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RESEARCH

The hypolipidemic activity of ferulic acid isolated from *Hordeum vulgare* through PPAR-γ/SIRT1/FOXO1/LXR-α mediated signaling pathway: a biochemical study on high fat diet induced obese male Sprague Dawley rats

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Introduction: Whole grains have been known for their medicinal properties for millennia. Numerous whole grains with antihyperlipidemic qualities have drawn attention from researchers as potential therapeutic adjuncts in lowering the incidence of cardiovascular disease. According to certain folklore and preliminary evidence, barley can lower body blood fat levels. The main goal of this study is to present scientific evidence of the lipid-lowering properties of hulled barley grain powder (HBGP) and ferulic acid (FA), its active ingredient.

Materials and methods: Male Sprague Dawley (SD) rats were provided with a high-fat diet (HFD) to develop hyperlipidemia. Groups 1 and 2 rats were given the standard feed. Rats in groups 4, 5, 6, and 7 were fed an HFD for 14 weeks. While Group 6 rats received 200 mg/kg body weight of FA, Group 5 rats started receiving 50% of hulled barley flour mixed into their feed in the third week in addition to 50% of regular and HFD meals. Furthermore, rats in Group 7 were given 10 mg/kg body weight of rosuvastatin. Rats were euthanized right after 14 weeks, and mRNA expression of peroxisome proliferator activated receptor gamma (PPAR-γ), silence information regulator 1 (SIRT1), forkhead box factors (FOXO1), and liver X receptor alpha (LXR-α) was assessed in liver and adipose tissue (AT) samples.

Results: It had been discovered that liver and AT samples of rats supplied with HFD had fewer mRNA expressions of SIRT1, PPAR-γ, FOXO1, and LXR-α than the liver and AT samples of experimental animals. In HFD + HBGP and HFD + FA fed groups, HBGP and FA co-administration significantly ($P = 0.001$) stimulated SIRT1, PPAR-γ, FOXO1, and LXR-α expression, therefore reducing adipocyte differentiation in obese conditions.

Conclusion: From our current results, it is highly evident that HBGP and its major compound FA have a hypolipidemic effect via increasing the mRNA expression of SIRT1, FOXO1, LXR-α, and PPAR-γ. The hypolipidemic outcome caused by HBGP may be explained by the presence of prominent phytochemicals like FA. FA and HBGP are undoubtedly promising drugs for the management of health issues associated with hyperlipidemia in the general human population.

Keywords: HBGP, FA, PPAR-γ, SIRT1, FOXO1, LXR-α

Graphical Abstract

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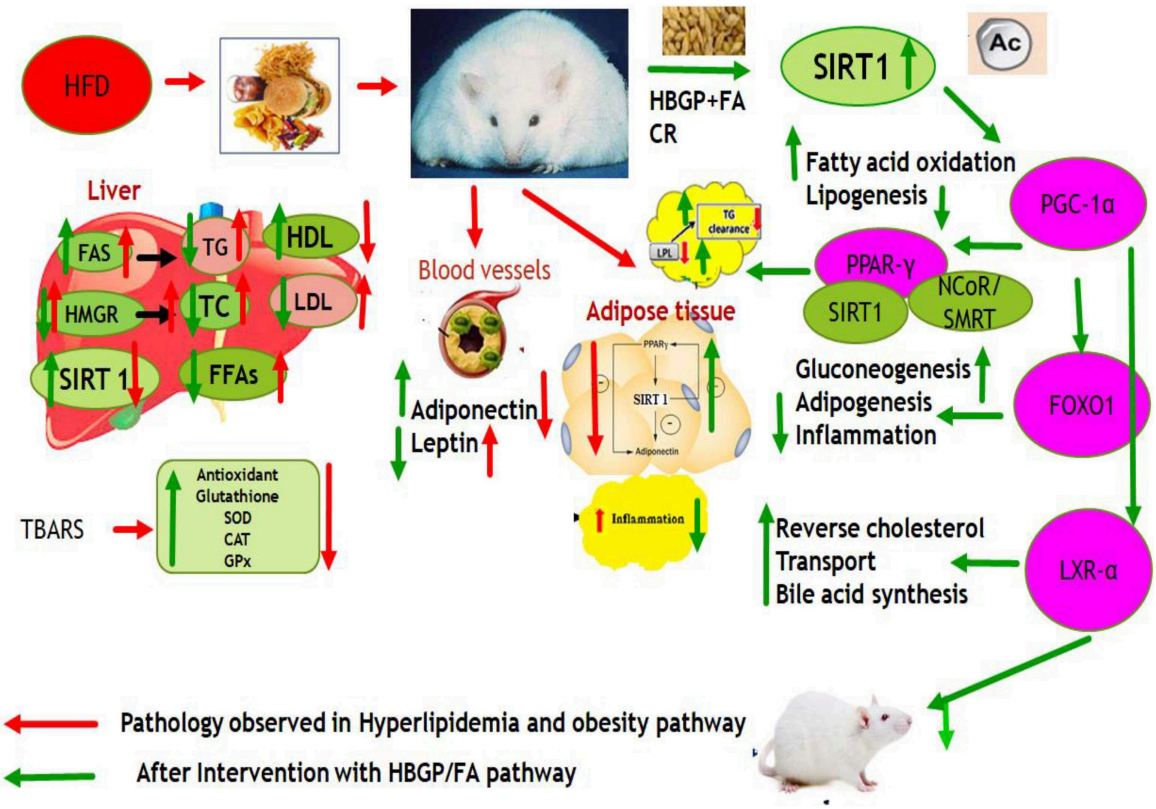

Introduction

Whole grains in general contain the original amounts of bran, germ, and endosperm from the entire grain seed. Whole grains should account for at least half of the total grain diet, as per the Dietary Guidelines for Americans 2020–2025. Because of its peculiar nutrition and abundance of bioactive components, hulled barley grain has attracted increasing interest as a major crop commonly produced. β-glucans, phenolics, tocols, and phytosterols were shown to be higher in whole grain than in most other grains (1).

The Prophet Muhammed (pbuh) stated that "Two of the greatest cures Allah has given for heart disease are whole wheat and barley." Both have numerous advantages in the fight against heart diseases. They decrease cholesterol while also improving the body's waste removal. They also help with normal blood coagulation and promote circulation and cellular health. Also, Prophet Muhammed (pbuh) usually consumes bread made up of barley (2). Yava or barley is described in detail in the Vedas, Upanishads, Grihya Sutra, and Shatapata Brahmana. Yava is utilized in numerous Ayurvedic medicines as well as in the diet for several santarpanajanyaroga, or disorders induced by overeating, such as diabetes. Yavakshara, an Ayurvedic medication made from the entire barley plant, is used to cure urinary issues and stomach pain and is found in many Ayurvedic classical preparations (3).

A major constituent of the phenolic acid family, FA is found in large quantities in a variety of fruits, veggies, and crops, including rice bran, oats, wheat, barley, brewed coffee, tomatoes, asparagus, berries, oranges, lemons, and the leaves of most plants. It is the dominant phytochemical present in hulled barley grains. Research experiments have demonstrated that FA possesses anti-inflammatory, anti-cancer, anti-atherogenic, antioxidant, antidiabetic, hypocholesterolemic, and hypoglycemic effects. In addition, FA can be easily absorbed and digested by the human body, and it has a low toxicity level. FA has also recently been proved to enhance lipid and glucose homeostasis in mice fed a high-fat diet (HFD) (4).

Sun et al. (5) demonstrated that FA may protect cardiomyocytes from oxidative stress by modulating the miR-499-5p/p21 signalling pathway. Moreover, Chowdary et al. (6) stated the protective role of ferulic acid (FA) by eliminating oxidative stress, inflammation, and autophagy in hyperglycemia-induced rats.

A member of the sirtuin family, silence information regulator 1 (SIRT1) affects the activity of metabolic

transcriptional regulators in a number of organs, including forkhead box factors (FOXO1), peroxisome proliferator activated receptor alpha (PPAR-α), PPAR-γ, and PPAR gamma coactivator (PGC-1α). By removing the acetyl group, SIRT1 stimulates PGC-1α in the liver, and it also interacts with FOXO1 to switch on gluconeogenesis while limiting glycolysis. SIRT1 increases fatty acid oxidation and mitochondrial biogenesis in muscle by stimulating PGC-1α. SIRT1 inhibits PPAR-γ transcriptional activity in white fat of adipose tissue (WAT), increasing its mobilization and decreasing adipogenesis. Fasting and restricting calories (CR), as well as exposure to resveratrol (polyphenol), stimulate SIRT1 production and cellular activity (7). It has been discovered that SIRT1 is dependent on nicotinamide adenosine dinucleotide (NAD+) and plays a significant role in the development and maturation of fatty tissue cells, metabolism of liver lipids, systemic inflammatory state, centralized nutritional monitoring, and rhythmic metabolism (8).

With the available data, this research mainly focuses on the potential of hulled barley grain powder (HBGP) and their active compound FA against hyperlipidemia induced in experimental rats. The lipid-lowering effect of test materials might be modulating the expression of molecular markers such as PPAR-γ, SIRT1, FOXO1, and liver X receptor alpha (LXR-α) on the gene level, the influencing factors of vascular inflammation during hyperlipidemia.

Methodology

Collating and analyzing barley grains with hulls

Herbal Parc in Chennai verified the legitimacy of the hulled barley grains, which had been obtained at Chengalpattu in Chennai (Voucher No. PARC/2015/3040). After being cleaned and allowed to air dry, the hulled barley grains were ground into a coarse flour and kept at ambient temperature.

Extraction of FA from HBGP

Ferulic acid was extracted from hulled barley grains according to the method described by Gamel and Abdel (9). For alkaline extraction, 15 ml of 2N sodium hydroxide was applied to 0.2 g of milled hulled barley grains. For 2 hours, the mixture was agitated at room temperature while exposed to nitrogen. After processing, 20 ml of anesthetic ether/acetic ether (1:1) were added, and 6N hydrochloric acid was used to bring the pH down to 2. Following ten minutes of shaking, the vials were then centrifuged for 10 minutes at 14000 × g. The water layer (bottom) was then removed by carefully transferring the supernatant into a separator funnel. A layer of anhydrous sodium sulfate was used to filter the organic solvent extraction (upper layer) into a round-bottomed flask. After two rounds of the organic extraction, all of the fractions were poured into a round-bottomed flask. The glass flask was vacuum-dried at 40°C in a rotary evaporator until it was completely dry. The result is a brown precipitate of FA. For analysis, the dry product was refrigerated after being reconstituted in 1 ml of 95% aqueous ethanol.

TABLE 1 | Experimental protocol of efficacy study.

S. no	Groups	Experimental design
1	Group 1	Control
2	Group 2	HBGP control
3	Group 3	FA control
4	Group 4 (diseased)	High-fat diet (HFD)
5	Group 5 (treatment 1 with HBGP)	HFD + HBGP
6	Group 6 (treatment 2 with FA)	HFD + FA
7	Group 7 (treatment 3 with standard rosuvastatin)	HFD + RT

Test animals

Healthy male SD rats weighing 150–200 g were housed in a light/dark cycle with a regulated atmospheric humidity of 44–55% and an average temperature of 22 ± 2°C. The Institutional Animal Ethics Committee (IAEC) granted its approval to this study protocol (XXIII/VELS/PCOL/14/2000/CPCSE/IAEC/07.02.2020).

Induction of hyperlipidemia

For 14 weeks, males of SD rats were given an HFD so as to develop obesity and hyperlipidemia.

High-fat diet (HFD) composition

The experimental animals were fed HFD in accordance with Nascimento et al. (10) recommendations. The HFD was composed of 439, 218, 129, 61, and 153 g/kg body weight of ground labina, browned peanuts, milk powder, corn oil, and french-fried potatoes, respectively. The standard rat chow feed was purchased from the Centre for Animal Nutrition, Kattupakkam, Chennai. All other ingredients were purchased from Agro Products of high quality and purity. They were pulverized before being supplemented with 1 or 2 capsules of vitamins and minerals. The blend was subsequently formed into balls and dried in a drying oven at a temperature of 55 ± 5°C.

Research protocol

To determine the antihyperlipidemic effect of HBGP and FA against HFD-induced hyperlipidemia, rats were split into seven equal-number groups (**Table 1**). A normal feed was

TABLE 2 | Primer sequences used for RT-PCR.

S. no	Gene	Primersequences	
		Forward(5'-3')	Reverse(3'-5')
1	RatPPAR-γ	CCCTGGCAAAGCATTTGTAT	ACTGGCACCCTTGAAAAATG
2	RatSIRT1	GCAGTAACAGTGACAGTGGC	CGAAACTGGCACCCTTGAAA
3	RatFOXO1	GGTGAAGAGTGTGCCCTACT	TCTTCTCCGGGGTGATTTCC
4	RatLXR-α	GTCAAGAAGAGGAGCAGGCT	AAGTCGGTCAGAGAAGGAGC
5	Ratβ-actin	CACCAACTGGGACGACAT	ACAGCCTGGATAGCAACG

administered to the rats in groups 1, 2, and 3. From the third week onwards, Group 2 rats were fed HBGP (50:50), whereas Group 3 rats were treated with 200 mg/kg body weight of FA till the end of the experiment. For 14 weeks, rats in groups 4, 5, 6, and 7 were fed an HFD. In addition to 50% of the regular and HFD meals, Group 5 rats started receiving 50% of HBGP added into their feed starting the third week. Rats in Group 6 received 200 mg of FA per kilogram of body weight. Additionally, rats in Group 7 received 10 mg/kg of body weight of rosuvastatin. The rats were given ethoxy ether at the end of the trial period, and they were then put to death via cervical decapitation. For gene expression research, liver and adipose tissue (AT) samples were immediately taken out and suspended in RNA later solution.

Gene expression studies

The mRNA transcript levels of PPAR-γ, SIRT1, FOXO1, and LXR-α was assessed in the liver and AT samples taken from test animals by RT-PCR analysis. The β-actin gene was selected as the housekeeping gene. Following animal sacrifice, 30 mg of the rat's liver and AT were removed and immediately suspended in the RNA later solution. By employing the RNeasy Miniprep kit, total RNA has been obtained from the study material.

RT-PCR quantitative analysis

Liver and AT samples were processed for RNA isolation prior to being converted to cDNA in order to perform qPCR. Total RNA was extracted using Trizol (11). Following the quantification of the RNA using a spectrophotometer, the samples' purity was evaluated using A260/280 values. To check for DNA contamination, samples were first treated using DNase I (Catalogue #M03035) from New England Biolabs and then run on an RNA gel. RNA was converted to DNA by Thermo Fisher Scientific, Mumbai, India (Catalogue #401425), using a cDNA reverse transcription kit. **Table 2** showed the specific primer sequences employed for real-time PCR. Agilent Technologies' Stratagene PCR equipment (Santa Clara, CA) was used for the PCR. The PCR conditions were as follows: Denaturation was carried out at forty rounds of 95°C for 10 minutes, and then thawing was done at 60°C for 60 seconds in a two-step real-time PCR. The data were quantified using the derived CT values.

Statistical data analysis

A post hoc Bonferroni test was performed following the one-way ANOVA method to assess statistical significance. In case the observed *P*-value was <0.05, statistical significance is then recorded.

Results

Impact of HBGP and FA on the molecular mechanism of PPAR-γ

PPAR-γ receptors primarily function in AT and control accumulation of fat by regulating the functioning of important genes involved in adipogenesis. **Figures 1** and **2**, respectively, depicted PPAR-γ activities in liver and AT samples of experimental rats. The concentration of PPAR-γ in both liver and AT samples was lowered ($P = 0.001$) in HFD-treated rats than in normal rats. In this investigation, PPAR-γ expression in HBGP and FA co-administered rats together showed ($P = 0.001$) more than rats treated with HFD.

Impact of HBG and FA on molecular mechanism of SIRT1

Silence information regulator 1 is a crucial biochemical sensor that directly connects environmental dietary cues to mammalian physiological regulation. It has been connected to insulin production in the pancreas, hepatic gluconeogenesis, and white fat mobilization. Expression of SIRT1 in liver and AT samples of experimental animals was depicted in **Figures 3** and **4**. SIRT1 expression was found to be downregulated in both the test samples of HFD treated rats. HBGP/FA co-administration significantly ($P = 0.001$) upregulated SIRT1 gene expression in HFD + HBGP and HFD + FA fed groups, therefore decreasing the adipocyte differentiation in obese conditions.

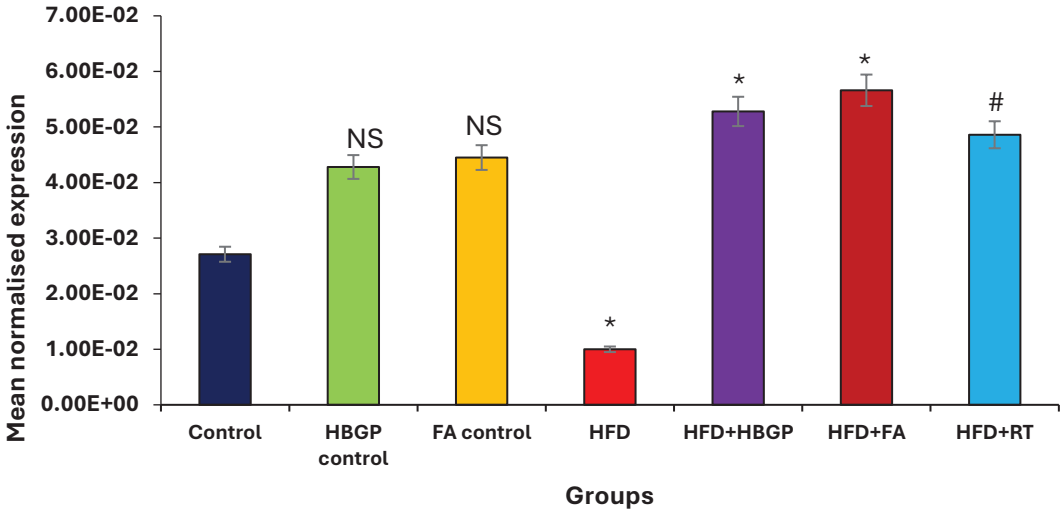

FIGURE 1 | mRNA expression of PPAR-γ in liver tissue of HBGP/FA-treated & untreated rats. For each group, data were presented as mean ± SD for six animals. One-way ANOVA and the post hoc Bonferroni test were used to analyze the data. The comparison of Control versus HBGP control, Control versus FA control, Control versus HFD, HFD versus HFD + HBGP, HFD versus HFD + FA, and HFD versus HFD + RT was used for determining statistical significance. #, $P = 0.01$; NS, non-significant; *, $P = 0.001$.

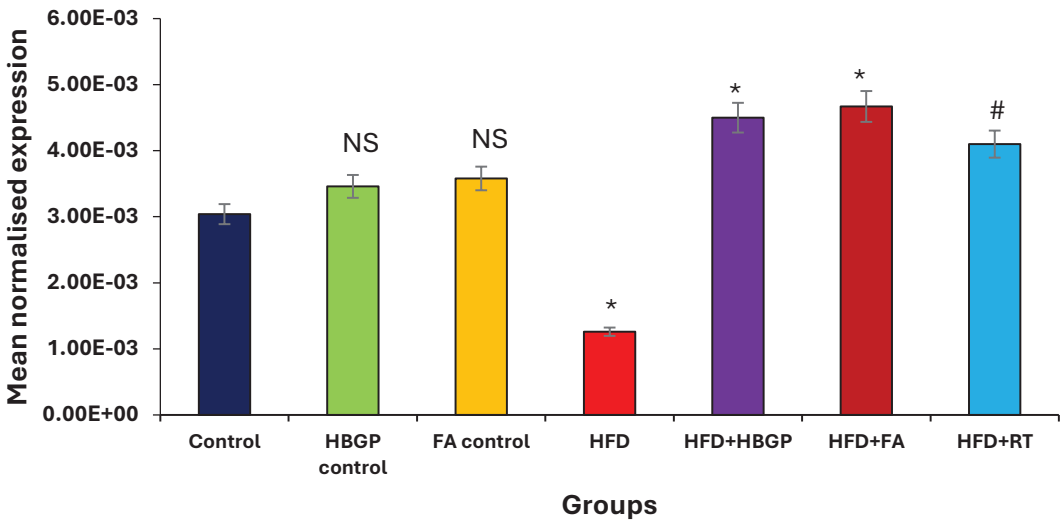

FIGURE 2 | mRNA expression of PPAR-γ in AT of HBGP/FA-treated & untreated rats. For each group, data were presented as mean ± SD for six animals. One-way ANOVA and the post hoc Bonferroni test were used to analyze the data. The comparison of Control versus HBGP control, Control versus FA control, Control versus HFD, HFD versus HFD + HBGP, HFD versus HFD + FA, and HFD versus HFD + RT was used for determining statistical significance. #, $P = 0.01$; *NS*, non-significant; *, $P = 0.001$.

Impact of HBGP and FA on molecular mechanism FOXO1

Forkhead box factors has been linked to glucose and lipid homeostasis in addition to the integration of hormonal and dietary signals for metabolic homeostasis. This gene was recently demonstrated with implications for sirtuin activity via influencing the expression of nicotinamide phosphoribosyl transferase (NAMPT), the enzyme that limits the rate at which NAD is produced and hence modulates sirtuin activity. In the liver, both SIRT1 and SIRT6 have been proven to suppress lipogenesis while promoting fatty acid oxidation. In this study, there was an upregulation in the expression of FOXO1 in the liver and AT samples of HFD-fed rats; on the contrary, FOXO1 activity was found to be restored in the control group as well as in HBGP/FA co-administered rats, as shown in **Figures 5** and **6**.

Impact of HBGP and FA on molecular mechanism of LXR-α

LXR-α is a well-known metabolic and cholesterol mobility regulator in the cells of the liver, intestine, and macrophages. It upregulates the gene regulation of HDL-associated apolipoprotein E (ApoE), Cyp7A, the enzyme that limits the rate of bile acid synthesis and the cholesterol transporters ABCA1, ABCG1, and ABCG5/ABCG8. The expression of

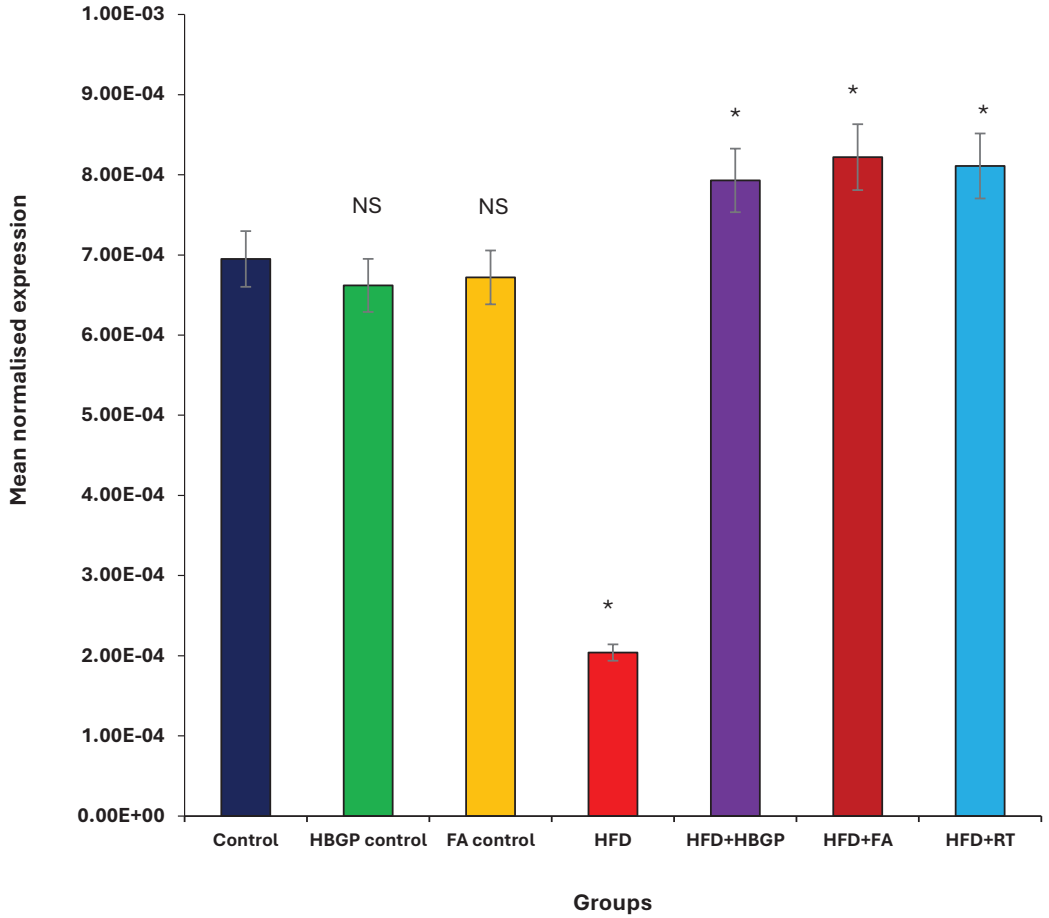

FIGURE 3 | mRNA expression of silence information regulator 1 (SIRT1) in liver tissue of HBGP/FA-treated & untreated rats. For each group, data were presented as mean ± SD for six animals. One-way ANOVA and the post hoc Bonferroni test were used to analyze the data. The comparison of Control versus HBGP control, Control versus FA control, Control versus HFD, HFD versus HFD + HBGP, HFD versus HFD + FA, and HFD versus HFD + RT was used for determining statistical significance. *, $P = 0.001$; NS, non-significant.

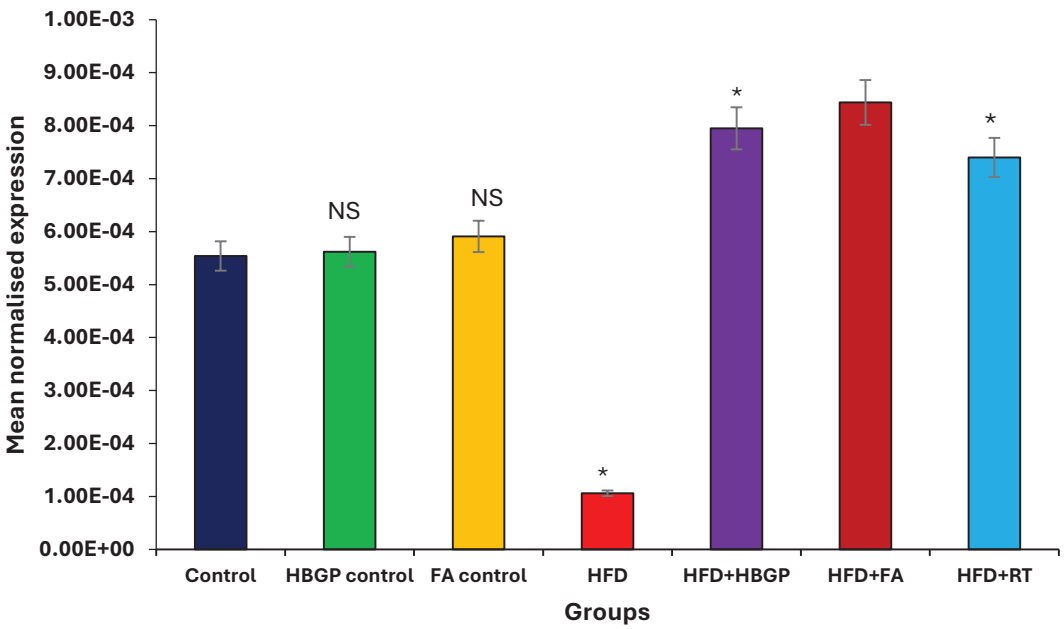

FIGURE 4 | mRNA expression of SIRT1 in AT of HBGP/FA-treated & untreated rats. For each group, data were presented as mean ± SD for six animals. One-way ANOVA and the post hoc Bonferroni test were used to analyze the data. The comparison of Control versus HBGP control, Control versus FA control, Control versus HFD, HFD versus HFD + HBGP, HFD versus HFD + FA, and HFD versus HFD + RT was used for determining statistical significance. *, $P = 0.001$; NS, non-significant.

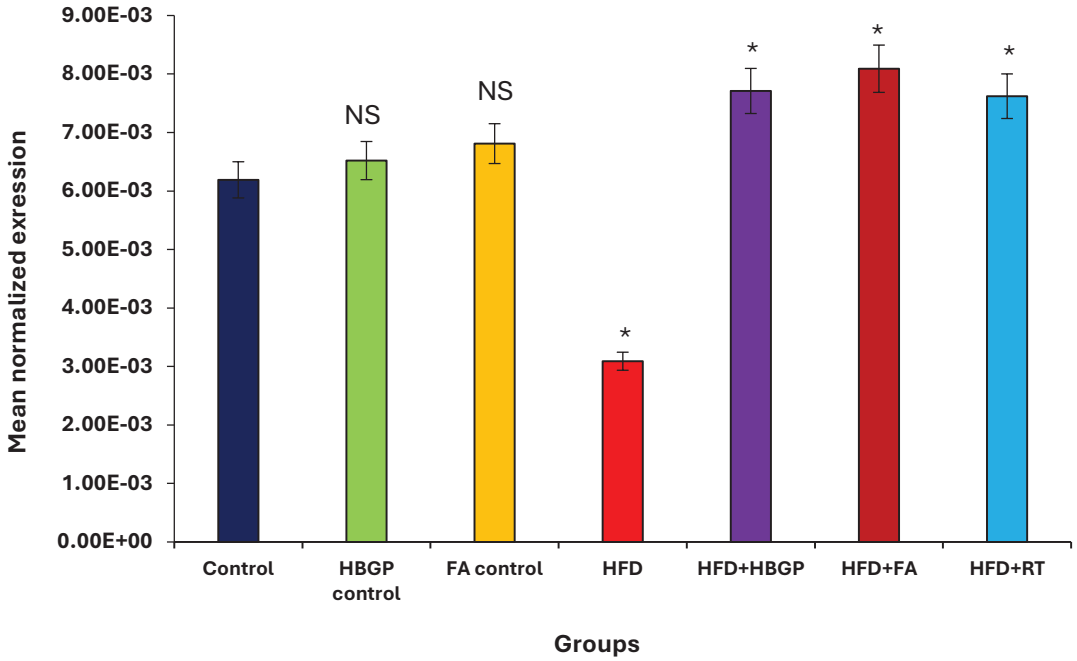

FIGURE 5 | mRNA expression of forkhead box factors (FOXO1) in liver tissue of HBGP/FA-treated & untreated rats. For each group, data were presented as mean ± SD for six animals. One-way ANOVA and the post hoc Bonferroni test were used to analyze the data. The comparison of Control versus HBGP control, Control versus FA control, Control versus HFD, HFD versus HFD + HBGP, HFD versus HFD + FA, and HFD versus HFD + RT was used for determining statistical significance. *, $P = 0.001$; NS, non-significant.

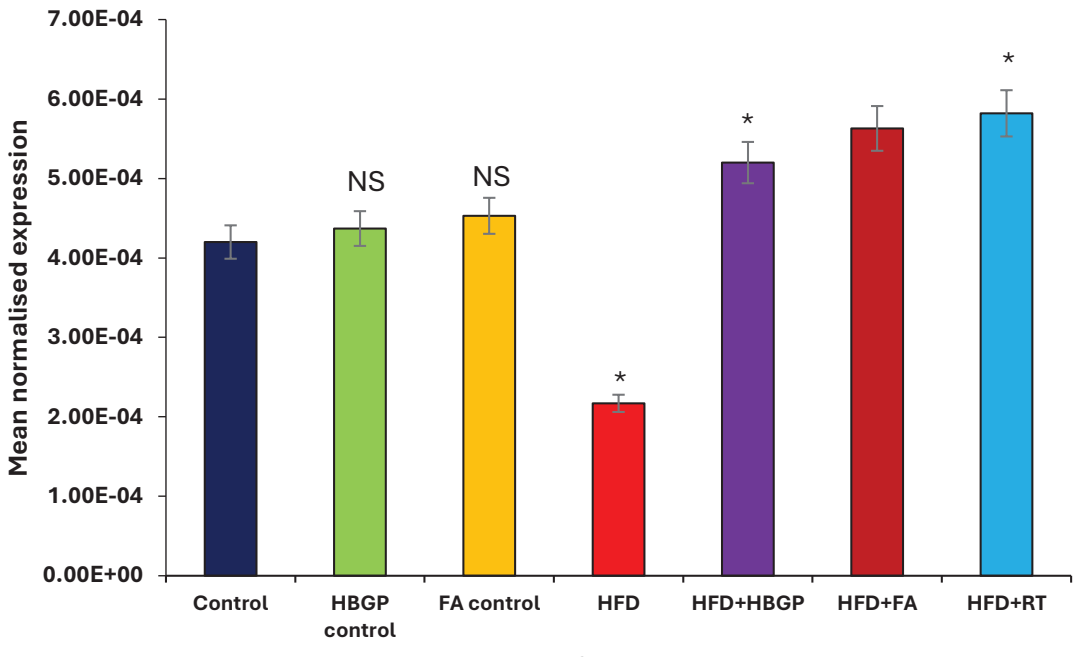

FIGURE 6 | mRNA expression of FOXO1 in AT of HBGP/FA-treated & untreated rats. For each group, data were presented as mean ± SD for six animals. One-way ANOVA and the post hoc Bonferroni test were used to analyze the data. The comparison of Control versus HBGP control, Control versus FA control, Control versus HFD, HFD versus HFD + HBGP, HFD versus HFD + FA, and HFD versus HFD + RT was used for determining statistical significance. *, $P = 0.001$; NS, non-significant.

LXR-α in liver and AT samples of experimental animals is depicted in **Figures 7** and **8**. LXR-α expression was found to be downregulated in liver as well as AT samples of HFD-given rats. HBGP and FA co-administration upregulated LXR-α gene expression in HFD + HBGP and HFD + FA fed groups, therefore regulating whole-body cholesterol homeostasis.

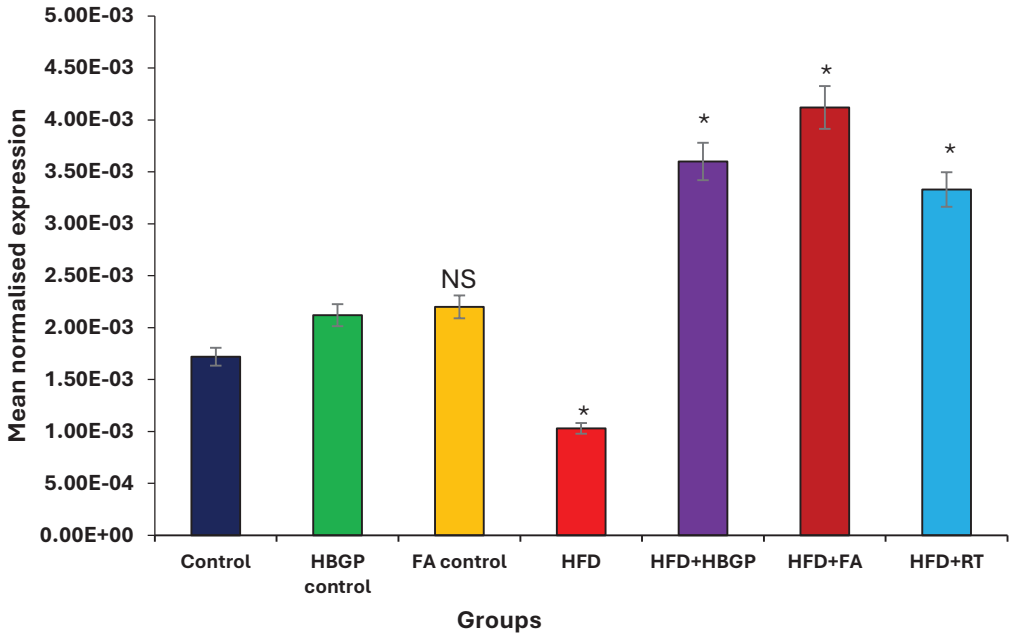

FIGURE 7 | mRNA expression of liver X receptor alpha (LXR-α) in liver tissue of HBGP/FA-treated & untreated rats. For each group, data were presented as mean ± SD for six animals. One-way ANOVA and the post hoc Bonferroni test were used to analyze the data. The comparison of Control versus HBGP control, Control versus FA control, Control versus HFD, HFD versus HFD + HBGP, HFD versus HFD + FA, and HFD versus HFD + RT was used for determining statistical significance. *, $P = 0.001$; NS, non-significant.

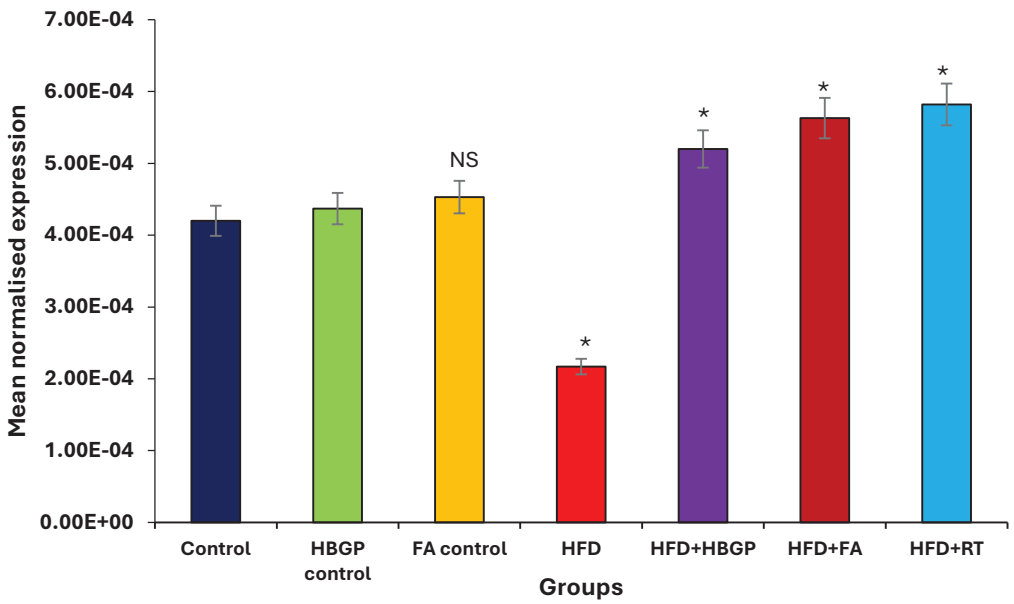

FIGURE 8 | mRNA expression of LXR-α in AT of HBGP/FA-treated & untreated rats. For each group, data were presented as mean ± SD for six animals. One-way ANOVA and the post hoc Bonferroni test were used to analyze the data. The comparison of Control versus HBGP control, Control versus FA control, Control versus HFD, HFD versus HFD + HBGP, HFD versus HFD + FA, and HFD versus HFD + RT was used for determining statistical significance. *, $P = 0.001$; NS, non-significant.

Discussion

Impact of HBGP and FA on molecular mechanism of PPAR-γ

The main transcription factor linked to adipogenesis among the other PPARs (α, γ, and δ) is PPAR-γ, which has two functions: it stimulates genes unique to AT and expresses the mature adipose phenotype. According to Tontonoz et al. (12), PPAR-γ reduces the incidence of atherosclerosis and fibrotic arteries by suppressing macrophage MMP-9 expression (12). It has been discovered that PPAR-γ is involved in the enhancement of adiponectin that takes place during adipocyte development. In our current study, the elevated levels of

PPAR-γ expression in the AT of HBGP- or FA-treated rats (**Figures 1** and **2**) may be due to PPAR-γ's major role in the elevation of adiponectin at the time of adipocyte maturation. On the other hand, decreased levels of PPAR-γ expression in HFD-given rats are due to increased calorie intake leading to fat accumulation and oxidative stress. Consequently, PPAR-γ is downregulated with diminished adiponectin concentration, the negative modulator in hyperlipidemic conditions.

High calorie consumption is usually associated with less mitochondrial membrane fluidity as well as more free radical production (13). In adipocytes, fat accumulation and increased oxygen species have been demonstrated to decrease PPAR-γ expression (14). As a result, medicinal drugs that exhibit an increase in the activity of PPAR-γ consequently elevate adiponectin depots, which may be advantageous in the management of hyperlipidemia and overweight. HBGP and its active compound FA are the natural therapy of choice for diet-induced obesity, as per the results of this study. Our findings show that HBGP/FA has hypolipidemic activity, most likely via upregulating PPAR-γ expression to increase the transcription of adiponectin, a negative regulator of obesity and hyperlipidemia.

Impact of HBGP and FA on molecular mechanism of SIRT1

SIRT1 stimulation can help to improve lipid levels. Nicotinic acid, a precursor of NAD+, elevates cellular NAD+ concentrations along with SIRT1 activation (15), which in turn is utilized to reduce cholesterol levels. Another mechanism to enhance SIRT1 is through CR. In a human investigation, CR was shown to lower TG and cholesterol levels and postpone the onset of many aging-related cardiovascular problems (16).

SIRT1 requires PPAR-γ as a substrate. The deacetylation of lysine residues (268 and 293 K) on PPAR-γ by SIRT1 is required for the co-repressors NCoR and SMRT to regulate its transcriptional activity. SIRT1 can thus limit white adipogenesis by stimulating the binding of PPAR-γ co-repressors NCoR and SMRT, which suppresses PPAR-γ transcriptional activity (17, 18).

Figures 3 and **4** display a decrease in the gene expression of SIRT1 in HFD-treated and untreated rats when compared with control rats. This decrease is an indicator of disturbed lipid and carbohydrate metabolism. Co-administration of HBGP/FA increased SIRT1 expression in the liver and AT of HFD + HBGP and HFD + FA group rats. The lines of evidence that SIRT1 regulates lipid homeostasis are numerous and are explained. CR, prolonged fasting, and natural polyphenolic products like resveratrol (19), fisetin, quercetin, and curcumin lead to the activation of SIRT1, which in turn serves as a checkpoint for the various stages of fat metabolism. In AT SIRT1 represses PPAR-γ via binding docking to the NCoR/SMRT proteins. The final product of NCoR/SMRT/SIRT1 collides with the sequences of DNA known as PPREs and decreases the molecular mechanism of PPAR-γ, thereby initiating fat mobilization (20). Chen and Li (21) reported that decreased SIRT1 levels may be accountable for reduced lipid profiles in chronic kidney failure.

Barley is a versatile grain that competes with other cereal grains in terms of phytochemical content and nutrition. It has high levels of active compounds that work in numerous ways to prevent diseases. The phytochemistry of HBGP reveals the presence of the dominant phenolic compound FA. Therefore, the hypolipidemic activity of HBGP may be due to the presence of FA. Moreover, our findings are supported by Chen et al (22), who demonstrated the potential role of FA in regulating muscle fiber formation through the activation of the SIRT1/AMPK pathway.

Impact of HBGP and FA on molecular mechanism of FOXO1

Figures 5 and **6** show the gene expression results of FOXO1 in both liver and AT samples of HGBP/FA-treated and untreated rats. FOXO1 expression turns out to be downregulated in HFD-treated rats. HBGP and FA co-administration increased the expression of FOXO1 in HFD + HBGP and HFD + FA fed rats. The mechanism involved in the upregulation of FOXO1 in liver and AT is hypothesized as follows. Firstly, in the liver, SIRT1 removes acetyl groups from gene transcription proteins such as transcription factors such as forkhead box O (FOXO), thus preventing cholesterol production. Moreover, the insulin response element (IRE) sequence in the SREBP-2 promoter region is uniquely recognized by FOXO1, which inhibits SREBP-2 transcription. Li and Wu (23) provided evidence to prove that the upregulation of fatty acids and LDL was detected in FOXO1 knockout mice. Its regulating factor in bile acid metabolism was also discovered in a recent study. In the liver, FOXO1 is necessary for the release of genes associated with bile acid production. On the other hand, inactivation of FOXO1 in hepatic tissues takes place, causing abnormal bile acid synthesis, thus affecting farnesoid x receptor (FXR) capacity to lower TG and cholesterol.

Secondly, in AT SIRT1 deacetylates PGC-1α and activates it. Activated PGC-1α combines with FOXO1, thereby influencing gluconeogenesis and glucose homeostasis in the case of the liver whereas, in the case of AT, FOXO1 upregulates adiponectin gene transcription. Activated FOXO1 binds to the FOXO1-responsive region in the mouse adiponectin promoter, which comprises two neighboring FOXO1 binding sites and also 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 mice (24). Increased adiponectin further stimulates cells to insulin and increases fatty acid

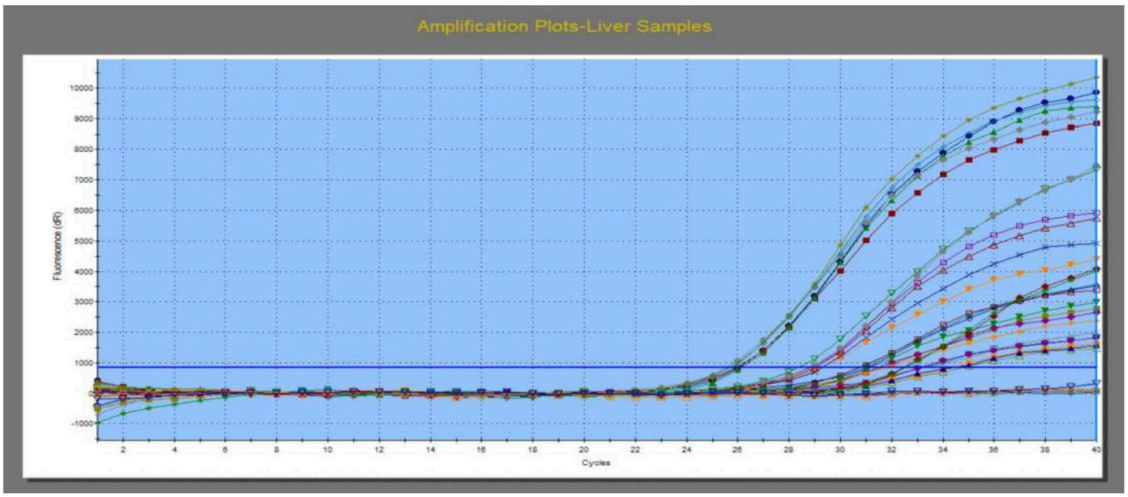

FIGURE 9 | Overall representation of fluorescent profile and amplification plot in liver samples of all the target genes during the PCR program.

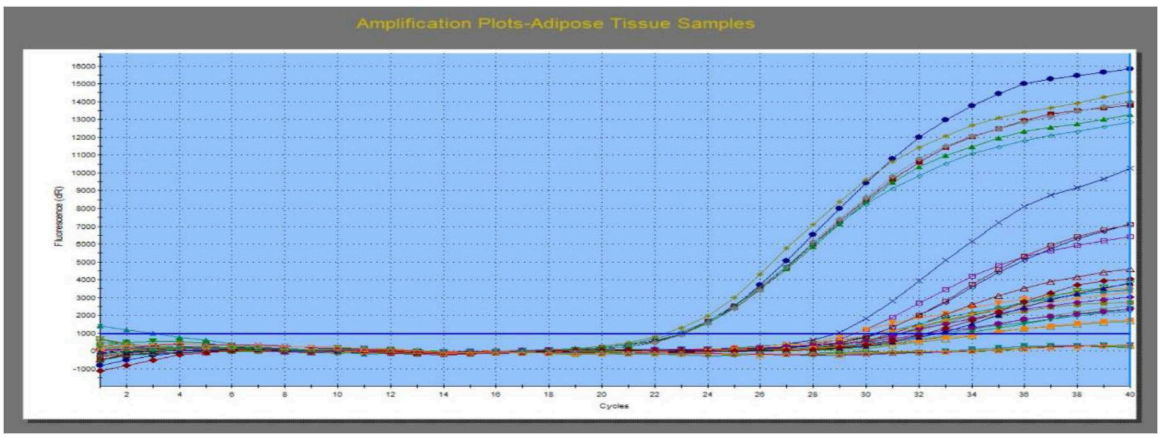

FIGURE 10 | Overall representation of fluorescent profile and amplification plot in AT samples of all the target genes during the PCR program.

oxidation, leading to new energy homeostasis. From the above results, it is highly evident that HBGP/FA co-administration maintained the activity of FOXO1, and this might be by modulating SIRT1 level by both the test drugs, underlining their protection against hyperlipidemia and obesity.

Impact of HBGP and FA on molecular mechanism of LXR-α

Figures 7 and **8** show the gene expression results of LXR-α in liver and AT samples of HBGP/FA-treated and untreated rats. LXR-α expression displayed a downregulation in HFD-treated rats. HBGP and FA co-administration increased the expression of LXR-α in HFD + HBGP and HFD + FA fed rats. Zeng et al. also found that SIRT1 can prevent atherosclerosis through activation of LXR-ABCA1/ABCG1/CCR7 and by deactivation of NF-κB signaling pathways (25).

In conjunction with fatty acid metabolism, SIRT1 is also shown to regulate LXR through deacetylation reaction. There are two types of LXRs, LXR-α and LXR-β. Human and mouse adipocytes contain both α and β form; however, α form is primarily increased during fat cell proliferation (26). Interestingly, the coregulator PGC-1α is needed for LXR-α activation, which may be beneficial for reducing gastrointestinal cholesterol uptake and increasing centripetal cholesterol flux. Cholesterol 7 α hydroxylase, the enzyme that controls the rate of bile acid synthesis, and ATP binding cassette (ABC) genes associated with hepatic and intestinal genes that LXR-α positively regulates and are necessary for the elimination of cholesterol from the body.

Taken together, we suggest that SIRT1 has a multifunctional role in the body, which is as follows: regulator of whole-body cholesterol and lipid metabolism by activation of PGC-1α, FOXO1, and LXR-α. The overall expression of the amplification plot and fluorescent profile of all target genes during PCR in liver and AT are represented in **Figures 9** and **10**.

Conclusion

It can be concluded that HBGP and its active compound FA can be a potent hypolipidemic drug by activating SIRT1, activated SIRT1 deacetylates FOXO1 and LXR-α which in turn influences PPAR-γ gene expression. FA, the major phytochemical present in HBGP might be accounted for Antihyperlipidemic activity. This research also acts as an eye opener for extending the need to pursue further clinical trials and investigations in human population to prove that HBGP/FA intervention could lower the severity of hyperlipidemia and obesity. Therefore, this study recommends the consumption of HBGP as a portion of the daily diet if one feels that fat restriction is not possible every time for each meal.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

References

1. Obadi M, Sun J, Xu B. Highland barley: chemical composition, bioactive compounds, health effects, and applications. *Food Res Int (Ottawa, Ont.).* (2021) 140:110065. doi: 10.1016/j.foodres.2020
2. Ali SA, Parveen N, Ali AS. Links between the Prophet Muhammad (PBUH) recommended foods and disease management: a review in the light of modern superfoods. *Int J Health Sci.* (2018) 12(2):61–9.
3. Shirke UJ, Jyotsna Y, Shirke JM, Udmale MM. Literary review of Yava (Barley). *J Ayurveda Integr Med Sci.* (2018) 3(3):165–8. doi: 10.21760/jaims.v3i03.417
4. de Melo TS, Lima PR, Carvalho KM, Fontenele TM, Solon FR, Tomé AR, et al. Ferulic acid lowers body weight and visceral fat accumulation via modulation of enzymatic, hormonal and inflammatory changes in a mouse model of high-fat diet-induced obesity. *Braz J Med Biol Res.* (2017) 50(1):e5630. doi: 10.1590/1414-431X20165630
5. Sun S, Ruan Y, Yan M, Xu K, Yang Y, Shen T, et al. Ferulic acid alleviates oxidative stress-induced cardiomyocyte injury by the regulation of miR-499-5p/p21 signal cascade. *Evid Based Complement Alternat Med.* (2021) 2021:1921457.
6. Chowdhury S, Ghosh S, Das AK, Sil PC. Ferulic acid protects hyperglycemia-induced kidney damage by regulating oxidative insult, inflammation and autophagy. *Front Pharmacol.* (2019) 10:27.
7. Iside C, Scafuro M, Nebbioso A, Altucci L. SIRT1 activation by natural phytochemicals: an overview. *Front Pharmacol.* (2020) 11:1225. doi: 10.3389/fphar.2020.01225
8. Alageel A, Tomasi J, Tersigni C, Brietzke E, Zuckerman H, Subramaniapillai M, et al. Evidence supporting a mechanistic role of sirtuins in mood and metabolic disorders. *Prog Neuropsychopharmacol Biol Psychiatry.* (2018) 86:95–101. doi: 10.1016/j.pnpbp.2018.05.017
9. Gamel T, Abdel-Aal E-SM. Phenolic acids and antioxidant properties of barley wholegrain and pearling fractions. *Agric Food Sci.* (2012) 21(2):118–31. doi: 10.23986/afsci.4727
10. Nascimento AF, Sugizaki MM, Leopoldo AS, Lima-Leopoldo AP, Luvizotto RA, Nogueira CR, et al. A hypercaloric pellet-diet cycle induces obesity and co-morbidities in Wistar rats. *Arq Bras Endocrinol Metabol.* (2008) 52(6):968–74. doi: 10.1590/s0004-27302008000600007
11. Chomczynski P, Mackey K. Short technical reports. Modification of the TRI reagent procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. *BioTechniques.* (1995) 19(6):942–5.
12. Tontonoz P, Nagy L, Alvarez JG, Thomazy VA, Evans RM. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell.* (1998) 93(2):241–52. doi: 10.1016/s0092-8674(00)81575-5
13. Esposito K, Giugliano F, Di Palo C, Giugliano G, Marfella R, D'Andrea F, et al. Effect of lifestyle changes on erectile dysfunction in obese men: a randomized controlled trial. *JAMA*. (2004) 291(24):2978–84. doi: 10.1001/jama.291.24.2978
14. Iwaki M, Matsuda M, Maeda N, Funahashi T, Matsuzawa Y, Makishima M, et al. Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes*. (2003) 52(7):1655–63. doi: 10.2337/diabetes.52.7.1655
15. Bogan KL, Brenner C. Nicotinic acid, nicotinamide, and nicotinamide riboside: a molecular evaluation of NAD+ precursor vitamins in human nutrition. *Ann Rev Nutr.* (2008) 28:115–30. doi: 10.1146/annurev.nutr.28.061807.155443
16. Most J, Tosti V, Redman LM, Fontana L. Calorie restriction in humans: an update. *Ageing Res Rev.* (2017) 39:36–45. doi: 10.1016/j.arr.2016.08.005
17. Picard F, Kurtev M, Chung N, Topark-Ngarm A, Senawong T, Machado De Oliveira R, et al. SIRT1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature.* (2004) 429(6993):771–6. doi: 10.1038/nature02583
18. Qiao L, Shao J. SIRT1 regulates adiponectin gene expression through FOXO1-C/enhancer-binding protein alpha transcriptional complex. *J Biol Chem.* (2006) 281(52):39915–24. doi: 10.1074/jbc.M607215200
19. Cheang WS, Wong WT, Wang L, Cheng CK, Lau CW, Ma RCW, et al. Resveratrol ameliorates endothelial dysfunction in diabetic and obese mice through sirtuin 1 and peroxisome proliferator-activated receptor δ. *Pharm Res.* (2019) 139:384–94. doi: 10.1016/j.phrs.2018.11.041
20. Liang N, Jakobsson T, Fan R, Treuter E. The nuclear receptor-co-repressor complex in control of liver metabolism and disease. *Front Endocrinol.* (2019) 10:411. doi: 10.3389/fendo.2019.00411
21. Chen G, Li X. The decreased SIRT1 level may account for the lipid profile in chronic kidney disease. *J Biol Res (Thessalonike, Greece).* (2019) 26:9. doi: 10.1186/s40709-019-0101-2
22. Chen X, Guo Y, Jia G, Zhao H, Liu G, Huang Z. Ferulic acid regulates muscle fiber type formation through the SIRT1/AMPK signaling pathway. *Food Funct.* (2019) 10(1):259–65. doi: 10.1039/c8fo01902a
23. Li Y, Wu S. Epigallocatechin gallate suppresses hepatic cholesterol synthesis by targeting SREBP-2 through SIRT1/FOXO1 signaling pathway. *Mol Cell Biochem.* (2018) 448(1–2):175–85. doi: 10.1007/s11010-018-3324-x
24. Li K, Zhang J, Yu J, Liu B, Guo Y, Deng J, et al. MicroRNA-214 suppresses gluconeogenesis by targeting activating transcriptional factor 4. *J Biol Chem.* (2015) 290(13):8185–95. doi: 10.1074/jbc.M114.633990
25. Zeng HT, Fu YC, Yu W, Lin JM, Zhou L, Liu L, et al. SIRT1 prevents atherosclerosis via liver-X-receptor and NF-κB signaling in a U937 cell model. *Mol Med Rep.* (2013) 8(1):23–8. doi: 10.3892/mmr.2013.1460
26. Li X, Zhang S, Blander G, Tse JG, Krieger M, Guarente L. SIRT1 deacetylates and positively regulates the nuclear receptor LXR. *Mol Cell.* (2007) 28(1):91–106. doi: 10.1016/j.molcel.2007.07.032