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## Hepatoprotective Effect of Olive and Argan Oils Supplemented with Tomato Lycopene in Wistar Rats

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**Abstract:** Lycopene, the most important carotenoids, is a red lipophilic pigment containing 11 conjugated double bonds make lycopene a powerful radical scavenger. It has the powerful antioxidant activity within carotenoids and is an effective free radical scavenger. Virgin olive oil (VOO) and argan oil (AO) contain a wide variety of phytochemicals which are considered as a functional foods. The present study intended to assess the effect of various dietary VOO and AO in combination with lycopene consumption on some liver function parameters, including liver enzymes such as alanine amino-transferase (ALT), aspartate-aminotransferase (AST), alkaline phosphatase (ALP) as well as on hepatic lycopene concentration of wistar rats. Results showed that ingestion of VOO and AO diminished ALT, AST, ALP levels in all the groups assayed. The lycopene enrichment VOO and AO significantly improved the beneficial effects derived from the consumption of both oils on serum liver enzymes and protect the liver tissue by enhancing the hepatic lycopene concentration, induced hepatoprotective effect by ameliorating the capacity antioxidant and function parameters of the liver tissue in wistar rats. These results suggest that the inclusion of lycopene in VOO and AO may be used as a natural antioxidant to protect the liver against damage.

**Key words:** Lycopene, virgin olive oil, argan oil, liver enzymes, hepatic lycopene

### INTRODUCTION

Anti-oxidative therapy, mainly using natural and synthetic antioxidants, represents a reasonable therapeutic approach for the prevention and treatment of liver diseases due to the role of oxidative stress in contributing to initiation and progression of hepatic damage (Sha *et al.*, 2015). Carotenoids have received considerable attention due to their antioxidant properties (Stahl and Sies, 2003). Lycopene is the most important carotenoids present in fruits and vegetables. It is important to note that the absorption of this carotenoid from food is dependent on several factors such as the type of dietary fat. In this sense, Lee *et al.* have reported that the consumption of tomato products with olive oil significantly raises the plasma antioxidant capacity whereas no effect was observed when the sunflower oil was used (Lee *et al.*, 2000). In addition, the liver injury induced by NDEA is a well-known model of hepatotoxicity commonly used for the screening of the hepatoprotective activity of natural compounds (Kujawska *et al.*, 2011; Kujawska *et al.*, 2009; Poojari *et al.*, 2010).

The present study was designed to evaluate the efficiency of lycopene-enriched oils (VOO, AO) in ameliorating the liver function parameters, including

liver enzymes such as alanine amino-transferase (ALT), aspartate-aminotransferase (AST), alkaline phosphatase (ALP), as well as on hepatic lycopene concentration of male Wistar-Albino rats.

### MATERIALS AND METHODS

**Animals and treatment:** Seventy-five male Wistar rats (*Rattus norvegicus*) at their weaning age (3-4 weeks) and weighed  $87.98 \pm 15.95$  g ( $n = 15$  per group) were obtained from Animal Laboratory (Institute Pasteur, Algiers, Algeria) as described previously (Aidoud *et al.*, 2014). 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 in accordance with the National Institutes of Health Guide (Reg. No. 488/160/1 999/CPCSEA). Rats were divided in five experimental groups and fed a different experimental diet each during 9 weeks: control group (C), virgin olive oil group (VOO), lycopene-enriched virgin olive oil (VOO+Lyc), argan oil group (AO) and lycopene-enriched argan oil (AO+Lyc). The composition of the different diets (Table 1) was prepared following the recommendations of Hochgraf *et al.* (1997), Sanchez-Muniz (1998) and Varela and Ruiz-Rosso (1998) and previously published (Aidoud *et al.*, 2014).

Table 1: Compositions of the diets (g 100/g of food) for the different rat groups Aidoud *et al.* (2014)

Ingredients	Control	VOO	VOO+Lyc	AO	AO+Lyc
Skimmed milk powder	42.11	42.11	42.11	42.11	42.11
Sucrose	7.37	7.37	7.37	7.37	7.37
Sunflower oil	10	-	-	-	-
Argan oil	-	-	-	10	10
Virgin olive oil	-	10	10	-	-
Lycopene	-	-	0.1	-	0.1
Mineral supplement*	3.84	3.84	3.84	3.84	3.84
Vitamin supplement**	1	1	1	1	1
Fibre (agar)	3	3	3	3	3
DL-Methionine	0.3	0.3	0.3	0.3	0.3
Corn starch	23.38	23.38	23.28	23.38	23.28
Humidity	9	9	9	9	9
Total	100	100	100	100	100
Energy (kcal)	359	359	359	359	359

Control, laboratory standard food diet; VOO, virgin olive oil diet; VOO+Lyc, lycopene-enriched virgin olive oil diet; AO, argan oil diet; AO+Lyc, lycopene-enriched argan oil diet

**Blood sampling collection:** Blood samples experiments were described previously (Aidoud *et al.*, 2014). Blood samples were collected 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 anaesthetized by intramuscular injection of 50 mg/kg 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 x g. Serum was carefully separated into clean dry tubes and kept frozen at -30°C until analysis.

**Determination of liver enzymes:** Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were determined in serum samples obtained from all groups of rats by colorimetric method according to the IFCC recommendations (Bergmeyer *et al.*, 1986; Eccls, 1999). Whereas alkaline phosphatase (ALP) activity was assayed by colorimetric method according to Tietz *et al.* (1983). The activities of ALT, AST and ALP were assayed using enzymatic kits from Abcam (Cambridge, MA, USA) on a Cobas Analyzer (Roche Diagnostics, Paris, France) and expressed as U/L.

**Analysis of hepatic lycopene:** Determination of hepatic lycopene concentration was in accordance with the method described by Miller *et al.* (1984). By using a 1200 Agilent HPLC instrument equipped with UV and fluorescence spectrometer detector (Agilent technologies, Waldbronn, Germany). Liver tissue was homogenized in 10% KOH and ethanol (1:5, v/v) containing 0.1 % butylated hydroxy toluene and incubated at 60°C for 30 min. Lycopene was extracted from the liver using hexane and distilled water. Each sample was centrifuged at 2,000 x g for 5 min and the upper hexane layer was removed to another amber

bottle. The hexane fractions were evaporated to dryness under nitrogen gas and the residue was redissolved in mixture of chloroform and methanol (2: 1, v/v). Lycopene was measured with a UV-Vis detector at 470 nm with a mixture of acetonitrile: methanol: chloroform (47:47:6) as the mobile phase. Trans- $\beta$ -Apo-8-carotenal (Sigma, St. Louis, MO, USA) was used as the international standard to determine extraction efficiency. An external lycopene standard (Wako Pure Chemical Co, Osaka, Japan) was used as the reference standard.

**Statistical analysis:** Data were expressed as mean $\pm$ SD of the number of determinations carried out in duplicate, unless otherwise indicated. To compare the different treatments, statistical significance was calculated by one-way analysis of Variance (ANOVA) followed by the Tukey post-hoc test. The degree of significance was set at  $p < 0.05$ . All analyses were performed using Graph Pad Prism (version 6.01, 2012; GraphPad Software Inc., San Diego, CA, USA).

## RESULTS

Figure 1 shows liver enzymes concentration levels, the alanine amino-transferase concentration levels represented in (Fig. 1a), aspartate amino-transferase in (Fig. 1b) and alkaline phosphatase concentrations in (Fig. 1c). ALT levels in VOO and VOO+Lyc groups were significantly ( $p < 0.05$ ) reduced since week 3 up to the end of the experiment with respect to the control group. This parameter was gradually further reduced in weeks 3, 6 and 9 in VOO+Lyc group ( $p < 0.05$ ) compared to the VOO and control groups. Related to AO, better results were obtained in the group AO+Lyc, since ALT levels were reduced ( $p < 0.05$ ) since week 3 of treatment, whereas in AO group, ALT levels were statistically ( $p < 0.05$ ) decreased only after 6 weeks of treatment compared to the control group. As for AST concentrations, it progressively decreased ( $p < 0.05$ ) throughout the study in both VOO-treated and VOO+Lyc-

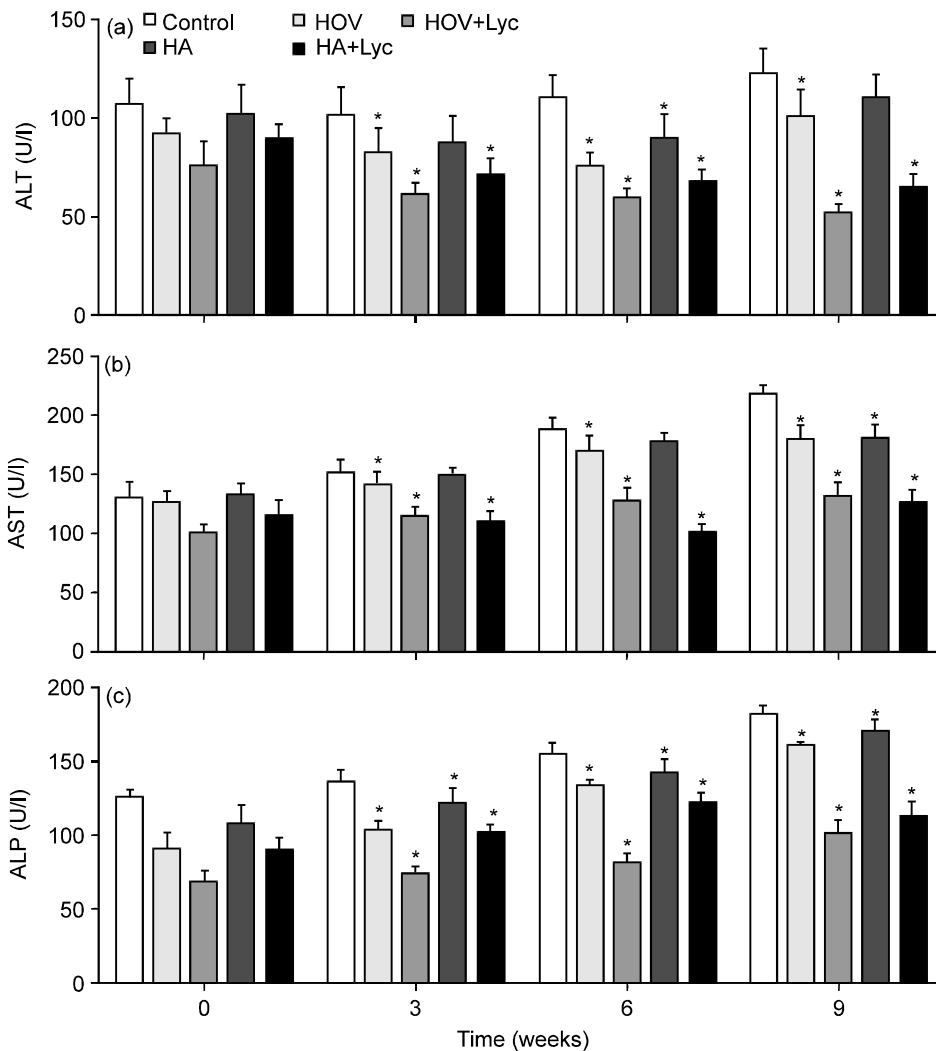


Fig. 1: Liver enzymes concentration levels in rats fed different oil diets supplemented with lycopene. (a) Alanine amino-transferase levels obtained after 3,6 and 9 weeks of treatment with virgin olive oil (VOO), lycopene-enriched virgin olive oil (VOO+Lyc), argan oil (AO) or lycopene-enriched argan oil (AO+Lyc), (b) Aspartate amino-transferase levels after 3, 6 and 9 weeks treatment with VOO, VOO+Lyc, AO or AO+Lyc, (c) Alkaline phosphatase levels found after 3,6 and 9 weeks treatment with VOO, VOO+Lyc, AO or AO+Lyc

treated groups with respect to the control group. In particular, the concentration levels of AST in AO group decreased significantly since week 9 of treatment with respect to the control group. However, the AST levels in AO+Lyc were reduced significantly ( $p < 0.05$ ) since week 3 up to the end of the study (week 9). As compared to the control group, the ALP concentration levels at weeks 3, 6 and 9 decreased significantly in all the treated groups ( $p < 0.05$ ) such as VOO, VOO+Lyc, AO, AO+Lyc. The more significant improvements of ALP concentrations were found in the groups with lycopene enriched oils (VOO+Lyc, AO+Lyc).

Figure 2 shows hepatic lycopene concentration after 9 weeks of lycopene supplementation with different diets

(VOO, VOO+Lyc, AO, AO+Lyc). The hepatic levels of lycopene in rats supplemented with lycopene-enriched oils (virgin olive oil, argan oil) significantly increased ( $p < 0.05$ ) with respect to the control group). However, no significant differences were observed in groups of rats fed with diets without lycopene. In addition, we observed that the hepatic lycopene concentration in (VOO+Lyc) group was slightly higher than that in (AO+Lyc) group.

## DISCUSSION

In this study, we found that the enrichment of VOO and AO with lycopene enhanced the beneficial effects on health. Several studies have suggested that lycopene is not detectable in the liver of animals consuming a diet

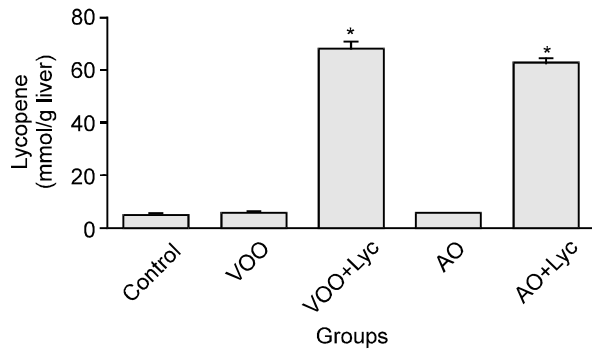


Fig. 2: Effect of lycopene supplementation on hepatic lycopene concentration in rats fed a diet supplemented with virgin olive oil (VOO), lycopene-enriched virgin olive oil (VOO+Lyc), argan oil (AO) or lycopene-enriched argan oil (AO+Lyc) after 9 weeks of treatment. Values represent mean±SD of 15 animals. \*p<0.05 with respect to the control group

without lycopene supplementation (Novak *et al.*, 2003; Simopoulos, 2005). However, lycopene supplementation causes a dose-dependent accumulation of lycopene in liver tissue (Soo-Kyong *et al.*, 2005), which was in accordance to our results. Although a small amount of lycopene was detected in the control, VOO and AO groups. In addition, in the groups of rats fed lycopene-enriched oils (virgin olive oil and argan oil) the hepatic lycopene concentration increased significantly more than 12 times higher in comparison with the control group. Soo-Kyong *et al.* (2005) showed that liver concentration of lycopene increased about four times higher in gerbils fed a high fat diet with lycopene than in gerbils fed a normal diet with lycopene. Also, Gitenay *et al.* (2007) reported that hepatic lycopene concentration increased in rat fed lycopene beadlets at 50 increase in hepatic lycopene concentration after animals have consumed lycopene with lipids such as sunflower oil, olive oil and avocado lipids, principally by stimulating bile production (Lee *et al.*, 2000; Unlu *et al.*, 2005).

It is important to note that in the current study the beneficial effects reported by the consumption of VOO and AO were further improved by the enrichment of both oils with lycopene. Several studies showed that the amount and type of dietary fat influence lycopene absorption (Tso *et al.*, 2001; Lee *et al.*, 2000; Unlu *et al.*, 2005). Additionally, Melendez-Martinez *et al.* found that supplementation with tomato extract, which contains mainly lycopene, decreases hepatic inflammation and plasma TC associated with high dietary fat intake (Melendez-Martinez *et al.*, 2013). In this study, the inclusion of lycopene into diet may improved the hepatic enzymes (AST, ALT and ALP) disorders and induced the promotive effect on lycopene absorption by enhancing the hepatic lycopene concentration in wistar rats.

**Conclusion:** This present study suggest that diets with olive and argan oils in combination with lycopene may contributed to ameliorate the liver function disorders by modulate the hepatic enzymes level and enhancing the antioxidant level of the liver tissue.

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