THE MODULATING EFFECT OF BARLEY β-GLUCAN EXTRACT ON HIGH FAT DIET INDUCED OBESITY-A BIOCHEMICAL STUDY IN ALBINO WISTAR RATS

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INTRODUCTION

Obesity is a pathological condition which is characterized by an increase in body weight that results in excessive fat accumulation. Obesity and hyperlipidemia are closely associated with the development of CVD, which is the main cause of death in developing countries [1].

Hyperlipidemia is a heterogeneous group of disorders characterized by an excess of lipids in the bloodstream. Frequent intake of HFD or increased endogenous biosynthesis of lipids are the major causes of hyperlipidemia. Increased lipogenesis is together with hyperlipidemia and increased, fat deposition contribute to obesity [2].

BMI is an index of weight for height that is widely used to identify overweight and obesity. It is obtained by dividing a person’s weight in kilograms by the square of the person’s height in meters.

Overweight and obesity are associated with more deaths worldwide and found to be an epidemic. People with obesity are at high risk of numerous health problems like heart attack from coronary heart failure, gallstones, congestive heart failure, stroke, high blood cholesterol, TG, hypertension and diabetes, which may cause sleeplessness and poor attention [3]. These multiple pathological alterations collectively facilitate the progress of atherosclerosis.

Oxidative stress is highly associated with a wide variety of inflammatory and metabolic disease states, including obesity [4]. It is highly correlated with cumulative damage in the body done by free radicals inadequately neutralized by antioxidants thiobarbituric acid-reacting substances (TBARS) and hydroperoxides levels are markers of oxidative damage to cell membranes. Furthermore, oxidative damage is aggravated by the decrease in antioxidant enzyme activities such as superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) and glutathione peroxidase (GPx) which acts as a free radical scavenger.

Adipose tissue (AT) is not only acting as a simple energy depository tissue, but also as an active endocrine organ, releasing a variety of biologically active molecules known as adipokines or adipokines. Adiponectin, the first cloned protein hormone from AT is decreased in obese subjects and also functions to inhibit inflammatory processes [5]. Since adiponectin plays a crucial role in the pathogenesis of obesity-related complications, therapeutic drugs for the treatment of obesity should have the capacity to enhance the level of adiponectin.

The chief goal of obesity treatment is to maintain a healthy weight. Use of allopathic and pharmacological drugs like Statin, Rimonabant and Shubtramine has become a popular means to overcome excess weight gain. Nevertheless, these drugs generally are effective with adverse side effects [6]. Hence naturally occurring phytochemicals such as dietary fibers present an exciting opportunity for the discovery of newer anti-obesity agents.

Barley (Hordeum vulgare L.), a member of the grass family is a major cereal and first cultivated grains. β-glucan, a lead compound located primarily in the endosperm cell walls of barley is actively involved in the metabolic response to barley products [7]. β-glucan extracted from barley have been reported to possess various biological functions such as reducing blood lipids [8,9], blood glucose levels, enhancing insulin response [10], protecting against stress response [11] and restraining allergic reactions [12]. The aim of the present study is to evaluate the effect of BRBG on the level of blood lipids, adiponectin and HMG CoA reductase activity in diet-induced hyperlipidemia.

OBJECTIVE

Diet-induced hyperlipidemia and obesity are the major risk factors for type II diabetes mellitus, hypertension, musculoskeletal and cardiovascular disorders (CVD). The objective of the present study is to furnish scientific proof for the lipid-lowering effect of β-glucan, a lead compound present in barley with a defined mechanism of action.

METHODS

Obesity was induced in male albino Wistar rats by feeding a high-fat diet (HFD) for 14 w and were randomly divided into four groups of equal number (n=6). Group 1 and 2 served as control fed with normal diet (5% fat). Group 3 and 4 were fed HFD (23% fat) for 14 w. In addition, group 2 and 4 rats were administered orally with 20 0 mg/kg body weight of barley β glucan (BRBG) from the third week. After 14 w, the rats were sacrificed, and serum/plasma levels of total cholesterol (TC), phospholipids, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL) and adiponectin were determined.

RESULTS: Biochemical changes were observed in weight gain, body mass index (BMI), adiposity index (ADI), total fat pad mass (TFP), blood lipids, LDL, lipid peroxides (LPO) and antioxidant enzyme activity of HFD fed rats when compared with BRBG co-administered rats. In addition, serum adiponectin levels and 3-hydroxy-3methyl-glutaryl-coenzyme A reductase (HMG CoA reductase) activity were elevated in rats administered BRBG along with HFD. Histological examination in HFD induced rats revealed a profound change in cell size with increased hypertrophy in visceral adipose tissue.

CONCLUSIONS: The results indicate that barley consumption could reverse most of these biochemical and histological changes in HFD fed rats owing to its hypolipidemic and antioxidant effect.

KEYWORDS: BRBG, HFD, BMI, Adiponectin, HMG CoA reductase, Blood lipids, Hyperlipidemia

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MATERIALS AND METHODS

Chemicals and reagents
ELISA kit used for the determination of serum adiponectin was purchased from Abcam, USA (ab108784). All other chemicals and reagents used were of analytical grade and were purchased from Sigma Aldrich, India and Excel Biosciences. Chennai-India.

Animal maintenance
Male Sprague Dawley rats (175-200 g) were individually housed in polycarbonate cage for 7 d under controlled conditions (22±2 °C/RH 50±5%) in a 12:12 h L: D cycle and fed a standard pellet diet containing required amount of minerals and vitamins obtained from M/s: Provimi Animal Nutrition India Pvt Ltd., Bangalore for acclimatization. During the period diet and water were provided ad libitum.

Experimental protocol
After the acclimatization period, rats were randomly divided into four groups (n=6). Group 1 and 2 rats were fed a normal diet containing 5% fat. Group 3 and 4 rats were fed HFD containing 23% saturated fat for a period of 14 w. HFD was prepared and administered to rats, according to the report of Nascimento et al. [13]. The high purity ingredients (ground labina-439g, roasted peanuts-218g, casein-129g, corn oil-61g, French fried potatoes-153g per kg; purchased from Spencers Food World, Chennai, India) were weighed and mixed with vitamins and minerals (1-1.2 g per 100g of HFD). The mixture was then made into pellets and dried in a ventilated drying oven at 55-65°C. Rats were allowed for free access to food and water. Care was taken to monitor the food intake by rats every day. Rats were divided into four Groups. Group 1 and 2 was fed with normal diet. Group 3 and 4 received HFD in 14 w. In addition group 2 and 4 rats were co-administered with 200 mg/kg body weight of BRBG by oral intubation from the third week onwards till the total experimental period of 14 w. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) (XIV/VELS/CO/43/CPSEA/IAEC/15.03.2013).

Body weight was measured weekly and the final body weight-to-length ratio was also calculated for the determination of BMI. Length was measured between the nose and anus, not counting the tail. BMI was calculated by using the formula: BMI=body weight (g)/(nose-anus) (cm). At the end of the experimental period, the rats were anesthetized by injecting 0.1 ml/10g b. wt. Of ketamine/xylazine mixture (prepared by combining 1.5 ml of 100 mg/ml xylazine and 10 ml of 100 mg/ml ketamine) and killed by cervical decapitation; immediately, blood was collected and the plasma/serum was separated by centrifugation and stored at 4 °C until analysis. AT was isolated and weighed from the epididymal, visceral and retroperitoneal fat pads. The sum of the entire fat pad mass was determined (TFP). ADI was also determined by using the formula: (sum of TFP/body weight) × 100 and expressed as adiposity percent (%). A portion of liver, heart and AT from each group was fixed in 10% formalin for 24 h. Ultra-thin sections of the tissues were cut from formalin-fixed paraffin-embedded sections and stained with hematoxylin-eosin and observed under a light microscope.

Statistical analysis
Data were analyzed by using a commercially available statistical software package (SPSS for window V.10). The statistical significance of mean values between different groups was determined by applying one way ANOVA with post hoc Bonferroni test and the P value<0.05 was considered as significant.

RESULTS

Effect of BRBG on BMI, TFP and ADI in experimental rats
Group 3 rats showed a significant (P<0.000) increase in BMI in HFD fed rats when compared to that of rats fed with normal diet (Group 1). A significant decrease in BMI, TFP, and ADI were observed in BRBG co-administered rats shown in table 1. Control rats fed with normal diet and BRBG did not show any significant difference in these parameters when compared to normal Group 1 rats.

Effect of BRBG on lipid profile
Table 2 depicts the plasma levels of TC, TG, HDL and LDL, which was found to be significant (P<0.000) increased in Group 3 rats when fed with HFD containing 23% fat. Group 3 rats showed a significant (P<0.000) increase in BMI in HFD fed rats when compared to that of rats fed with normal diet (Group 1). A significant decrease in BMI, TFP, and ADI were observed in BRBG co-administered rats shown in table 1. Control rats fed with normal diet and BRBG did not show any significant difference in these parameters when compared to normal Group 1 rats.

Histopathological examinations
Liver, heart and AT samples were fixed at 10% neutral buffered formalin for 24 h. Ultra-thin sections of the tissues were cut from embedded tissue blocks. The sections were then stained with hematoxylin-eosin and observed under a light microscope.

Statistical analysis
Data were analyzed by using a commercially available statistical software package (SPSS for window V.10). The statistical significance of mean values between different groups was determined by applying one way ANOVA with post hoc Bonferroni test and the P value<0.05 was considered as significant.

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compared with Group 1 rats and a non-significant decrease in HDL cholesterol level was observed in rats fed with HFD when compared to control rats. BRBG co-administered rats showed a significant (P=0.000) increase in HDL cholesterol than in rats fed only HFD.

Table 1: Effect of BRBG on weight gain, BMI, TFP mass and ADI in control and experimental rats

| Groups         | Net weight gain [g] (final-initial) | BMI [g/cm²] | TFP [g] | ADI (%) |
|----------------|------------------------------------|-------------|---------|---------|
| Control        | 155.4±12.3                         | 0.61±1.2    | 15.3±1.2 | 4.3±1.2  |
| BRBG Control   | 152.3±12.2**                       | 0.60±1.2^4  | 13.7±1.4 | 4.2±1.0  |
| HFD            | 285±12.6^7                         | 1.50±11.4^7 | 27.4±13.6| 6.9±10.6^7|
| HFD+BRBG       | 177.4±12.8^*                       | 0.76±11.6^* | 12.8±13.8| 5.7±10.8^*|

Data were expressed by one-way ANOVA followed by post hoc Bonferroni test. Values are expressed as mean±SD for 6 animals in each group. Statistical significance was calculated by comparing control Vs BRBG control, control Vs HFD and HFD Vs HFD+BRBG. *P=0.000, NS= non-significant

Table 2: Effect of BRBG on serum TC, TG and lipoprotein levels

| Groups         | TC [mg/dl] | TG [mg/dl] | HDL [mg/dl] | LDL [mg/dl] | LDL-HDL ratio |
|----------------|------------|------------|-------------|-------------|---------------|
| Control        | 69.4±9.37  | 96.3±9.72  | 27.3±3.49   | 15.7±2.33   | 0.58±0.07     |
| BRBG Control   | 67.1±8.26^NS| 94.6±12.96^NS| 28.4±2.87^NS| 14.1±1.62^NS| 0.50±0.06 NS^NS|
| HFD            | 144.65±20.83^*| 155.1±19.23^*| 15.9±2.20^* | 25.3±3.47^* | 1.59±0.18^*   |
| HFD+BRBG       | 102.19±12.26^*| 117.4±12.06^*| 21.5±2.45^* | 20.8±2.58^* | 0.96±0.14^*   |

Data were expressed by one-way ANOVA followed by post hoc Bonferroni test. Values are expressed as mean±SD for 6 animals in each group. Statistical significance was calculated by comparing control Vs BRBG control, control Vs HFD and HFD Vs HFD+BRBG. *P=0.000, NS= non-significant

Effect of BRBG on activity of HMG CoA reductase

The activity of HMG CoA reductase was assayed indirectly by assessing the ratio of HMG CoA to mevalonate in hepatic tissue as shown in table 3. The lower ratio indicates higher enzyme activity and vice versa. In the present investigation, this ratio was found to be decreased in HFD fed rats when compared with BRBG co-administered rats. There was no significant difference (P=1.000) between Group1 and Group 2 rats.

Table 3: Activity levels, of HMG CoA reductase in the liver tissue of different groups of rats

| Groups         | Liver (Ratio of HMG CoA to mevalonate) |
|----------------|---------------------------------------|
| Control        | 4.2±0.47                              |
| BRBG Control   | 3.7±0.48^NS                           |
| HFD            | 2.6±0.36^*                            |
| HFD+BRBG       | 6±0.20^*                              |

Data were expressed by one-way ANOVA followed by post hoc Bonferroni test. Values are expressed as mean±SD for 6 animals in each group. Statistical significance was calculated by comparing control Vs BRBG control, control Vs HFD and HFD Vs HFD+BRBG. **Lower ratio indicates higher enzyme activity and vice versa. *P=0.000, NS= non-significant

Effect of BRBG on serum adiponectin levels

The serum adiponectin level is shown in fig. 1. A significant decrease in adiponectin level was found in HFD fed control rats. The BRBG co-administration was identified to increase the serum adiponectin level significantly (P=0.77).

In table 4 and 5 it is shown that there is a strong correlation between the occurrence of obesity and free radical production, as in untreated HFD induced rats (Group 3), the levels of LPO and GSH was found to be decreased which serves as an effective indicator of oxidative stress. Interestingly, the present study showed that barley consumption significantly increased (P=0.000) the level of LPO and GSH in experimental animals.

Histopathological examinations

The light microscopic examination of liver tissue of control is shown in fig. 2A and drug control (fig. 2B) group revealed almost same histological changes. A high degree of steatosis, fat deposition, inflammation and accumulation of lipid droplets (fig. 2C) were found in group 3 rats. Fig. 2D shows that the administration of BRBG along with HFD restored the normal appearance of liver tissue by reducing necrosis and inflammation.

Fig. 1: Values are expressed as mean±SD for 6 animals in each group. Statistically significant variations were derived when Group 1 was compared with Group 2 and Group 3, and Group 3 was compared with Group 4. Values were expressed as *P=0.000, NS = non-significant
Table 4: Levels of lipid peroxidation products in serum, heart and liver of experimental animals

| Groups        | Serum (μM/ml) | Heart nm MDA/mg of protein | Liver nm MDA/mg of protein |
|---------------|--------------|----------------------------|---------------------------|
| Control       | 8.33±0.866   | 59.2±6.98                  | 89.5±9.75                 |
| BRBG Control  | 7.9±0.97     | 58.4±7.41<sup>NS</sup>     | 89±12.56<sup>NS</sup>     |
| HFD           | 10.8±1.34*   | 70.3±9.56                  | 109±14.72<sup>a</sup>     |
| HFD+BRBG      | 8.23±1.06*   | 58.9±8.65<sup>c</sup>      | 90.2±10.28<sup>d</sup>    |

Data were expressed by one-way ANOVA followed by post hoc Bonferroni test. Values are expressed as mean±SD for 6 animals in each group. Statistical significance was calculated by comparing control Vs BRBG control, control Vs HFD and HFD Vs HFD+BRBG. *P=0.000, †P=. 022, ‡P=. 007, §P=. 018, ‰P=0.010, NS = non-significant.

Table 5: Levels of reduced GSH in the serum, heart and liver of experimental animals

| Groups        | Serum mg/g of protein | Liver mg/g of protein | Heart mg/g of protein |
|---------------|-----------------------|-----------------------|-----------------------|
| Control       | 4±0.245               | 16.33±1.93            | 9.69±1.32             |
| BRBG Control  | 3.33±0.35 <sup>NS</sup> | 15±2.13<sup>NS</sup> | 8.21±1.2<sup>NS</sup> |
| HFD           | 2±0.22*               | 7.85±1.1<sup>*</sup>  | 3.51±0.36<sup>**</sup> |
| HFD+BRBG      | 5.33±0.564*           | 16.20±2.1<sup>*</sup> | 8.90±1.3<sup>*</sup>  |

Data were expressed by one-way ANOVA followed by post hoc Bonferroni test. Values are expressed as mean±SD for 6 animals in each group. Statistical significance was calculated by comparing control Vs BRBG control, control Vs HFD and HFD Vs HFD+BRBG. *P=0.000, **P=. 124, NS = non-significant.

Fig. 2: Photomicrograph of liver showing normal tissue architecture in control rats (A) and no significant pathological alteration in BRBG control rats (B). A high degree of steatosis and fatty infiltration in HFD fed rats (C). Very mild inflammation and fatty changes in HFD and BRBG treated rats (D)

Fig. 3: The light microscopic examination showing normal tissue architecture of the heart in control rats (A). BRBG control rats showing normal tissue with no pathological changes (B). Rats which received HFD (C) showing fat deposition and degenerated necrotic cells, whereas BRBG (D) co-administration showing only mild degeneration were observed in the heart sections
The tissue architecture of heart tissue of control (fig. 3A) and BRBG control (fig. 3B) rats show normal tissue with no pathological changes. Rats which received HFD (fig. 3C) showed fat deposition and degenerated necrotic cells when compared with BRBG co-administered rats (fig. 3D). Visceral adipose tissue has shown an abnormal increase in adipocyte cell size (fig. 4C) in HFD fed rats, whereas BRBG co-administered rats showed an improved adipocyte size (fig. 4D).

DISCUSSION

Obesity is a strong risk factor for developing dyslipidemia, diabetes mellitus and CVD. Feeding of HFD to rats was found to be a useful model of putative effects of dietary fats in humans. In the present study, the weight gained by rats fed HFD formula was significantly more than those gained by a control group of rats fed a normal diet. The BMI, TFP and ADI were found well maintained in rats that received BRBG probably by its active components which exert hypolipidemic activity [25].

HFD resulted in dyslipidemic changes as illustrated by increasing serum levels of TG, TC, LDL cholesterol and low level of HDL cholesterol as compared with control and BRBG co-administered rats. In addition, the enzyme HMG CoA reductase converts HMG CoA to mevalonate in cholesterol biosynthesis. The lower ratio points to higher enzyme activity and vice versa. In the current study, this ratio was found to be decreased in HFD fed rats when compared with BRBG received rats.

The main mechanism for BRBG cholesterol-lowering effect is thought to be dependent on its ability to entrap whole micelles containing bile acid in the intestinal contents due to its viscosity and excluding them from the required interaction with the luminal membrane transporters on the intestinal epithelium, thereby decreasing the absorption or re-absorption of fats, including cholesterol and bile acid, which leads to an increased fecal output and catabolism of cholesterol. This further leads to increased activities of 7α-hydroxylase and HMG CoA reductase to compensate for the loss of bile acids and cholesterol from liver stores. Similar results were observed by Ab et al. [26] who reported that the supplementation of diet with either oat or barley bran resulted in a significant decrease in the level of serum total lipid, TC, TG, LDL, VLDL, and LDL/HDL ratio with an increase in the level of HDL compared with those fed high cholesterol alone.

Adiponectin is the most abundant adipokine secreted by adipose cells that may couple regulation of insulin sensitivity with energy metabolism. Adiponectin is a 30 kD protein that consists of a N-terminal collagenous domain and a C-terminal globular domain. This globular domain of adiponectin is pharmacologically active and can regulate body weight and fatty acid oxidation [27]. From the current study, it is clear that the level of adiponectin has been inversely correlated with obesity. Reduced serum adiponectin levels are observed in patients with diabetes mellitus, metabolic syndrome and coronary heart disease and this may play a key role in the development of obesity. Increased adiponectin level in BRBG treated rats (Group 4) support the concept that it has a distinct potential for being a therapeutic drug for the treatment of obesity-related diseases, indicates the metabolic syndrome to malignancies.

Oxidative stress is closely associated with a wide variety of inflammatory and metabolic disease states, including obesity. TBARS and hydroperoxides levels are markers of oxidative damage of cell membranes. Lipid peroxidation is considered as a component of obesity-induced pathology. The data reported in this study showed increased lipid peroxidation in serum, hepatic and cardiac tissues as expressed by increased levels of malondialdehyde (MDA). The current results are in basic agreement with the results of Amirkhizzi et al., [28] who showed that obesity is associated with an increase in lipid peroxidation and the decrease in the activity of cytoprotective enzymes. The potential mechanism for increased lipid peroxidation in cardiac tissue may be a result of increased lipid substrate within the myocardium, which serves as a larger target for oxidation by free radicals. Hence the oxidized LDL, was attracted by monocytes/macrophages, resulting in atherosclerotic plaques.

GSH is a water-soluble tripeptide composed of the amino acids glutamine, cysteine, and glycine. GSH also functions as a free radical scavenger and in the repair of radical caused biological damage. Reduced GSH in liver, heart and blood in HFD fed rats represented increased utilization due to oxidative stress. On the contrary BRBG succeeded in antagonizing the oxidative stress induced by HFD to a greater extent [29].

Histological changes induced by HFD in liver, heart and AT of experimental rats were consistently alleviated by BRBG proving its hepatoprotective and hypolipidemic property.

CONCLUSION

In conclusion, the present study showed that β-glucan of barley can be accounted for the hypolipidemic and anti-obesity effect of barley. It can be recommended that barley can be included in the everyday diet of hyperlipidemia and obese individuals to minimize the...
complications such as hypertension, type II diabetes mellitus and CVD. The low cost and easy availability of barley can replace high cost statin drugs with side effects on long term consumption.

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AUTHORS CONTRIBUTIONS

Performed the experiment: Mumtaz khan Mohamed, Monika Padmanabhan, Wrote the paper: Mumtaz khan Mohamed, revised the paper: Mumtaz khan Mohamed

CONFLICTS OF INTERESTS

Declared none

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