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RESEARCH ARTICLE

Effect of Hulled Barley Grains (*Hordeum vulgare*) Powder on the Gene Level Expression of PPAR-γ and SIRT1 in Rats Subjected to Experimental Hyperlipidemia

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Abstract

Objectives: To determine the mode of hypolipidemic action of Hulled Barley Grains Powder (HBGP) on the gene level expression of PPAR-γ and SIRT1 in High-Fat Diet (HFD) induced hyperlipidemic rats. **Methods:** The HBGP was subjected to High-Performance Liquid Chromatography (HPLC) to quantify the major phytochemicals. Male Sprague Dawley rats were fed an 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, serum levels of adiponectin and leptin were measured. The mRNA expression of PPAR-γ and SIRT1were also measured. **Findings:** HBGP was found to contain phenolics: Ferulic acid-9.11, benzoic acid-3.58, cinnamic acid-1.191, vanillic acid-2.43, flavonoids: quercetin-6.53, kaempferol-1.04, and myricetin-1.443 (mg/g). HFD+HBGP fed rats showed a significant decrease in blood lipids and leptin levels. Furthermore, serum adiponectin levels and mRNA expression of PPAR-γ and SIRT1 were found to be quantitatively increased in HBGP co-administered rats when compared to HFD fed rats. **Novelty:** HBGP exhibits hypolipidemic activity by elevating the mRNA expression of PPAR-γ and SIRT1. The presence of various phytochemicals such as ferulic acid, quercetin, benzoic acid, vanillic acid, myricetin, cinnamic acid, and kaempferol might be accounted for the hypolipidemic effect of HBGP.

Keywords: Hyperlipidemia; HFD; HBGP; HPLC; Adiponectin; Leptin; PPAR-γ; SIRT1

1 Introduction

Hyperlipidemia is defined as an elevation of one or more lipids such as total cholesterol (C), triglycerides (TG), free fatty acids, phospholipids, cholesterol esters, and LDL. Increased tissue deposition of fats together with hyperlipidemia contributes to obesity. The prevalence of hyperlipidemia is reaching its peak every year, both in developed and developing countries. The main causes are a sedentary lifestyle, frequent consumption of fast foods rich in trans fats, and lack of physical exercise. Although the pathogenesis of hyperlipidemia is difficult to understand, many experimental animal models have been developed to enrich a better understanding of the mechanisms involved in hyperlipidemia and for the development of novel herbal-based therapeutic drugs.

Drug therapy for treating hyperlipidemia includes an allopathic formulation consisting of statins, bile acids, fibrates, and nicotinic acids. Intensive statin therapy, medication lowered cholesterol levels in a large-scale, prospective, randomized study, However, the major adverse side effect is empathy, which can develop into fatal or non-fatal rhabdomyolysis. Herbal medicinal therapy is the unique alternative for the treatment of hyperlipidemia. Evidence is emerging to support that increasing consumption of dietary phytochemicals is an effective strategy to overcome hyperlipidemia.

Barley (*Hordeum vulgare*) is one of the oldest edible crops still farmed today, and it ranks fourth among the most significant cereal grain crops in the world. Nutritionally, barley is classified as hulled grains, which have an outer caryopsis covered with a hull, and hulless grains without a hull. Hulled barley grains have greater starch levels, carbohydrates, and total dietary fiber compared to hulless barley types. Hulled barley grains can combat more than 20 chronic diseases, including diabetes mellitus, metabolic syndrome, cancer, atherosclerosis, and hyperlipidemia. The therapeutic potentials of hulled barley grains are attributed to the presence of functional nutritional ingredients such as fiber, phenolic acids, flavonoids, phytosterols, alkylresorcinols, benzoxazinoids, lignans, tocol, and folate, which have antidiabetes, anticancer, antiobesity, cardioprotective, antioxidant, antiproliferative, and cholesterol-lowering properties[(1)].

Phenolics are the dominant phytochemicals present in the outer layer of the kernel of hulled barley grains. It exists in free, conjugated, and bound forms, and the highest concentration is obtained in the bound form, followed by the conjugated and free forms, respectively. The most abundant phenolic acids in hulled barley grains are p-hydroxybenzoic (17.6%), p-coumaric (15.2%), and ferulic acids (54.4%). Ferulic acid (4-hydroxy-3-methoxy-cinnamic acid) is a hydroxycinnamic acid derivative with many potential health benefits because of its antioxidant and anti-inflammatory activity. Ferulic acid is also known to reduce acute liver cell inflammation in mice by reducing intrahepatic inflammation and liver apoptosis. Based on the evidence shown above, it is obvious that ferulic acid may be effective in the treatment of diabetes mellitus, cardiovascular disease, cancer, Alzheimer's disease, and hyperlipidemia, most of which are disorders related to inflammation and oxidative stress[(2)].

Flavonoids are the largest group of natural polyphenols and are of three types: flavonols, anthocyanins, and proanthocyanidins. Quercetin is one of the most powerful antioxidants found in plants. It is a member of the flavonols class of flavonoids, which is a prominent polyphenol class. Based on the numerous clinical studies on humans, quercetin was found to possess anti-carcinogenic, anti-inflammatory, anti-infective, and psychostimulant agents. It also promotes mitochondrial biogenesis and prevents lipid peroxidation and platelet aggregation[(1)]. Hence, the biologically active components ferulic acid, vanillic acid, quercetin, and benzoic acid may be accounted for their nutritive and therapeutic effects.

Adipose Tissue (AT) is the major energy storage organ in mammals and mainly consists of adipocytes. In hyperlipidemic conditions, enlargement of adipocytes occurs with elevated production of pro-inflammatory cytokines, leading to enhanced infiltration of immune cells resulting in AT dysfunction. The effect of adipokines such as adiponectin, leptin, resistin, and visfatin on skeletal muscle, insulin resistance, and cardiovascular function has been reported. White AT produces and secretes leptin, a 167-amino-acid peptide. Plasma leptin levels are directly proportional to the quantity of body fat. Moreover, leptin levels are increased in response to both starvation and overfeeding. This pulsatile behavior of leptin secretion is similar in obese as well as lean people, but a higher amplitude is observed in obesity. Unlike leptin, plasma adiponectin levels are significantly decreased in hyperlipidemia and obesity. Adiponectin levels in cerebrospinal fluid are increased in response to fasting and decreased after refeeding, suggesting that adiponectin acts as a starvation signal[(3)].

SIRT1 is a member of the sirtuin family and it is a Nicotinamide Adenosine Dinucleotide (NAD)-dependent deacetylase that removes acetyl groups from various histone and non-histone proteins. SIRT1 can deacetylate a wide range of substrates and is thus implicated in a wide range of physiological processes, including gene regulation, metabolism, and aging. SIRT1 catalyzes the reaction that produces nicotinamide and transfers the acetyl group of the substrate to cleaved NAD, resulting in a unique metabolite known as O-acetyl-ADP-ribose. SIRT1 substrates include the tumor suppressor protein p53, members of the FOXO family (forkhead box factors controlled by insulin/Akt), PPAR-γ, p300, PGC-1α (PPAR gamma coactivator), and NF-kappa B (nuclear factor kappa B). PPAR-γ is a nuclear receptor transcription factor that is activated by ligands and greatly influences lipid metabolism. PPAR-γ is mostly expressed in insulin-responsive tissues, where it plays a key role in adipocyte differentiation and the production of adipogenesis-related genes. Hence, this research is focused on evaluating the

hypolipidemic effect of HBGP on the gene level expression of PPAR-γ and SIRT1, which play a crucial role in regulating lipid metabolism at HFD-induced hyperlipidemia [4].

2 Methodology

2.1 Chemicals

ELISA kits for the measurement of serum adiponectin and leptin were obtained from Abcam, USA. All other chemicals used in the analysis were analytical grade and purchased through authorized Merck distributors in India.

2.2. Preparation of HBGP

The fresh hulled barley grains were 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, and ground to a fine powder (HBGP), and stored at room temperature.

2.3. HPLC analysis of HBGP

HPLC analysis was carried out on Shimadzu HPLC CLASS-VPTM series equipped with 2489 UV/Visible detector for the quantitative determination of phytochemicals in HBGP. The EZChrom Elite Information System is used to acquire, process, and control HPLC and report generation.

2.3.1. Identification and quantification of phenolic acids

A C18 column (5 m, 100 x 4.0 mm) was used to fractionate the phenolic acids in HBGP. The resulting fraction was subjected to reverse-phase HPLC (RP-HPLC). As a stationary phase, octadecylsilyl silica gel was used. The mobile phase phosphoric acid, water, and acetonitrile were mixed in the ratio of 90:10:0.5. Reference standards include coumaric acid, vanillic acid, cinnamic acid, benzoic acid, and ferulic acid. A UV detector was set at 330 nm with a flow rate of 1.0 ml/min for a 20 μl sample.

2.3.2 Identification and quantification of flavonoids

HPLC was used to detect the presence of different flavonoids in HBGP, with octadecylsilyl silica gel as the stationary phase and acetonitrile and sodium dihydrogen phosphate with dilute orthophosphoric acid as the mobile phase (50:50:0.5). Standards for comparison included quercetin, kaempferol, myricetin, and rutin. The UV detector was set at 350 nm with a flow rate of 0.5 ml/min for the 20 μl sample.

2.4 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%. Water and food were given ad libitum.

2.4.1 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 [5] et al., [Table 1]. 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). Bodyweight was recorded once a week, and body mass index (BMI) was determined using the following formula: BMI = weight (g)/l^2 (nose-anus) (cm^2). 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.

Table 1. Composition of normal and HFD

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

*Others: vitamins, minerals, cinders and water

2.5 Biochemical investigations

2.5.1 Lipid profile

Plasma was estimated for cholesterol [6], triglycerides (TG) [7], high density lipoprotein (HDL) [8],and low density lipoprotein (LDL) [9].

2.5.2 Serum adiponectin and leptin determination

A quantitative ELISA kit was used to detect the concentrations of adiponectin and leptin in the blood (Abcam, USA). The assay was carried out according to the directions included in the kit.

2.5.3 Quantitative RT-PCR analysis

To perform qPCR, liver and AT samples were first processed for RNA isolation before getting converted to cDNA. Trizol was used to extract total RNA [10]. The purity of the samples was assessed using A260/280 values after the RNA was quantified using a spectrophotometer. After being processed with DNase *I* from New England Biolabs (Catalogue#M03035), samples were run on an RNA gel to check for DNA contamination. Thermofisher Scientific, Mumbai, India, (Calalogue#401425) used a cDNA reverse transcription kit to convert RNA to DNA. Specific primer sequences used for real-time PCR are depicted in Table 2. PCR was carried out using astratagene PCR equipment from Agilent Technologies (Santa Clara, CA). PCR conditions were as follows: Denaturation at 40 cycles of 95·C for 10 minutes, followed by annealing at 60·C for 60 seconds in a two-step real-time PCR. The Ct values obtained were used to quantify the data.

Table 2. Primer sequence

Gene	Primer sequence Forward (5'-3')	Primer sequence Forward ('3'-'5')
Rat PPAR-γ	AGGGCGATCTTGACAGGAAA	GCAAACTGGCACCCTTGAAA
Rat SIRT1	GCAGTAACAGTGACAGTGGC	AACTGCCTCTTGATCCCCTC

2.6 Statistical analysis

A 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.

3 Results and discussion

3.1 HPLC-UV analysis of HBGP

3.1.1. Identification and quantification of phenolic acids

HBGP had four peaks at 330 nm on the HPLC-UV chromatogram, with retention times of 5.300, 7.916, 9.833, and 15.316 minutes, respectively Figure 1A. The presence of ferulic acid, benzoic acid, cinnamic acid, and vanillic acid at concentrations of 9.11 mg/g, 3.58 mg/g, 1.19 mg/g, and 2.42 mg/g was established by the UV spectra of these peaks when compared to the retention time of corresponding standards [Figure 1B]. The compounds identified were confirmed by comparing their retention times to those of the standards. Natural phenolic acids are secondary metabolites of plants having probable optimistic physiological effects. They exhibit several roles like lowering blood pressure, reducing body weight, and preventing the formation of singlet oxygen as well as dyslipidemia [11]. Ferulic acid is abundant in various cereals that have beneficial effects on preventing cardiovascular diseases, possibly due to its antihypertensive and antioxidant activity. HPLC-UV spectra confirmed that HBGP is rich in ferulic acid. Also, ferulic acid has been shown to reduce blood cholesterol levels in experimental models of hyperlipidemia [2].

3.1.2 Identification and quantification of flavonoids

Figure 1C and Figure 1D show the UV spectra of the flavonoids in HBGP obtained using the most reliable approach, HPLC, after extracting the flavonoids using the C18 Hypersil gold column. The flavonoids quercetin (6.53 mg/g), kaempferol (1.04 mg/g), and myricetin (1.44 mg/g) were shown in ascending order of concentration Table 3. Flavonoids are plant-derived polyphenolic components with a variety of biological functions including anti-allergic, anti-bacterial, anti-viral, anti-inflammatory, and anti-tumor activities. In human diets, quercetin can be found in fruits, vegetables, tea, red wine, coffee, beer, and a variety of medicinal

herbs. Kaempferol is a flavonol found in a wide range of culinary plants as well as traditional medicines. The presence of these flavonoids in HBGP could explain the putative lipid-lowering effect observed in this study[11].

Table 3. Concentration of phenolic acids and flavonoids in HBGP

S. no	Phytochemicals	mg/g of HBGP
Phenolic acids 1 2 3 4	Ferulic acid Vanillic acid Benzoic acid Cinnamic acid	9.11, 2.42, 3.58, 1.19
Flavonoids 5 6 7	Kaempherol Quercetin Myricetin	1.045, 6.53, 1.44

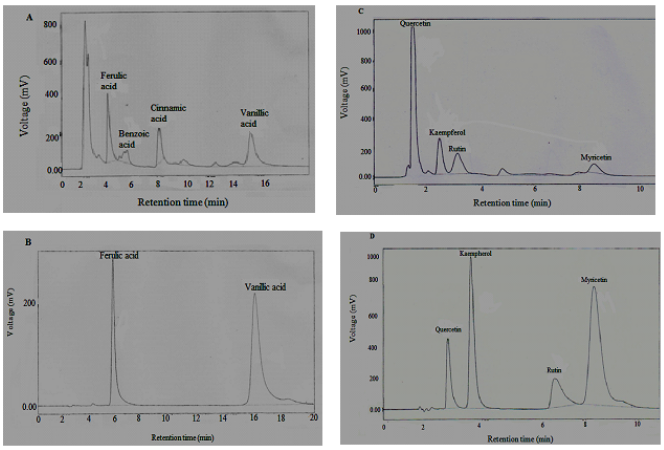

Fig 1. HPLC chromatogram of HBGP showing phenolic acids (A, B) and flavonoids (C, D) where (B and D) denote the corresponding standards

3.2 Effect of HBGP 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 Figure 2. In HFD+HBGP fed rats, HBGP efficiently decreased plasma C, TG, and LDL and significantly increased HDL levels (P = 0.000).

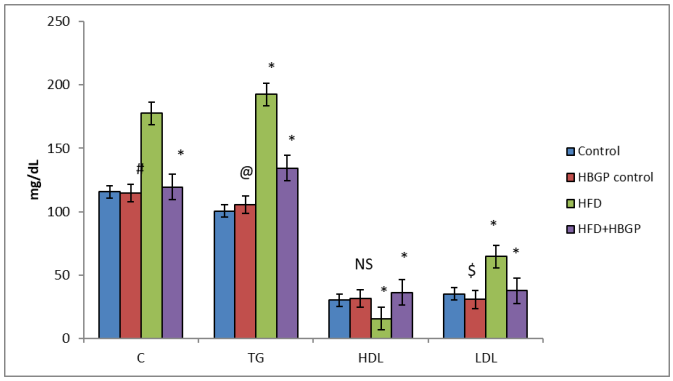

Fig 2. Effect of HBGP on blood lipids. *[Values are expressed as mean ± S.D. for six animals in each group. Control vs. HBGP control, Control vs. HFD, HFD vs. HFD+HBGP were compared for statistical significance.* $^{\#}P = 0.140$, $^{*}P = 0.000$, $^{@}P = 0.001$, $^{\$}P = 0.006$, *NS = Non-significant]*

Lipid deposition is a major complication of hyperlipidemia. HFD administration for 14 weeks was found to increase blood lipid levels. The saturated fats present in HFD serve as a precursor for endogenous lipid synthesis and are accountable for elevated blood lipids. Elevated blood cholesterol and TG levels in HFD fed rats are due to increased availability of free fatty acids and denovo synthesis of lipids in the liver. Furthermore, enhanced plasma LDL levels in HFD fed rats are possibly due

to increased uptake of endogenous cholesterol and disruption of LDL receptors with decreased cholesterol catabolism and production of bile acids. The concurrent supplementation of HBGP to HFD+HBGP rats significantly improved the lipid profile. HBGP is rich in soluble fiber β-glucan that helps in reducing intestinal absorption of cholesterol and bile acids by binding to glucan, thus shifting the liver from cholesterol synthesis to bile acid production and the net impact is a decrease in blood cholesterol. Barley fiber is an excellent source of vitamin B3 (niacin) that decreases platelet aggregation, which is responsible for the formation of blood clots, otherwise lead to blockage in arteries and decrease total cholesterol, lipoprotein as well as free radicals that causes oxidation of LDL. Deng et al.,[12] who demonstrated the hypolipidemic effects of barley whole grains in experimentally induced hyperlipidemic rats, supports our study. In this investigation, we also found that HBGP, when ingested as whole grains, exhibits effective hypolipidemic activity.

3.3. Effect of HBGP on BMI and serum adiponectin and leptin levels

Table 4 shows BMI, adiponectin, and leptin levels in the blood. When comparedto normal diet-fed rats, HFD fed rats had a significant (P = 0.000) elevation in BMI. BMI of rats fed with 50% HBGP decreased significantly. When compared to normal rats, HFD fed rats had considerably lower levels of adiponectin. The HBGP co-administration was found to significantly raise serum adiponectin (P = 0.027). Serum leptin levels, on the other hand, were considerably (P = 0.000) higher in group 3 rats than in group 1. In HBGP co-administered rats, this effect was greatly reduced (P = 0.000).Adiponectin is a 30 KD multimeric protein that influences whole-body and glucose homeostasis at the organ and systemic levels. Adiponectin promotes adipocyte cell development through its autocrine action. The amount of adiponectin in the bloodstream is inversely related to body fat content and plays an important role in reflecting its metabolic action in adipocytes. Overexpression of adiponectin protects mice from the acute and chronic consequences of HFD-induced lipotoxicity[13]. Leptin is a satiety hormone that regulates fat storage in the body. It is produced in fat cells and is primarily responsible for FI inhibition and energy expenditure stimulation. When HBGP co-administered group 4 rats were compared to HFD fed group 3 rats, the results showed a significant increase in adiponectin and a significant decrease in leptin. These results run in parallel with the findings of Wang et al.,[14] suggested that ferulic acid and γ-oryzanol treatment enhanced adiponectin levels and lowered the levels of leptin in rats fed with high-fat, high-fructose diet-induced metabolic syndrome As a result, HBGP has the potential to be a novel therapeutic agent for the treatment of hyperlipidemia.

Table 4. Effect of HBGP on BMI, serum adiponectin and leptin levels

Groups	BMI (g/cm)	Adiponectin (μg / ml)	Leptin (pg /ml)
Group 1 (Control)	6.12 ± 0.73	6.12 ± 0.73	15.96± 1.91
Group 2 (HBGP control)	6.2 ± 0.75^{NS}	6.2 ± 0.75^{NS}	15.08±1.80^{NS}
Group 3 (HFD)	4.86 ± 0.58*	4.86 ± 0.58*	50.22± 6.02*
Group 4 (HFD + HBGP)	5.18 ± 0.62$^{\#}$	5.18 ± 0.62$^{\#}$	33.26±3.99*

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, $^{\#}$P = 0.027, NS = Non-significant.

3.4 Effect of HBGP on the expression of PPAR-γ and SIRT1 in AT

The expression of PPAR-γ and SIRT1 was measured by RT-PCR in AT Figures 3 and 4. When compared with normal rats, HFD fed rats showed a significant decrease (P=0.000) in the levels of PPAR-γ and SIRT1. In contrast, PPAR-γ and SIRT1were significantly increased in HBGP co-administered rats.PPAR-γ is mostly expressed in AT and it regulates fat storage by controlling the expression of key genes involved in adipogenesis. In this investigation, PPAR-γ expression in HBGP co-administered rats was significantly increased (P=0.000) when compared with HFD fed rats. Elevated levels of PPAR-γ expression in AT of HFD+HBGP treated rats may be due to its prominent role in the upregulation of adiponectin, which occurs during adipocyte differentiation. On the other hand, decreased levels of PPAR-γ expression in HFD fed rats are due to increased calorie intake leading to fat accumulation and oxidative stress. Consequently, PPAR-γ is downregulated with low levels of adiponectin, the negative modulator of hyperlipidemia.

SIRT1 activity is tightly regulated in response to many environmental signals, which is not surprising. Caloric Restriction (CR), which is a 20–40% reduction in calories ingested below ad libitum intake without starvation, is one feeding regimen known to increase SIRT1 activation. Interestingly, CR is linked to greater physical activity in mice. However, this increase does not increase in SIRT1 knockout mice subjected to CR, indicating that SIRT1 is required for the consequences of CR. The lines of evidence that SIRT1 regulates lipid homeostasis are numerous and are explained below.Firstly, CR, prolonged fasting and

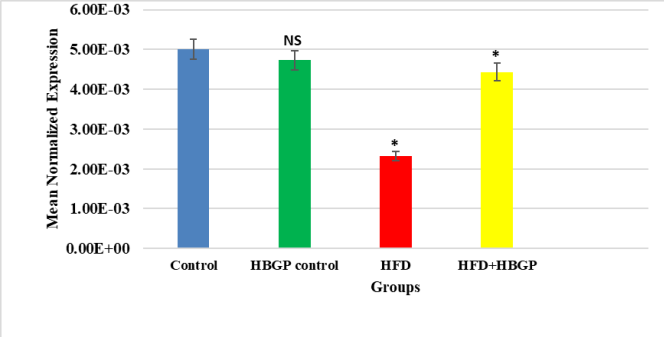

Fig 3. PPAR-γ gene expression in AT of experimental rats. *[Values are expressed as mean ± SD for six animals in each group. Data were analysed by one way ANOVA followed by post hoc Bonferroni test. Statistical significance was calculated by comparing Control vs. HBGP control, Control vs. HFD, HFD vs. HFD+HBGP were compared for statistical significance. *P = 0.000,NS = Non-significant]*

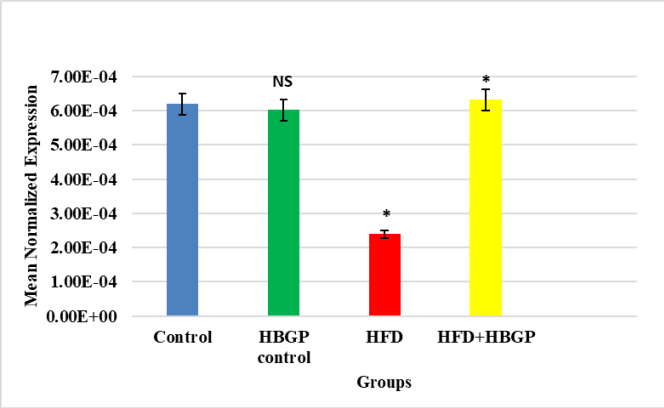

Fig 4. SIRT1 gene expression in AT of experimental rats. *[Values are expressed as mean ± SD for six animals in each group. Data were analysed by one way ANOVA followed by post hoc Bonferroni test. Statistical significance was calculated by comparing Control vs. HBGP control, Control vs. HFD, HFD vs. HFD+HBGP were compared for statistical significance. *P = 0.000,NS = Non-significant]*

natural polyphenolic products like resveratrol[15], fisetin, quercetin, and curcumin lead to the activation of SIRT1, which in turn modulates fat metabolism at several levels. In AT, SIRT1 represses PPAR-γ by docking with the nuclear receptor corepressor (NCoR) and the silencing mediator for retinoid and thyroid hormone receptors (SMRT). The resulting SIRT1/NCoR/SMRT complex binds to DNA sequences referred to as PPAR-γ response elements (PPREs) and thus represses the expression of PPAR-γ target genes, thereby causing increased fat mobilization and decreased adipogenesis.

Secondly, SIRT1 deacetylates PGC-1α and activates it. Activated PGC-1α binds and further activates FOXO1, leading to stimulation of gluconeogenesis thus improving glucose homeostasis in the liver whereas, in AT FOXO1 up regulates adiponectin gene transcription. Activated FOXO1 binds to the FOXO1-responsive region in the mouse adiponectin promoter, which comprises two neighboring FOXO1 binding sites, CCAAT/enhancer-binding protein α (C/EBPα). Therefore, FOXO1 forms a transcription complex with C/EBPα at the mouse adiponectin promoter improving the expression and secretion of adiponectin in adipocytes, thus increasing serum adiponectin levels in mouse[16]. Increased adiponectin further stimulates cells to insulin and increases fatty acid oxidation leading to new energy homeostasis. Furthermore, activated SIRT1 can also directly deacetylate FOXO1, which, in turn, interacts with PPAR-γ as well. To bind to the target region of DNA, PPAR-γ forms a heterodimer with the retinoid X receptor (RXR), and FOXO1 binding to PPAR-γ is thought to disrupt this PPAR-γ /RXR complex, resulting in PPAR-γ inability to bind DNA. FOXO1 signaling is antagonized by PPAR-γ, suggesting a reciprocal antagonistic connection between FOXO1 and PPAR-γ. FOXO1 functions are anti-adipogenic in adipocytes, whereas insulin and PPAR-γ functions are pro-adipogenic. In preadipocytes, FOXO1 activation suppresses adipocyte proliferation whereas PPAR-γ has the opposite effect[4]. Thus, FOXO1 regulates adipogenesis in a stage-dependent manner.

Thirdly, SIRT1 has been demonstrated to control cholesterol metabolism by deacetylation of Liver X receptor (LXR), in addition to fatty acid metabolism. LXR consists of two members, LXR-α, and LXR-β. Both α and β LXRs are expressed

in murine and human adipocytes but LXR-α is predominantly up regulated during fat cell differentiation. Interestingly, the coregulator PGC-1α is needed for LXR-α activation and is advantageous because it not only reduces intestinal cholesterol uptake but also increases reverse cholesterol transport. LXR-α positively regulates several hepatic and intestinal genes required for cholesterol excretion from the body including Cyp7a, the rate limiting enzyme for bile acid synthesis, and ATP binding cassette (ABC) genes involved in the liver and intestine. The overall impact is SIRT1 is a positive regulator of LXR-α and thus regulates whole body cholesterol homeostasis.

In our current investigation, SIRT1 expression in HBGP co-administered rats was found to be increased when compared with HFD fed rats. Thus, it is highly evident that the dominant phytochemical ferulic acid present in HBGP might have acted as an activator of SIRT1, exerting its hypolipidemic effects that are similar to CR. Also, Chen et al.,[17] proved that ferulic acid regulates muscle fiber type formation through the activation of Sirt1/AMPK signalling pathway supports our study. Further evidence for the role of SIRT1 in hyperlipidemia comes from experiments with resveratrol, a polyphenolic compound isolated from wine that is associated with a decrease in white AT and smaller adipocytes in mice fed an HFD[15]. Taken together, we suggest that SIRT1 is involved in the regulation of lipid metabolism by activating PGC-1α, FOXO1 and LXR-α proteins.

4 Conclusions

From the above study, it can be concluded that HBGP acted as a potent hypolipidemic drug by activating SIRT1, which influences PPAR-γ gene expression. Ferulic acid, the major phytochemical present in HBGP might be accounted for its hypolipidemic activity. An Insilco research can be conducted further to confirm the drug-like interaction property of ferulic acid on SIRT1. In the future, this unique therapeutic potential of HBGP and ferulic acid can probably serve as an innovative approach for the treatment of hyperlipidemia-related health complications in the human population.

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