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ORIGINAL ARTICLE

Effect of virgin olive and *Pistacia lentiscus* oils fortified with tomato lycopene on biochemical parameters in Wistar rats

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Abstract

Background: *Pistacia lentiscus* oil (PLO) and virgin olive oil (VOO) contain a large variety of phytochemicals providing beneficial effects. Lycopene is the main carotenoid with antioxidant properties. The consumption of lycopene containing foods may fight against cardiovascular diseases. **Aims**: The present study aims to evaluate the effects of fortified oils (VOO and PLO) with lycopene on some biochemical parameters in Wistar rats. **Material and Methods:** The experimentation included 50 male Wistar rats from the Algerian Pasteur Institute for the duration of 9 weeks of treatment. Rats were divided into five experimental groups (n=10) and fed a different experimental diet each for 9 weeks: control group (C), *Pistacia lentiscus* oil group (PLO), lycopene-enriched *Pistacia lentiscus* oil group (PLO-Lyc), virgin olive oil group (VOO) and lycopene-enriched virgin olive oil (VOO-Lyc). Total Cholesterol (TC) concentration was determined by the enzymatic method CHOD-PAP, High-density lipoprotein-cholesterol (HDL-C) with Biotrol diagnostic, the levels of low-density lipoprotein-cholesterol (LDL-C) were calculated using the Friedewald formula (LDL-C=TC–HDL-C–TGs/5). Triglycerides (TG) were determined by the enzymatic method PAP-1000 and Serum phospholipids (PL) were determined by an enzymatic colorimetric method. The plasma atherogenic index (PAI) was calculated as (TC/HDL-C). **Results:** Results showed that ingestion of PLO and VOO diminished TC, LDL-C, TG, and PL levels, whereas the HDL-C levels raised in all the groups assayed. Moreover, the lowest level of plasma atherogenic index (PAI) was shown in the VOO-Lyc group after 3, 6, and 9 weeks of treatment. **Conclusions**: The enrichment of PLO and VOO with lycopene improved the beneficial effects derived from the consumption of both oils on serum biochemical parameters. These findings suggest that lycopene enriched PLO and VOO may be used as a natural product to defend against some cardiovascular diseases (CVD) as hyperlipidemic and hypercholesterolemic acquired disorders.

Keywords: lycopene, *Pistacia lentiscus* oil, virgin olive oil, LDL-C, HDL-C, triglycerides.

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1 Introduction

Lycopene is the most important carotenoid in the Mediterranean diet, being abundant in red fruits such as tomato and tomato-based products, and other fruits including watermelon, papaya, guava, grapefruit, and apricot [1]. Lycopene is an antioxidant that has high biological activity in the human body because it is able to act and eliminate the free radicals which generate oxidative stress causing long-term several diseases. Therefore, it must be consumed in a daily diet [2]. Consumption of tomato products with olive oil significantly increases the antioxidant capacity of plasma, while no effect was observed when sunflower oil was used [3]. Previous studies in humans have shown that olive oil compared to seed oil has the ability to prevent peroxidation of lipids due to its high content of monounsaturated fatty acids (MUFA) [4]. In fact, foods fortified with olive oil have been shown to be effective in reducing total cholesterol (TC) and low-density lipoprotein-cholesterol (LDL-C) than conventional dietary treatments that do not contain significant MUFA [5]. *Pistacia lentiscus* is widely distributed tree in the extreme ecosystem of the Mediterranean basin [6]. It grows wild in Algeria, Turkey, Morocco, Tunisia, France, Spain, Italy, and Greece [7]. *Pistacia lentiscus* is known worldwide for its various therapeutic properties such as its antifungal [8], antimicrobial [9], antioxidant [10], and antiproliferative effects [11]. The fruit of *Pistacia lentiscus* provides edible oil which is rich in unsaturated fatty acids as oleic and linoleic [12] and used in traditional medicine, especially in the treatment of scabies, rheumatism, and in the manufacture of anti-diarrheal pills [13]. Also, this oil protects against mercury poisoning as in the case of alkaline phosphatase, aspartate aminotransferase, and urea. This oil is also considered as a nutritional source participating in the maintenance of TC and LDL-C in its normal values [14]. In recent years, there has been a growing interest in lycopene's health benefits. Its beneficial effects in the prevention and treatment of a wide variety of diseases have been assessed by several systematic reviews and meta-analyses [15].

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The main objective of this work was to investigate the consumption effects of diets based on *Pistacia lentiscus* oil (PLO) and virgin olive oil (VOO) in combination with tomato lycopene on some serum biochemical parameters such as TC, HDL-C, LDL-C, TG, PL and their effect on the plasma atherogenic index (PAI) of Wistar rats.

2 Material and Methods

2.1 Animals

Fifty male Wistar rats from Pasteur Institute (Algiers, Algeria) were included in the experimentation. They were individually housed under controlled environmental conditions (22˚C; 50% humidity), subjected to a regime of 12/12 h light/dark cycle. Food and water were available ad libitum and changed daily for 9 weeks. All experiments were carried out according to a procedure approved by local ethics committees (Ref. no. PDT 08A008), in accordance with the current guidelines for the care of laboratory animals, and the National Institutes of Health Guide (Reg. No. 488/160/1999/CPCSEA).

2.2 Animal treatments

Animals were allowed 1 week of in-house acclimatization. Rats were divided into five experimental groups (n=10) and fed a different experimental diet each for 9 weeks: control group (C), *Pistacia lentiscus* oil group (PLO), lycopene-enriched *Pistacia lentiscus* oil group (PLO-Lyc), virgin olive oil group (VOO) and lycopene-enriched virgin olive oil (VOO-Lyc). The VOO and PLO were purchased from the local market in Algeria and lycopene was acquired from DSM Inc. (Istanbul, Turkey). Control animals consumed standard food and water, whereas treatment groups received a standard food supplemented with: 10% of *Pistacia lentiscus* oil (PLO); 10% *Pistacia lentiscus* oil-rich plus 0.1% of tomato lycopene (PLO-Lyc); 10% of virgin olive oil (VOO), and 10% of virgin olive oil plus 0.1% of tomato lycopene (VOO-Lyc), respectively. The composition of the diets was prepared following the recommendations of previous studies [16-19].

2.3 Blood sampling collection

Blood samples were drawn by cutting the tail of all rats before the beginning of the assay (basal conditions), as well as after 3 and 6 weeks of administration of the different dietary treatments. At the end of the 9 weeks of experimental treatment, the animals were deprived of food overnight, and then anesthetized by intramuscular injection of 50 mg kg^{-1} ketamine and sacrificed to obtain blood samples by puncturing the heart ventricle. Blood samples (2 mL) were placed in dry clean centrifuge tubes and then centrifuged for 10 min at 900 × g. Serum was carefully separated into clean dry tubes by using a Pasteur pipette and kept frozen at -30˚C until analysis.

2.4 Plasma Biochemical analysis

TC was determined by enzymatic method CHOD-PAP (Diagnostic Merck), HDL-C by Biotrol Diagnosis, and TGs were determined by enzymatic method PAP-1000 (Bio Mérieux method) on a Cobas Analyzer (Roche Diagnostics, Paris, France). Levels of LDL-C were calculated using the Friedewald formula (LDL-C=TC–HDL-C–TGs/5) [20]. The Serum phospholipids were determined by an enzymatic colorimetric method (Bio-Direct, Taunton, MA, USA). The plasma atherogenic index (PAI), calculated as (TC/HDL-C) [21, 22].

2.5 Statistical analysis

Data were expressed as mean ± SD (n=10) of the number of determinations carried out in triplicate. To compare the different treatments, statistical significance was calculated by one-way analysis of variance (ANOVA). The degree of significance was set at $P < 0.05$. All analyses were performed using Graph Pad Prism (version 5.0, 2007; GraphPad Software, Inc.; San Diego, CA).

3 Results

Figure 1 shows concentrations levels of TC (Figure 1A), HDL-C (Figure 1B), and LDL-C (Figure 1C). TC concentrations in VOO-Lyc and PLO-Lyc groups were significantly ($P < 0.05$) reduced at week 6 by approximately 6% and 7% respectively and at week 9 by 17% and 15% respectively with respect to the control group. In addition, in the VOO group, TC levels showed a statistically significant decrease ($P < 0.05$) only after 9 weeks of treatment by 8% compared with the control group, whereas, for the PLO group, the level of TC was slightly reduced by 6% after 9 weeks of treatment with respect to the control group.

In Figure 1B, better results for HDL-C concentrations were found in groups treated with diets enriched lycopene (VOO-Lyc and PLO-Lyc) than in groups treated with oil alone. Moreover, in VOO-Lyc and PLO-Lyc, HDL-C levels started to increase significantly ($P < 0.05$) after 9 weeks of treatment with respect to the control group, while in the PLO group, no significant difference was recorded for HDL-C level, compared to the control group. In Figure 1C, the most important result of LDL-C concentrations was observed in the VOO-Lyc group, recorded the lowest value in weeks 6 and 9 compared to the control group. However, there was a significant decrease ($P \leq 0.05$) in the LDL-C level in all treated groups (VOO, VOO-Lyc, PLO, PLO-Lyc) after 9 weeks with respect to the control group.

The reduction of TG concentrations (Figure 2A) appeared in VOO-Lyc and PLO-Lyc groups after 6 and 9 weeks of treatment compared to the control group. The major reduction ($P < 0.05$) was observed in the VOO-Lyc group after 9 weeks of treatment compared with the control group.

The phospholipids (PL) levels decreased significantly ($P < 0.05$) after 6 and 9 weeks in the VOO-Lyc and PLO-Lyc with respect to the control group, whereas, the PL diminution in the PLO group appeared after 9 weeks of treatment, while the reduction of PL level in VOO group was observed at 6 weeks of treatment with respect to the control group. In Figure 3 the lowest level of plasma atherogenic index (PAI) was shown in the VOO-Lyc group after 3, 6, and 9 weeks of treatment compared with the control group.

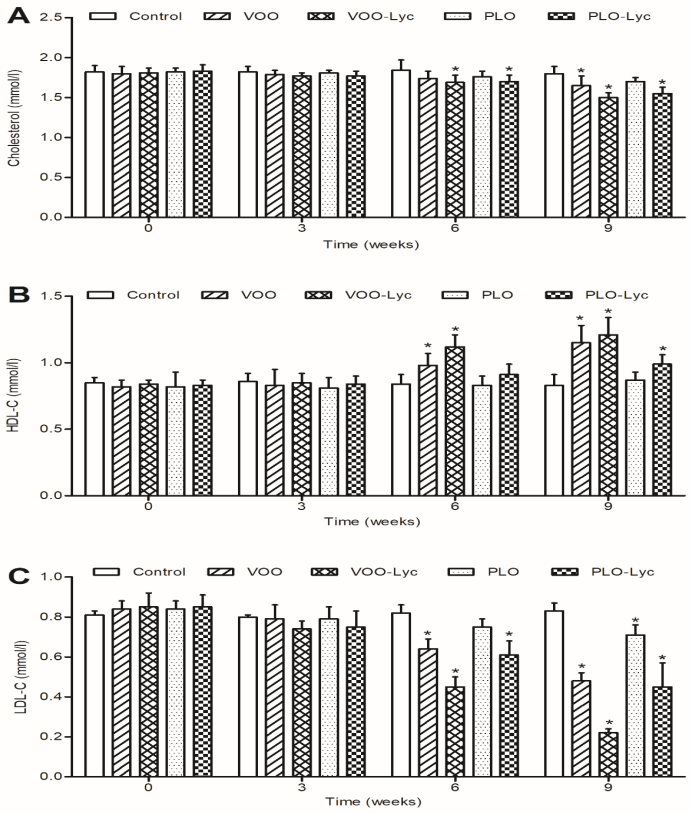

Figure 1: Effect of lycopene supplementation on cholesterol levels. (A) Total cholesterol concentration obtained after 3, 6 and 9 weeks of treatment with virgin olive oil (VOO), lycopene-enriched virgin olive oil (VOO-Lyc), *Pistacia lentiscus* oil (PLO) or lycopene-enriched *Pistacia lentiscus* oil (PLO-Lyc). (B) Hight density lipoprotein-cholesterol (HDL-C) levels reached after 3, 6 and 9 weeks of treatment with VOO, VOO-Lyc, PLO or PLO-Lyc. (C) Low density lipoprotein-cholesterol (LDL-C) found after 3,6 and 9 weeks of treatment with VOO, VOO-Lyc, PLO or PLO-Lyc. Values represent mean ± SD of 10 animals. *p <0.05 with respect to the control group.

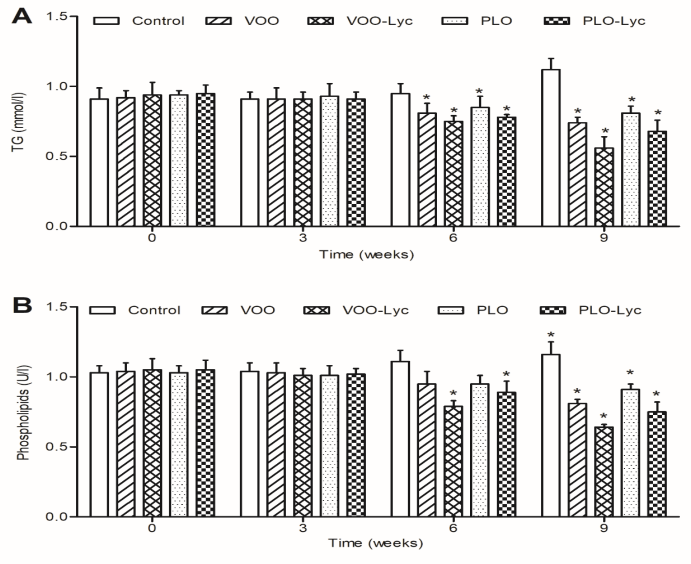

Figure 2: Effect of lycopene supplementation on lipid serum parameters
(A) Triglycerides concentration obtained after 3, 6 and 9 weeks of treatment with virgin olive oil (VOO), lycopene-enriched virgin olive oil (VOO-Lyc), *Pistacia lentiscus* oil (PLO) or lycopene-enriched *Pistacia lentiscus* oil (PLO-Lyc). (B) Phospholipids levels reached after 3, 6 and 9 weeks of treatment with VOO, VOO-Lyc, PLO or PLO-Lyc. Values represent mean ± SD of 10 animals. *p <0.05 with respect to the control

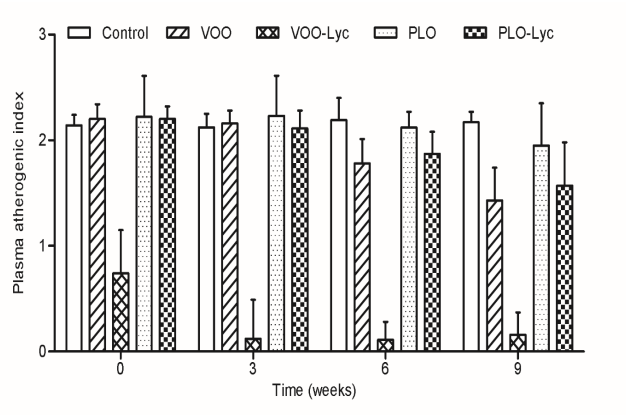

Figure 3: Effect of lycopene supplementation on plasma atherogenic index (PAI). PAI measured after 3, 6 and 9 weeks of treatment with virgin olive oil (VOO), lycopene-enriched virgin olive oil (VOO-Lyc), *Pistacia lentiscus* oil (PLO) or lycopene-enriched *Pistacia Lentiscus* oil (PLO-Lyc)..

4 Discussion

Epidemiological studies related to tomato consumption enhance beneficial effects on health, especially in decreasing the risk of CVD [23], and prostate cancer [24, 25]. PLO has a good nutritive quality because of its content in unsaturated fatty acids (70%) and saturated fatty acids (26%) [26]. PLO and VOO oils present a high amount of polyphenol content (810 mg GAE/kg oil and 1085.92-1406.40 mg GAE/kg oil respectively) [27]. These characteristics offer these oils their beneficial effects on human health.

The present study aimed to investigate the effects of lycopene – enriched oils such as VOO and PLO upon some biochemical parameters in Wistar rats. Our results showed that the fatty acid profile of *Pistacia lentiscus* oil indicates the dominance of MUFAs (55.76%), as well as its high oleic acid content [14]. Oleic acid (C18: 1) is a MUFA that induces a cholesterol-lowering effect, thus reducing the risk of cardiovascular disease as well as a significant decrease in systolic and diastolic blood pressure in susceptible populations [28]. High levels of HDL-C provide anti-inflammatory properties and protection against cardiovascular disease [29]. The results showed that the consumption of VOO and PLO decreased TC and LDL-C in the rat population, while HDL-C concentration increased significantly. Moreover, these oils improve the serum lipid parameters such as TG and PL.

The phenolic compounds present in olive oil protect against LDL-C *in vitro* [30] and *in vivo* [19] studies. Also, animal experimentation studies on rats have shown that ingestion of VOO rich in phenols decreases the concentrations of TC, LDL-C, and TG [31] and substantially increased the levels of HDL-C [32]. In addition, a study on Wistar rats showed that lycopene supplementation decreases serum concentrations of TC and LDL-C and increases levels of HDL-C [19]. The protective effect of PLO may be due to the high content of natural antioxidants such as terpenes and unsaturated fatty acids (UFA) [14]. Conjugated linoleic acids are considered to be powerful new anti-atherogenic fatty acids in animal models of atherosclerosis [33]. Several authors present the antioxidant properties of phenols and terpenes. Also, most *in vitro* studies indicate the protective effect of the experimented oils against oxidation of LDL-C [34-36]. The decrease in serum PL concentrations in VOO and PLO groups may be due to the effect of PUFAs contains in oils. These results corroborate the work of Toyoshima *et al.* [37] who showed that α-linoleic acid decreased the concentration of PL serum in the same animal model.

5 Conclusions

The enrichment of virgin olive and *Pistacia lentiscus* oils with tomato lycopene increases the hypolipidemic and hypocholesterolemic effects. These biological properties can have a significant impact on human health and particularly on problems related to the increase in TG serum, decrease in HDL-C, and high oxidation of LDL-C. In fact, fortifying VOO and PLO could have a positive effect on the risk of cardiovascular disease and mortality.

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