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Antihyperlipidemic Effect of Hulled Barley Grains (*Hordeum vulgare*) Powder in Rats Subjected to High-Fat Diet-Induced Hyperlipidemia

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A B S T R A C T

Hulled barley grains powder (HBGP) contains high amounts of fiber and bioactive phytochemicals that exhibit lipid-lowering effects. The objective of this study was to provide scientific proof for the hypolipidemic activity of HBGP. Male Sprague Dawley rats were fed a high-fat diet (HFD) for 14 weeks to induce Hyperlipidemia. The rats in groups 1 and 2 were fed a standard diet. Group 3 and 4 rats were fed HFD for 14 weeks. From the third week onwards, Group 2 and 4 rats received 50% of HBGP mixed in the feed, in addition to 50% of the normal and HFD diets. The rats were euthanized after 14 weeks, and plasma lipids, lipid peroxides, lipid metabolizing enzyme activity, and endogenous antioxidants were determined. HFD treatment significantly increased body mass index (BMI), blood lipids, low-density lipoprotein (LDL), lipid peroxides, HMG CoA reductase, and fatty acid synthase activity. HFD+HBGP fed rats showed a significant decrease in BMI, blood lipids, HMG CoA reductase, fatty acid synthase with an increase in antioxidants and lipoprotein lipase enzyme activity. Visceral adipocytes in group 3 rats increased in cell size with hypertrophy when compared to HBGP co-administered rats. The results of this study show that HBGP probably acts as a hypolipidemic agent by modulating the activities of HMG CoA reductase and fatty acid synthase in the liver.

***Key words*:** Hyperlipidemia, HFD, HBGP, HMG CoA reductase, Fatty acid synthase

Hyperlipidemia can be defined as a condition in which there is an increase in one or more lipids, such as total cholesterol (C), triglycerides (TG), free fatty acids, phospholipids, cholesterol esters, and LDL. Obesity is caused by increased fat tissue deposition coupled with hyperlipidemia. Every year, the frequency of hyperlipidemia rises in both advanced and emerging countries [1]. Sedentary behavior, frequent eating of fast foods high in trans fats, and a lack of physical activity are the primary causes. Although the pathophysiology of hyperlipidemia is complex, various experimental animal models have been established to understand the mechanisms involved in hyperlipidemia and to help researchers develop new herbal-based treatment medicines. Various investigations have found that a formulated HFD can easily induce experimental hyperlipidemia [2] that is similar to human hyperlipidemia. The rate-limiting enzyme in cholesterol production is hydroxymethyl glutaryl (HMG) CoA reductase [3]. Statins are commonly used hypolipidemic medications that target this enzyme. Fatty acid synthase (FAS) is a multi-enzyme complex containing seven enzymes, including thioesterase. HFD offers precursors for endogenous fatty acid production for the up regulation of FAS. During aberrant lipid metabolism, hepatic lipoprotein lipase (LPL) hydrolyzes triacylglycerol and down-regulates. Commonly, hypolipidemic medications are evaluated for their effectiveness in modifying their effect on the major enzymes indicated above. Oxidative stress is highly correlated with hyperlipidemia-induced pathology. It can cause an increase in lipid peroxidation by progressive and cumulative cell injury as a result of large body mass. Cell injury worsens the disease by generating more cytokines, particularly tumor necrotic factor-alpha (TNF-α) which generates reactive oxygen species in the tissues, causing lipid peroxidation [4]. Therefore, thiobarbituric acid reacting substances (TBARS) and hydroperoxides levels serve as a marker for oxidative cell damage, whereas superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and glutathione peroxidase (GPX) acts as effective free radical scavenger.

An allopathic formulation such as statins, bile acids, fibrates, and nicotinic acids have been used for treating hyperlipidemia. As a consequence, these drugs produce deleterious side effects on long-term use. Therefore, finding an herbal medicinal alternative is found to be important. Dietary phytochemicals are natural antihyperlipidemic agents because they suppress the growth of the adipose tissue, inhibit differentiation of preadipocytes, stimulate lipolysis and induce

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apoptosis of existing adipocytes, thereby reducing adipose tissue mass [5]. Barley (*Hordeum vulgare*) is the first cultivated cereal grain. According to FAOSTAT [6] data, barley is the world's fourth most important cereal grain crop. There are generally two types of barley. Hulled barley grains (HBG), which have an outer caryopsis covered by a hull, and hulless grains, which do not have a hull. When compared to hulless barley variants, HBG provides more starch, carbohydrates, and total dietary fiber. Diabetes mellitus, metabolic syndrome, cancer, atherosclerosis, and hyperlipidemia are just a few of the chronic conditions that HBG can prevent [7]. Fiber, phenolic acids, flavonoids, phytosterols, alkylresorcinols, benzoxazinoids, lignans, tocol, and folate are functional dietary components present in HBG with anti-diabetes, anti-cancer, anti-obesity, cardioprotective, antioxidant, antiproliferative, and cholesterol-lowering properties [8]. The focus of this research was to investigate whether HBGP could reduce oxidative stress and alter the activities of HMG CoA reductase and FAS associated with hyperlipidemia in male Sprague Dawley rats fed with HFD.

MATERIALS AND METHODS

Chemicals

All chemicals used in the analysis were analytical grade and purchased through authorized Merck distributors in India.

Preparation of HBG

The fresh HBG was purchased from the local market in Chennai and authenticated by Dr. P. Jayaraman, Taxonomist, Plant anatomy research center (PARC). The grains were washed, air-dried, ground to a fine powder, and stored at room temperature.

Animals

Male Sprague-Dawley (150-200 g) rats were housed in a light/dark cycle in a regulated temperature (22±2°C) setting with a relative humidity of 44-55 percent. Water and food were given ad libitum.

Experimental protocol

The rats were split into four groups after a week of acclimatization. The rats in groups 1 and 2 were used as controls and were fed a normal diet. HFD was given to rats in groups 3 and 4 for 14 weeks. The HFD diet was prepared and fed according to the method of Nascimento *et al*. [9] (Table1). The average diet provides 3.48 kcal/g of energy, while the HFD provides 4.6 kcal/g. In addition, from the third week onwards, Group 2 and 4 rats were given 50% HBGP. The Institutional Animal Ethics Committee (IAEC) accepted the study protocol (XXIII/ VELS/ PCOL/ 14/2000/ CPCSE/ IAEC/ 07.02.2020). Once in a week, body weight was measured and BMI was calculated using the formula: BMI = weight (g)/(nose-anus) (cm^2). Daily food intake (FI), calorie intake (CI), body weight gain (BWG) and feed efficiency ratio (FER) per group was calculated by using the following equations:

$$CI = \frac{\text{Daily FI (g)} \times \text{Total energy of food (kcal/g)}}{1000}$$

$$BWG\ \% = \frac{\text{Final body weight} - \text{Initial body weight}}{\text{Initial body weight}} \times 100$$

$$FER = \frac{\text{Gain in body weight (g)}}{\text{Feed consumed (g)}} \quad [10]$$

Table 1 Composition of normal and HFD

Components	Normal diet	HFD
Protein %	26	28
Carbohydrates %	54	36
Fat %	3	23
Others %	17	13
Calories kcal/g	3.5	4.6

*Others: vitamins, minerals, cinders and water

Rats were anesthetized with diethyl ether and euthanized by cervical decapitation at the end of the experimental period. Blood was taken instantly, the plasma/serum separated, and refrigerated at 4°C until analysis. Heart, liver, and visceral AT were removed immediately. A part of these organs from each rat was fixed in 10% formalin saline for 24 hours and subjected to processing for histopathological examinations.

Extraction of lipids

A sample of liver tissue was homogenized in cold 0.15 mol KCl and extracted with (chloroform) $CHCl_3$:CH_3OH (methanol) (2 percent v/v) [11]. The lipid residue's weight was calculated.

Biochemical investigations

Lipid profile

The plasma was estimated for cholesterol [12], triglycerides (TG) [13], high density lipoprotein (HDL) [14] and low-density lipoprotein (LDL) [15].

Assay of lipid metabolic key enzymes

The activity of HMG CoA reductase was measured indirectly using the Philipp and Shapiro method [16] of determining the ratio of HMG CoA to mevalonate in the liver. The malonyl CoA-dependent oxidation of NADPH at 37°C was used to evaluate FAS activity in the liver [17]. At 37°C, one unit of enzyme activity equals 1 mM of NADPH oxidized per minute. The concentration of protein was measured using bovine serum albumin as a standard [18]. The activity of LPL was calculated using the Korn method [19] with some modifications. The results were represented as moles of glycerol liberated /hour/gram of tissue.

Estimation of lipid peroxides, GSH, and antioxidant enzymes

Thiobarbituric acid reacting (TBARS) substances were used to measure the level of lipid peroxides in plasma by the method of Draper and Hardley [20]. The TBARS concentration was measured in nanograms per milliliter of plasma. GSH level was determined by the method of Moron et al [21]. The activity of GPX was estimated using the Flohe and Gunzler (1984) [22] method. SOD and CAT activity were measured according to the method of Kakkar *et al*. [23] and Aebi [24].

Histopathological examinations

For histological examination, heart, liver, and visceral AT were fixed in 10% (v/v) formalin saline. It was cleaned in methyl benzoate, paraffin methyl benzoate, and paraffin-embedded for light microscopic investigation after passing through a graded sequence of alcohol washing, cut at a thickness of 5M. Hematoxylin and eosin were used to stain paraffin-embedded tissue slices. The morphological evaluation was carried out using a light microscope at a magnification of 400x [25].

Statistical analysis

The statistical software package was used to analyze the data (SPSS for Windows v. 10). A one-way ANOVA with a post

hoc Bonferroni test was used to examine the statistical significance of mean values between groups, with a P-value of 0.05 considered significant.

RESULTS AND DISCUSSSION

Effect of HBGP on BMG, BMI, FI, CI, and FER

Data depicted in (Table 2) shows the BWG, FI, CI, and FER in control and experimental rats. There was a significant (P = 0.000) increase in the BWG and BMI of HFD fed rats when compared to normal diet-fed rats. HBGP co-administered rats showed a significant decrease in BWG and BMI. Moreover, HFD fed rats consumed significantly (P = 0.000) more food and calories compared to control rats. HBGP supplementation decreased the FI and CI of HFD + HBGP diet-fed rats. Bioactive phytochemicals, naturally occurring in cereals, fruits and vegetables have enormous potential in regulating lipid metabolism, adipocyte biology and thus studied for possible hypolipidemic effects, based on that the fact it reduces FI, CI, or fat absorption, increase energy expenses, or inhibits energy storage. BWG and BMI are important parameters for monitoring the effect of HFD on the development of hyperlipidemia. It mainly depends on the FI and CI in every animal species and must be recorded. Thus, in this study, BWG, BMI, FI, CI, and FER were significantly more in HFD fed rats when compared with a control group of rats fed a normal diet. BWG and BMI were well maintained in rats that received HBG probably by its active phytonutrients FA which exert hypolipidemic activity.

Table 2 Effect of HBGP on BWG, BMI, FI, CI and FER

Groups	BWG (%)	BMI (g/cm^2)	FI (g)	CI (kcal/g)	FER
Group 1(Control)	100 ± 12	0.50 ± 0.06	17.50 ± 2.10	61 ± 7.32	6.07 ± 0.72
Group 2 (HBGP Control)	115.78 ± 13.89$^{\#}$	0.52 ± 0.06 ^{NS}	20.24 ± 2.42 $^{@}$	70.5 ± 8.46*	4.74 ± 0.56^{NS}
Group 3 (HFD)	280 ± 33.6*	1.50 ± 0.08*	33.33 ± 3.99*	150 ± 17.98*	6.68 ± 0.80*
Group 4 (HFD + HBGP)	126.01 ±15.12*	0.55 ± 0.06*	18.73 ± 2.24*	76.85 ± 9.22*	5.80 ± 0.69*

[Values are expressed as mean ± S.D. for six animals in each group. Statistical significance was calculated by comparing Control vs. HBGP control, control vs. HFD, HFD vs. HFD + HBGP. $^{\#}$P = 0.027, *P = 0.000, $^{@}$P = 0.006, NS = non-significant

Effect of HBG on blood lipids

When compared to normal rats, serum C, TG, and LDL concentrations of group 3 rats were significantly higher (P = 0.000), while HDL levels were significantly lower (Table 3). In HFD + HBGP fed rats, HBGP efficiently decreased plasma C, TG, and LDL and significantly increased HDL levels (P = 0.000). The HFD was found to raise blood lipid levels after 14 weeks of consumption. The saturated fats in the HFD act as a precursor for endogenous lipid production, which is the reason for high blood lipids in HFD fed rats. Increased availability of free fatty acids and denovo lipid synthesis in the liver cause elevated blood cholesterol and TG levels in HFD fed rats. Furthermore, elevated plasma LDL levels in HFD fed rats may be attributed to increased endogenous cholesterol absorption and disruption of LDL receptors, resulting in reduced cholesterol catabolism and bile acid generation. The lipid profile of HFD + HBGP rats was dramatically improved when HBGP was given concurrently. HBGP has shown promising lipid-lowering effects, especially on cholesterol metabolism. HBGP is rich in Beta-glucan that helps in decreasing intestinal absorption of cholesterol and bile acids [26]. Swelim *et al*. [27] who demonstrated the hypolipidemic effects of barley-β-glucan in experimentally induced hyperlipidemic rats, supports our study.

Table 3 Effect of HBGP on blood lipids

Groups	C (mg/dl)	TG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Group 1 (Control)	115.5 ± 13.86	100.5 ± 12.06	30 ± 3.60	35 ± 4.20
Group 2 (HBGP Control)	114.5 ± 13.74$^{\#}$	105.5 ± 12.66$^{@}$	31.5 ± 3.78^{NS}	30.5 ± 3.66
Group 3 (HFD)	177.5 ± 21.3*	192.5 ± 23.10*	15.6 ± 1.87*	64.5 ± 7.74*
Group 4 (HFD + HBGP)	119.5 ± 14.34*	134.3 ± 16.11*	36.2 ± 4.34*	37.5 ± 4.50*

Values are expressed as mean ± S.D. for six animals in each group. Control vs. HBGP control, Control vs. HFD, HFD vs. HFD + HBGP were compared for statistical significance. $^{\#}$P = 0.140, *P = 0.000, $^{@}$P = 0.001, $^{\$}$P = 0.006, NS = Non-significant

Table 4 Effect of HBGP on key enzymes of lipid metabolism

Groups	HMG CoA Reductase (Ratio of HMG CoA to mevalonate)***	Lipoprotein lipase (µ mol. of glycerol liberated/h/g protein)	Fatty acid synthase (mU/g tissue)
Group 1 (Control)	3.75 ± 0.45	70.6 ± 8.47	1264.5 ± 151.74
Group 2 (HBGP Control)	4.58 ± 0.54^{NS}	72.5 ± 8.70^{NS}	1249.5 ± 149.94^{NS}
Group 3 (HFD)	3.16 ± 0.37^{NS}	41.5 ± 4.98*	2192.5 ± 263.1*
Group 4 (HFD + HBGP)	5.5 ± 0.66$^{\#}$	73.5 ± 8.82*	1474.5 ± 176.94*

Values are expressed as mean ± S.D. for six animals in each group. Control vs. HBGP control, Control vs. HFD, HFD vs. HFD + HBGP were compared for statistical significance. ***Lower ratio indicates higher enzyme activity and vice versa. *P = 0.000, NS = Non-significant

Effect of HBG on key enzymes of lipid metabolism

Data presented in (Table 4) represents the activities of FAS, LPL, and HMG CoA reductase in the liver of control and experimental rats. We observed a significant increase (p=0.000) in the activity of HMG CoA reductase, FAS and a significant decrease (p=0.000) in the activity of lipoprotein lipase in HFD fed rats compared to the normal diet-fed rats. HBGP co-administered rats showed a significant (p=0.000) decrease in the activities of HMG CoA reductase and FAS. LPL activity in HFD+HBGP rats showed a significant (p=0.000) increase. FAS is a multienzyme complex that is involved in the biosynthesis of fatty acids. FAS activity was shown to be much higher in HFD-fed rats than in HFD+HBGP fed rats. HMG CoA reductase is a catalytic enzyme that converts HMG CoA to mevalonate. The lower ratio indicates greater enzyme activity and vice versa. The significant increase in HMG CoA: mevalonate ratio is observed in HFD rats suggests that HMG CoA reductase activity is higher in HFD control rats than in

HFD+HBGP fed rats. Furthermore, these findings are further supported by the elevated blood cholesterol in the HFD control rat group [3]. This research clearly shows that HBGP inhibits HMG CoA reductase. In addition, the activity of LPL was shown to be much lower in HFD control rats. LPL is required for the breakdown of triacylglycerol and the release of free fatty acids from lipoproteins. The enzyme activity is significantly higher in HBGP co-administered rats than in HFD fed rats.

Effect of HBGP on lipid peroxides and antioxidant enzymes

Data presented in (Table 5) shows the serum levels of TBARS, GSH, GPX, SOD, and CAT in experimental rats. The level of lipid peroxide in the HFD + HBGP group was significantly lower (P = 0.000) than in the HFD group. Antioxidant enzyme levels, on the other hand, were found to be considerably lower (P = 0.000) in group 3 rats. Both the HFD and the HBGP improved the levels of these cellular antioxidants in group 4 rats. The pathogenesis of HFD-induced hyperlipidemia involves reactive oxygen species from both endogenous and exogenous sources. In the current study, the increased levels of TBARS in HFD fed rats could be linked to an excess of free radicals and lipid peroxide activation in tissues, resulting in cell damage. HBGP on the other hand was able to counteract the oxidative damage caused by the HFD. GSH is a water-soluble tripeptide with a powerful reducing agent in the form of a thiol group. GSH is the principal antioxidant and plays a vital role in the detoxification of a variety of electrophilic ions and peroxides. SOD, CAT, and GPX are antioxidant enzymes that provide a first-line barrier against the disposal of superoxide anions and hydrogen peroxides. Increased utilization owing to oxidative stress is shown by a decrease in the level of these enzymes in HFD control rats. The presence of a high level of antioxidants and phytochemicals in HBGP resulted in a permanent increase in the activity of these enzymes in HFD+HBGP rats. The current results are in basic agreement with the results of Ahmed *et al.* [28] who showed that Citrus limetta has a protective effect against hyperlipidemia in diabetic and non-diabetic rats.

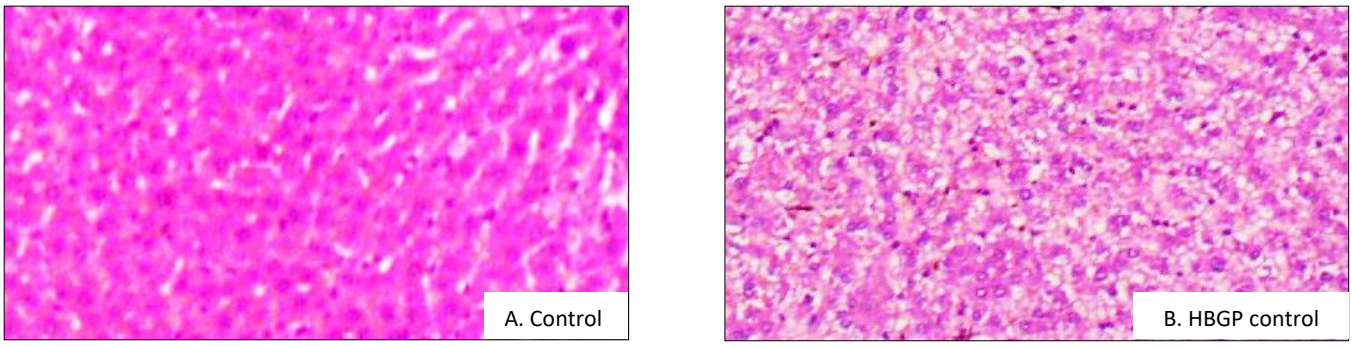
Table 5 Effect of HBGP on the serum levels of TBARS, GSH, GPX, SOD and CAT

Groups	TBARS (µM / ml)	GSH (mg/g of protein)	GPX (nM of GSH oxidized/ml)	SOD (U / ml)	CAT (U / ml)
Group 1 (Control)	8.33 ± 0.86	4 ± 0.24	14.75 ± 1.77	17.36 ± 2.08	6.95 ± 0.83
Group 2 (HBGP Control)	7.9 ± 0.97 ^{NS}	3.33 ± 0.35*	15.77 ± 1.89^{NS}	17.61 ± 2.11^{NS}	7.34 ± 0.88^{NS}
Group 3 (HFD)	10.8 ± 1.34*	2 ± 0.22*	7.46 ± 0.89*	11.03 ± 1.32*	4.67 ± 0.56*
Group 4 (HFD + HBGP)	8.23 ± 1.06*	5.33 ± 0.56*	12.8 ± 1.53*	14.42 ± 1.73*	5.53 ± 0.66*

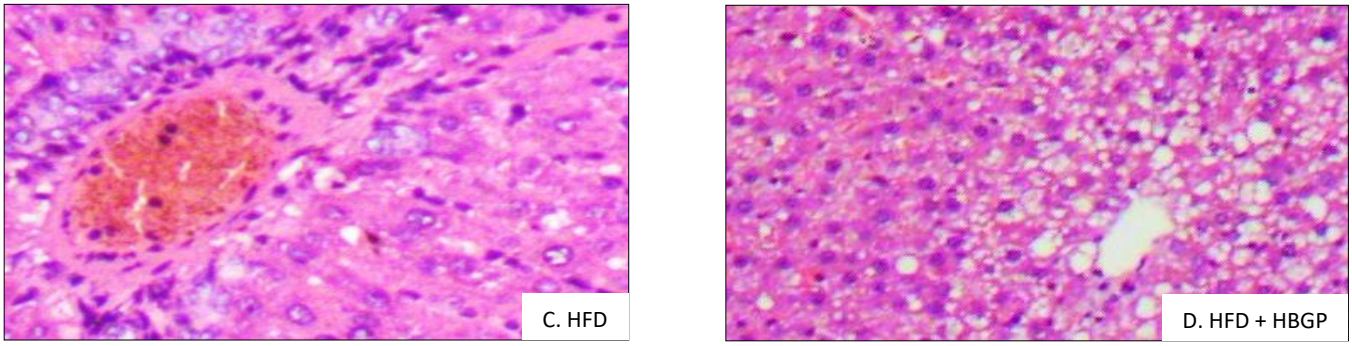
Values are expressed as mean ± S.D. for six animals in each group. Control vs. HBGP control, Control vs. HFD, HFD vs. HFD + HBGP were compared for statistical significance. *P = 0.000, NS = Non-significant

Histopathological observations

The light microscopic examination showed normal tissue architecture of the liver (Fig 1A), heart (Fig 2A), and visceral AT (Fig 3A) in control rats. Rats that received HFD showed degeneration of hepatocytes with multifocal mononuclear cell (MNC) infiltration, steatosis, and accumulation of lipid droplets (Fig 1C). HBGP co-administered rats showed mild degenerative changes and fat accumulation (Fig 1D). Congestion and perivascular MNC infiltration with fat vacuoles were observed in the heart sections of HFD fed rats (Fig 2C), whereas HBGP co-administration resulted in only mild cardiocytes degeneration (Fig 2D). The size of visceral AT was reduced in HBGP co-administered rats (Fig 3D) when compared to HFD control rats (Fig 3C).

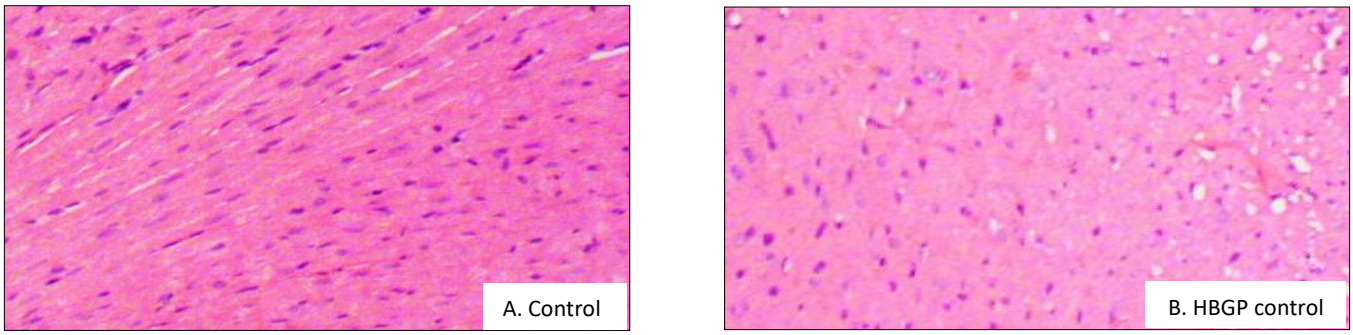


A) and B) Liver sections from the control rats and rats treated with hulled barley grains alone show pristine tissue architecture

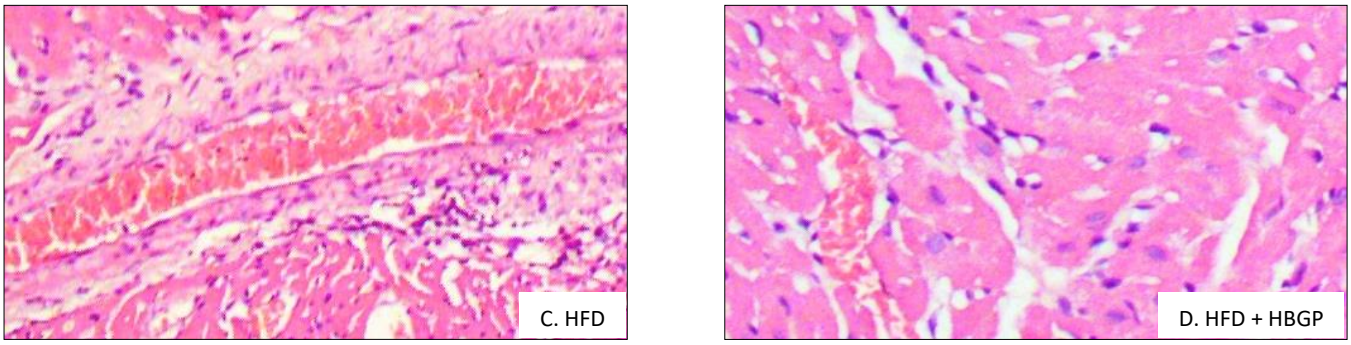


C) Sections from rats administered with HFD showing MNC infiltration with congestion, macro to microvesicular steatosis; D) Minimal mononuclear cell infiltration, moderate macro to microvesicular steatosis was observed in HFD and HBGP treated rats

Fig 1 Histology of liver tissue sections (H&E stain, 400X)

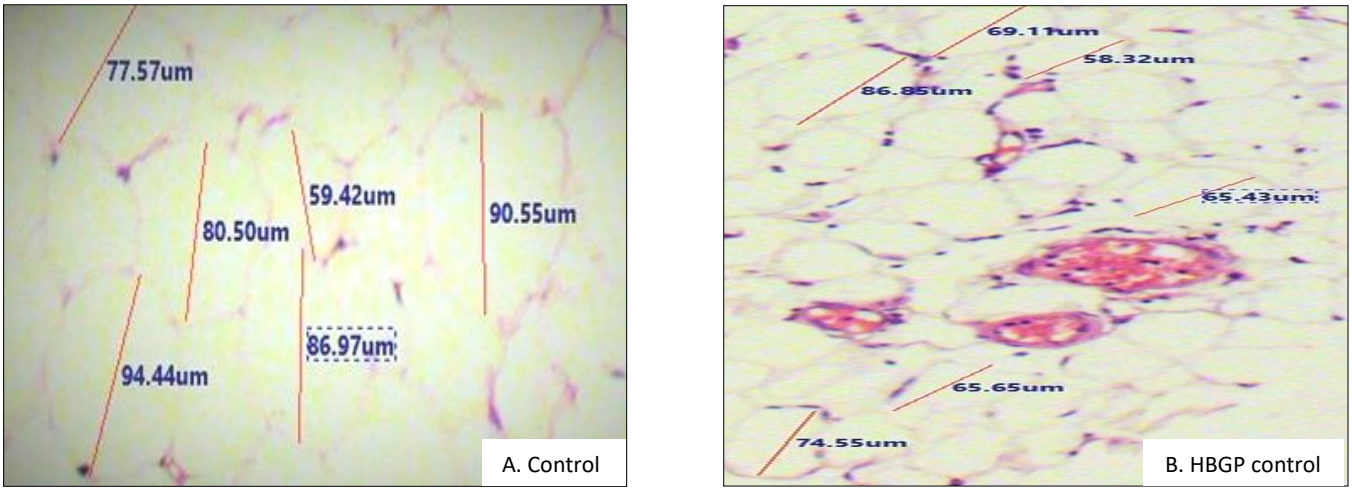


A) and B) Heart sections the control rats and rats treated with HBGP alone showed undisturbed structure

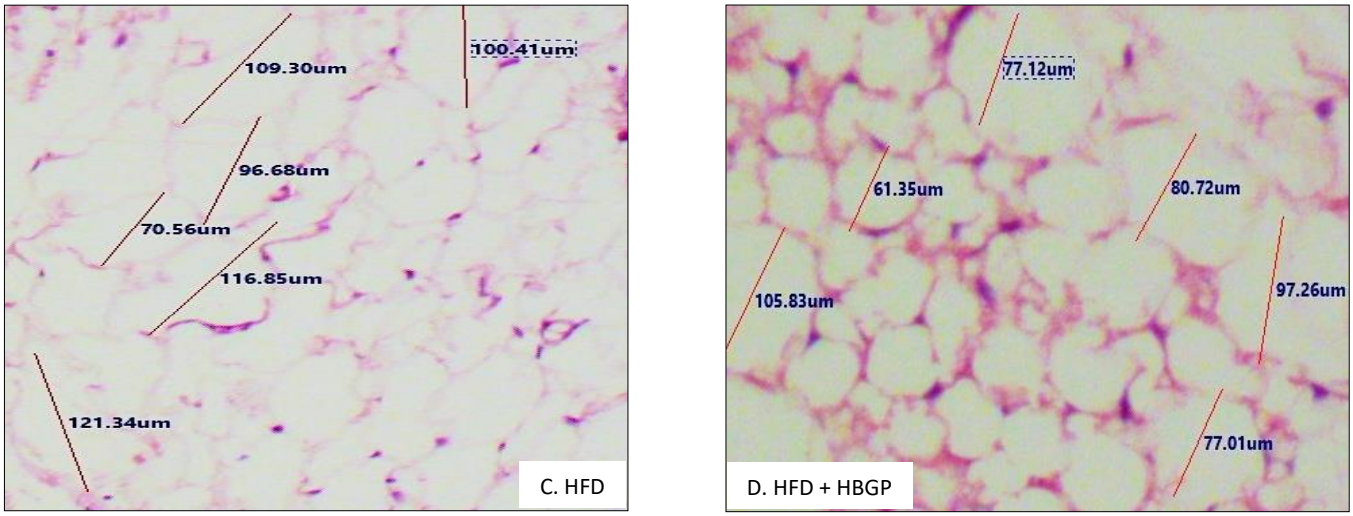


C) Heart tissues from rats administered with HFD showing congestion, perivascular mild MNC infiltration with fat vacuoles; (D) heart sections from rats co-administered with HBGP show mild degeneration of cardiocytes and decrease in fat vacuoles and necrosis

Fig 2 Histology of heart sections (H&E stain, 400X)



A) and B) Visceral AT sections from the control rats and rats treated with HBGP alone showing the normal size of adipocytes with an average diameter of 79.57 µm and 69.98 µm respectively



C) Hypertrophy condition with an increased adipocyte cell size of 102.46 µm exists; HBG co-administered rats showed an improved adipocyte size of 83.21 µm

Fig 3 Histology of visceral AT sections (H&E stain, 400X)

CONCLUSION

To conclude, the present study clearly demonstrated that HBGP exhibit potent hypolipidemic activity probably by decreasing the activities of HMG CoA reductase and FAS, the key metabolic enzyme enzymes of liver which regulate lipid metabolism.

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Conflict of interest

There is no conflict of interest among us.

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