Effects of endurance training and herb supplementation on tissue nesfatin-1/nucleobindin-2 and ghrelin mRNA expression

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ABSTRACT: The aim of present study was to investigate the effects of aerobic training with and without Pistacia-atlantica (Bane) extraction as a plant rich in fatty acids, on rat tissues nesfatin-1/nucleobindin-2 and ghrelin gene expression and also on plasma lipid and lipoproteins profiles. Twenty healthy and intact Wistar rats were obtain and randomly were assigned into saline-control (SC), saline-training (ST), Bane-control (BC), and Bane-training (BT) groups. Training groups ran on a motor driven treadmill (at intensity 25m/min, 5days/wk.) for 8 weeks. Rats were orally received saline or bene solution (100 mg/kg and 7.5ul/g of body weight). Blood was collected to determine biochemical variables and liver and visceral fat were also obtain for Nesfatin-1/nucleobindin-2 and ghrelin mRNA expressions by using a Real-time PCR technique. A higher and significant nesfatin-1 mRNA expressions in liver and visceral fat and liver estradiol were observed in trained groups. However, the level of ghrelin expression was lower in trained groups when compared to control rats. In general, lower tissues nesfatin-1 mRNA expression and lower plasma and higher estradiol HDL-C were observed in bane-treated rats. A negative and positive correlation between liver estradiol and liver ghrelin expression, plasma estradiol, HDL-C and visceral fat nesfatin-1 expression were found respectively. The results of present study indicate that exercise combined with bane-treatment induced considerable changes on liver and visceral fat nesfatin-1 and ghrelin expression which was accompanied with a significant change in plasma HDL-C as a cardiovascular risk factor.

KEY WORDS: Pistacia-atlantica (Bane), Aerobic training, Nesfatin-1, ghrelin, HDL-C, Treadmill, Estradiol

INTRODUCTION

The effects of physical exercise at different forms, modes, and intensity on appetite and its related peptides in human and animal central system and peripheral tissues, have inclusively been studies by several investigators [1-8]. Unfortunately, most of these research focused on plasma less on tissues appetite peptides such as ; leptin, orexins, NPY, POMC, PYY, ghrelin, AgRP,vaspin, omentin, nesfatin-1, visfatin, resistin, irisin, and other related peptides. On the other hand, some of these studies also concentrated their directions on the effect of nutritional status, nutrient effects on these peptides [9-11]. With consider to the effects of aerobic exercise training on the expression of appetite peptides in animal tissues, particularly; ghrelin and Nesfatin-1 with or without herbs supplementation less information is existed.
 Nesfatin-1, a recently discovered protein derived from postranslational processing of the nucleobindin 2 (NUCB2) gene is expressed in the appetite-control hypothalamic nuclei in rats [12]. Nesfatin/NUCB2 is composed of a signal N-terminal peptide of 24 amino acids, and a protein structure containing 396 amino acids [13]. It has been reported that nucleobindin-2 (NUCB2) is classified into the N-terminal nesfatin-1, nesfatin-2 and the C-terminal nesfatin-3 by prohormone –convertase1/3 [14, 15]. According to the published data, only nesfatin-1 not nesfatin-2 or nesfatin-3 effectively reduced food intake and body weight after injection to the third brain ventricle and other brain areas in rats and mice. Nesfatin-1 is also expressed in extra-hypothalamic tissues such as; rat gastric oxyntic mucosa or gastric XI/A like cells, digestive system, pancreatic beta cells, adipose tissue[9]. In addition to nesfatin-1 gene expression in different tissues, the presence of nesfatin-1 in different biological fluids such as human breast milk, saliva, serum/plasma, cerebrospinal fluid [9, 16-18]. Although the action of nesfatin-1 on food intake behavior and body weight get much more attention, but on the basis of published data nesfatin-1 could act as a biomarkers and also has an anti-hyperglycemic, anti-inflammatory, neuroendocrine regulator, and lowering body fat via appetite suppression, and metabolic and cardiovascular actions [9, 19-23].

The levels of tissue and plasma nesfatin-1 could be affected under nutritional status (fasting and fed states, restraint stress abdominal surgery, high fat diets, glycemic state, different nutrient manipulation [9, 24, 25]. In contrast to the nesfatin-1 whose recognized and discovered as an orexigenic peptide, ghrelin is discovered in peripheral tissue (fundus part of stomach) and introduced as an orexigenic peptide, but according to the several published studies this peptide also centrally expresses [26-28].

Ghrelin is a 28-amino-acid peptide which discovered, purified from rat stomach and introduced to peptide world by Kojima et al in 1999 [29]. The Structurally, ghrelin is made from a preproghrelin with 117 amino acids which contains a 23 AA signal peptide and a 66 AA carboxyterminal peptide called C-ghrelin and this segment of preproghrelin is further processed to a 23AA peptide named obestatin [30]. It has to be noted that nascent ghrelin peptide, derived from the ghrelin gene transcription and translation are subjected to a posttranslational modification process consisting in acylation of the hydroxyl group of the serine 3, and two form of ghrelin acylated and nonacylated has been recognized [29-31]. The enzyme responsible for ghrelin acylation is ghrelin O-acyltransferase (GOAT) belonging to the family of membrane O-acyl transferase [31]. In contrast to nesfatin-1, ghrelin as an orexigenic peptide increase food intake behavior during energy deficiencies status/negative energy balance situations (starvation, fasting, cachexia, malnutrition, weight reduction, calorie restriction, eating disorders) [32-34]. In addition, to energy regulation matter, it has been shown that different nutrients manipulation has also impact on ghrelin levels and gene expression [35, 36]. A varieties of actions for ghrelin on different bodily system, energy regulation, metabolic functions, regulatory action on some appetite peptides, and some other function have been mentioned [26, 37]. With considering nesfatin-1 and ghrelin mRNA expression and their response to natural nutrient and herbs supplementation which taking more attention by who prefer alternative medicine and traditional medicinal plant for their health and longevity, weight and appetite control. In this regards, water extraction of zizyphus jujube (An-nab) administration (at a dose of 1.25mL/100g of body weight) resulted in a higher significant increase in plasma and liver nesfatin-1 levels at rest in rats treated with zizyphus. Data also indicate the levels of nesfatin-1 collectively higher in control and trained-zizyphus treated groups (Ghanbari-Niaki 2013) [9]. The effect of water extraction of zizyphus jujube at the same dose on the fundus nesfatin-1 level in female rats was reported by Ghanbari-Niaki et al (2013) [38]. In this study the levels of nesfatin-1 was higher in zizyphus treated rats when compared to saline groups and data also revealed that treadmill running significantly increased fundus nesfatin-1 in trained-zizyphus treated rats when compared to control-treatd groups. According to Farhangi et al. (2016) report the consumption of Nigella sativa powder (Siah-daneh) in form capsule (1g/day) for 7 weeks the level of serum nesfatin-1 remained unchanged [39]. The effect of oral administration of aqueous extraction of Pistacia-atlantica (Bane) at dose 100mg/kg of body weight into female rats, significantly decreased small intestine nesfatin-1 concentration in contro-Baneh treated rats when compared to control-salin group. However, in this study, in despite a treadmill running-induced increase in small intestine nesfatin-1 in trained-Bane treated rats, but collectively a lower concentration of nesfatin-1 was observed in Bane-treated rats. On the other hand, the levels of small intestine visfatin significantly higher in Bane-treated groups [40]. In relation to the ghrelin response to herbal plants most of research focused on the effects of Rikkunshito is an herbal medicine (in Japanese traditional medicine) on ghrelin secretion in human and animal subjects [41-44]. It has been reported that Rikkunshito is prepared by compounding eight herbal medicines listed in the Japanese Pharmacopoeia: Atractylodis Lanceae Rhizoma, Ginseng Radix, Pinelliae Tuber, Hoelen, Ziziphi Fructus, Aurantii Nobilis Pericarpium, Glycyrrhizae Radix and Zingiberis Rhizoma and stimulates secretion of the orexigenic peptide, ghrelin, from the stomach [41, 42]. Although, there are a considerable and growing information and report about the impact of exercise on ghrelin and nesfatin-1, but there is very little data in relation to the effects of exercise combined with herbal plant supplementation on plasma and tissues ghrelin concentrations and its mRNA expression in different species. On the other hand, as mentioned above, a high fatty acids content diets or nutrients [24, 25]. Pistacia-Atlantica (in Persian named Bane) is a family of pistachio trees which is existed wild and cultivated in different areas and provinces in Iran. The ripe and unripe of pistacia-
atlanitca (Baneh) fruit is consuming during growing and ripen time by local people and cultivars an herbal seed, as a part of habitual foods (such as Eshkenh and O-Baneh), and is also export to other Iranian cities as good. In addition Pistachia atlantica (Bane) as a member of Anacardiaceae family has been reported to be rich in essential fatty acids and antioxidants being found in nuts and it has also been measured that total amount of essential oils obtained from Pistachio-atlanitca was higher than that from any other Pistachio species [45-47]. The effects of an aqueous extraction of pistachia-atlanitca on HDL transporter factors such as ABCG8 and ABCG4 in female rats small intestine and kidney and also its effect on small intestine nesfatin-1 and visfatin with or without exercise have been reported elsewhere [40, 48-50]. With consider to the somewhat controversial reports and also the potential of ghrelin and Nesfatin-1 responses to any positive /negative change in energy balance, high-fat diet, and nutrients ,the present study was designed to investigate the effect of a treadmill running program combined with aqueous extraction of Pistacia-atlanitca on rats liver and visceral fat tissues ghrelin and nesfatin-1 mRNA expression. The second aim was to see the pattern of responses of two orexigenic (ghrelin) and anorexigenic (nesfatin-1) peptides in both liver and fat tissues which are recognized as metabolically active tissues and with different content of energy sources.

METHODS

Plant material
Collection of the ripped Pistacia atlantica (Baneh) samples was done in Maybod fields of Yazd province, Iran, the materials of which were then identified by the herbarium of the Department of Biology, Faculty of Sciences, Mazandarn University, Iran and stored at −18°C to be used later.

Extract preparation
Based on method of Hamdan et al. (2004) [51], the extraction was prepared by coarsely powdering Pistacia-atlanitca ripped and dried fruit (10g), mixing with 150 ml of tap water, boiling for 45 min, cooling at room temperature filtering twice with a Whatman filter (No. 4 filter), And enhancing the volume to 100 ml with tap water so that 1 ml of the solution was equivalent to 100 mg of the starting material [51]. However, no distilled water was used based on herbalists’ recommendation. Then, at the end of the training session, a fresh extract was immediately given at a dose of 100 mg/kg (7.5ul/g of body weight) orally. The same amount was administered for the control groups.

GCMS analysis
GCMS analysis was performed using the method previously described [48].

Animals
Based on the Iranian convention concerning the protection of vertebrate animals employed for experimental and scientific purposes, this experiment was performed and approved by the Ethics Committee of Sciences, University of Mazandaran (UMZ) and Babol University of Medical Sciences (BUMS, Mazandaran, Iran). 20 Wistar female rats (6-8 weeks old and 125-135 g) were acquired from Pasteur's Institute (Amol, Mazandaran) and kept in the Central Animal House of Faculty of Physical Education and Sports Science of UMZ. Each cage of 12-hour light-dark cycle housed 5 rats (46-L, volume). Humidity and temperature were kept at 55.6% ± 4.0% and 22°C ± 1.4°C, respectively. Diets (in a pellet form) and water were provided in ad libitum. The rats were randomly assigned to training (n = 10) and control (n = 10) groups. They were also divided into saline-training (ST), saline-control (SC), Baneh-training (BT), and Baneh-control (BC) groups. The training group received a moderate running exercise program, while the control group remained sedentary.

Exercise training protocol
The rats were first familiarized with the 14-lane motorized-driven treadmill apparatus designed by the primary author (UMZ, Babolsar, Mazandaran, Iran) for 4 days. Using the training methods previously described, the exercise group was trained for 8 weeks [52, 53]. Running at 25 m/min for 60 minutes, 5 d/wk, the rats were scarified 72 hours after the last session of exercises. Yet, 4 hours before the sacrifices, food was removed from their cages, but not water. Taking vaginal smears through vaginal lavage each morning, the estrous cycle was determined in the intact female rats. To identify the existing cell types and estrous cycle stage, the smears were analyzed under a microscope [54, 55] so as to only employ the female rats with at least two consecutive estrous cycles of 4 or 5 days. To select the days of the experiment, the estrous cycle established in each female was utilized, during which its stage was corroborated by vaginal smear.

Tissue biopsy
Using intra-peritoneal administration of ketamine (30–50 mg/kg body weight) and xylazine (3–5 mg / kg body weight) mixture, the rats were anesthetized 72 hours after the last training session. Excision and cleaning of liver and visceral fat tissues were done to be then divided into two pieces and washed in ice-cold saline. At last, they were stored at -80°C until the extraction of RNA after freezing them immediately in liquid nitrogen. To prepare plasma, the blood collected in EDTA test tubes as anticoagulant was immediately centrifuged at 3000 rpm for 10 min. It was then stored at -80°C for future analysis.
Isolation of RNA, cDNA synthesis, and Real-time PCR
Using RNA purification kits (Accu Zol, Bioneer Company), total RNA was extracted from 80 to 100 mg of tissues. Then, using cDNA synthesis kit (AccuPower RT PreMix) based on the manufacturer’s instructions, extension of complementary DNA (cDNA) from oligo-(dt) 18 primers (0.25 g per reaction) was conducted. Finally, by the use of QuantiFast SYBR Green PCR Kit (Cat. No. 204052; Qiagen, GmbH, Germany), a real-time quantitative PCR was performed on light Cycler apparatus (Corbet) for the reactions. Each 15 μl of reaction sample contained 0.5 μl of single-strand cDNA, 7.5 μl of Master Mix, 1 μl of each forward and reverse primers (5 pmol/μl), and 5 μl of dH2O. Nesfatin-1/NUCB2, ghrelin, and β-actin sense and antisense primers used as normalizer genes were represented as 5'-TTTGAACACCTGAACCACCA-3', 5'-TGCAAACTTGGCCTTC-CT-3' (211bp; accession no: Q9JI85), 5'-CAGGTTCCAGCTTCTGA-3', 5'-GACAGCGTTGATGCCA- ACA-3 (AB029433, 191 bp), 5'-TATCGGCAATGAGCGGTTCC-3', and 5'-CACTGTGTTGGCATAGAGG-3' (NM_031144, 145 bp), respectively [56].

Glucose, Glycogen, Lipids and lipoproteins
Table 1 shows the measurement method and the kit used, as well as intra-assay coefficient of variation and sensitivity of each variable. All the methods and kits were taken from Pars Azmoun, Tehran, Iran. By subtracting HDL-c and VLDL-c (or TG/5) from the total cholesterol (LDL-c=TC–TG/5–HDL-c) based on Friedwald’s equation and using equation VLDL=TG/5 [57], the low-density lipoprotein cholesterol (LDL-c) serum and VLDL-c values were calculated, respectively.

Table 1. The measurement method and the kit, Intra-assay coefficient of variation and sensitivity of the variable

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Intra-assay coefficient</th>
<th>Method</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.2%</td>
<td>Enzymatic, colorimetric (glucose oxidase-amino antipyrine)</td>
<td>Plasma glucose</td>
<td></td>
</tr>
<tr>
<td>48%</td>
<td>Glycogen Colorimetric kit (Nanjing, China)</td>
<td>Tissue glycogen</td>
<td></td>
</tr>
<tr>
<td>1.2%</td>
<td>Direct Immuno method (HDL-C Immuno FS)</td>
<td>Plasma high density lipoprotein cholesterol (HDL)</td>
<td></td>
</tr>
</tbody>
</table>

Statistical analysis
Using the comparative threshold cycle method (CT) to determine each sample with the help of Rotor-Gene 3000 Software, the data were analyzed. ΔCT and ΔΔCT values were calculated by taking the target gene CT and subtracting it from β-actin CT and subtracting ΔCT (sample) from ΔCT (control) values, respectively, and then, the relative quantification was performed via the expression of 2^-ΔΔCT [58]. Using Kolmogorov-Smirnov test, the variables were found to be normally distributed and the results were expressed as means ± SEM. Furthermore, using a two-way analysis of variance, least significant difference post hoc test, and Pearson Product Moment correlation, the statistical analyses were performed with the help of SPSS software (Version 13; SPSS, Chicago, IL) and significant F ratio (P < .05) and correlation between tissue gene expressions and other variables were calculated, respectively.

STATISTICAL RESULTS
Using GC-MS analysis were shown in Table.2.

Table 2. The main components of the whole fruits of Pistacia atlantica (Baneh) extracted by GC-MS analysis.

<table>
<thead>
<tr>
<th>RT [min.]</th>
<th>Library/ID</th>
<th>Area %</th>
<th>Ref</th>
<th>Qual</th>
</tr>
</thead>
<tbody>
<tr>
<td>39.200</td>
<td>Elaidic acid (trans-9-Octadecenoic acid) (E-Oleic acid)</td>
<td>49.28</td>
<td>228773</td>
<td>99</td>
</tr>
<tr>
<td>36.056</td>
<td>Palmitic acid</td>
<td>28.86</td>
<td>195439</td>
<td>99</td>
</tr>
<tr>
<td>35.196</td>
<td>Hexadecenoic acid</td>
<td>7.52</td>
<td>192904</td>
<td>97</td>
</tr>
</tbody>
</table>
Liver and Visceral Fat Tissues gene Expression

Nesfatin-1/Nucleobindin-2 mRNA expression were observed in liver and visceral fat tissues in all experimental groups. The results also showed that the nesfatin-1mRNA expression in saline groups was significantly higher compared to Bane-treated rats (F=10.562, P<0.001), but this increase was much higher in saline-trained rats when compared to other groups. The results indicate that training program restored nesfatin-1 mRNA expression suppression to somewhat in visceral fat tissue in Bane-treated rats. (Fig. 1).

A similar pattern of change was also found in liver nesfatin-1 mRNA expression (F=4.338, P=0.022) (Fig. 2). It has to be noted that the magnitude of nesfatin-1 mRNA expression was much higher in liver saline-trained rats (3fold) compared to visceral fat tissue in saline-trained rats (1.5 fold).

It seems using an aqueous Bane extraction reducing nesfatin-1 mRNA expression in both liver and visceral fat tissues in female rats. As results show ghrelin was expressed in all experimental groups. Data also revealed a lower and significant (F=5.149, P=0.011) ghrelin mRNA expression in trained groups in both solution-treated rats (Fig.3).

The results showed a modest increase in visceral fat ghrelin mRNA expression but, these changes were no significant in all treated groups (F=0.767, P=0.529) (Fig.4).
Tissues Metabolites and plasma Biochemical Variables

Liver and visceral fat glycogen content remained unchanged in all experimental groups (Table.3). However, significant change has been observed in the liver glucose concentration \( F=3.956, P=0.028 \). A higher liver glucose levels were observed in Bane-control and Bane-trained rats when compared with saline-control group (Fig.5). A modest and nonsignificant and increase were observed in the levels of estradiol in visceral fat-trained and liver-trained tissues (Fig.6 and Table.3). The levels of TC and TG in liver and visceral fat tissue remained unchanged (Table.3).

Changes in plasma HDL-C concentrations were significantly different between the groups \( F=3.608, P=0.037 \). Further analysis of plasma data by the following post hoc test revealed that the saline-trained rats had significantly higher plasma HDL-C compared to Baneh-control and trained groups at \( P=0.01 \) (Fig. 7). The difference between saline and Baneh treated groups was also significant \( P=0.02 \). Overall, the levels of plasma HDL-C were significantly low in Baneh as compared to the saline group (Fig.7). The levels of plasma LDL-C, and VLDL-C were also remained unchanged (Table.3).

A significant change was observed in the plasma estradiol concentrations \( F=3.060, P=0.049 \). Using a suitable following post hoc test revealed a lower plasma estradiol concentrations in Baneh-treated rats when compared to saline-treated groups. A significant differences was
observed between saline-trained compared to Bane-trained rats (P<0.013, qnd P<0.03) (Fig.8). However, Bane suppressed training effect on plasma estradiol level in Bane-trained rats.

The correlations between liver and visceral fat gene expression with other measured variables were shown in Table.4 and 5. There was a negative and significant correlation was (r = -550, p <0.012) found between liver ghrelin mRNA expression with liver estradiol concentration (Table.4). We also observed a positive and significant (r = 0.586, P<0.007) correlation between visceral fat nesfatin-1mRNA expression with plasma HDL-C and estradiol concentrations (Table.5)

Table 3. Variables means ± SEM

<table>
<thead>
<tr>
<th>P value</th>
<th>F</th>
<th>BT</th>
<th>BC</th>
<th>ST</th>
<th>SC</th>
<th>Variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.50</td>
<td>0.822</td>
<td>2.06±0.11</td>
<td>1.92±0.07</td>
<td>2.16±0.13</td>
<td>2.04±0.10</td>
<td>Visceral fat cholesterol (mg/g)</td>
</tr>
<tr>
<td>0.41</td>
<td>1.019</td>
<td>147±36.9</td>
<td>103±36.02</td>
<td>104±36.2</td>
<td>116±15.4</td>
<td>Visceral fat triglyceride (mg/g)</td>
</tr>
<tr>
<td>0.50</td>
<td>0.807</td>
<td>15±1.76</td>
<td>14.6±1.56</td>
<td>15.8±2</td>
<td>17.8±0.6</td>
<td>Visceral fat estradiol (Pg/g)</td>
</tr>
<tr>
<td>0.70</td>
<td>0.476</td>
<td>0.82±0.03</td>
<td>0.86±0.02</td>
<td>0.80±0.04</td>
<td>0.84±0.04</td>
<td>Visceral fat glycogen (mg/g)</td>
</tr>
<tr>
<td>0.22</td>
<td>1.603</td>
<td>3.40±0.21</td>
<td>3.26±0.20</td>
<td>2.82±0.21</td>
<td>3.06±0.15</td>
<td>Liver cholesterol (mg/g)</td>
</tr>
<tr>
<td>0.10</td>
<td>2.429</td>
<td>24±1.48</td>
<td>25.40±0.97</td>
<td>25.40±0.81</td>
<td>21.80±0.96</td>
<td>Liver triglyceride (mg/g)</td>
</tr>
<tr>
<td>0.94</td>
<td>0.129</td>
<td>4.38±0.34</td>
<td>4.44±0.37</td>
<td>4.28±0.27</td>
<td>4.54±0.18</td>
<td>Liver glycogen (mg/g)</td>
</tr>
<tr>
<td>0.43</td>
<td>0.096</td>
<td>97±6.4</td>
<td>86±3.7</td>
<td>92.4±6.8</td>
<td>85.6±4.7</td>
<td>Plasma cholesterol (mg/dl)</td>
</tr>
<tr>
<td>0.08</td>
<td>2.70</td>
<td>124±7.5</td>
<td>146±8.8</td>
<td>123.2±5.8</td>
<td>120.6±7.8</td>
<td>Plasma glucose (mg/dl)</td>
</tr>
<tr>
<td>0.41</td>
<td>1.00</td>
<td>86±7.3</td>
<td>94±7.8</td>
<td>84±8.3</td>
<td>77±4.01</td>
<td>Plasma triglyceride (mg/dl)</td>
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<tr>
<td>0.33</td>
<td>1.21</td>
<td>34.2±5.6</td>
<td>20.9±2.3</td>
<td>21.8±7.5</td>
<td>22.6±5.41</td>
<td>Plasma LDL (mg/dl)</td>
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<tr>
<td>0.41</td>
<td>1.00</td>
<td>17.3±1.4</td>
<td>18.9±1.5</td>
<td>16.8±1.6</td>
<td>15.5±0.8</td>
<td>Plasma VLDL (mg/dl)</td>
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Table 4. Correlations between liver ghrelin and nesfatin-1 with other variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Liver ghrelin</th>
<th>Liver Nesfatin-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>r Value</td>
<td>P value</td>
<td>r Value</td>
</tr>
<tr>
<td>Liver cholesterol (mg/g)</td>
<td>0.065</td>
<td>0.785</td>
</tr>
<tr>
<td>Liver triglyceride (mg/g)</td>
<td>-0.037</td>
<td>0.012</td>
</tr>
<tr>
<td>Liver estradiol (Pg/g)</td>
<td>-0.350</td>
<td>0.870</td>
</tr>
<tr>
<td>Liver glycogen (mg/g)</td>
<td>0.039</td>
<td>0.106</td>
</tr>
<tr>
<td>Plasma Cholesterol (mg/dl)</td>
<td>-0.372</td>
<td>0.428</td>
</tr>
<tr>
<td>Plasma Estradiol (Pg/ml)</td>
<td>0.037</td>
<td>0.952</td>
</tr>
<tr>
<td>Plasma Glucose (mg/dl)</td>
<td>-0.058</td>
<td>0.174</td>
</tr>
<tr>
<td>Plasma HDL-C (mg/dl)</td>
<td>0.001</td>
<td>0.003</td>
</tr>
<tr>
<td>Plasma LDL (mg/dl)</td>
<td>-0.334</td>
<td>0.361</td>
</tr>
<tr>
<td>Plasma VLDL (mg/dl)</td>
<td>0.001</td>
<td>0.147</td>
</tr>
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</table>

Table 5. Correlations between visceral fat ghrelin and nesfatin-1 with other variables

<table>
<thead>
<tr>
<th>Variables</th>
<th>Visceral fat ghrelin</th>
<th>Visceral fat Nesfatin-1</th>
</tr>
</thead>
<tbody>
<tr>
<td>r Value</td>
<td>P value</td>
<td>r Value</td>
</tr>
<tr>
<td>Visceral fat cholesterol (mg/g)</td>
<td>-0.233</td>
<td>0.345</td>
</tr>
<tr>
<td>Visceral fat triglyceride (mg/g)</td>
<td>0.217</td>
<td>0.357</td>
</tr>
<tr>
<td>Visceral fat estradiol (Pg/g)</td>
<td>-0.077</td>
<td>0.237</td>
</tr>
<tr>
<td>Visceral fat glycogen (mg/g)</td>
<td>-0.170</td>
<td>0.475</td>
</tr>
<tr>
<td>Plasma Cholesterol (mg/dl)</td>
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<td>0.070</td>
</tr>
<tr>
<td>Plasma Estradiol (Pg/ml)</td>
<td>-0.084</td>
<td>0.386</td>
</tr>
<tr>
<td>Plasma Glucose (mg/dl)</td>
<td>0.167</td>
<td>0.475</td>
</tr>
<tr>
<td>Plasma Triglyceride (mg/dl)</td>
<td>0.186</td>
<td>0.482</td>
</tr>
<tr>
<td>Plasma HDL-C (mg/dl)</td>
<td>0.109</td>
<td>0.070</td>
</tr>
<tr>
<td>Plasma LDL (mg/dl)</td>
<td>0.325</td>
<td>0.070</td>
</tr>
<tr>
<td>Plasma VLDL (mg/dl)</td>
<td>0.186</td>
<td>0.482</td>
</tr>
</tbody>
</table>

DISCUSSION

The first aim of the present study was to examine the effects of treadmill running program at moderate intensity combined with orally administration of an aqueous extraction of pistacia-atlantica (Bane) on female rats liver and visceral fat tissues ghrelin and nesfatin-1mRNA expression. The second purpose this study was to see patterns of ghrelin and nesfatine-1gene expression in two metabolically active tissues with different content of glycogen and other energy sources. In this regards, the main findings of the present study were as follows; 1) ghrelin and nesfatin-1 mRNA were expressed in all experimental groups, 2) running training program resulted in an higher Nesfatin-1mRNA expression in both tissue regardless solution treatments; 3) an inverse pattern of change was observed in liver ghrelin mRNA expression, while exercise resulted a modest and non-significant increase in visceral fat ghrelin mRNA expression; 4) a little and non-significant reduction were observed in both liver and visceral fat-trained glycogen, 5) the levels of visceral fat estradiol were lower in bane-treated and saline exercise rats.
while a higher liver estradiol were observed in bane-treated and saline-trained rats. 6) plasma HDL-C and estradiol concentrations significantly lower in Bane-treated rats, but an higher plasma HDL-C and estradiol concentrations were also observed in saline-trained rats.

Nesfatin-1 is the N-terminal fragment of nucleobindin-2 and the antibody against nesfatin-1 recognized both full length on nUCB-2 and nesfatin, thus the immunolabeling represent NUCB-2/nesfatin-1 [59]. The expression on nesfatin-1 in central nervous system and it is highly expressed in gastric mucosa than endocrine cells, heart, pancreas, skeletal muscle, lung, liver, kidney, pituitary, pancreas, adrenal gland, testis and visceral adipose tissue, small and large intestines [15, 25, 59-62]. Recently Ramajananeya et al (2010) [63] who reported a high nesfatin-1 mRNA expression in brain. Relatively high in subcutaneous fat tissue, and low level in omamental fat tissue in human and mice and the levels of subcutaneous fat tissue nesfatin-1 was significantly higher than omamental fat tissue nesfatin-1 in both human and mice. In our study, we observed an expression in rat liver and visceral fat adipose tissues but a higher level nesfatin-1 expression was found in rat liver. It has been showed that an aerobic exercise training resulted in a significant increase in liver, kidney and small intestine nesfatin-1 mRNA expression but the level of plasma nesfatin-1 remained unchanged [64]. Rahmati-Ahmadianad et al (2012)[40] who was observed a lower nesfatin-1 mRNA expression in rat small intestine treated with an aqueous Bane-extraction. The pattern of changes in small intestine nesfatin-1 expression in mentioned study was the similar as we observed in our present study. The effects of nutrients on plasma nesfatine-1 and its mRNA expression, particularly glucose administration and zizyphus jujube aqueous extraction has been reported by several investigators [9, 38, 64]. The effect of high fat content nutrients and high fat diet on deficient of nesfatin-1 immunopositive neurons in PVN and SON of hypothalamus and increased immunopositive cell in gastric mucosa also reported by ( Dong et al. 2013)[65]. Mohan et al (2014)[25] who found that NUCB2 mRNA expression significantly reduced in cells treated with 1, 10, and 100 µM oleic acid in comparison to the control. No changes in NUCB2 mRNA were observed in cells treated with linolenic and octanoic acid. Further, NUCB2/nesfatin-1 secretion was unaltered in cells treated with different doses of linolenic acid, octanoic acid and oleic acid. Bertucci et al (2017) [24] who investigated the effects of different nutrients on goldfish tissue ghrelin and NUCB2/nesfatin-1 gene expression. They reported that ghrelin and Nucleobindin-2/nesfatin-1 have co-localization in the cytoplasm of cells in the goldfish hepatopancreas and they also suggested that in intestine the highest glucose concentration decrease in preproghrelin and NUCB2/nesfatin-1 after 2 and 4h of all glucose treatment. It has been found that 10 uM oleic acid treatment led to a significant increase in both preproghrelin and NUCB2/nesfatin-1 mRNA expression at 2 qad 4hours. Four hours after treatment with 1uM oleic acid preproghrelin mRNA expressions significantly increased, while NUCB2/nesfatin-1 mRNA expression levels were decreased. However, an increase in preproghrelin and a reduction in NUCB2/nesfatin-1 mRNA expression levels were observed 4hours after linolenic acid (1uM and 100uM) treatment [24]. Hagshenas et al (2014)[66] who reported that a similar pattern of changes in plasma nesfatin-1 in normal and high-fat diet with and without aerobic training program. In this study high-fat diet induced reduction was restore somewhat by exercise in high-fat diet trained rats. Nishi et al (2005)[67] who showed that a high-fat diet resulted in a reduction in stomach total ghrelin in mice, and they also suggested that in their experiment condition faint, but significant increasein the proportion of stomach-octanoyl ghrelin in conjunction with a decrease in the levels of des-acyl ghrelin. Lee et al (2002) [68] who reported that stomach ghrelin mRNA levels were significantly lower in rats fed a high-fat diet, compared with control rats. They also mentioned that the plasma ghrelin levels were significantly lower in rats fed the high-fat diet, whereas plasma ghrelin levels were significantly higher in rats fed the low-protein diet, compared with rats fed the control diet. Plasma ghrelin levels in rats fed the low-protein diet were approximately 2-fold greater when compared with control rats.

Pistacia-atlantica (Bane) GC-MS data analysis shown a following components such as ; trans-Oleic acid (49.28%), Palmitic acid (28.86%), Hexadecenoic acid (7.52%), n-Octadecanoic acid (3.87%), 3-pentadecyl-Phenol (2.69%), α-Pinene (0.71%), Limonon (0.54%), Oleic acid (0.2%), 9-Octadecenoic acid (Z) (0.18%) ,which confirm the Bane can be considered as seed with high fatty acids contents. Thus, lower and significant changes in liver and visceral fat tissue nesfatin-1 mRNA expression in Bane-treated rats might be attributed to high level fatty acids which exist in Bane extraction. Our present study results indicate that exercise training program restored somewhat Bane-suppressed nesfatin-mRNA expression in both liver and visceral fat tissues, in the other words the levels of nesfatin-1 mRNA expression were higher in both liver and visceral fat trained tissues in trained rats. Our data are consistent with those studies who’s investigated the effects of different mode acute/ chronic exercise training on plasma nesfatin-1 mRNA expression in human and animal subjects [69-72]. In our study, we observed a lower ghrelin mRNA expression in both saline and Bane-trained liver, not in visceral fat tissue. Unfortunately, to our knowledge and research on different data base, we think there is no published data in relation to pistachio-atlantica aqueous extraction combined with treadmill running program on liver ghrelin mRNA expression. However, our present study data are partially consistent to those results previously reported by the several investigators [8, 73-79]. A lower liver ghrelin RNA expression in trained rats regardless of the solutions treatment, might be attributed to lower plasma estradiol concentrations [80, 81]. We found a lower and significant increase plasma HDL-C in saline trained rats and lower HDL-C concentrations in Bane-treated rats. Our data are consistent with previously reported results elsewhere [9, 48-50, 82].
In summary, up to now on the basis of our knowledge, for the first time we showed the effects of a herbal plant seeds (Pistacia-Atlantica/Bane) aqueous extraction on female rats liver and visceral fat ghrelin and nUCB2/nesfatin-1 mRNA expression combined with a treadmill running program (25m/min, 60min/day, 5days/week, for 8 weeks). The present data indicate that ghrelin and nesfatin-1 were expressed in both tissues in all experimental groups. A higher visfatin-1 mRNA were observed in saline than Bane treated rats and also in trained liver and visceral fat tissues. The results also revealed a higher Nesfatin-1 mRNA expression in liver than visceral fat tissues. Interestingly, running training program significantly decreased ghrelin mRNA expression in both saline and Bane-treated rats which similar to reduction on plasma and tissue ghrelin levels immediately after acute and chronic exercise and training in human and rat subjects. However, an exercise-induced change in visceral fat tissue ghrelin mRNA expression was no significant. A lower plasma HDL-C and estradiol concentrations was also observed. The data also revealed a negative correlation between liver estradiol with liver ghrelin and between plasma estradiol with visceral fat nesfatin-1 mRNA expression. The results of the present study also indicate positive correlations between plasma estradiol with liver nesfatin-1 mRNA expression and between plasma HDL-C with visceral fat nesfatin-1 mRNA expression. In conclusion, on the basis of our findings and experimental conditions, we could come to conclusion that Bane extraction combined with exercise might have much anorexigenic potential than only Bane-treatment in female rats. Other point is that using much aqueous extraction of Pistacia-atlantica (Bane) reduces cardiovascular health indices such as plasma HDL-C and estradiol concentration. We also think exercise training combined Bane extraction might be important to prevent tissue-specific orexigenic behavior for regain energy losing and switching to ward might fat. If it would be true training with Bane-treatment might be applicable for nonalcoholic fatty liver.

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REFERENCES
