Original article

Effect of 6-gingerol on AMPK- NF-κB axis in high fat diet fed rats

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Objectives: Adenosine monophosphate (AMP)-activated protein kinase (AMPK) plays a central role in metabolic homeostasis and regulation of inflammatory responses through attenuation of nuclear factor kappa-B (NF-κB). Thus AMPK may be a promising pharmacologic target for the treatment of various chronic inflammatory diseases. We examined the effect of 6-gingerol, an active ingredient of ginger on AMPK-NF-κB pathway in high fat diet (HFD) rats in comparison to fish oil.

Methods: Protein levels of AMPK-α1 and phosphorylated AMPK-α1 were measured by western blot while Sirtuin 6 (Sirt-6), resistin and P65 were estimated by RT-PCR. TNF-α was determined by ELISA, FFAs were estimated chemically as well as the enzymatic determination of the metabolic parameters.

Results: 6-Gingerol substantially enhanced phosphorylated AMPK-α1 more than fish oil and reduced the P65 via upregulation of Sirt-6 and downregulation of resistin, and resulted in attenuation of the inflammatory molecules P65, FFAs and TNF-α more than fish oil treated groups but in an insignificant statistical manner, those effects were accompanied by a substantial hypoglycemic effect.

Conclusion: Gingerol treatment effectively modulated the state of inflammatory privilege in HFD group and the metabolic disorders via targeting the AMPK-NF-κB pathway, through an increment in the SIRT-6 and substantial decrement in resistin levels.

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1. Introduction

A defect in AMPK function has been found in various cells in animals with metabolic diseases. In diabetes and obesity, it is likely that AMPK activation is compromised in inflammation-related cells and leads to the development of inflammatory diseases. Thus AMPK may be a promising pharmacologic target for the treatment of various chronic inflammatory diseases [1].

The transcription factor NF-κB controls the expression of genes involved in inflammation [2]. It is found in many types of cells, including adipocytes and macrophages [3,4]. The common form of NF-κB contains two subunits: p50 and p65, the later contains the transactivation domain and mediates transcriptional activation of target genes while p50 inhibits the transcriptional activity of p65 [5]. NF-κB activity is increased by either p65 overexpression or p50 knocking-out [6].

AMPK can suppress the activation of NF-κB and expression of proinflammatory cytokines in multiple cell types [5–10]. It is activated via allosteric regulation of increased AMP concentration and by the phosphorylation of α subunit (Thr172) [11–13]. Inflammatory stimuli and a fatty acid rich-diet decrease the expression and activity of AMPK in mouse adipose tissue and macrophages and triggered TNF-α expression [14]. Because activation of AMPK has anti-inflammatory functions, in the present studies we compared the effect of the ginger extract, 6-gingerol with fish oil on the AMPK-NF-κB axis. SIRT 6 is an NAD(+) –dependent deacetylase and mono-ADP-riboseyltransferase. It is known to interfere with the NF-κB signaling pathway and thereby has an antiinflammatory function. It has been postulated to act as upstream signalling molecule of AMPK-NF-κB [15,16]. Resistin is another deactivating pivotal molecule for AMPK pathway, it is a secretory cysteine-rich protein that is characterized as an insulin resistance factor found in rodent model of type II diabetes and obesity, [17–19].

Ginger (Zingiber officinale Roscoe) is a rhizomatous perennial plant that has been used as a spice in foods and beverages, its
extract contains the pungent phenolic components, 6-shogaol and 6-gingerol, the latter have been demonstrated to have anti-inflammatory, antioxidant and anticancer activity [20,21]. Nevertheless its effect on AMPK pathway and inflammation in obesity has not been elucidated yet. In contrary fish oil has been proven recently to activate AMPK and decrease adipose tissue inflammation pathway in mice [22].

The main objective of this study is to throw the light on the effect of 6-gingerol on AMPK-NF-κB axis in comparison to fish oil on the inflammatory state provoked by HFD and to evaluate the role of resistin and Sirt-6 in modulation of this inflammatory status.

2. Materials and methods

2.1. Plant collection and plant extraction

Fresh rhizomes of *Zingiber officinale* Roscoe, *Zingiberaceae* (ginger) were purchased and authenticated by Dr. Therese Labib, a Senior botanist in El-Orman garden, Egypt. A voucher specimen (no. BUPD 37) and deposited in Pharmacognosy Department, Faculty of Pharmacy, Beni-Suef University, Egypt. The fresh rhizomes (2 kg) were cut into small pieces using a knife and macerated on cold ethanol (95%) at room temperature till exhaustion. The combined ethanolic extracts were filtered and distilled off using a rotary evaporator, at 55 °C, under reduced pressure, affording a brown semisolid residue.

2.2. Phytochemical screening

The total ethanolic extract was screened for phytoconstituents; Molisch’s test for carbohydrates, Salkowski test for phytosterols and/or terpenes, froth test for saponins, FeCl₃ test for tannins, alkali test for flavonoids and Dragendorff’s test for alkaloids according to [23,24].

2.3. Qualitative detection of 6-gingerol in the ginger ethanolic extract using thin layer chromatography (TLC)

The total ethanolic extract was screened for the presence of 6-gingerol using TLC F254, readymade plates of silica gel (Fluka, Germany) and n-hexane/ethyl acetate (4:6 v/v) as the solvent system. Ferric chloride spray reagent was used to display the presence of the phenolic compounds, including gingerols. The appearance of a blue spot for at Rf value of 0.33 indicates the presence of 6-gingerol [25].

2.4. Animals

Sixty male Albino Wister rats weighing 96 ± 10 gm were used in the present study, they were purchased from the animal house of research institute of ophthalmology (Giza, Egypt). Rats had free access to water ad libitum and a standard laboratory diet; all animals were subjected to controlled conditions of temperature with a 12 h light-dark cycle. All studies were conducted in accordance with the Animal Care and Use Committee of the Biochemistry Department, Faculty of Pharmacy, Beni-Suef University.

2.5. Experimental design

One week after acclimatization, half of rats were fed HFD consisting of 25% fat “beef tallow” + 10% sucrose + 20% corn starch + 45% normal chow with 30% sucrose in drinking water [24], while the other half was maintained on standard normal chow diet (ATMID Company, Egypt) which composed of Soyabean, Corn, soyabean oil, calcium carbonate, dicalcium phosphate, sodium chloride, lecithin, methionine, and vitamins/minerals mixture, the diet contains 55–70% carbohydrates, 23% protein, 6% fat, 3% fibers and 1–4% vitamins/minerals mixture, feeding volume was 10% (w/w) of the experimental animals. After 18 weeks, HF-G group (n = 10) administered ginger extract (200 mg/kg body weight) via oral gavage and HFD, HF-F group (n = 10) administered fish oil (18% EPA and 12% DHA; Sedico, Egypt) in a dose level (0.8 gm/kg body weight) [12,26] and HFD, HF group (n = 10) received only HFD, NG group (n = 10) received standard Chow diet and 6-gingerol (200 mg/kg body weight) via oral gavage, NF group (n = 10) treated with standard Chow diet and fish oil, and normal control (N) group (n = 10) received standard normal Chow diet only. Two weeks later, the final body weight of rats was recorded, then the rats were sacrificed and blood samples were collected via retro-orbital bleeding into tubes containing EDTA and centrifuged at 1500 rpm for 30 min, Fasting blood glucose was estimated and the blood aliquots were stored at −20 °C for further analysis. Liver and visceral white adipose tissue were separated, excised, washed with 0.9% physiological saline, dried using dry filter paper then weighed and photographed. Harvested liver and adipose tissues were stored at −80 °C for further mRNA extraction and western blotting.

2.6. Metabolic markers

Enzymatic methods using commercially available kits (Spinreact, Santa Coloma, Spain) were used for colorimetric determination of plasma levels of glucose [27], triglycerides [28], total cholesterol [29], HDL-C [30], FFA level was determined according to the chemical method [31]. The relative adipose tissue weight was expressed as: (absolute adipose tissue weight/final body weight × 100) [32].

2.7. Determination of TNF-α

TNF-α was determined using ELISA kit supplied by RayBiotech Inc. (Norcross, GA, United States) according to manufacturer’s instructions.

2.8. Detection of resistin, p65, sirt-6 and CPT-1 gene expression by real time-polymerase chain reaction (RT-PCR)

Total RNA was isolated from visceral adipose tissue homogenates using RNeasy Purification Reagent (Qiagen, Valencia, CA) according to manufacturer’s instructions. The concentration of RNA was measured using a UV spectrophotometer.

2.8.1. cDNA synthesis

Five-microgram RNA was reverse transcribed using oligonucleotide (dT)₁₈ primer (final concentration, 0.2 mM) and was denatured at 70 °C for 2 min. Denatured RNA was placed on ice and reverse transcription mixture containing 50 mM KCl, 50 mM Tris HCl (pH 8.3), 0.5 mM of deoxyribonucleotide triphosphate (dNTP), 3 mM MgCl₂, 1 U/ml RNase inhibitor, and 200 units of moloney murine leukemia virus reverse transcriptase. The reaction tube was placed at 42 °C for 1 hour, followed by heating to 92 °C to stop the reaction.

2.8.2. Real-time quantitative polymerase chain reaction (PCR)

For real-time quantitative PCR, 5 μl of first-strand cDNA was used in a total volume of 25 μl containing 12.5 μl 2x SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) and 200 ng of each primer as shown in Table 1. PCR reactions consisting of 95 °C for 10 min (1 cycle), 94 °C for 15 s, and 60 °C for 1 min (40 cycles), were performed on step one plus Real Time PCR system
(Applied Biosystems). Data were analyzed with the ABI Prism 7500 sequence detection system software and quantified using the v1.7 Sequence Detection Software from PE Biosystems (Foster City, CA). Relative expression of studied genes was calculated using the comparative threshold cycle method. All values were normalized to the beta actin genes, all these steps were described by [33].

2.9. Detection of AMPK-α1 and phosphorylated AMPK-α1 by western blot technique

Briefly, liver tissue (50 mg) was homogenized using a polytron homogenizer in 1.5 mL cold lysis buffer (50 mMol/L Tris-HCl, pH 8.0, 150 mmol/L NaCl, 1% NP40, 0.5% sodium deoxycholate, 0.1% SDS and 0.5 mmol/L phenylmethylsulfonylfluoride). The homogenate was centrifuged for 20 min at 4°C and the supernatant was collected. Samples were stored at −80°C until use. After boiling at 95°C for 5 min, samples (20 μg/lane) were subjected to 7% SDS-PAGE gel and then transferred to nitrocellulose membranes (Bio-Rad). The membranes were blocked in 5% BSA in TBS-T for 1 h at room temperature, then incubated overnight at 4°C with primary antibodies specific for AMPK-α1 and phosphorylated AMPK-α1 (Thr172) at dilution factor 1:1000 (Cell Signalling Technology, Beverly, MA, USA). The membranes were washed 3x/10 min with TBS-T and incubated with a secondary horseradish peroxidase-conjugated anti-rabbit IgG antibody (1: 5000, Bio-Rad, Hercules, CA, USA) for 1 h at room temperature, followed by 3x/10 min washing with TBS-T. Proteins were visualized by enhanced chemiluminescence (ECL plus; Amersham, Arlington Heights, IL, USA) and quantified using densitometry and Molecular Analyst Software (Bio-Rad, Richmond, CA, USA). Protein level was expressed relative to beta actin [34].

2.10. Statistical analysis

Data are presented as means ± SEM values. The results were analyzed statistically by one-way analysis of variance (ANOVA) with subsequent multiple comparisons using Tukey multiple comparison Post-Hoc test. The p-values less than 0.05 were considered significant. Correlations between variables were assessed by Pearson’s correlation test.

All calculations were made using the computer program SPSS 16.0 (SPSS, Chicago, III, USA). The data were graphed using GraphPad Prism 6 (GraphPad Software, Inc., USA) and Microsoft Office Excel computer programs.

3. Results

3.1. Phytochemical study

3.1.1. Phytochemical screening

The phytochemical screening of total ethanolic extract of ginger indicates the presence of carbohydrates, alkaloids, tannins, flavonoids, saponin, terpenoids and sterols (Table 2). The results of these tests have been shown also in Fig. 1.

Table 1
The sequence of primers of studied genes.

<table>
<thead>
<tr>
<th>Genes</th>
<th>Primer sequence (5’–3’)</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resistin</td>
<td>Forward primer TGTGCCCCATGGATGAAGCC</td>
<td>Product length: 157</td>
</tr>
<tr>
<td></td>
<td>Reverse primer GAGCAGCTAGCAGCTGTG</td>
<td>Gene bank accession number: NM_001031649.1</td>
</tr>
<tr>
<td>p65</td>
<td>Forward primer CACTTCTCCCTAGACAGCCAGC</td>
<td>Product length: 135</td>
</tr>
<tr>
<td></td>
<td>Reverse primer GTGTCCTCTCCCTCCCCAGAAAT</td>
<td>Gene bank accession number: AF079314.1</td>
</tr>
<tr>
<td>SIRT6</td>
<td>Forward primer GACCTACGGAAGCGTTGGA</td>
<td>Product length: 163</td>
</tr>
<tr>
<td></td>
<td>Reverse primer CCTGCCGCGTACATTTTGT</td>
<td>Gene bank accession number: NM_001031649.1</td>
</tr>
<tr>
<td>CPT-1</td>
<td>Forward primer AACAGATCTGCGGTGGCGG</td>
<td>Product length: 123</td>
</tr>
<tr>
<td></td>
<td>Reverse primer CACACCCACACCCAGATAA</td>
<td>Gene bank accession number: NM_031559.2</td>
</tr>
</tbody>
</table>

3.1.2. Qualitative detection of 6-gingerol in ginger ethanolic extract

The phenolic compound (6-gingerol) was detected by TLC as a blue spot with Rf value = 0.33 (Fig. 1).

3.2. Visceral adipose tissue morphology

NC group, NG group and NF group showed similarities in weight of the visceral adipose tissue as in Fig. 2 (A–C). HF group showed a very big mass of visceral adipose tissue with significant increase in weight compared to NC as in figure (D). HF-G group and HF-F group showed a decrease in visceral adipose tissue weight compared to HF group (figure E,F).

3.3. Detection of absolute and relative adipose tissue weight and final body weight

The morphological observation was also reflected by Table 3 which showed that administration of HFD successively induced obesity as indicated by the significant increment in both absolute and relative adipose tissue weight and final body weight as compared to the control group, while either HF-G or HF-F group recorded a significant decrease in those parameters in comparison to HF group. It is worth to mention that either NG or NF group pinpointed no harmless effect on weight, indeed there was no significant difference in either ginger extract or fish oil treated rats when compared with the normal one.

3.4. Detection of plasma metabolic markers

HFD induced a potential metabolic disturbance as evidenced by the significant increment in the levels of glucose, triglycerides, FFAs and total cholesterol with a little effect on HDL-C compared to those of normal control group. Administration of either ginger extract or fish oil resulted in a considerable statistical improvement as indicated by the significant decrement in the levels of glucose, triglycerides, free fatty acid and total cholesterol with a little effect on HDL-C as compared to HF group. NG and NF group showed no significant difference in these metabolic parameters in comparison with the normal rats (Table 4 and Fig. 3).

Table 2
Phytochemical screening of total ethanolic extract of ginger.

<table>
<thead>
<tr>
<th>Constituents</th>
<th>Ethanol extract of ginger</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrates</td>
<td>+</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
</tr>
<tr>
<td>Terpenes and sterols</td>
<td>+</td>
</tr>
</tbody>
</table>

● + = Present.
● − = Not present.
3.5. Determination of TNF-α level

Fig. 4 showed that measurement of the level of TNF-α of rats of the tested groups revealed that there was a significant increase in TNF-α level by 78.11% in HF group as compared to the control group, while either NG or NF group showed non-significant effects in comparison with the normal one. Either HF-G or HF-F group recorded a significant decrease in TNF-α level by 57.73%, 51.42% in comparison to HF group respectively.

3.6. Determination of molecular markers

A great activation of the inflammatory pathway was observed in HFD induced obesity as manifested by the significant increase in the levels of Resistin and P65 with significant reduction in Sirt-6 and CPT-1 as compared to the control rats. Nevertheless, the treatment by either ginger extract or fish oil recorded a statistically significant increase in Sirt-6 and CPT-1 level by with a significant decrease in the levels of Resistin by 83.72%, 76.16% and NF-κB by 85.91%, 71.82% in comparison to HF group respectively. NG and NF group revealed no significant difference in the molecular pattern as compared to the normal rats (Figs. 5, 6, 7 and 8).

3.7. Determination of AMPK-α1 and phosphorylated AMPK-α1

Figs. 9 and 10 showed that measurement of liver AMPK-α1 and phosphorylated AMPK-α1 of rats of the tested groups revealed that there was a significant increase in liver AMPK-α1 and a significant decrease in phosphorylated AMPK-α1 and phosphorylated AMPK-α1/AMPK-α1 ratio by western blot in HF group as compared to the control group, while NG and NF group showed non-significant effects in comparison with the normal one. Either HF-G or HF-F group recorded a significant decrease in liver AMPK-α1 and a significant increase in phosphorylated AMPK-α1 in comparison to HF group. HF-G group exhibited a significant elevation in phosphorylated AMPK-α1/AMPK-α1 ratio while HF-F group showed non-significant elevation compared to HF group. There was a significant difference between effect of HF-G group and HF-F group in those parameters (Figs. 11 and 12).

4. Discussion

In the HFD the excess nutrients can be sensed by the nutrient sensor AMPK, which may function as a cellular link between nutrient metabolism and inflammation [35]. A plethora of studies...
has demonstrated that the activation of AMPK signaling down regulates the function of NF-κB system [35–39]. Down regulation in the phosphorylation levels of the AMPK activity increased the levels of FFAs and amplified the inflammatory status and resulted in a hyperglycemia in the HFD. It is well known that FFAs levels are usually elevated in obesity because the enlarged adipose tissue mass releases more FFAs, we reported increased percentage of visceral adipose tissue and enhanced accumulation of TAG in addition to the reduced FFAs clearance whereas, the key enzyme in transferring acyl-CoA into mitochondria for β-oxidation, CPT-I

### Table 3
Changes in absolute and relative adipose tissue weight and body weight in different studied groups.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>NG</th>
<th>NF</th>
<th>HF</th>
<th>HF-G</th>
<th>HF-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute adipose tissue weight (gm)</td>
<td>5.00 ± 0.34</td>
<td>3.18 ± 0.55</td>
<td>3.42 ± 0.49</td>
<td>9.87 ± 0.91*</td>
<td>4.96 ± 0.40b</td>
<td>5.65 ± 0.43b</td>
</tr>
<tr>
<td>Relative adipose tissue weight</td>
<td>1.60 ± 0.09</td>
<td>1.10 ± 0.16</td>
<td>1.13 ± 0.15</td>
<td>2.61 ± 0.22*</td>
<td>1.78 ± 0.11b</td>
<td>1.98 ± 0.08b</td>
</tr>
<tr>
<td>Final body weight (gm)</td>
<td>300.75 ± 5.28</td>
<td>281.25 ± 11.13</td>
<td>288.12 ± 9.90</td>
<td>376.23 ± 12.07*</td>
<td>280.12 ± 15.47b</td>
<td>285.12 ± 12.80b</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 10).
N, normal control; NG, normal group treated with ginger; NF, normal group treated with fish oil; HF, HFD group; HF-G, HFD group treated with ginger; HF-F, HFD group treated with fish oil.

*P < 0.001 compared with normal control group.
**P < 0.001 compared with high-fat fed group.
***P < 0.01 compared with high-fat fed group.
****P < 0.05 compared with normal control group.

### Table 4
Changes in metabolic parameters in different studied groups.

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>NG</th>
<th>NF</th>
<th>HF</th>
<th>HF-G</th>
<th>HF-F</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting plasma glucose (mg/dl)</td>
<td>90.13 ± 3.92</td>
<td>83.55 ± 2.60</td>
<td>88.52 ± 2.99</td>
<td>132.26 ± 2.31*</td>
<td>93.88 ± 2.52b</td>
<td>101.00 ± 4.11b</td>
</tr>
<tr>
<td>Plasma triglycerides (mg/dl)</td>
<td>81.04 ± 15.47</td>
<td>59.28 ± 3.039</td>
<td>64.02 ± 3.16</td>
<td>143.56 ± 18.94e</td>
<td>74.12 ± 6.34e</td>
<td>86.65 ± 7.81e</td>
</tr>
<tr>
<td>Plasma FFAs (mg/dl)</td>
<td>51.07 ± 5.70</td>
<td>47.45 ± 1.49</td>
<td>49.72 ± 1.81</td>
<td>90.77 ± 4.04a</td>
<td>55.93 ± 2.53b</td>
<td>62.58 ± 3.89b</td>
</tr>
<tr>
<td>Plasma total cholesterol (mg/dl)</td>
<td>143.03 ± 4.12</td>
<td>147.21 ± 4.62</td>
<td>142.55 ± 4.86</td>
<td>170.35 ± 6.59***</td>
<td>144.13 ± 5.52***</td>
<td>141.65 ± 9.32***</td>
</tr>
<tr>
<td>Plasma HDL-C (mg/dl)</td>
<td>41.51 ± 2.65</td>
<td>41.91 ± 3.59</td>
<td>42.02 ± 2.54</td>
<td>38.35 ± 3.06</td>
<td>43.66 ± 2.22</td>
<td>44.28 ± 3.56</td>
</tr>
</tbody>
</table>

Data are means ± SEM (n = 10).
N, normal control; NG, normal group treated with ginger; NF, normal group treated with fish oil; HF, HFD group; HF-G, HFD group treated with ginger; HF-F, HFD group treated with fish oil.

*P < 0.001 compared with normal control group.
**P < 0.001 compared with high-fat fed group.
***P < 0.01 compared with normal control group.
****P < 0.05 compared with normal control group.
Administration of 6-gingerol and fish oil effectively decreased the levels of FFAs through increasing their oxidation as estimated by increased CPT-1 gene expression level and decreased percentage of visceral adipose tissue and TAG as well. Okamoto and his coworkers showed that Supplementation of HFD mice with 6-gingerol significantly reduces body weight gain, fat accumulation, and the extent of lipogenesis by downregulating of the mRNA levels of sterol regulatory element-binding protein 1c (SREBP-1c) and acetyl-CoA carboxylase [43].

Moreover both dietary supplements showed a significant hypoglycemic effect, this effect can be attributed to the stimulating effect on the phosphorylated AMPKα1 that affects FFAs and the accompanying state of inflammation in the HFD rats. Many studies reported that FFAs are a major link between obesity and insulin resistance/type 2 diabetes, the elevated FFAs levels inhibits insulin’s anti-lipolytic action, which will further increase the rate of FFAs release into the circulation [44] and stimulate the proinflammatory NF-κB pathway. We recorded a substantial increase in the gene expression levels of P65 to reveal significant activation in NF-κB pathway and enhanced production of TNF-α from adipose tissue, a recent study has stated that chronic activation of AMPK by the hormone adiponectin, reduces TNF-α production [45,46].

Despite being statistically insignificant, 6-gingerol group showed anti-inflammatory effects more than fish oil one as manifested by decreased levels of P65 and TNF-α in addition to FFAs. Recently, 6-gingerol and fish oil have been proven to exert anti-inflammatory effects through AMPK activation [22,47]. 6-gingerol increases cytosolic Ca2+ concentration and enhances the levels of phosphorylated AMPKα via modulation by Ca2+/calmodulin-dependent protein kinase kinases while the G protein-coupled receptor 120 functions as an omega-3 fatty acid receptor/sensor [48,49] through which the fish oil increases AMPK phosphorylation and exerts their antiinflammatory effects [50].

In the present study 6-gingerol enhanced the phosphorylation levels of AMPK and increased the ratio of phosphorylated AMPKα1/AMPKα1 more significantly than fish oil, AMPK may be a promising pharmacologic target for the treatment of various chronic inflammatory diseases [1]. Increasing AMPK activity with AICAR, or by transfection of a constitutively active AMPK catalytic subunit, blunts the ability of FFAs or TNF-α to activate NF-κB [51]. Furthermore, a number of studies demonstrated that AMPK signaling could inhibit the inflammatory responses induced by NF-κB.

6-gingerol has been shown to increase the gene expression of Sirt-6 more than fish oil, this may be considered as a novel effect for both nutraceuticals in modulating the NF-κB axis. More recently, Sirt-6 has been postulated to act as upstream of AMPK signaling [15] to play an important role in metabolic homeostasis and inflammation through attenuation of NF-κB signaling [16]. Sirt-6 interacts with the p65 subunit of NF-κB and deacetylates H3K9 at target promoters. It has been demonstrated that deficiency of Sirt-6 in mice results in increased NF-κB-driven gene expression programs, moreover, the knockdown of SIRT-6 resulted in the increased expression of proinflammatory cytokines [52]. In ECs, the loss of Sirt-6 was associated with an increased expression of NF-κB, whereas overexpression of Sirt-6 was associated with decreased NF-κB transcriptional activity [52], indicating that Sirt-6 may be associated with the upregulation of genes involved in inflammation. Interestingly, when a HFD, transgenic mice overexpressing Sirt-6, in comparison to their wild-type littermates, accumulate significantly less visceral LDL cholesterol, and TAG [53].
It is worth mentioning that the present study recorded significant increment in the lipid profile in HFD implicating the role of AMPK and Sirt-6 in affecting the metabolic status. Balestrieri and his coauthors reported that the increased Sirt-6 expression observed in diabetic plaques from the current incretin users suggests a low inflammatory activity linked to decreased NF-κB activation [54].

Nutraceuticals treated groups resulted in a substantial decrement in the levels of resistin, additionally, 6- gingeroi treatment down regulated the elevated levels of resistin in HFD group more than fish oil treatment, many studies reported that resistin level rise during obesity and promote hyperglycemia and insulin resistance in HFD, wild-type mice [55], furthermore, it has been shown that resistin treatment can reduce AMPK phosphorylation in both liver [56,57] and skeletal muscle [58], and this may be a key intermediate molecule in resistin action [59]. Moreover the adipokine, resistin activates NF-κB, and leads to the translocation of p65 subunits of NF-κB from the cytoplasm to the nucleus [60]. The hypoglycemic effect in nutraceuticals treated group may also be linked to the reduced resistin effect, Muse and his colleagues recorded that reducing plasma resistin with a resistin-specific
antisense oligonucleotide normalizes fasting plasma glucose levels in high fat-fed mice, this improvement is accompanied by an increase in hepatic AMPK activation [61]. High fat-fed, resistin knockout mice display improvements in indices of hepatic glucose production and elevated AMPK activation [62].

5. Conclusion

The present study showed that gingerol treatment effectively modulated the state of inflammatory privilege in HF group and the metabolic disorders via targeting the AMPK- NF-κB pathway. Through an increment in the Sirt-6 and substantial decrement in resistin levels, there was a significant decrease in the levels of the inflammatory signalling molecules p65, FFAs, and TNF-α.

Conflict of interest

Authors declare that they have no conflict of interest.

Acknowledgement

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jbiopharm.2017.01.035.

References