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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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ABSTRACT

Introduction: Whole grains are known for its medicinal properties for millennia. Numerous whole grains with antihyperlipidemic qualities have drawn attention for researchers as potential therapeutic adjuncts in lowering the incidence of cardiovascular disease. According to certain folklore and preliminary evidence, barley can lower blood body 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 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 a HFD for fourteen 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 3rd 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 fourteen weeks, and mRNA expression of peroxisome proliferator activated receptor

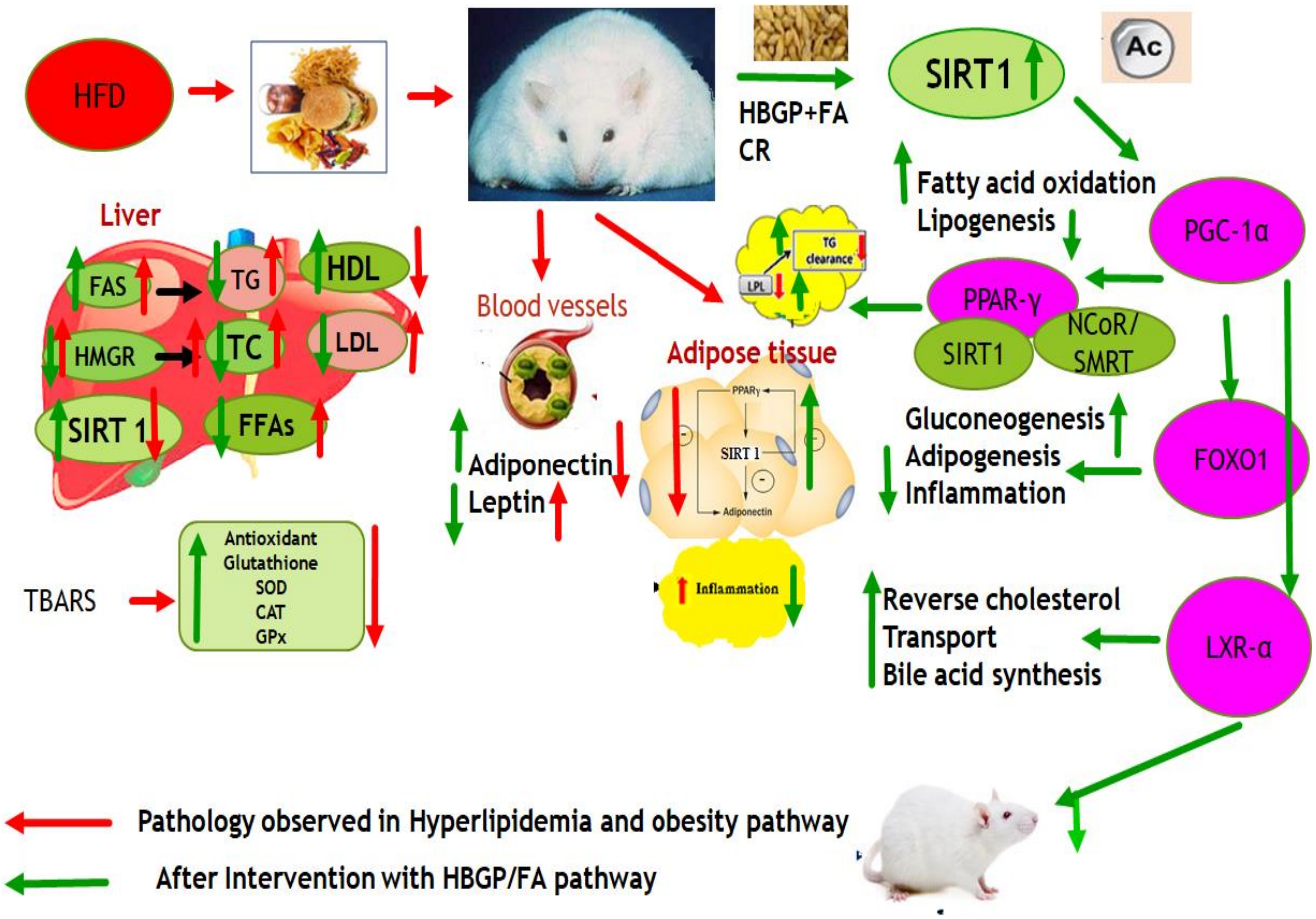
gamma (PPAR-γ), silence information regulator 1 (SIRT1), forkhead box factors (FOXO1), and liver X receptor alpha (LXR-α) was assessed in liver and adipose tissue (AT) samples.

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 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


HFD
HBGP+FA
CR
SIRT1
Ac
Fatty acid oxidation
Lipogenesis
PGC-1α
Liver
FAS
TG
HDL
HMGR
TC
LDL
SIRT 1
FFAs
Blood vessels
TG clearance
LPL
PPAR-γ
SIRT1
NCoR/
SMRT
Adipose tissue
PPARγ
SIRT 1
Adiponectin
Adiponectin
Leptin
Gluconeogenesis
Adipogenesis
Inflammation
FOXO1
Inflammation
TBARS
Antioxidant
Glutathione
SOD
CAT
GPx
Reverse cholesterol
Transport
Bile acid synthesis
LXR-α
Pathology observed in Hyperlipidemia and obesity pathway
After Intervention with HBGP/FA pathway

1. 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 total grain diet, as per the Dietary Guidelines for Americans 2020–2025. Because of its peculiar nutrition and abundance of bioactive components hulled barley grains 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, 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 utilised 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].

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 HFD [4].

Sun et al. 2021[5] demonstrated that FA may protect cardiomyocytes from oxidative stress by modulating the miR-499-5p/p21 signalling pathway. Moreover, Chowdary et al. (2019) [6] stated the protective role of ferulic acid by eliminating oxidative stress, inflammation and autophagy in hyperglycaemia-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 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. SIRT1inhibits 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 SIRT 1 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, systemically inflammatory state, centralized nutritional monitoring and rhythmic metabolism [8].

With the available data, this research mainly focuses on the potential of 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 LXR-α on gene level, the influencing factors of vascular inflammation during hyperlipidemia.

2. METHODOLOGY

2.1 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.

2.1.2 Extraction of FA from HBGP

Ferulic acid was extracted from hulled barley grains according to the method described by Gamel and Abdel (2012) [9]. For alkaline extraction, 15 ml of 2N sodium hydroxide were applied to 0.2 grams of milled hulled barley grains. For two 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 ten minutes at 14000 x 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.

2.2. Test animals

Healthy male SD rats weighing 150–200g 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).

2.3 Induction of hyperlipidemia

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

2.3.1. High-fat diet (HFD) composition

The experimental animals were fed HFD in accordance with Nascimento et al.,. (2008) [10] recommendations. The HFD was composed of 439, 218, 129, 61, and 153 grams per kilogram body weight of ground labina, browned peanuts, milk powder, corn oil, and french-fried potatoes, respectively. The standard rat chow feed was purchased from 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 vitamin and minerals. The blend was subsequently formed into balls and dried in a drying oven at a temperature of 55±5°C.

2.4. 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 administered to the rats in groups 1, 2 and 3 rats. From 3rd week onwards, Group 2 rats were fed HBGP (50:50) whereas Group 3 rats were treated with 200mg/kg body weight of FA till the end of the experiment. For 14 weeks, rats in groups 4, 5, 6, and 7 were fed a 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 per kilogram 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.

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)	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

2.5 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, 30mg 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.

2.5.1 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) New England Biolabs and then ran on an RNA gel. RNA was converted to DNA by Thermofisher Scientific, Mumbai, India (Calalogue#401425), using a cDNA reverse transcription kit. Table 2 showed the specific primer sequences employed for real-time PCR. Agilent Technologies' astratagene 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 degrees Celsius for 10 minutes, and then thawing was done at sixty degrees Celsius for 60 seconds in a two-step real-time PCR. The data was quantified using the derived Ct values.

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

2.6 Statistical Data Analysis

A post hoc Bonferroni test was performed following the one-way ANOVA method to assess statistical significance. In case observed p-value was less than 0.05, statistical significance is

then recorded.

3. RESULTS

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

PPAR-γ receptors primarily functions in AT, controls accumulation of fat by regulating the functioning of important genes involved in adipogenesis, Figure 1 and Figure 1.1, respectively, depicted PPAR-γ activities in liver and AT samples of experimental rats. The concentration of PPAR-γ in both liver and AT samples were 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) than rats treated with HFD.

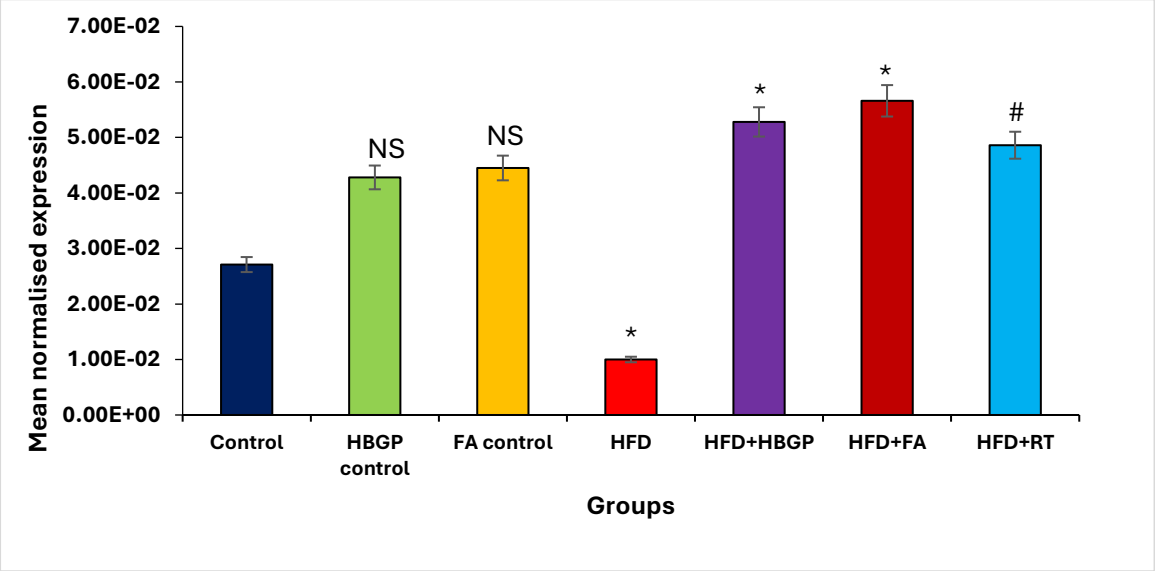


Figure 1. mRNA expression of PPAR-γ in liver tissue of HBGP/FA treated & untreated rats.

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.

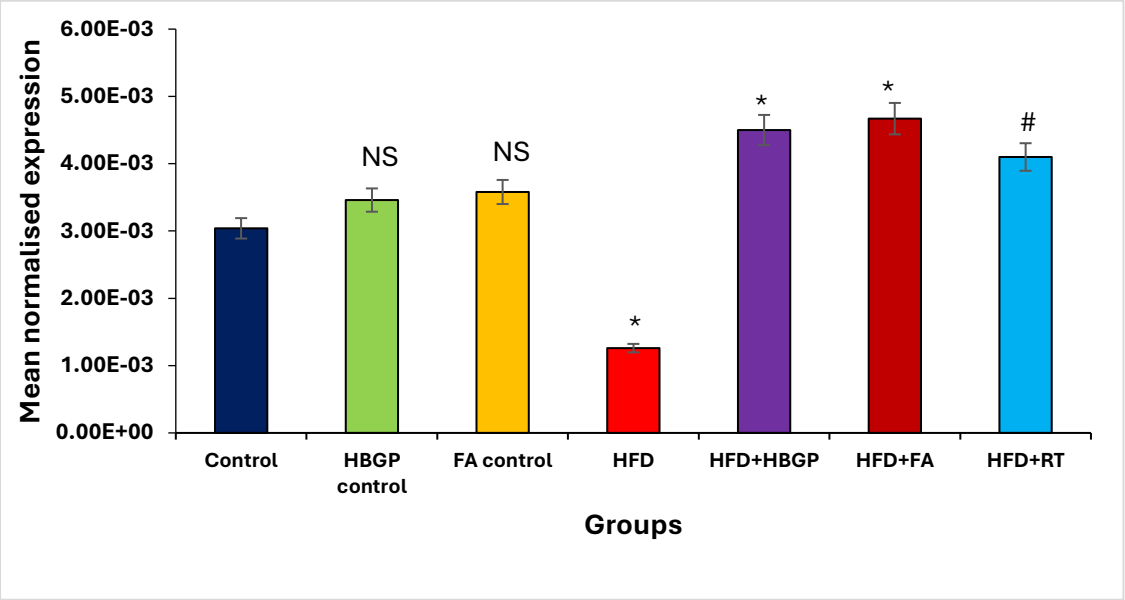


Figure 1.1 mRNA expression of PPAR-γ in AT of HBGP/FA treated & untreated rats.

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.

3.2 Impact of HBG and FA on molecular mechanism of SIRT1

SIRT1 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 Figure 2 & 2.1 SIRT1 expression was found to be downregulated in both the test samples of HFD treated rats. HBGP/FA co-administration significantly (P=0.001) up regulated SIRT1 gene expression in HFD+HBGP and HFD+FA fed groups, therefore decreasing the adipocyte differentiation in obese conditions.

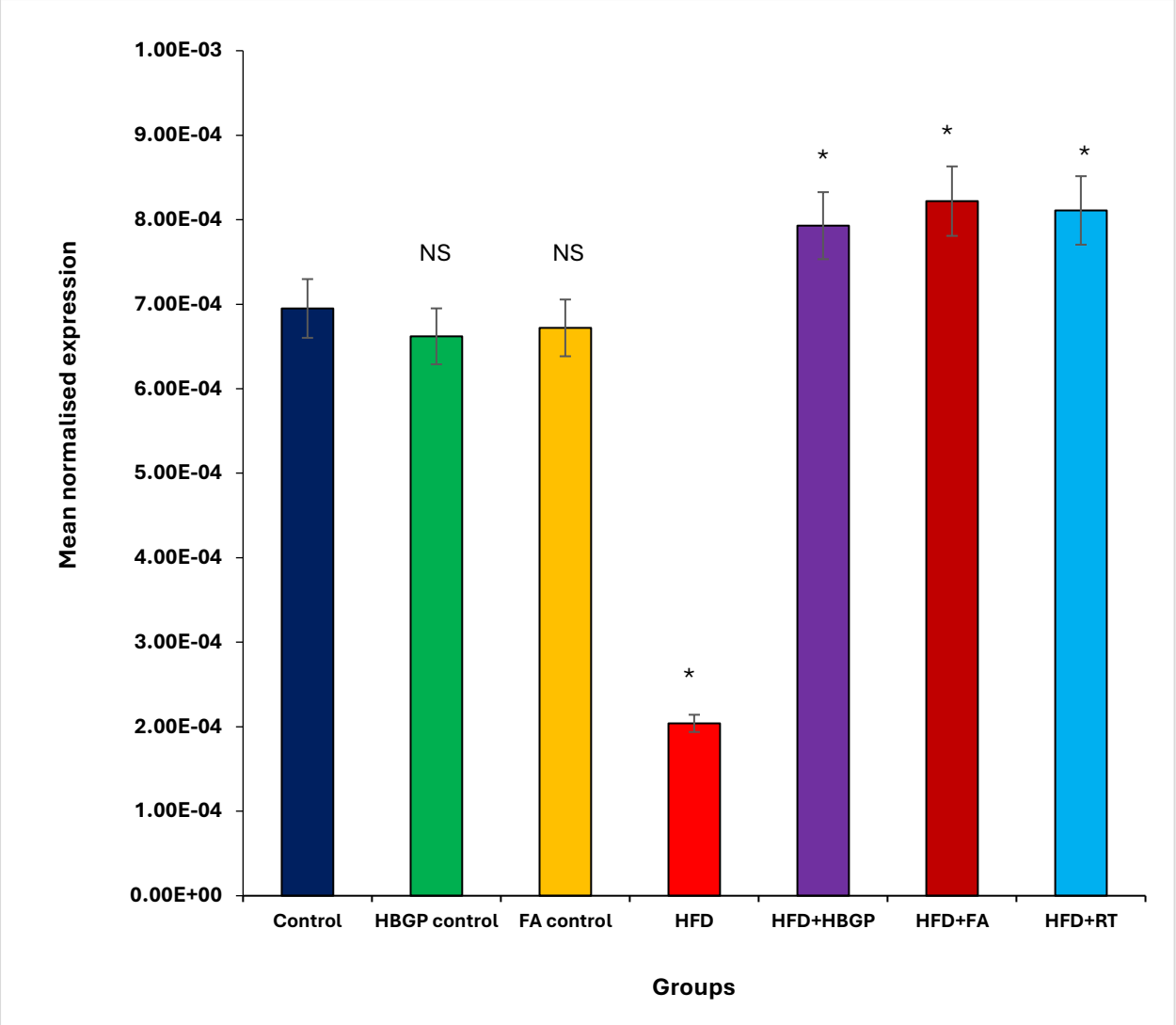


Figure 2. mRNA expression of SIRT1 in liver tissue of HBGP/FA treated & untreated rats.

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.

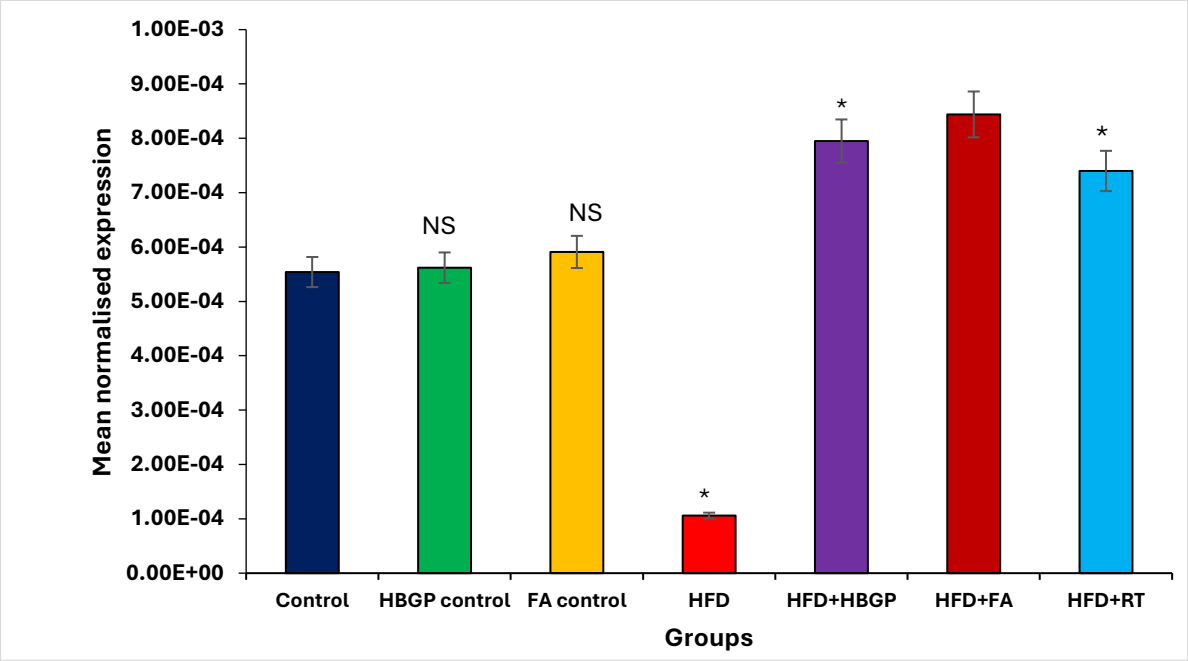


Figure 2.1 mRNA expression of SIRT1 in AT of HBGP/FA treated & untreated rats.

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.

3.3 Impact of HBGP and FA on molecular mechanism FOXO1

FOXO1 have 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 modulate sirtuin activity. In liver, both SIRT1and SIRT6 have been proven to suppress lipogenesis while promoting fatty acid oxidation. In this study, there was an up regulation 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 shown in Figure 3 and 3.1.

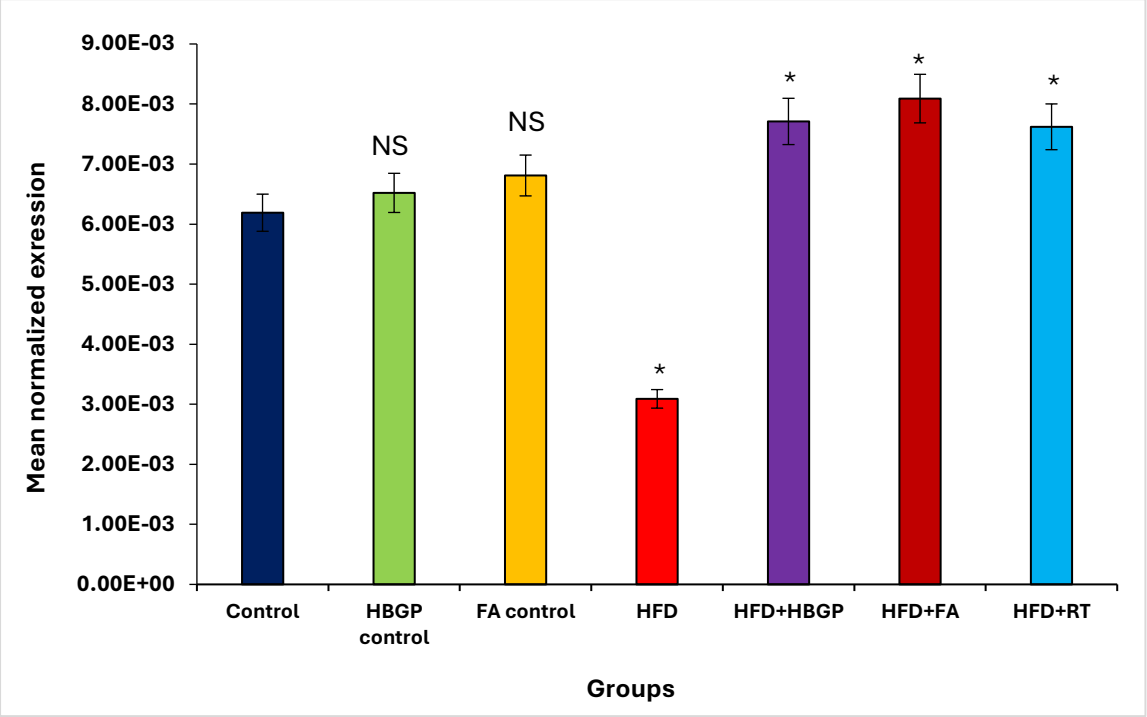


Figure 3. mRNA expression of FOXO1 in liver tissue of HBGP/FA treated & untreated rats.

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.

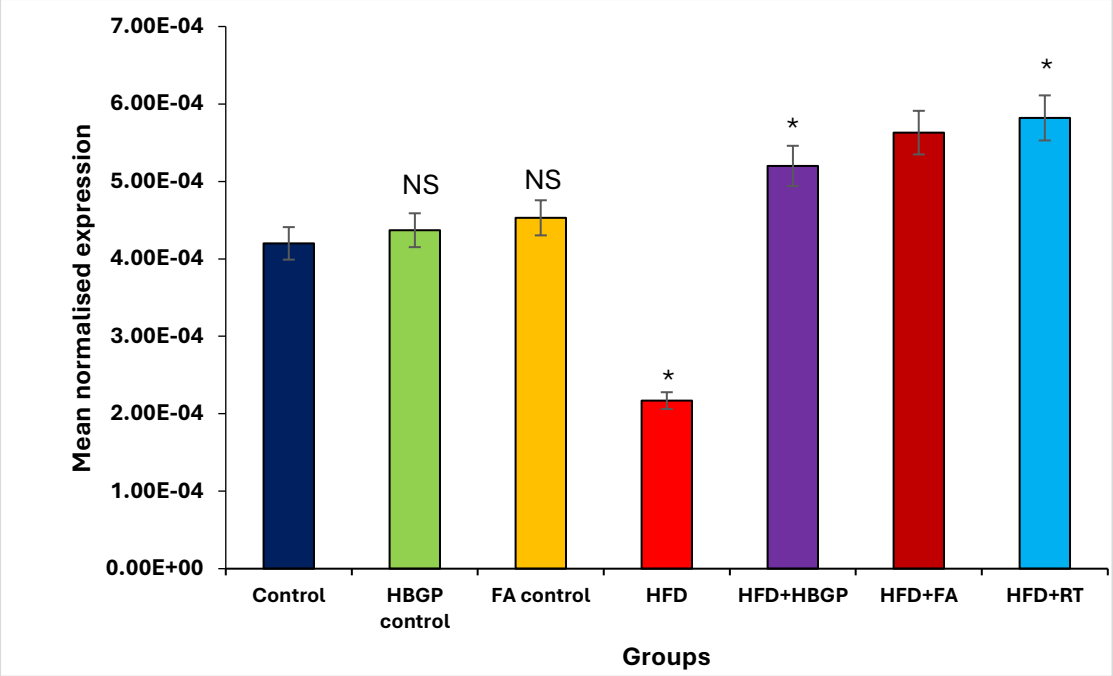


Figure 3.1 mRNA expression of FOXO1 in AT of HBGP/FA treated & untreated rats.

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.

3.4 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, ABCG5/ABCG8.The expression of LXR-α in liver and AT samples of experimental animals are depicted is Figure 4 and 4.1.LXR-α expression was found to be down regulated in liver as well as AT samples of HFD given rats. HBGP and

FA co-administration up regulated LXR-α gene expression in HFD+HBGP and HFD+FA fed groups, therefore regulating whole body cholesterol homeostasis.

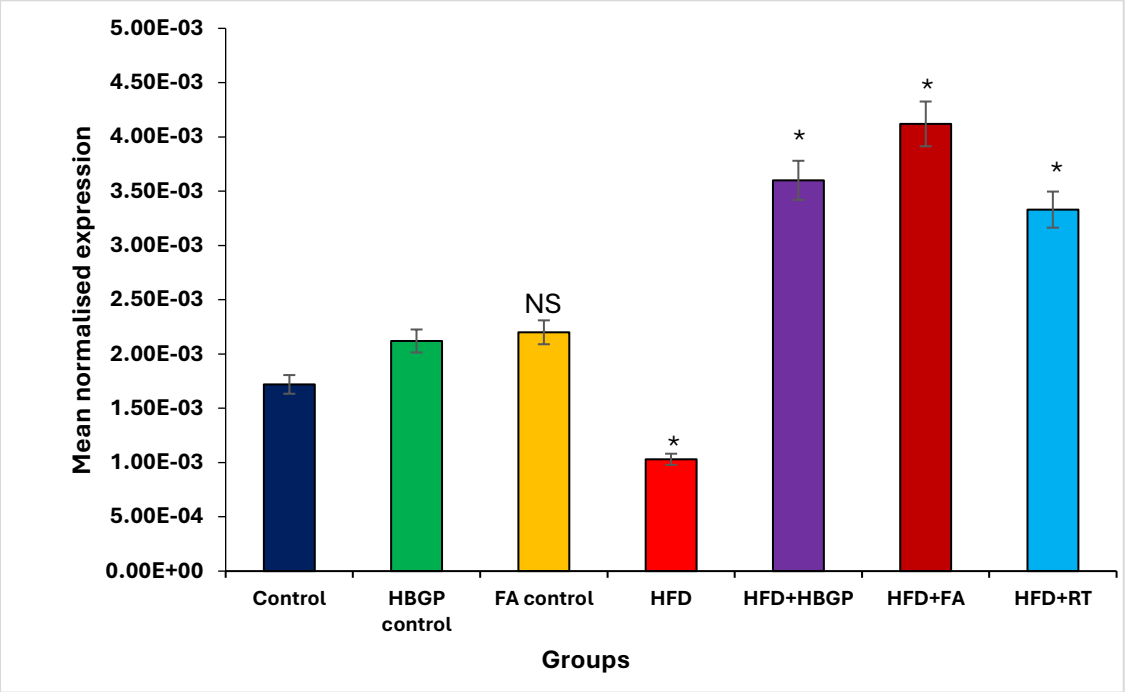


Figure 4 mRNA expression of LXR-α in liver tissue of HBGP/FA treated & untreated rats. For each group, data were presented as mean ± SD for six animals. One-way ANOVA and the post hoc Bonferroni test were used to analyze the data. The comparison of Control versus HBGP control, Control versus FA control, 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.

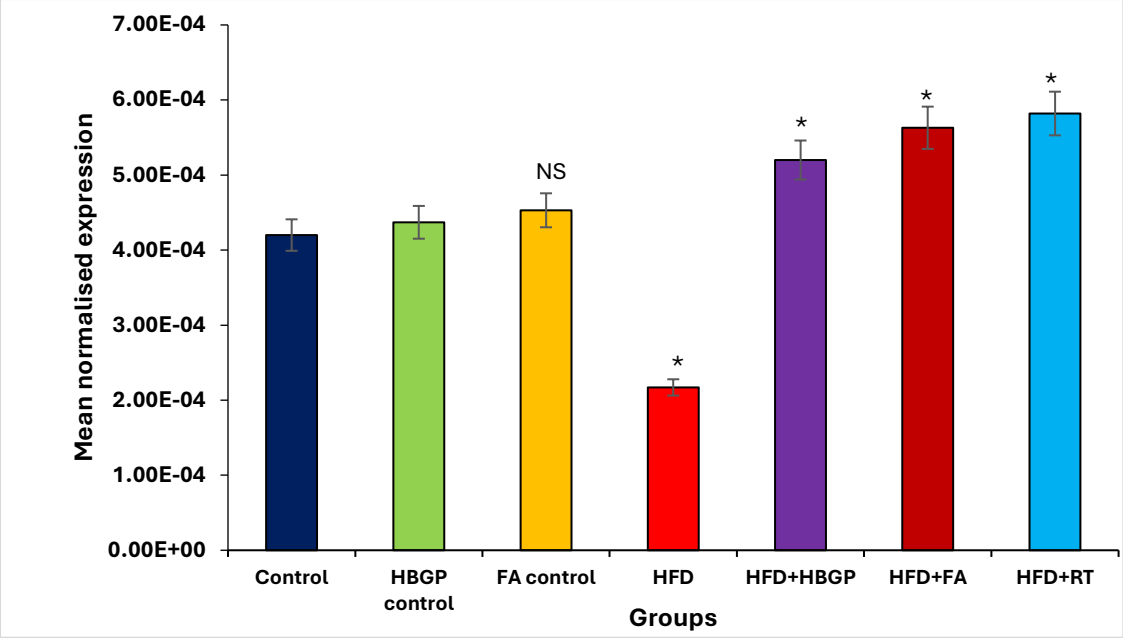


Figure 4.1 mRNA expression of LXR-α in AT of HBGP/FA treated & untreated rats.

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.

4. DISCUSSION

4.1 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 adipose tissue and expresses the mature adipose phenotype. According to Tontonoz et al. (1998), 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 (Figure 1 & 1.1) may be due to PPAR-γ 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 down regulated 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 exhibits increase in the activity of PPAR-γ consequently elevate adiponectin depot 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.

4.2 Impact of HBGP and FA on molecular mechanism of SIRT1

SIRT1 stimulation can help to improve lipid levels. Nicotinic acid, a precursor of NAD+ that elevates cellular NAD+ concentrations along with SIRT1 activation [15], which in turn 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 postpones the onset of many aging- related cardio vascular 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].

Figure 2 and 2.1 displays 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 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 collide 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 profile 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 works in numerous routes to prevent diseases. The phytochemistry of HBGP reveals the presence of dominant phenolic compound FA. Therefore, the hypolipidemic activity of HBGP may be due to the presence of FA. Moreover, our findings are supported with Chen et al [22] who demonstrated the potential role of FA on regulating muscle fiber formation through the activation of SIRT1/AMPK pathway.

4.3 Impact of HBGP and FA on molecular mechanism of FOXO1

Figure 3 and 3.1 shows 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 liver, SIRT1

removes acetyl group from gene transcription proteins such as transcription factors such as forkhead box O (FOXO), thus preventing cholesterol production. Moreover, IRE (insulin response element) sequence in the SREBP-2 promoter region is uniquely recognized by FOXO1 which inhibits SREBP-2 transcription. Li and Wu [23] provided evidences to prove that the upregulated of fatty acids and LDL were detected in FOXO1 knockout mice. Its regulating factor in bile acid metabolism was also discovered in a recent study. In liver, FOXO1 is necessary for the release of gene 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's) 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 case of liver whereas, in 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 mouse [24]. Increased adiponectin further stimulates cells to insulin and increases fatty acid 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.

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

Figure 4 and 4.1 shows 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, SIRT1also shown to regulate LXR through deacetylation reaction. There are two types of LXR's, 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, 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 Figure 5 & 5.1.

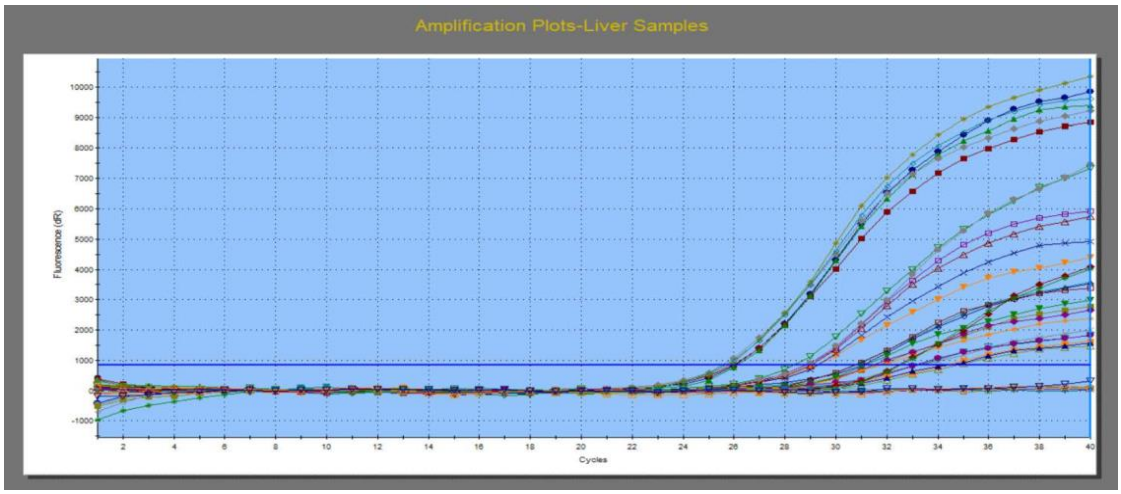


Figure 5. Overall representation of fluorescent profile and amplification plot in Liver samples of all the target genes during PCR program

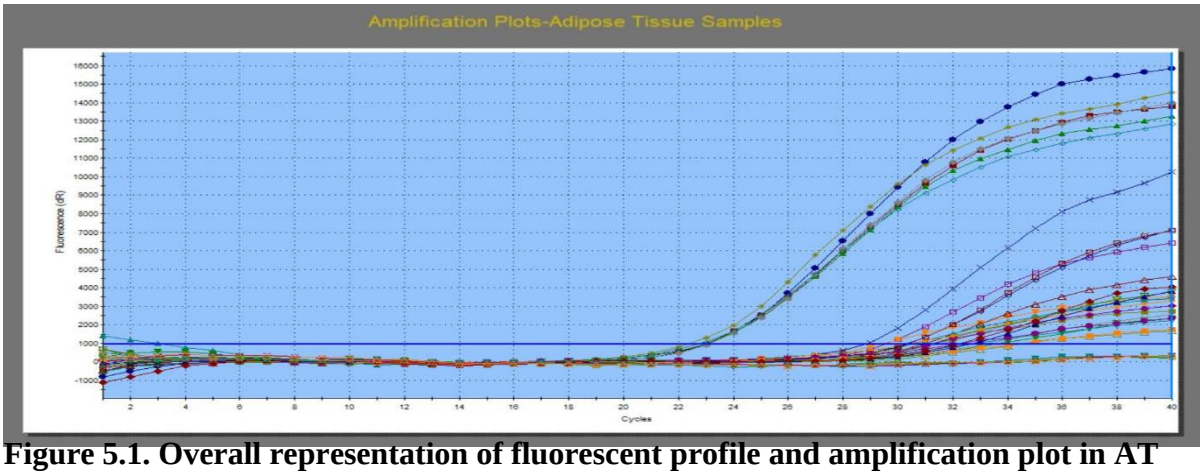


Figure 5.1. Overall representation of fluorescent profile and amplification plot in AT samples of all the target genes during PCR program

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: There is no conflict of interest among us

REFERENCES

1. Obadi, M., Sun, J., & Xu, B. (2021). Highland barley: Chemical composition, bioactive compounds, health effects, and applications. *Food research international (Ottawa, Ont.)*, *140*, 110065. https://doi.org/10.1016/j.foodres.2020.

2. Ali, S. A., Parveen, N., & Ali, A. S. (2018). Links between the Prophet Muhammad (PBUH) recommended foods and disease management: A review in the light of modern superfoods. *International journal of health sciences*, *12*(2), 61–69.

3. Dr. Shirke UJ, Dr. Yadav Jyotsna, Dr. Shirke JM, & Dr. Udmale MM. (2018).Literary review of Yava (Barley). *Journal of Ayurveda and Integrated Medical Sciences*, *3*(03), 165-168. https://doi.org/10.21760/jaims.v3i03.417.

4. de Melo, T. S., Lima, P. R., Carvalho, K. M., Fontenele, T. M., Solon, F. R., Tomé, A. R., de Lemos, T. L., da Cruz Fonseca, S. G., Santos, F. A., Rao, V. S., & de Queiroz, M. G. (2017). 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. *Brazilian journal of medical and biological research = Revistabrasileira de pesquisasmedicas e biologicas*, *50*(1), e5630. https://doi.org/10.1590/1414-431X20165630

5. Sun, S., Ruan, Y., Yan, M., Xu, K., Yang, Y., Shen, T., & Jin, Z. 2021. Ferulic Acid Alleviates Oxidative Stress-Induced Cardiomyocyte Injury by the Regulation of miR-499-5p/p21 Signal Cascade. *Evid Based Complement Alternat Med.* 2021: 1921457.

6. Chowdhury, S., Ghosh, S., Das, A.K., Sil, P.C. 2019. Ferulic acid protects hyperglycemia-induced kidney damage by regulating oxidative insult, inflammation and autophagy. *Front Pharmacol*. **10:** 27.

7. Iside, C., Scafuro, M., Nebbioso, A., &Altucci, L. (2020). SIRT1 Activation by Natural Phytochemicals: An Overview. *Frontiers in pharmacology*, *11*, 1225. https://doi.org/10.3389/fphar.2020.01225

8. Alageel, A., Tomasi, J., Tersigni, C., Brietzke, E., Zuckerman, H., Subramaniapillai, M., Lee, Y., Iacobucci, M., Rosenblat, J. D., Mansur, R. B., & McIntyre, R. S. (2018). Evidence supporting a mechanistic role of sirtuins in mood and metabolic disorders. *Progress in neuro-psychopharmacology & biological psychiatry*, *86*, 95–101. https://doi.org/10.1016/j.pnpbp.2018.05.017

9. Gamel, T., & Abdel-Aal, E.-S. M. (2012). Phenolic acids and antioxidant properties of barley wholegrain and pearling fractions. *Agricultural and Food Science*, *21*(2), 118–131. https://doi.org/10.23986/afsci.4727

10. Nascimento, A. F., Sugizaki, M. M., Leopoldo, A. S., Lima-Leopoldo, A. P., Luvizotto, R. A., Nogueira, C. R., & Cicogna, A. C. (2008). A hypercaloric pellet-diet cycle induces obesity and co-morbidities in Wistar rats. *Arquivosbrasileiros de endocrinologia e metabologia*, *52*(6), 968–974. https://doi.org/10.1590/s0004-

11. Chomczynski, P., & Mackey, K. (1995). Short technical reports. Modification of the TRI reagent procedure for isolation of RNA from polysaccharide- and proteoglycan-rich sources. *BioTechniques*, *19*(6), 942–945.

12. Tontonoz, P., Nagy, L., Alvarez, J. G., Thomazy, V. A., & Evans, R. M. (1998). PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell*, *93*(2), 241–252. https://doi.org/10.1016/s0092-8674(00)81575-

13. Esposito, K., Giugliano, F., Di Palo, C., Giugliano, G., Marfella, R., D'Andrea, F., D'Armiento, M., & Giugliano, D. (2004). Effect of lifestyle changes on erectile dysfunction in obese men: a randomized controlled trial. *JAMA*, *291*(24), 2978–2984. https://doi.org/10.1001/jama.291.24.2978

14. Iwaki, M., Matsuda, M., Maeda, N., Funahashi, T., Matsuzawa, Y., Makishima, M., & Shimomura, I. (2003). Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes*, *52*(7), 1655–1663. https://doi.org/10.2337/diabetes.52.7.1655

15. Bogan, K. L., & Brenner, C. (2008). Nicotinic acid, nicotinamide, and nicotinamide riboside:

a molecular evaluation of NAD+ precursor vitamins in human nutrition. *Annual review of nutrition*, *28*, 115–130. https://doi.org/10.1146/annurev.nutr.28.061807.155443

16. Most, J., Tosti, V., Redman, L. M., & Fontana, L. (2017). Calorie restriction in humans: An update. *Ageing research reviews*, *39*, 36–45. https://doi.org/10.1016/j.arr.2016.08.005

17. Picard, F., Kurtev, M., Chung, N., Topark-Ngarm, A., Senawong, T., Machado De Oliveira, R., Leid, M., McBurney, M. W., & Guarente, L. (2004). Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR-gamma. *Nature*, *429*(6993), 771–776. https://doi.org/10.1038/nature02583

18. Qiao, L., & Shao, J. (2006). SIRT1 regulates adiponectin gene expression through Foxo1-C/enhancer-binding protein alpha transcriptional complex. *The Journal of biological chemistry*, *281*(52), 39915–39924. https://doi.org/10.1074/jbc.M607215200

19. Cheang, W. S., Wong, W. T., Wang, L., Cheng, C. K., Lau, C. W., Ma, R. C. W., Xu, A., Wang, N., Huang, Y., & Tian, X. Y. (2019). Resveratrol ameliorates endothelial dysfunction in diabetic and obese mice through sirtuin 1 and peroxisome proliferator-activated receptor δ. *Pharmacological research*, *139*, 384–394. https://doi.org/10.1016/j.phrs.2018.11.041

20. Liang, N., Jakobsson, T., Fan, R., & Treuter, E. (2019). The Nuclear Receptor-Co-repressor Complex in Control of Liver Metabolism and Disease. *Frontiers in endocrinology*, *10*, 411. https://doi.org/10.3389/fendo.2019.00411

21. Chen, G., & Li, X. (2019). The decreased SIRT1 level may account for the lipid profile in chronic kidney disease. *Journal of biological research (Thessalonike, Greece)*, *26*, 9. https://doi.org/10.1186/s40709-019-0101-2

22. Chen, X., , Guo, Y., , Jia, G., , Zhao, H., , Liu, G., , & Huang, Z., (2019). Ferulic acid regulates muscle fiber type formation through the Sirt1/AMPK signaling pathway. *Food & function*, *10*(1), 259–265. https://doi.org/10.1039/c8fo01902a

23. Li, Y., & Wu, S. (2018). Epigallocatechin gallate suppresses hepatic cholesterol synthesis by

targeting SREBP-2 through SIRT1/FOXO1 signaling pathway. *Molecular and cellular biochemistry*, *448*(1-2), 175–185. https://doi.org/10.1007/s11010-018-3324-x

24. Li, K., Zhang, J., Yu, J., Liu, B., Guo, Y., Deng, J., Chen, S., Wang, C., & Guo, F. (2015). MicroRNA-214 suppresses gluconeogenesis by targeting activating transcriptional factor 4. *The Journal of biological chemistry*, *290*(13), 8185–8195. https://doi.org/10.1074/jbc.M114.633990

25. Zeng, H. T., Fu, Y. C., Yu, W., Lin, J. M., Zhou, L., Liu, L., & Wang, W. (2013). SIRT1 prevents atherosclerosis via liver-X-receptor and NF-κB signaling in a U937 cell model. *Molecular medicine reports*, *8*(1), 23–28. https://doi.org/10.3892/mmr.2013.1460

26. Li, X., Zhang, S., Blander, G., Tse, J. G., Krieger, M., & Guarente, L. (2007). SIRT1 deacetylates and positively regulates the nuclear receptor LXR. *Molecular cell*, *28*(1), 91–106. https://doi.org/10.1016/j.molcel.2007.07.032