Anti-Obesity Synergistic Effect of Pomegranate Seed Oil (PSO) and Arabic Gum (AG) in Albino Rats

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ABSTRACT

Obesity has become a global concern worldwide, incidence of obesity is more likely in developing countries. Punicic acid (PA) is the major fatty acids proportion in PSO, it possesses numerous health benefits. On other hand, dietary fibers induce satiation, remodeling motility of gastric emptying and even lead to control body weight. AG blocked glucose absorption of intestine, this current investigation studies the anti-obesity effect PSO and AG integration on induced obese albino rats by high fat diet (HFD). Forty Dawley male rats (149±16g) allocated into: G1 - standard group: rats fed normal standard diet, G2- HFD group: rats fed on a high fat diet, G3- HFD+PSO group: rats fed on HDF and received PSO (2 ml/kg of BW daily), G4- HFD+ AG group: rats fed on HDF incorporated with Arabic Gum (5%) and G5- HFD+PSO+AG group: rats fed on HDF and received (PSO and AG) as previous varied ratio. Co-administration of PSO+AG reduced body weight gain, suppressing intestinal glucose absorption, improve lipid profile, its effect on gut hormone secretion plus antioxidant activity. It concluded that anti-obesity benefits make PSO & AG are useful combination in controlling body weight gain or treat obesity.

Key words: Pomegranate seed oil, Arabic Gum, High fat diet, Leptin, Obesity, Cardiovascular disease

INTRODUCTION

Obesity is the real nidus of several risk factors leading to sever diseases and may be postulated to be the first episode in the slow death and also as initiator to several metabolic disorders (Wahba and Mak, 2007), such as insulin resistance, risk factor for type 2 diabetes (Ye, 2013), dyslipidaemia accompanied with reduced high density lipoprotein cholesterol (HDL-c) (Velic et al., 2018).

Obesity also may increases prevalence of other serious diseases such as hypertension (Hosick et al., 2014), cardiovascular diseases (coronary artery disease, stroke) and end stage renal disease (Hotamisligil, 2006).

Obesity not only affect health status but also affect quality of life leading to psychiatric disorders and depression (Lykouras and Michopoulos, 2011; Rajan and Menon, 2017).

The most important factors that determine fatty acids deposition in adipose tissues and fat depot include many mechanisms: the rate of fatty acids uptake (Turcotte et al., 2001), fatty acid metabolism (Strible and Ntambi, 2010), triacylglyceried level in the blood (Guo and Jensen, 2003).

The induction of obesity not only affected by amount of dietary fat intake but dietary fats type also plays a central role in weight gain, body composition and adipose tissue cellularity, where the recent reports revealed presence of intense relationship between dietary fat intake and obesity. This relationship has affected by genetics, sex, biochemical basis and the roles of hormones such as leptin, insulin and ghrelin (Hariri and Thibault, 2010).

The Leptin is protein/cytokines secreted by adipocytes, mainly by the white adipose tissue of the human body; human leptin gene encoded to 167 amino acids which is the core component of leptin. The amount of leptin circulating in the body is proportional to the amount of fat ratio (adipose tissue) of an individual (Kelesidis et al., 2010). Mainly, the Leptin target is to fulfill an energy basis and the roles of hormones such as leptin, insulin and ghrelin (Hariri and Thibault, 2010).

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Nature is a huge source of remedies to control all of human kind insults, especially after failure of modern medicine in controlling the majority of diseases due to drugs toxicity. It has shown that natural antioxidant effect is the central role in effects associated with phytocompounds (Abeet et al., 2018).
*Punica granatum* (Pg), known as pomegranate (Pg), is an important member of the monogeneric family, Punicaceae. It found in arid and semiarid regions due to its ability to adapt to bad ecological conditions (Sheidai *et al*., 2007). It can be cultivated throughout the Mediterranean region (Facciola, 1990).

Pg and its chemical components possess various pharmacological properties including antioxidant, anti-inflammatory, and anti-angiogenesis activities. They also show inhibitory effects on vital enzymes especially cyclooxygenase (COX), lipoxygenase (LOX), phospholipase A2 (PLA2), (Rahimi *et al*., 2012).

Although all people prefer eating of *Pomegranate* (*Punica granatum L*) fruits or drinking its juice, they suppose pomegranate seeds as a waste products of processing, this seeds have great interest, because of their rich oil composition (Kýralan *et al*., 2009).

In the same time, the beneficial effects of PSO are summarized in its composition of polyunsaturated fatty acid especially punicic acid (PA) ~55% of its composition. PA also known as trichosanic acid and/or omega-5 as mentioned by Melo *et al*., (2014). PA has chemical form similar to conjugated α-linolenic and linoleic acid especially in double bonds numbers and atomic arrangement, it contains a ω-5 bond, it is considered as conjugated α-linolenic acid isomer (Melo *et al*., 2014).

Primarily, PA is observed to be useful in controlling of obesity related diseases such as: diabetics, dyslipidemia and metabolic disorders. (Yang *et al*., 2005; Zarfeshany *et al*., 2014).

Up till now, Arabic Gum (AG) is natural dried transparent or translucent sticky exudates sprinkled from Acacia trees, meanwhile is richest content into natural non-viscous soluble fiber content. It is an edible fiber mainly used into foodstuff products and pharmaceutical formula as an emulsifier and/or preservative agent (Ali *et al*., 2009). In Middle East and North Africa used for many centuries as an oral hygiene substance in conventional medicine (Tyler, 1977). Actually, more intake of dietary fiber had a useful role in fat metabolism (Slavin, 2003). Dietary fiber dominantly induced satiety, reduce glycemic index, alter gastric motility and gut hormone secretions. Accordingly, AG play important role in management of obesity and body weight gain (Chandalia *et al*., 2000). In many cases of human and animal focused on the anti-obesity important role of AG on body mass index and fat deposition in adipose tissue distribution over all the body (Ushida *et al*., 2011).

So, the current study focusing on the correlation between PSO effect and the AG function potentiality to control obesity in male rats, which due to its role in reducing obesity. Furthermore, study evaluated the essential role of leptin and the action to minimize obesity in rat−induced with high fat diet. Meanwhile, the glucose level in blood and insulin conducted as indicators to the obesity complication, lipid profiles and oxidative stress enzymes in obese experimental rats.

**MATERIALS AND METHODS**

**Plant**

Pomegranate whole plants were collected from rural area of villages (Assuit governorate), the plants and its fully ripened fruits were identified and authenticated as plants of Pomegranate by the taxonomy section of the Department of Biological Sciences, Cairo University, Egypt. Fruits were transferred to the laboratory, thoroughly washed, dried and kept refrigerated before subjected to subsequent study.

**Preparation of Pomegranate Seeds (PS)**

This extract was obtained by the following steps: Fruits were cut into pieces using sharp knife, then the arils containing seeds embedded in the surrounded juice were manual separated from the white endocarp. The seeds were carefully splitting by hand, and then washed several times by tap water, dried in an oven at 40°C for 24 hrs. Dried seeds were kept at −20°C until extracting pomegranate seed oil (PSO).

**Extraction of PSO using organic solvents**

The normal stirring extraction method described by Abbasi *et al.* (2008) with slight modifications, was performed to obtain PSO. Briefly, PS was crushed using the Moulinex Blender just before starting the experiment. Ten g of grinded PS was added to 100 ml hexane in a conical flask plugged with cotton plug and then kept on a rotary shaker at 120 rpm for 24 h. Thereafter, filtration, centrifugation at 5000 rpm at 4°C for 15 min, the collected supernatant was and solvent was evaporated using a rotary vacuum evaporator to obtain PSO. The residues were weighed and the yield was calculated expressed as percent. PSO just obtained was stored refrigerated at 4°C in an air tight glass bottles until used to determine fatty acid profile.

**Animals**

The experiment was carried out on 40 adult male Sprague Dawley male rats (weighing 149±16 g) were obtained from the animal house of the National Research Centre. All experiments were carried out according to the ethical guidelines approved by the Ethical Committee of NRC (1993).

The induction of the obesity was carried out by the feeding of The High Fat Diet (HFD) for 6 weeks (start first of June 2018) by substitution of soybean oil in standard diet by 20% (hydrogenated palm oil), compromised as (19%, 1%) of (butter oil, soybean oil, respectively) + 20% sucrose.

**GC analysis**

Gas chromatographic (GC) analyses of the methanol extract essential oil was performed by using a Perkin Elmer Auto System XL equipped with flame ionization detector (FID).

**Antioxidant analysis**

The DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical: method was performed according to Brand-Williams *et al.* (1995), β-Carotene / Linoleic Acid Method was determined according to (Kaur and Kapoor, 2002) and ABTS⁺ Scavenging Activity (total antioxidant activity) was measured using method of (Djeridane *et al*., 2006).

**Experimental design**

Animals were allocated into five groups, each of eight animals as follow:

- G 1: (standard group): rats fed with normal standard diet (AIN 1993),
- G 2: (HFD group): rats fed with a high
fat-diet (HFD), G 3. (HFD+ PSO); rats fed with HFD and received PSO (0.8 ml/kg of BW daily) (Hamid et al., 2016). G 4. (HFD+ AG); rats fed with HFD and received Arabic Gum (5% added to HFD). (Al-Majed et al., 2002) and G 5 (HFD+PSO+AG); rats fed with HFD and received pomegranate seed oil and Arabic Gum.

**Body weight and food intake tracking**

Body weight and food intake were measured twice weekly (digital scale). Report the average of these as body weight and food intake.

**Blood sampling and Biochemical analysis**

Blood sampling is carried out by open heart puncture under slight ether anesthesia, note: (animals were fasted overnight), then centrifugation for 10 minutes at 3500 r.p.m. serum was separated and kept at -20EC till used in chemicals analysis.

Assay of lipid peroxidation and antioxidant: Malondialdehyde (MDA), Superoxide dismutase (SOD), glutathione peroxidase (GPx), Catalase (CAT) and reduced glutathione (GSH) according to (Weinhold et al., 1990), respectively. (Biodiagnostic®, Giza, Egypt.)

Lipid profile assay: Total cholesterol, serum triglycerides, LDL-C and HDL-C evaluated by the technique described by (Fossati and Prencipe, 1982), respectively. (Biodiagnostic®, Giza, Egypt.)

Hormonal assay: Insulin and Leptin hormones detected using ELISA technique (ImmunoSec Corporation., USA).

**Statistical analysis**

Result expressed as mean ± SD. Data analysis was carried out by Co-Stat 6.303 Software Computer Program 2004 hypothesis testing methods, the difference is compared by one way analysis of variance (ANOVA), followed by Duncan Test (COSTAT-C, 1988). Difference was considered significant when P<0.05.

**RESULTS**

As shown in Table (1) and Figure (1) the scavenging activity of PSO against DPPH radicals *In vitro*, was firstly examined to evaluate its antioxidant activity, scavenging effect against DPPH radicals amounted to (percentile) 44.62% and B-carotene 22.52% at concentration 100 µL. The POS antioxidants higher than B-carotene, but slightly decline less than BHA and TBHQ the value 65.7% and 72.3% respectively.

The PSO fatty acid composition evaluated by GC showed that the major component of fatty acid was punicic acid amounted to 74.61%, it polyunsaturated fatty acid (three conjugated double bonds). It is called ω-5 and chemically analogous to the conjugated linoleic acids. The high ratio of polyunsaturated cleared in Table (2) of total fatty acids content in PSO nearly over 85% of fatty acids pattern.

Table 3 determined the final weight of albino rats, spontaneously the highest weight (Expressed as mean±SE) in induction group G2 rats fed with HFD reach to 217.60±16.80g meanwhile, the final weight reduced slightly in group G3 value 197.6±13.29g, obviously decline in final weight in group G4 rats fed with high fat diet in SOD, Gpx, GR, GSH and Catalase mean±SE 31.00±0.79 μ/mg, 151.00±2.26 μ/mg, 183±1.66, 31.40±0.72μ/mg, mmol/g and 42.40±4.04U/mg respectively, this group rats fed with a high fat diet, PSO and Arabic Gum, the finding amounted to 181.00±13.82 g. In the same time, the slightly exceed in leptin amount in groups G3, G4 and G5 was 2.74±0.08 µg/l, 2.40±0.20 µg/l and 2.76±0.23 µg/l respectively.

The relationship between the significant reduce of insulin value in groups G3, G4 and G5 7.20±0.55 μIU/mL and 7.90±0.43 μIU/mL, respectively and the significant increasing in leptin value is very important observation should take in account.

From Table 4 noticed that G5 the lowest value in cholesterol and triglyceride (mean±SE)144±0.96 mg/dl, 137±1.91 mg/dl and increased in HDL to 45.60±0.66 mg/dl, respectively, this group rats fed with a high fat-diet, PSO and Arabic Gum. Indeed, this conduct of cooperation influence of PSO and Arabic Gum to elevated HDL and decreased of cholesterol and triglyceride.

In Table 5 our findings assured that Malondialdehyed value (mean±SE) in G5 to 214±1.51 mmol/ml equal with normal control group (G1) level of Malondialdehyed also the results illustrated the antioxidant enzymes values in blood serum was decreased in group G2 (induction group with high fat diet) in SOD, Gpx, GR, GSH and Catalase mean±SE 31.00±0.79 μ/mg, 151.00±2.26 μ/mg, 183±1.66, 31.40±0.72μ/mg, mmol/g and 42.40±4.04U/mg respectively, than group G1 or normal control which feed on standard normal diet. But, noticed ameliorate and increased in G3, G4 and G5 in all antioxidant enzymes regard to feeding rats on PSO, GA and PSO + GA.

**DISCUSSION**

Antioxidant activity assessment of PSO *In vitro* by DPPH assay which was considered accurate, a well-known...
trap for other radicals and broad utilized to evaluation antiradical or antioxidant potentiality of natural compounds in foods or biological systems. Antioxidant activity of PSO with respect to the abundance of n-3 PUFAs was notably punicic fatty acids high score ratio value 74.61% and γ-tocopherol. The influence of Punicic acids on DPPH scavenging and TBHQ and BHA were used as reference to the assay of scavenging activity against DPPH.

No doubt, the induction of obesity in this study with HFD not only increased final weight but also impaired cognition and increased brain inflammation as mentioned in other study by Pistell et al. (2010) and Mohamed & Elkhamsiy, (2018). Moreover, Zhang et al., 2005 has reported that elevated free radicals as a mediator lead to tocopherols and polyphenolic compound contents the current results agreed with Elfalleh et al. (2011) findings. While PSO somewhat decline body overweight, insulin and leptin in blood, improves peripheral insulin tolerance, increases carbohydrate metabolism, and prevent the complication of type 2 diabetes (Vroegrijk et al., 2011). Otherwise, PSO Antioxidant efficacy was potent and contributed conducted to tocopherols and polyphenolic compound contents the current result agreed with Elfalleh et al. (2011) findings.

In order to PA has a very chemical structural resemblance cis and trans isomer of the carbon backbone chain double bonds with ratio of 2:1 and a lot of previous researches have distinct that conjugated linoleic acids and α-linolenic acids acquired numerous health advantages essentially as like as anti-inflammatory, anti-atherogenic, anti-estrogen and improve lipid profile (Bassaganya-Riera, 2014). The main properties of PSO mentioned of numerous studies that Antioxidant and anti-inflammatory activities which outcome of depressed of lipid peroxidation and atherogenic, anti-inflammatory, anti-atherogenic, anti-estrogen, and improve lipid profile (Bassaganya-Riera, 2014). The main properties of PSO mentioned of numerous studies that Antioxidant and anti-inflammatory activities which outcome of depressed of lipid peroxidation and atherogenic, anti-inflammatory, anti-atherogenic, anti-estrogen, and improve lipid profile (Bassaganya-Riera, 2014). The main properties of PSO mentioned of numerous studies that Antioxidant and anti-inflammatory activities which outcome of depressed of lipid peroxidation and atherogenic, anti-inflammatory, anti-atherogenic, anti-estrogen, and improve lipid profile (Bassaganya-Riera, 2014).

Table 3: indicate the relation of leptin hormone with rats weight, level of glucose and insulin.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Initial weight (g)</th>
<th>Final weight (g)</th>
<th>Glucose (mg/dl)</th>
<th>Insulin (µU/mL)</th>
<th>Leptin (µg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>149.60±6.21</td>
<td>198.60±11.53</td>
<td>106±8.89</td>
<td>8.14±0.53</td>
<td>2.66±0.16</td>
</tr>
<tr>
<td>G2</td>
<td>148.20±7.81</td>
<td>217.60±16.80</td>
<td>133±9.33</td>
<td>7.84±0.64</td>
<td>2.28±0.13</td>
</tr>
<tr>
<td>G3</td>
<td>146.40±10.19</td>
<td>197.60±13.29</td>
<td>127±0.96</td>
<td>7.20±0.55</td>
<td>2.74±0.08</td>
</tr>
<tr>
<td>G4</td>
<td>146.40±5.89</td>
<td>185.20±16.87</td>
<td>128±1.37</td>
<td>8.66±0.25</td>
<td>2.40±0.20</td>
</tr>
<tr>
<td>G5</td>
<td>147.20±10.47</td>
<td>181.00±13.82</td>
<td>126±1.07</td>
<td>7.90±0.43</td>
<td>2.76±0.23</td>
</tr>
</tbody>
</table>

Table 4: the obesity rats blood lipid profile.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>138±1.26</td>
<td>133±0.99</td>
<td>40.40±0.97</td>
<td>71.00±0.99</td>
</tr>
<tr>
<td>G2</td>
<td>171±0.54</td>
<td>179±2.67</td>
<td>40.00±1.20</td>
<td>94.00±1.12</td>
</tr>
<tr>
<td>G3</td>
<td>158±0.86</td>
<td>167±1.24</td>
<td>41.20±1.33</td>
<td>84.60±0.82</td>
</tr>
<tr>
<td>G4</td>
<td>163±0.94</td>
<td>155±1.71</td>
<td>43.00±0.83</td>
<td>86.40±0.10</td>
</tr>
<tr>
<td>G5</td>
<td>144±0.96</td>
<td>137±1.91</td>
<td>45.60±0.66</td>
<td>73.60±0.76</td>
</tr>
</tbody>
</table>

Table 5: the serum oxidative stress enzymes of obesity rat’s blood.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Malondialdehyde (µmol/ml)</th>
<th>SOD (µ /mg)</th>
<th>GPx (µ /mg)</th>
<th>GR (µ /mg)</th>
<th>GSH (µmol/g)</th>
<th>Catalase (U/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>G1</td>
<td>214±2.27</td>
<td>37.90±0.48</td>
<td>167.00±1.96</td>
<td>202.3±28</td>
<td>28.00±0.87</td>
<td>46.80±3.73</td>
</tr>
<tr>
<td>G2</td>
<td>227±1.88</td>
<td>31.00±0.79</td>
<td>151.00±2.26</td>
<td>183±1.66</td>
<td>31.40±0.72</td>
<td>42.40±0.40</td>
</tr>
<tr>
<td>G3</td>
<td>215±1.49</td>
<td>35.80±1.29</td>
<td>158.60±0.49</td>
<td>189±4.71</td>
<td>28.00±1.37</td>
<td>45.60±3.31</td>
</tr>
<tr>
<td>G4</td>
<td>223±1.12</td>
<td>35.80±0.53</td>
<td>163.00±0.83</td>
<td>185±3.10</td>
<td>28.20±0.91</td>
<td>44.40±3.87</td>
</tr>
<tr>
<td>G5</td>
<td>214±1.51</td>
<td>32.80±0.54</td>
<td>177.80±1.77</td>
<td>210±4.96</td>
<td>27.40±1.05</td>
<td>46.60±0.95</td>
</tr>
</tbody>
</table>

abc Means ± SE in each column having the same letter were not significantly different. Values with different superscript letters are significantly different at P<0.05.
leptin to linkage with receptors of the hypothalamus gland and influence on centers of hunger and satiety in rats obesity to homeostasis the hunger and decline appetite to reduce obesity. These regard to PSO antioxidant and anti-inflammatory activities and reduced oxidative stress on cells that make a protection of produce of cytokines pro-inflammatory agents (TNF-alpha, IL-1β, and IL-6). Also, reduction-oxidation process should stimulate and deactivate specific genes that confirmed many secondary stages in disease circumstance (Valko et al., 2007). The NF-κB and AP-1 are two models of this instance. Reactive oxygen species (ROS) act as ‘signal transduction messengers to enhance the activity of the cytokines (Gloire et al., 2006). Also, our result in the same line, Zarfeshany et al., 2014 whose report discussed that PA and alphatocopherol content of PSO made a significant reduction in LDL-C and lipid peroxidation with alloxan model.

Moreover, PSO also reduced insulin in rat blood with no significant difference in blood serum glucose curve as compared to control rats group (Yang et al., 2005).

Generally, Dietary fibers induce satiation, reduce glycemic index, remodeling gastric motility or emptying, gut secretion of the hormone and even lead to control weight (Chandelia et al., 2000). Previous study has described that AG blocked glucose absorption and metabolite in intestine through interaction with membrane multitude of Sodium-glucose transport proteins (SGLT1) in experimental animal (Nasir et al., 2010). AG observed from current result significantly eliminated the increasing in body weight, glucose of plasma and insulin level through feeding rats HFD. But, Leptin raised weight lack by twice uneven mechanisms. It decreased appetite, and even food intake, and in the same way elevates energy expenditure balance in addition, intake of dietary fiber was correlated inversely with leptin concentration in young Japanese (Kuroda et al., 2010). So, It was clear that the integration potent effect (synergistic effect) between pomegranate seed oil (PA) which may effect on leptin receptors of the hypothalamus satiety center caused decreased appetite and AG suppressed intestinal glucose absorption with effect of gut hormone secretion. Finally, the study demonstrated that nutraceutical or pharmaceutical as anti-obesity potential should contain PSO & AG to useful synergistic effect on controlling gain body weight or treat obesity.

Conclusions
The findings of our study suggest that rich proportion of puninic acid in PSO has a basic role in increment of leptin to linkage with receptors of the hypothalamus gland and influence on centers of hunger and satiety in obesity rats to homeostasis the hunger and appetite to reduce obesity in addition of its antioxidant effect that detoxifies the risk effects of obesity and prevent its aggravation by HFD and that it is also involved in regeneration processes, preventing from organ damage and necrosis. Moreover, AG has a significant role in blockage of intestinal absorption of glucose and its metabolite, this action observed from significant reduction in the elevated body weight. Therefore, dietary supplementation of POS and AG offer integration potent effect (synergistic anti-obesity effect) by decreased appetite and suppression of intestinal glucose absorption and presents a new promising pharmaceutical and food supplement with obese patients.

REFERENCES


