OnLine Journal of Biological Sciences

Original Research Paper

Effets of *Pistacia lentiscus* Virgin Fatty Oil on Lipidemic Profile and Carcass Characteristics in Hyperlipidemic *Oryctolagus cuniculus* Rabbits

[1,2]Zouhir Djerrou, [1]Noudjoud Boutobza, [1]Saida Bouzeguine, [1]Besma Khelfa, [1]Ilhem Brighet, [1]Imane Mokhbi, [1]Khadidja Saci Hadef and [2]Youcef Hamdi Pacha

[1]Department of Sciences of Nature and Life, Faculty of Sciences, University of August 20th 1955, Skikda, Algeria
[2]Laboratory of Pharmacology and Toxicology, University of Mentouri Constantine, Algeria

Article history
Received: 14-05-2016
Revised: 22-06-2016
Accepted: 24-06-2016

Corresponding Author:
Zouhir Djerrou
Department of Sciences of Nature and Life, Faculty of Sciences, University of August 20th 1955, Skikda, Algeria
E-mail: z.djerrou@univ-skikda.dz
zouhir21265@yahoo.fr

Abstract: The study aimed to evaluate *Pistacia Lentiscus* Virgin Fatty Oil (PLVFO) effects on lipidemic profile and carcass characteristics in rabbits. 15 male adult rabbits were allocated in three groups of 5 each. The first was not treated and served as control (CRL), the second was gavaged by Egg Yolk (EY) at a dose of 7 mL kg^{-1} BW (6/7 days), the third was treated as EY and then PLVFO was applied at a dose of 1 mL kg^{-1} BW (6/7 days). At 28th day of experiment, the biochemical profiles were determined and then animals were sacrified for anatomo-pathological studies and carcass characteristics investigation. PLVFO has resulted in non significant decrease of total cholesterol, a significant decrease of LDL-C (PLVFO Vs EY, ANOVA, $p<0.01$) and a significant increase of HDL-C (PLVFO Vs EY, ANOVA, $p<0.001$) with a significant amelioration of atherogenic index (PLVFO Vs EY, ANOVA, $p<0.01$). This oil has also increased non significantly ALT with a significant increase of AST (PLVFO Vs EY, ANOVA, $p<0.01$). Anatomo pathological investigations have not shown significant disturbances by PLVFO, this later has reduced significantly dissectible fat (PLVFO Vs EY, ANOVA, $p<0.05$). The study concludes that PLVFO prevent hyperlipidemia and obesity at tested dose. However, its prolonged use may increase transaminases enzymes activities.

Keywords: *Pistacia lentiscus* L., Virgin Fatty Oil, Hyperlipidemia, Atherogenic Index, Carcass Characteristics

Introduction

Lentisk oil is a fatty oil extracted from berries of *Pistacia lentiscus* L. plant which is largely distributed in the Mediterranean area. In North African regions, this oil is used traditionally to treat burns and wounds, respiratory allergies and lombalgies (Boukef and Souissi, 1982). It is consumed also for its nutritive value. Some pharmacological properties of this oil were recently investigated as cicatrizing activity (Djerrou *et al*., 2010), protection of mercury intoxication (Tounes *et al*., 2008), investigation of hepatoprotective effect against carbontetrachloride (Maameri *et al*., 2015). To ascertain the safety of this vegetable oil for its consumers, some studies were conducted as effects on glycemic index, liver and renal functions in rabbits (Djerrou *et al*., 2011), acute toxicity in mice (Boukeloua *et al*., 2012), irritancy potential and subacute dermal toxicity in rabbits (Djerrou *et al*., 2013), subchronic oral toxicity in rabbits (Maameri *et al*., 2016).

In a previous report, we have studied the anti-hyperlipidemic effect of this oil in female rabbits following a hyperlipidemic diet; at a dose of 2 mL kg^{-1} BW, this oil has resulted in reducing total cholesterol, LDL-cholesterol and triglycerides after 6 weeks of treatment (Djerrou, 2014).

The aim of the present study is to evaluate the anti-hyperlipidemic effect of *P. lentiscus* virgin fatty oil at a lower dose (1 mL kg^{-1} BW) and to investigate its possible side effects on hepatorenal functions and carcass characteristics in male rabbits.

Materials and Methods

Chemicals and Materials

Pistacia lentiscus Virgin Fatty Oil

The fruits of *Pistacia lentiscus* L. were harvested from Tamalous region (Latitude: 36°50.2578′, Nord, Longitude: 6°38.4108′ Est.) in the second half of December 2015, during sunny days. The virgin fatty oil, extracted traditionally, was stored in well-filled glass bottle and well sealed. It was kept cool to protect from light until use.

Eggs

These eggs were purchased from the local market. The yellow of eggs were separated manually, grouped together in a clean container and mixed.

Animals and Housing Conditions

This study was conducted on 15 *Oryctolagus cuniculus* local rabbits, males, healthy adults, weighing between 1750 g and 2190 g at the beginning of the experiment. The animals were housed 2 or 3 per cage in a standardized environment at room temperature with a light-dark cycle of 12 h. Food and water were provided *ad libitum*. The study was approved by Faculty of sciences, University of Skikda, Algeria.

Experimental Design

The animals were divided into three groups of five rabbits each:

- Normal control (CRL): This group received a normal diet
- Hyperlipidemic control (EY): This group received a normal diet + 7 mL kg^{-1} body weight of egg yolk
- Tested group (PLVFO): This group received a normal diet +7 mL kg^{-1} body weight of egg yolk + PLVFO at a dose of 1 mL kg^{-1} body weight

Rabbits were gavaged using a stomach tube (egg yolk and oil), once daily, 6 days a week for 28 consecutive days. PLVFO was administered 15 min after administration of egg yolk to avoid interaction. All animals were controlled for their general state and were weighted weekly until 28th day.

At the end of the experiment, the animals were fasted overnight and for biochemical analysis, samples of blood from the marginal ear vein were collected into heparinized tubes.

Biochemical Assays

Blood samples were centrifuged at 4000 rpm for 4 min and then plasma was separated. Selected blood parameters were carried out by an automatic analyzer and included Total Cholesterol (TC), Triglycerides (TG), High Density Lipoprotein (HDL), Aspartateamino Transferase (AST), Alanine Transaminase (ALT), creatinine, urea and uric acid. Low Density Lipoproteins (LDL) was calculated using Friedewald equation, the atherogenic index was calculated as reported by Hua *et al*. (2009): Atherosclerosis index = (serum TC- HDL-C)/HDL-C.

Anatomo-Pathological Study and Carcass Characteristics

After slaughtering animals on the 29th day of the experiment, they were dissected to examine the internal organs: Liver, kidneys, lungs, spleen, adrenals and heart. These organs were subjected to a quantitative and qualitative macroscopic examination (external structure, color, consistency and texture). These organs were weighed immediately to avoid their desiccation. Other zoo technical and carcass quality parameters were investigated: Live weight, slaughter weight, dressed weight, dressing percentage, head and pelt with lambs weight, full gastrointestinal tract and dissectible fat weights.

Statistical Analysis

Statistical data were presented as mean with SD and analyzed by one-way analysis of variance (ANOVA). The level of significance was $p<0.05$.

Results

Throughout the trial period, no deaths have been reported and no serious clinical signs of toxicity were observed in animals. All rabbits remained healthy and were available for evaluation.

Body Weight

Weekly weighed rabbits have shown that the difference between the averages of the different groups was not significant.

The average weight of the control group (CRL) has evolved gradually from 1855±108.972 g to 2028.66±202.02 g towards the end of the experimental period.

The group fed by the Egg Yolk (EY) has shown a gradual increase until the 3rd week (1958±189.233 g to 2061.6±225.22 g); thereafter there was a slight decrease recording 2006±189.145 g on the 28th day. While the PLVFO group recorded an increase in weight during the first 3 weeks (1908.33±289.233 g on day 0 and 1974±268.813 g at 21st day) followed by a more interesting decrease compared to EY group, but this decrease was not significant by scoring a weight of 1904.66±356.875 g at the end of the experiment.

Biochemical Analysis

Lipidemic Profile

Statistical analysis of biochemical parameters was represented in Fig. 1-5. The rabbits gavaged Egg Yolk (EY group) showed a very significant increase in cholesterol (TC) (P = 5,75678E^{-4}) compared with CRL group. Hypercholesterolemia induced by egg yolk has been reduced, but not significantly in PLVFO group.

The HDL was increased very significantly in the PLVFO group compared to the CRL and EY groups (p = 6,50094E^{-7} and p = 7,46127E^{-4} respectively).

The LDL was increased very significantly in EY group compared to CRL group (p = 2,0814E^{-4}). The administration of the vegetable oil in PLVFO group resulted in a significant decrease of this parameter (p = 0.0174), the difference between this group and CRL group was not significant. For triglycerides, there was no significant difference between all the three groups.

The atherogenic risk was significantly increased in rabbits from EY group compared to CRL group. In PLVFO group, this risk was significantly decreased (P = 0.00385) compared to the group fed with egg yolk, noting that the difference with CRL group was not significant.

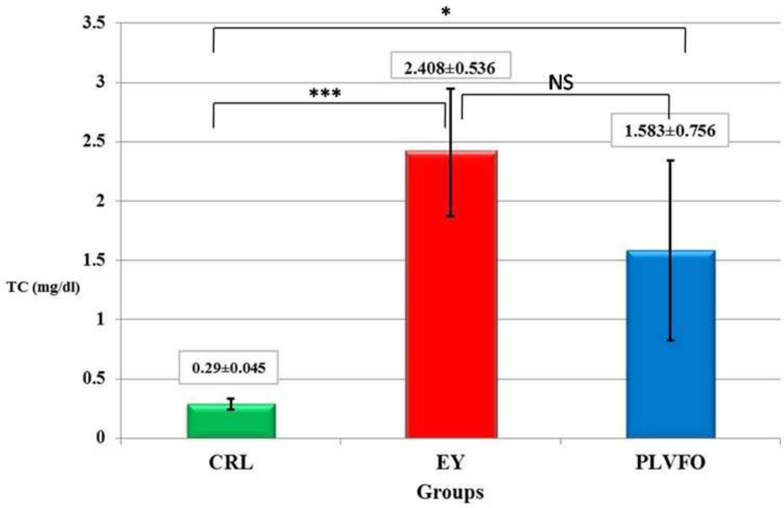

Fig. 1. Comparison of plasma total cholesterol levels among the different rabbits groups at 28th day of experiment (Mean ± SD), CRL: Control or untreated rabbits, EY: Rabbit's gavaged egg yolk, PLVFO: Rabbit's gavaged egg yolk and treated with *Pistacia lentiscus* virgin fatty oil. NS: no significant (P>0.05), * (P<0.05), *** (p<0.001), n = 5

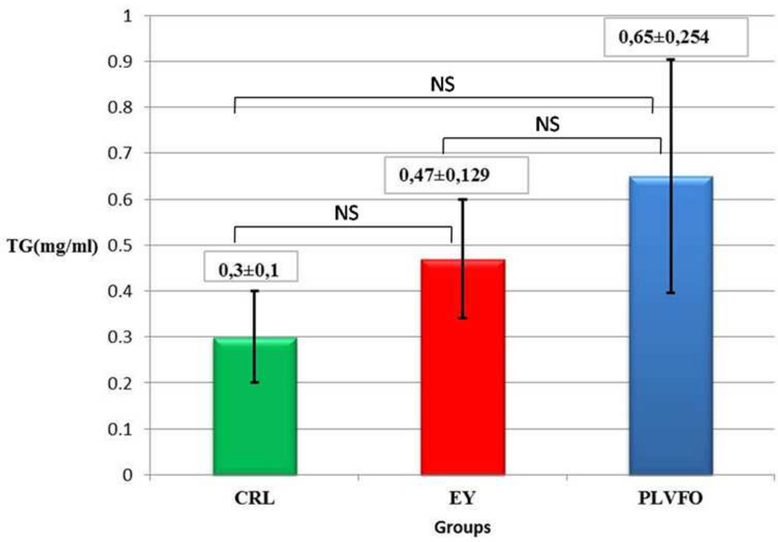

Fig. 2. Comparison of plasma triglycerides levels among the different rabbits groups at 28th day of experiment (Mean ± SD), CRL: Control or untreated rabbits, EY: Rabbit's gavaged egg yolk, PLVFO: Rabbit's gavaged egg yolk and treated with *Pistacia lentiscus* virgin fatty oil. NS: No significant (P>0.05), n = 5

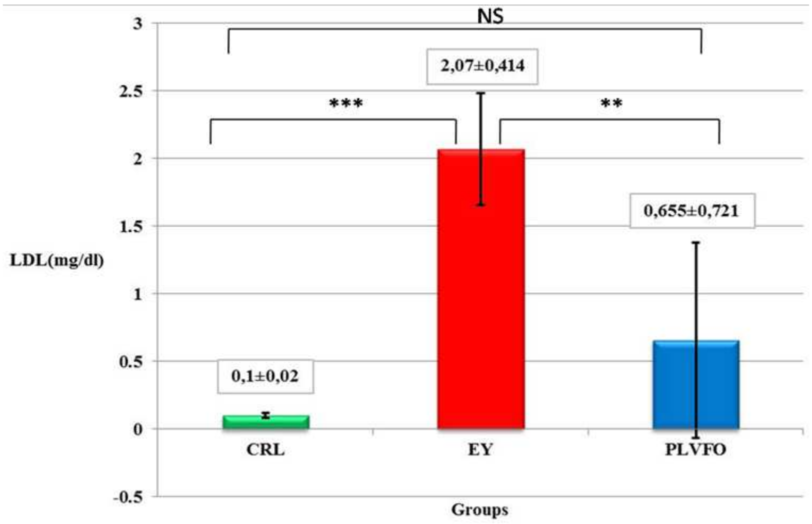

Fig. 3. Comparison of plasma LDL_Cholesterol levels among the different rabbits groups at 28th day of experiment (Mean ± SD), CRL: Control or untreated rabbits, EY: Rabbit's gavaged egg yolk, PLVFO: Rabbit's gavaged egg yolk and treated with *Pistacia lentiscus* virgin fatty oil. NS: No significant (P>0.05), ** (P<0.01), *** (p<0.001), n = 5

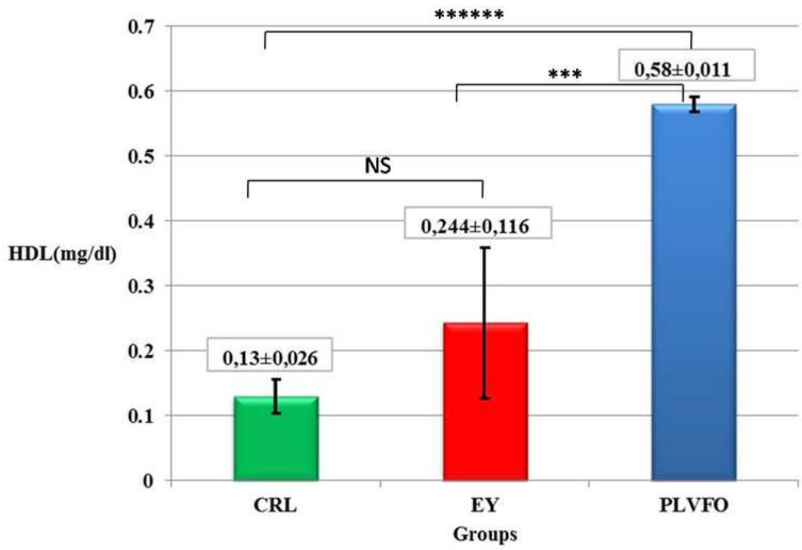

Fig. 4. Comparison of plasma HDL_Cholesterol levels among the different rabbits groups at 28th day of experiment (Mean ± SD), CRL: Control or untreated rabbits, EY: Rabbit's gavaged egg yolk, PLVFO: Rabbit's gavaged egg yolk and treated with *Pistacia lentiscus* virgin fatty oil. NS: No significant (P>0.05), *** (p<0.001), ****** (P<0.000001), n = 5

Hepatorenal Profiles

The results of hepatic and renal functions were recorded in Table 1. Aspartate Aminotransferase (AST) showed a significant increase in PLVFO group compared to CRL and EY groups (p = 0.00339 and p = 0.00588 respectively), in the EY group a less significant increase in that of PLVFO group was noted.

Alanine Aminotransferase (ALT) was increased but not significantly among PLVFO rabbits compared to other animals of CRL and EY groups.

The two parameters, creatinine and urea marked a slight increase but not significantly in EY and PLVFO groups. However, uric acid showed a significant increase in the animals of both groups compared to CRL group (P<0.001).

Anatomo-Pathological Study and Carcass Characteristics

The general appearance of rabbit's organs of different groups and their relative weights were normal. Statistical analysis (Table 2 and 3) did not show significant differences, except for abdominal dissectible fat, which was significantly decreased in the PLVFO group compared to CRL and EY groups (p<0, 01 and p<0, 05 respectively).

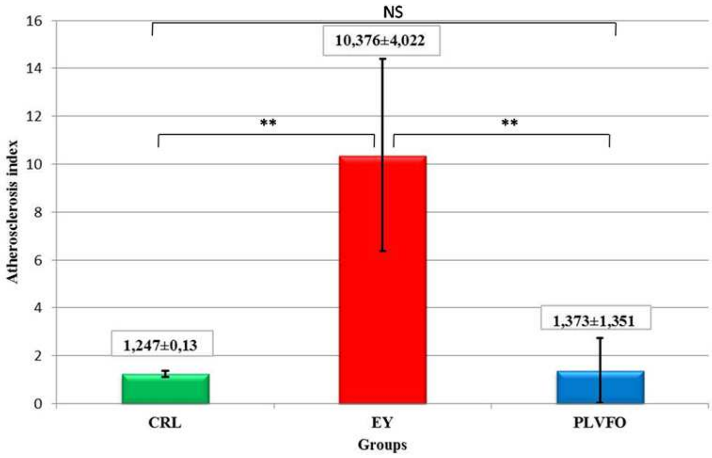

Fig. 5. Comparison of atherogenic index among the different rabbits groups at 28th day of experiment (Mean ± SD), CRL: Control or untreated rabbits, EY: Rabbit's gavaged egg yolk, PLVFO: Rabbit's gavaged egg yolk and treated with *Pistacia lentiscus* virgin fatty oil. NS: No significant (P>0.05), ** (p<0.01), n = 5

Table 1. Hepatorenal profile of different rabbit groups at 28th day of experiment

	Blood parameters				
Animal groups	ALT	AST	Creatinine	Urea	Uric acid
CRL	36,33±2,516	19,33±2,081	4,85±4,454	0,36±0,181	1,865±0,780
EY	43,33±6,429	31,33±5,773	6±2,645	0,376±0,147	6,998±1,398
PLVFO	86,75±48,671	124,25±33,876	7,9±7,353	0,24±0,01	7,112±1,418
Statistical data (*P value*)					
EY Vs CRL	0,153	0,027	0,732	0,898	3.29E-01
PLVFO Vs EY	0,194	0,005	0,691	0,184	0,906
PLVFO Vs CRL	0,140	0,003	0,665	0,310	6.40E+00

Values are expressed as Mean ± SD (n = 5)

Table 2. Relative organ weights in different rabbit groups at 29th day of experiment

	Organ weights						
Animal groups	Liver	Kidneys (2)	Heart	Adrenals (2)	Spleen	Lungs	Testes (2)
CRL	0,02945 ±0,00549	0,00558 ±0,00117	0,00263 ±1,457E^{-4}	4,446E^{-4} ±5,008E^{-5}	5,113E^{-4} ±6,33E^{-5}	0,00585 ±8,62E^{-4}	0,00409 ±3E^{-4}
EY	0,03589 ±0,00244	0,00515 ±6,785E^{-4}	0,00238 ±4,64E^{-4}	4,303E^{-4} ±1,82E^{-5}	4,373E^{-4} ±2,28E^{-5}	0,00615 ±0,00139	0,00379 ±7,422E^{-4}
PLVFO	0,02729 ±0,00479	0,00509 ±1,65E^{-4}	0,00264 ±4,00042E^{-4}	4,873E^{-4} ±9,171E^{-5}	4,71E^{-4} ±3,143E^{-5}	0,00649 ±0,00183	0,00404 ±8,706E^{-4}
Statistical data (*P value*)							
EY Vs CRL	0,13661	0,60914	0,43553	0,66559	0,12974	0,76614	0,5517
PLVFO Vs EY	0,0504	0,88892	0,51373	0,35063	0,20764	0,81368	0,72431
PLVFO Vs CRL	0,6357	0,51059	0,9695	0,51848	0,3792	0,61611	0,9296

Values are expressed as Mean ± SD (n = 5)

Table 3. Carcass characteristics of different rabbit groups at 29th day of experiment

	Groups			Statistical data (*P value*)		
Parameters	CRL	EY	PLVFO	EY Vs CRL	PLVFO Vs EY	PLVFO Vs CRL
Live weight (g)	2028,66±202,02	2006±189,14	1904,6±35,87	0,894	0,686	0,6281
Slaughter weight (g)	1995,33±204,02	1975±190,49	1873,66±343,8	0,905	0,678	0,626
Dressed weight (g)	1078,33±65,89	961,33±34,78	907±132,02	0,053	0,528	0,114
Dressing %	54,58±8,532	48,86±3,032	48,643±1,99	0,335	0,922	0,305
Head (g)	210±26	185,66±1,04	199,33±33,50	0,226	0,550	0,685
Pelt with lambs (g)	298,33±66,42	270,33±25,92	245± 58,96	0,533	0,533	0,357
Full gastrointestinal tract (g)	250,33±40,26	390±100,13	333,6±51,31	0,088	0,434	0,091
Dissectible fat (g)	3,83±1,04	19,49±5,75	8,33±2,88	0,009	0,039	0,063

Values are expressed as Mean ± SD (n = 5)

Discussion

In the present study, the gavage of rabbits during 28 days by *Pistacia lentiscus* virgin fatty oil has resulted in a slight reduction of body weight at the end of experiment accompanied by a significant reduction of dissectible fat; which suggest that this vegetable oil may be consumed to prevent obesity. This natural product has recorded a non significant decrease of total cholesterol but a significant decrease of LDL-C and a significant increase of HDL-C with an important amelioration of atherogenic index. In our previous study (Djerrou, 2014), this oil has decreased significantly total cholesterol in female rabbits, when applied at a dose of 2 mL kg^{-1} BW during 6 weeks. We note that in the current study conducted on males rabbits, the dose tested was only 1 mL kg^{-1} BW during 28 days, the egg yolk was gavaged at a dose higher than the previous study (7 mL kg^{-1} BW). The vegetable oil extracted from the same region in 2014 has shown a high level of Monounsaturated Fatty Acids (MUFA: 55.76%), a 20.45% of Poly Unsaturated Fatty Acids (PUFA), a 23.53% of Saturated Fatty Acids (SFA) with a good ratio of PUFA/SFA (ratio = 0.86) (Djerrou, 2014). The unsaponifiable fraction of *P. lentiscus* fatty oil contains sterols (cholesterol, campesterol, stigmasterol and β-sitosterol), tocopherols and phenolic components (Djerrou *et al*., 2010; Trabelsi *et al*., 2012). Several components existing in this oil were investigated and confirmed to be implicated in preventing hyperlipidemia, LDL oxidation and prevention of atherosclerosis as: Oleic acid (C18:1), Omega-3 PUFAs, Conjugated Linoleic Acids (CLAs), α-tocopherol, squalenes and phenolic components (Harris *et al*., 1997; Newmark, 1997; Kohr *et al*., 1998; Kris-Etherton, 1999; Visioli *et al*., 2002).

The current study has also shown for the first time that the application of *P. lentiscus* fatty oil for 28 days may increase transaminases enzymes activities particularly AST; noting here that several hypolipidemic drugs were known to increase transaminases activities.

Conclusion

The study concludes that *Pistacia lentiscus* virgin fatty oil may be consumed to prevent hyperlipidemia and obesity. However, it should be taken with precaution because it may increase transaminases enzymes activities in prolonged use.

Acknowledgement

We would like to thank the staff of Department of Sciences of Nature and Life, University of August 20th 1955 Skikda, for help in achieving the experiment. A particular thank to N. Boutobza (J. GR) for assistance in biochemical analysis.

Author’s Contributions

Zouhir Djerrou: The first author designed and supervised the study and assisted in data analysis and manuscript preparation.

Noudjoud Boutobza: Sample collection and laboratory experiments and participated in data analysis.

Saida Bouzeguine and Khadidja Saci Hadef: Sample collection and participated in laboratory experiments.

Ilhem Brighet, Besma Khelfa and Imane Mokhbi: Participated in laboratory experiments.

Youcef Hamdi Pacha: Participated in results interpretation.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

References

Boukef, K. and H.R. Souissi, 1982. Contribution à l’étude des plantes médicinales en médecine populaire en Tunisie. Rev. Soc. Pham. Tunisie., 2: 34-35.

Boukeloua, A., A. Belkhiri, Z. Djerrou, L. Bahri and N. Boulebda *et al*., 2012. Acute toxicity of *Opuntia ficus indica and Pistacia lentiscus* seed oils. Afr. J. Tradit. Compl. Altern. Med., 9: 607-611. DOI: 10.4314/ajtcam.v9i4.19

Djerrou, Z., 2014. Anti-hypercholesterolemic effect of *Pistacia lentiscus* fatty oil in egg yolk-fed rabbits: A comparative study with simvastatin. Chinese J. Natural Med., 12: 0561-0566. DOI: 10.1016/S1875-5364(14)60086-8

Djerrou, Z., H. Djaalab, F. Riachi, M. Serakta and A. Chettoum *et al*., 2013. Irritancy potential and sub acute dermal toxicity study of *Pistacia lentiscus* fatty oil as a topical traditional remedy. Afr. J. Tradit. Complement Altern Med., 10: 480-489. DOI: 10.4314/ajtcam.v10i3.15

Djerrou, Z., Y. Hamdi-Pacha, A.M. Belkhiri, H. Djaalab and F. Riachi *et al*., 2011. Evaluation of *Pistacia lentiscus* fatty oil effects on glycemic index, liver functions and kidney functions of New Zealand rabbits. Afr. J. Tradit. Complement Altern Med., 8: 214-219. DOI: 10.4314/ajtcam.v8i5S.27

Djerrou, Z., Z. Maamari, Y. Hamdi-Pacha, M. Serakta and F. Riachi *et al*., 2010. Effect of virgin fatty oil of *Pistacia lentiscus* on experimental burn wound’s healing in rabbits. Afr. J. Trad. CAM., 7: 258-263. DOI: 10.4314/ajtcam.v7i3.54788

Harris, W.S., G. Lu, G.S. Rambjor, A.I. Walen and J.A. Ontko *et al*., 1997. Influence of n-3 fatty acid supplementation on the endogenous activities of plasma lipases. Am. J. Clin. Nutr., 66: 254-260.

Hua, C., L. Li-Jun, Z. Jian-Jun, X. Bo and L. Rui, 2009. Effect of soybean oligosaccharides on blood lipid, glucose levels and antioxidant enzymes activity in high fat rats. Food Chem., 4: 1633-1636. DOI: 10.1016/j.foodchem.2009.09.056

Kohr, H.T., R. Rajendran and M. Gopalakrishnan, 1998. The role of unsaponifiable components in the lipidemic property of olive oil. Malayas J. Nutr., 4: 73-80. PMID: 22692344

Kris-Etherton, P.M., 1999. Monounsaturated fatty acids and risk of cardiovascular disease. Circulation, 100: 1253-1258. DOI: 10.1161/01.CIR.100.11.1253

Maameri, Z., Z. Djerrou, S. Halmi, H. Djaalab and F. Riachi *et al*., 2015. Evaluation of hepatoprotective effect of *Pistacia lentiscus* L. fatty oil in rats intoxicated by carbon tetrachloride. Int. J. Pharmacognosy Phytochem. Res., 7: 251-254.

Maameri, Z., Z. Djerrou, S. Habibatni, F. Riachi and H. Djaalab *et al*., 2016. Physicochemical characteristics and sub chronic oral toxicity of *Pistacia lentiscus* L. vegetable oil in rabbits. Online J. Biol. Sci., 16: 43-48. DOI: 0.3844/ojbsci.2016.43.48

Newmark, H.L., 1997. Squalene, olive oil and cancer risk: A review and hypothesis. Cancer Epidem Biomar., 6: 1101-1103. PMID: 9419410

Tounes, M., C. Abdennour and N. Houaine, 2008. Influence of *Pistacia lentiscus* oil on serum biochemical parameters of domestic rabbits *Oryctolagus cuniculus* in mercury induced toxicity. Eur. J. Sci. Res., 24: 591-600.

Trabelsi, H., O.A. Cherif, F. Sakouhi, P. Villeneuve and J. Renaud *et al*., 2012. Total lipid content, fatty acids and 4-desmethylsterols accumulation in developing fruit of *Pistacia lentiscus* L. growing wild in Tunisia. Food Chem., 131: 434-440. DOI: 10.1016/j.foodchem.2011.08.083

Visioli, F., A. Poli and C. Gall, 2002. Antioxidant and other biological activities of phenols from olives and olive oil. Med. Res. Rev., 22: 65-75. DOI: 10.1002/med.1028