Anti-obesity effects of Taif and Egyptian pomegranates: molecular study

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Anti-obesity effects of Taif and Egyptian pomegranates: molecular study

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The present study investigated the anti-obesity effects of pomegranate (Punica granatum) juices from the two Saudi Arabian, Taif red, Taif white, and Egyptian pomegranates in high-fat diet (HFD)-induced obese rats. Administering any of the used juices decreased the body weight gain, food consumption, and serum levels of lipid, leptin, and glucose, while it increased serum insulin level. Histologically, all types of juices decreased the number and size of lipid droplets in hepatocytes compared to the obese, non-treated animals. All juices types upregulated the hepatic mRNA expression of hormone-sensitive lipase, pyruvate kinase, and adiponectin in obese rats; the genes were all suppressed by HFD feeding. Additionally, the expression of fatty acid synthase, sterol regulatory element-binding protein-1c, and acetyl-CoA carboxylase1 was also upregulated by all types of juices. Conversely, ghrelin mRNA expression was downregulated by all used juices’ types. These findings demonstrate that all types of tested juices protect against the HFD-induced obesity in rats.

Key words: obesity; pomegranate; Taif; Egyptian; histology; rat

Obesity is a chronic metabolic disorder that results from an energy imbalance where energy intake is greater than energy expenditure. It is characterized by excessive fat mass and elevated lipid concentration in blood.1,2 Globally, obesity has reached epidemic rates where more than one billion adults worldwide are overweight and at least 300 million of them are clinically obese.3 The worldwide prevalence of childhood overweight and obesity have increased from 4.2% in 1990 to 6.7% in 2010 and this trend is expected to reach 9.1% in 2020.1,4 Importantly, obesity is a predisposing factor for and often associated with many diseases including coronary vascular disease, hypertension, type 2 diabetes mellitus, respiratory diseases, metabolic syndrome, dislipidemia, fatty liver, and some cancers.5 In addition, there is a significant correlation between body mass index (BMI) and metabolic syndrome in children and adolescents.6–9

As obesity is associated with many serious health problems; it is necessary to treat obese individuals pharmacologically, surgically, or through lifestyle interventions.10 Despite short-term benefits of pharmaceutical treatment of obesity, it is often associated with undesired side effects from the medication and rebound weight gain after the cessation of drug use.5 Moreover, surgical procedures of obesity treatment increase incidence of micronutrients and macronutrients deficiencies.11 As there are several risks associated with the pharmacologic and surgical interventions of obesity, it is suggested that a dietary intervention maybe the safest and most cost-effective option for those who are moderately obese.3,12 This paid more attention and concern to study natural sources having body weight lowering effects with minimal side effects.13 This may be an excellent alternative strategy for developing effective and safe anti-obesity drugs in the future.14,15

Among herbal products that were investigated for their anti-obesity activities are pomegranate extracts (leaf, seed, flower, and juice). Extracts from the different parts of pomegranate have been researched for numerous pharmacologic activities, such as anti-tumor, anti-bacterial, astringent, anti-diarrheal, and anti-obesity activities.16 Pomegranate extracts (juice, seed oil, and flower extracts) are known to possess enormous anti-diabetic, anti-inflammatory, anti-oxidant, and anti-tumor effects in vivo and in vitro.17 The juice is known to be a rich source of antioxidants from polyphenols, tannins, and anthocyanins in addition to vitamin C, vitamin E,
Obesity was caused by the increase in blood lipid parameters and body weight. The effect of pomegranate juice on cholesterol accumulation in macrophages and on cholesterol biosynthesis was studied in a J774.1 macrophage-like cell line. The HFD obese mice treated with the gallic acid (GA), lipoic acid (LA), and combination of GA and LA (1% of diet) for 7 weeks showed body weight decreases of 12.8, 6.8, and 12.20% for GA, LA, and GA and LA, respectively. Cerdá et al. investigated the effects of 6% punicagelin found in pomegranate juice in female rats for 37 days. Their results revealed that an intake of 4800 mg kg$^{-1}$d$^{-1}$ had a lowering effect on the food consumption and body weight of the animals. In Saudi Arabia, there are two native cultivars of pomegranate known as Taif Red pomegranate which has reddish peel and juice and Taif White pomegranate with greenish white peel and whitish juice. Both cultivars belong to Punica granatum species. As mentioned above, anthocyanine is responsible for giving color to the pomegranate fruit and juice. Therefore, the anthocyanine content must be different between the two native cultivars of Taif pomegranate. In this study, we compared the anti-obesity effect of pomegranate juice from the two native cultivars of Taif pomegranate and the Egyptian pomegranate (Hegazy cultivar) which belongs to the same species ($P$. granatum). The present study aimed to evaluate the efficacy of Taif and Egyptian pomegranate juice in treating the diet-induced obesity, and regulating body weight and food intake, and to elucidate this efficacy at the molecular level.

Materials and methods

Animals and experimental design

Thirty-five adult male Wistar rats of seven weeks old (140–150 g) were purchased from experimental animal center, Faculty of Pharmacy, King Saud University, Saudi Arabia. The animals were housed in the animal facility of the College of Science, Taif University, Saudi Arabia, at 22 °C and 55% humidity with 12 h light/12 h dark cycle. All procedures were approved by the Animal Care Committee of Taif University for project #2574/1434/1.

Induction of obesity

After one-week acclimatization, the rats were divided into two main groups; a control group of seven rats was fed a standard pellet diet and water ad libitum, and high-fat diet (HFD)-fed group of 28 rats that was fed HFD and gained free access to water for 10 weeks. The HFD is composed of 15.5% protein, 38.8% fat, and 45.7% carbohydrates (Table 1) based on the previous study with replacement of lard with beef tallow. Obesity was confirmed by the increase in blood lipid parameters and body weight.

Preparation of the juice. Fresh Egyptian pomegranate, Taif red, and Taif white pomegranates were purchased from local market in Taif City. Fresh fruits were peeled, and the edible portion was squeezed. The juice was filtered through Whatman No. 1 filter paper, and the filtrate was divided into 10 mL aliquots in 15 mL Falcon tubes and stored at −80 °C until used.

Animal treatment. High-fat diet-fed rats were gained free access to HFD throughout the experimental period and were subdivided into the following four groups of seven each. Obese, non-treated group, gained free access to normal drinking water and HFD; Egyptian pomegranate group, given Egyptian pomegranate juice and HFD; Taif Red pomegranate group, given Taif Red pomegranate juice and HFD, and Taif White pomegranate group, given Taif White pomegranate juice and HFD. Animals received normal drinking water and served as controls. The different types of pomegranate juices were given in drinking water at a dose 0.15% pomegranate juice (wt/vol). The dose of pomegranate juice was chosen based on a previous study. Water bottles were changed every other day. The treatment was continued for 4 weeks. Animal body weight and average daily food consumption were recorded weekly from the onset and throughout the experimental period.

Sampling. At the end of the experiment, animals were prevented from eating for 10 h, anaesthetized using diethyl ether, and blood samples were collected from medial canthus of the eyes. Blood samples were allowed to clot, centrifuged, and serum samples were collected and stored at −80 °C until used for biochemical assays. Then rats were sacrificed by head decapitation. Immediately, tissue samples from liver, stomach, and white adipose tissue were collected for analysis of gene expression. Another tissue samples from liver were collected for histological examination. Samples for histological study were kept in 10% neutral buffered formalin (NBF) while those for molecular assays were kept in Qiagen Inc., Valencia, CA, USA) at −80 °C until use.

Biochemical assays. Serum triacylglycerol (TAG), total cholesterol (TC), and glucose were measured spectrophotometrically using specific commercial kits (HUMAN Gesellschaft für Biochemica und Diagnostica mbH, Wiesbaden, Germany) according to manufacturer’s instructions. Serum levels of insulin and leptin were measured by ELISA using commercial kits (R&D, Inc., USA) according to the manufacturer’s instructions.

Histological examination. Small pieces of liver from rats in different animal groups were fixed in 10% NBF for at least 24 h, then processed, sectioned, mounted on glass slides, and stained with hematoxylin and eosin for studying the general histology. Other NBF-fixed pieces (2 mm thick) were processed according to Tracy and Walia for demonstration of lipid droplets by oil red O staining. This technique, in brief, depends on the immersion of the tissue slices in 1% solution of linoleic acid and 0.4% lecithin in 70% ethylene glycol at 56 °C for 72 h. Tissue slices were...
then rinsed in several changes of 70% ethanol and water (8 h each), respectively. Tissue slices were immersed in 2% chromic acid for 24 h at 4 °C, rinsed in water for 24 h, followed by the immersion in 5% sodium bicarbonate for another 24 h, and finally rinsed in water for at least 8 h. Tissue slices were then processed as in routine histological techniques.24) The sections (7 μm thick) were dewaxed, dehydrated, and brought to distilled water. Slides were then placed into filtered 0.5% oil red O in dextrin for 20 min, briefly rinsed with running water, and counterstained with Gill II hematoxylin for 30 s. Finally, they were rinsed with water and covered with cover slip with aqueous mounting media.24)

Analysis of gene expression

RNA extraction and cDNA synthesis. Total RNA was extracted from 100 mg of each tissue sample using QIAzol lysis reagent (QIAGEN Inc., Valencia, CA) according to the manufacturer’s instructions and as detailed previously.26) Integrity of the prepared RNA was checked by electrophoresis. RNA concentration and purity were determined spectrophotometrically at 260 and 280 nm. The ratio of OD260/280 of all RNA samples was 1.7–1.9. Two μg RNA were reverse transcribed with oligo-dT primer and Moloney murine leukemia virus (M-MuLV) reverse transcriptase (SibEnzyme Ltd. AK, Novosibirsk, Russia) as previously described.26) The resultant cDNA was preserved at −20 °C until used.

Semi-quantitative polymerase chain reaction. The expression of some genes involved in energy metabolism was tested by semi-quantitative polymerase chain reaction (PCR) using specific primers for these genes (Table 2). The tested genes were glucose transporter-2 (GLUT-2), pyruvate kinase (PK), hormone-sensitive lipase (HSL), sterol regulatory element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), and acetyl carboxylase 1 (ACC1). In addition, leptin and adiponectin genes expressions were also tested. The used primers were designed using Oligo-4 program and nucleotide sequences published in the Gene bank (Table 2). Primers were synthesized by Macrogen (Macrogen Company, GAsa-dong, Geumcheon-gu, Korea).

PCR was conducted in a final volume of 25 μL using PCR GoTaq® Green Master Mix (Promega Corporation, Madison, WI) as previously described with a modification of the annealing temperatures, that corresponding to each primer (see Table 1). The number of cycle was 25–35 cycles (Table 2). As a reference, expression of β-actin mRNA was tested using specific primers (Table 2).

Statistical analysis. Statistical analysis for the obtained results was performed using analysis of variance and Scheffe’s protected least significant difference test, by using SPSS software (SPSS version 13.0, IBM, Chicago, IL, USA) with p < 0.05 regarded as statistically significant. The results were expressed as means ± standard errors of means.

Results

Induction of obesity

Obesity was induced by feeding the animal on HFD. As shown in Fig. 1, average body weight in the HFD-fed group was higher than that of the control group. The difference in body weight was clear starting from the third week of experiment and continued until the 10th week.

Effect of pomegranate juice on food consumption and body weight

Food consumption was decreased with varying degrees in response to administration of the three types of juice as shown in Fig. 2(a) and the highest effect was induced by Taif Red pomegranate juice (RJ) while the lowest was due to Taif White pomegranate juice (WJ). After 10 weeks of treatment with the different types of juices, body weight of the treated animal groups was decreased significantly compared to obese, non-treated group with no different effects among the three types of juices-treated groups. However, the body weight in different types of juice-treated obese groups was still higher than that of control one (Fig. 2(b)).

Effect of pomegranate juice on serum lipid profile

Obesity significantly increased serum level of TAG and cholesterol (Fig. 3(a) and (b)). This increase was inhibited by pomegranate juices treatment with the highest effect observed in Egyptian pomegranate juice (EJ) and the lowest effect observed in WJ. On the contrary, WJ showed the strongest cholesterol lowering effect.

Effect of pomegranate juice treatment on the expression of genes related to lipid metabolism in liver

HFD significantly inhibited the mRNA expression of FAS to less than half of the level of that of the control group. This inhibition was counteracted by different

Table 1. Components of diet induced obesity.

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Food (g/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal pellet diet (NPD)</td>
<td>365</td>
</tr>
<tr>
<td>Beef tallow</td>
<td>310</td>
</tr>
<tr>
<td>Casein</td>
<td>250</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>10</td>
</tr>
<tr>
<td>Vitamins and mineral mix</td>
<td>60</td>
</tr>
<tr>
<td>D, L. Methionine</td>
<td>3</td>
</tr>
<tr>
<td>Yeast powder</td>
<td>1</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>1</td>
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</tbody>
</table>
types of pomegranate juice treatment (Fig. 4(a)). Furthermore, FAS mRNA expression was significantly upregulated (0.38 in EJ and 0.28 in RJ-treated groups) compared to the control, while it did reach the significance in case of WJ-treated group. Similarly, obesity downregulated the mRNA expression of SREP-1c. This downregulation was significantly counteracted by pomegranate juices treatment which increased expression level (0.63 in EJ, 0.59 in RJ, and 0.38-folds of increase) compared to the control (Fig. 4(b)).

**Effect of pomegranate juice treatment on the leptin mRNA expression and serum level of leptin, insulin, and glucose**

Leptin mRNA expression was significantly upregulated (more than two folds) in obese, non-treated group compared to control. This upregulation was inhibited in response to pomegranate juices treatment. WJ had obviously more inhibitory effects on leptin mRNA than the other two types (Fig. 5(b)). In accordance to leptin mRNA expression, the level of serum leptin in HFD-fed group was greatly increased (almost three folds of control level), while pomegranate juices treatment inhibited this increase with the strongest inhibitory effect seen in WJ-treated group (Fig. 5(c)). HFD significantly reduced serum insulin level (about 35% decrease compared to control). This reduction was inhibited by pomegranate juices treatment especially in case of EJ and WJ (Fig. 5(d)). Serum glucose values in HFD-fed group were significantly increased; the effect that was counteracted by the different types of pomegranate juices was tested. The strongest effect was observed in WJ-treated group (Fig. 5(e)).

**Effect of pomegranate juice treatment on the ghrelin and adiponectin mRNA expression**

Adiponectin mRNA expression was significantly downregulated (50% decrease compared to control level) in obese, non-treated group. This downregulation was inhibited in response to the treatment with pomegranate juices, with the strongest effect observed in WJ-treated group (Fig. 6(a)). HFD significantly induced ghrelin mRNA expression (0.25% increase compared to control). This induction was counteracted by pomegranate juices treatment. Both EJ and WJ treatments significantly inhibited ghrelin mRNA expression compared to either control or obese, non-treated group (Fig. 6(b)).
Effect of pomegranate juice treatment on mRNA expression of genes related to glucose metabolism in liver

HFD stimulated mRNA expression of GLUT-2 while the three types of juices inhibited this stimulation. The strongest inhibitory effect was shown in WJ-treated group (Fig. 6(c)). Furthermore, obesity suppressed PK mRNA expression (more than 50% reduction comparing to control), while pomegranate juices treatment counteracted this suppression and further increased the expression level in varying degrees being higher (about 65% increase of control level) for both EJ and RJ than that for WJ (about 35% increase of control level) as shown in Fig. 6(d).

Histological findings

The fatty degeneration of hepatocytes (steatosis) was observed in liver sections from rats in groups received HFD (Fig. 7(c)), but not in the control group (Fig. 7(a)). Oil red O staining of liver tissue from rats in groups received HFD without pomegranate juice showed remarkable lipid accumulation (Fig. 7(d)) compared to control (Fig. 7(b)). Existence of variable-sized vacuoles (lipid droplets in oil red O staining) and pleomorphic nuclei were more prominent in hepatic sections from rats-fed HFD compared to all other groups (Fig. 7(d)). Hepatic sections from rats-fed HFD revealed the presence of a large number of lipid droplets. These droplets were markedly reduced both in size and number, restricted to sporadic cells in liver sections from rats in groups received HFD concurrently with pomegranate juice (Fig. 7(e)-(j)).

Discussion

Obesity is metabolic disease resulting from energy imbalance where energy intake exceeds energy expenditure. The etiology of obesity is complex including
genetic, environmental causes. However, dietary factors, particularly the consumption of a HFD, are important predisposing factors for its development. In the present study, feeding on HFD increased body weight significantly compared to feeding on the normal diet; a result that goes in accordance with that of previous reports. Development of obesity in response to HFD feeding could be explained by the ability of HFD to induce a positive-energy balance that results in an increased visceral fat deposition. Moreover, HFD feeding was reported to be accompanied by molecular adaptations that favor fat storage in muscle rather than oxidation. The increased body weight in HFD group was accompanied with an increase in serum levels of TAG and TC compared to control. Interestingly, administration of the different types of pomegranate juices reversed the effects of feeding HFD, where either of EJ, RJ, or WJ could decrease the body weight significantly compared to the obesity, non-treated group. However there was no significant difference between the effects of the three types of juices. The body weight lowering effect of juices was accompanied by decrease in the food consumption of the treated groups with the strongest effect shown by RJ while WJ showed the least effect.

The anorexogenic effect of the tested pomegranate juices could be explained by their inhibitory effect on ghrelin expression. Ghrelin is known to stimulate appetite and food intake. The results also showed that
Body weight was positively correlated with serum leptin levels and leptin mRNA expression. The serum leptin levels of obese, non-treated group were higher than those of the control group, while the leptin levels were significantly lowered by supplementation with any of the three types of juice. Since adiposity is positively correlated to the plasma leptin concentration in rodents and humans, the reduced plasma leptin level found in

Fig. 5. Effects of EJ, RJ, and WJ on HSL (a) and leptin (b) mRNA expression in hepatic tissue of rat.
Notes: Representative blots and results of densitometric analyses of at least five independent experiments are shown. (c): Effects of EJ, RJ, and WJ on the serum leptin level, (d): Effects of EJ, RJ, and WJ on the serum insulin level, and (e): Effects of EJ, RJ, and WJ on the serum glucose level. Values are mean ± SE (n = 6). Cont, control group; Ob, obesity (HFD) non-treated group; EJ, HFD + Egyptian pomegranate juice group; RJ, HFD + Taif red pomegranate juice group; WJ, HFD + Taif white pomegranate juice group. *p < 0.05 vs. control group. §p < 0.05 vs. obesity, non-treated group.
the juices-treated obese groups compared to the obese, non-treated group can be attributed to reduced visceral adiposity after juices treatment. Leptin is an adipokine secreted by adipocyte and its level is well known to proportionate with body fat mass.33,34 The results of the current study agree with those previously reported by Zhou et al.35 Moreover, it is possible that pomegranate juices decreased the body weight through improving leptin sensitivity in the treated groups, which resulted in increased energy expenditure and decreased food consumption and consequently reduced body weight.

Lipogenic transcription factor, SREBP-1c, is a key regulator of lipid metabolism through controlling the gene expressions of enzymes for fatty acid synthesis and uptake and TAG synthesis.36,37 FAS and ACC1 are enzymes for de novo fatty acid synthesis and energy homeostasis.38 Fatty acids are critical substrates for biosynthesis of triglycerides.39 Our results showed that the mRNA expressions of adipogenesis-related genes such as SREBP-1c, FAS, and ACC1 were decreased in liver of rats fed with HFD, and this down-regulation was counteracted after administration of any of the tested juices. These results agree to that of Kim et al.40 who demonstrated that prolonged HFD consumption decreased the mRNA levels of SREBP-1c and FAS in rat, and correspond to previous observation that the expressions of PPAR-γ, SREBP1c, and their target genes (FAS and ACC) decreased in the adipose tissue of a long-term HFD-fed dog41 or ob/ob mice42 compared with their lean controls and to that of the earlier study by Shillabeer et al.43, who reported that HFD suppresses hepatic and lipogenic enzyme mRNA expression and also insulin level comparing to normal diet-fed control group. At the early stage of obesity, animals seemed to be in a state that favors high adipogenesis and high-energy metabolism. In the present study, the animals were fed HFD for long term and they had been fully obese for several weeks. Thus, feeding HFD for long period may induce an adaptive response in animals that intends to limit further fat
deposition. This adaptive response in adipogenesis-related genes was also confirmed in the adipose tissue of obese human subjects. Furthermore, either of EJ, RJ, or WJ increased the hepatic mRNA expression of lipolysis-related gene, HSL, compared to either obesity or control group. These results suggest that the anti-obesity effects of pomegranate juice are possibly in part due to the suppression of the mRNA expression levels of genes involved in fatty acid synthesis and upregulation of that related to lipolysis.

Adiponectin is an adipocytokine synthesized in adipose tissue. It is an insulin-sensitive hormone, playing a central role in glucose and lipid metabolism. In addition, it increases fatty acid oxidation in the muscle and potentiates insulin inhibition of hepatic gluconeogenesis. It is well documented that plasma adiponectin concentrations and mRNA expression have been shown to be decreased in obesity and insulin resistance. The present results showed that feeding of HFD increased serum glucose and decreased serum

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Fig. 7. Effect of pomegranate juices (EJ, RJ, and WJ) on lipid accumulation in liver.

Notes: The left column shows hematoxyline and eosin-stained (H&E) hepatic sections from all groups, whereas the right column shows oil red O-stained hepatic sections from all groups at the same order. (a), H&E-stained and (b), oil red O-stained hepatic sections from control group (Cont.); notice absence of vacuoles (HE) or red droplets (oil red O) in hepatocytes. (c), H&E-stained and (d), oil red O-stained hepatic sections from group fed on HFD; notice variable-sized vacuoles (HE) and red droplets (arrowheads, oil red O) in hepatocytes. (e), H&E-stained and (f), oil red O-stained hepatic sections from group fed on HFD concurrently with juice of Egyptian pomegranate (HF-EJ); notice the reduction in number of vacuoles (HE), and large (arrowhead) and small (arrow) red droplets (oil red O) in hepatocytes. (g), H&E-stained and (h), oil red O-stained hepatic sections from group fed on HFD concurrently with juice of Red Taif pomegranate (HF-RJ); notice the reduction in number of vacuoles (HE) and red droplets (arrowhead, oil red O) in hepatocytes. (i), H&E-stained and (j), oil red O-stained hepatic sections from group fed on HFD concurrently with juice of White Taif pomegranate (HF-WJ); notice the reduction in number of vacuoles (HE), and large (arrowhead) and small (arrow) red droplets (oil red O) in hepatocytes. Original magnification: X 400.
insulin levels which was associated with decreased adiponectin mRNA expression in subcutaneous adipose tissue, which are similar to results obtained by de Oliveira et al.\(^4\) Our results also showed that adiponectin mRNA expression is suppressed in HFD-induced obesity. This suppression was inhibited and the expression level was significantly restored after pomegranate juices treatment. This upregulated expression of adiponectin could explain at least in part the decreased serum glucose level, which may have resulted from decreased hepatic gluconeogenesis and improved insulin sensitivity.\(^5\)

GLUT-2 is a transmembrane carrier protein that facilitates transport of glucose across hepatic plasma membrane. GLUT-2 regulates passage of glucose between liver and blood, and it is responsible for renal glucose reabsorption.\(^5\) In the present study, obesity induced the expression of GLUT-2 and this induction was normalized to the level of control group after pomegranate juice treatment especially for either RJ or WJ. These results coincide with that of serum glucose level which maintained a strong relationship with previous studies,\(^3\) histological examination revealed fatty degeneration of hepatocytes (steatosis) in hepatic sections stained by oil red O from rats in groups received HFD, but not in the control group. Hepatic sections from rats in groups received HFD concurrently with pomegranate juices revealed remarkable reduction in the size and number of lipid droplets. Similar to previous studies on rats-fed HFD concurrently with ethanol extract of \(Punica granatum\) \((Kotalahimbutu)\) aqueous extract and cyclodextrin\(^6\) and hesperidin,\(^5\) our findings suggest that pomegranate juice treatment could effectively inhibit lipid accumulation in liver.

**Conclusion**

In conclusion, the current study demonstrated that juices from either types of Taif pomegranate or Egyptian pomegranate have beneficial effects for the suppression of HFD-induced obesity. These data provided evidence that supplementation of any type of the tested juices decreases body weight gain, food intake, serum lipid levels, and hepatic lipid accumulation in HFD-induced obese rats. EJ, RJ, and WJ appear to show such activities by modulating the lipid metabolism through the decreased activity in lipogenesis as well as the increase in both lipolysis and glucose oxidation at the transcriptional level, suggesting that any of the tested pomegranate juice has an excellent potential as an effective anti-obesity agent with no obvious toxicity.

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