estimation of BG

The percentage of BG extracted from different sources; baker's yeast (*Saccharomyces cerevisiae*), oats (*Avena sativa*), Barley (*Hordeum vulgare*) was 6.5%, 2.8% and 3.4%, respectively.

Effects of BGs on serum lipid components of induced hypercholesterolemic rats

It was observed that serum total cholesterol levels were reduced significantly in test groups than control group (Table 2). Remarkable lower level of serum total cholesterol (40 mg/dl) was observed when the rats were fed with BG extracted from baker's yeast than that fed with oat and barley extracted BG and control (basal diet). The researchers also reported similar effects of BG obtained from yeast-like fungus on the serum lipid profile of hamster models with induced hyperlipemia [16].

LDL-cholesterol levels were measured by the Fried Ewald equation [17]. Among the all test groups, the LDL-cholesterol level was lowest in rats fed with yeast extracted BG supplemented diet (8 mg/dl) whereas the levels observed in rats fed with barley and oat extracted BG with basal diet were

27 and 10 mg/dl, respectively. These results were in concordance with the report of others who observed a reduction in LDL-cholesterol levels with the use of oat BGs in hamsters [18].

On the other hand, the rat fed with oat extracted BG had a 32% increased HDL-cholesterol level compared to control while other groups fed with yeast and barley extracted BGs showed no influence on their HDL-cholesterol levels. The rise in HDL-cholesterol with the incorporation of BG is in accordance with the previous reports where the HDL-cholesterol levels were enhanced with the use of water-soluble oat gum. On the contrary, some researchers have also reported no significant effect of BG enriched diets on serum HDL-cholesterol levels of rats [19]. Furthermore, significantly the lowest level of triglycerides was observed in the serum of rats fed with BG diet derived from yeast extract (30 mg/dl) compared to that derived from barley (130 mg/dl), from oat (170 mg/dl) and control (180 mg/dl) respectively. These results were in concordance with the previous studies however, some reports are also available regarding the no effect or increasing effects of BG in different experimental subjects [20].

Serum VLDL level of rats fed with yeast extracted supplemented diet (30 mg/dl) were also found to be significantly low than in rats given others supplemented diets including barley (120 mg/dl), oat (150mg/dl) and control (160 mg/dl).

ANOVA analysis showed that BGs extracted from all the sources including barley, oat and Baker's yeast had statistically significant effects on levels of the total cholesterol, LDL-C, HDL-C, VLDL-C and triglyceride in the serum of induced hypercholesterolaemic rats.

Table 2. Effects of BG extracted from barley, oat and yeast on the total cholesterol, LDL cholesterol, HDL cholesterol, VLDL cholesterol and triglycerides of serum of albino rats

Dietary groups	Total cholesterol (mg / dl)	LDL cholesterol 1 (mg / dl)	HDL cholesterol (mg/ dl)	VLDL cholesterol (mg / dl)	Triglycerides (mg / dl)
Group 1	180	30	40	160	180
Group 2	125	27	40	120	130
Group 3	165	10	125	150	170
Group 4	40	8	40	30	30
Significance (p-value > 0.05)	0.000	0.000	0.000	0.000	0.000

Conclusion

Beta-glucan extracted from barley, oat and yeast may lower the serum concentrations of low-density lipoprotein (LDL) and raise the high-density lipoprotein (HDL) in hypercholesterolaemic rats, respectively. In this study, the efficacy of oat, barley and yeast extracted BG in depressing the levels of induced hypercholesterolemia and hyperlipidaemia in the serum of Albino rats (*Rattus rattus*) was calculated. Feeding rats on a cholesterol-enriched basal diet ominously augmented serum total cholesterol, low-density lipoprotein, and very-low-density lipoprotein and triglyceride and reduced serum high-density lipoprotein. In conclusion, BG extracted from oat, barley and yeast cells had protective effects against induced hyperlipidaemia, though, BG extracted from *Saccharomyces cerevisiae* seemed more effective than oat and barley to lower the lipid profile levels in hypercholesterolemic rats.

Authors' contributions

Conceived the idea and designed the experiments: MM Javed, Performed the experiments: MA Khan, Analysed the data contributed to analysis tools: K Iqbal & S Zahoor, Wrote the paper: MA Khan.

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