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# Bioscience Research

Print ISSN: 1811-9506 Online ISSN: 2218-3973

*Journal by Innovative Scientific Information & Services Network*

**RESEARCH ARTICLE** BIOSCIENCE RESEARCH, 2024 21(4): 726-737. **OPEN ACCESS**

# Physicochemical properties and hepatoprotective effect of *Pistacia lentiscus L.* Oil against carbon tetrachloride-induced liver damage in rats

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Pressed oil extracted from *Pistacia lentiscus* fruit was investigated for its hepatoprotective effect, using $CCl_4$ intoxicated rats. The oil was administrated intraperitoneally at 5 and 10 g $kg^{-1}$ of body weight. It was first characterized, and then its antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl scavenging assay. Serum catalase, superoxide dismutase, thio-barbituric acid-reactive substances activities, glutathione levels, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, glutamyl transferase, and bilirubin levels were investigated *in vivo*. The oil physicochemical characterization showed high carotenoids and polyphenols contents, with oil antioxidant activity ($IC_{50}$) of 3.5 mg $ml^{-1}$. *In vivo*, the $CCl_4$-intoxicated rats showed high levels of all the serum parameters, except the SOD activity. *Pistacia lentiscus* oil administration at 5 and 10 mg $kg^{-1}$ body weight, significantly decreased the serum parameters and restored the antioxidant effects. The results exhibited *Pistacia lentiscus* oil nutraceutical properties beneficial for medicinal and agro-food industries.

**Keywords: *Pistacia lentiscus* L. oil, hepatotoxicity, antioxidant activity, $IC_{50}$, serum parameters.**

## INTRODUCTION

*Pistacia lentiscus* L., commonly referred to as pistachio, pertains to the family of Anacardiaceae and grows in soil characterized by scarcity of water and nutrients and exposure to high solar radiation and temperatures for long periods (Gamli and Hayoglu, 2007; Bampouli et al. 2015; Mezni et al. 2020). *Pistacia lentiscus* L. has green leaves that attribute a strong aroma; which is widely available in the Mediterranean countries, especially in Tunisia (Trabelsi et al. 2012; Khedir et al. 2018; Mezni et al. 2018). The oil's active biological substance, like polyphenols, flavonoids, and essential fatty acids, provide it with significant therapeutic benefits, including anti-inflammatory, antioxidant, and antimicrobial properties (Bampouli et al. 2015; Trabelsi et al. 2012; Brahmi et al. 2020; Mezni et al. 2020). This functional ingredient is widely used in various foods and dietary supplements due to its health-promoting qualities. *Pistacia* species play a prominent role in traditional medicine, valued for their potent antioxidant, antimicrobial, anti-cholinesterase, anti-inflammatory, and cytotoxic effects, primarily owing to their phenolic and flavonoid component (Gardeli et al. 2008; El Bishbishy et al. 2020; Brahmi et al. 2020; Mezni et al. 2018). Specifically, *Pistacia* L*entiscus L. leaves have been utilized in the treatment of various ailments* notably eczema, paralysis, diarrhea, ulcers, jaundice, kidney stones, throat infections, asthma, stomachaches, diabetes, acute hypertension, fevers, indigestion, and pectoral and stimulant (Bampouli et al. 2015; Cherbal et al. 2017; El Bishbishy et al. 2020; Chebab et al. 2016). By neutralizing free radicals, Antioxidants are crucial in slowing or preventing oxidative stress. Recently, the strategy of incorporating antioxidants, especially those from natural sources, into diets has gained traction as a protective measure against oxidative damage (Hamidi et al. 2020). Organisms have evolved complex regenerative systems to safeguard functional molecules and structures

from the harmful effects of both external and internally generated free radicals during metabolic processes. The elimination of intracellular by-products by aerobic metabolism includes enzymatic processes such as catalase (CAT), superoxide dismutase (SOD), and the glutathione peroxidase-glutathione system (Zachara et al. 2006). Normally, the natural protective systems of organisms can face up to the radicals and keep the process in equilibrium by controlling their hazardous effects. However, this equilibrium may be disturbed by exogenous factors in some metabolic conditions. Oxidative stress, marked by an accumulation of reactive compounds, arises from an imbalance between nitrogen species and the formation and elimination of reactive oxygen species (ROS), contributing to various serious diseases and the aging process (Valko et al. 2016). Recently, the intake of vegetables, fruits, and plants high in antioxidants has been proven to reduce the risk of cardiovascular and cancer diseases (Janakat & Al Merie, 2002; Brahmi et al. 2020). This has spurred interest in natural antioxidants as alternatives to synthetic antioxidants, with a particular focus on plant-derived secondary metabolites. The protective effects of these plants are attributed to polyphenols, flavonoids, and anthocyanins, which can neutralize free radicals like superoxide, peroxyl, hydroxyl, and alkoxyl through hydrogen transfer reactions (Gardeli et al. 2008; Bampouli et al. 2015; Bouyahya et al. 2018; Brahmi et al. 2020). Beyond directly scavenging free radicals, these compounds may also inhibit enzymes or chelate trace metals that catalyze ROS formation (Valko et al. 2016).

Multiple studies have investigated the chemical characteristic of *P. lentiscus* in Mediterranean countries, highlighting its antioxidant properties, which have been linked to Its capacity to eliminate free radicals like DPPH (Trabelsi et al. 2012; Belyagoubi-Benhammou et al. 2018; Bouyahya et al. 2018; Mezni et al. 2018; Brahmi et al. 2020; Ljubuncic et al. 2005; Gardeli et al. 2008; Bampouli et al. 2015). The fruits are also edible and contain unsaturated fatty acids and other bioactive compounds, reducing risks associated with coronary heart disease, hypertension, inflammation, cancer, and diabetes. A study conducted in the K562 cell line found that gallic acid, its derivatives, and 1,2,3,4,6- pentagalloylglucose protected against $H_2O_2$-induced lipid peroxidation (Abdelwahed et al. 2007). However, studies on the antioxidant properties of the *P. lentiscus* extract remain limited (Ljubuncic et al. 2005; Gardeli et al. 2008; Bampouli et al. 2015).

The aims of the current study were to characterize the physical-chemical properties of vegetative oil extracted from *Pistacia lentiscus* L. fruit and evaluate its efficiency against $CCl_4$ rat liver damage.

## MATERIALS AND METHODS

### Plant material

The *Pistacia lentiscus* L. fruit was obtained from wild bushes growing in the Ain Drahem city in northwest Tunisia (36°48′N-10°11′E), an area noted for its humid climate and high average annual rainfall of 1534 mm. An identification of the plant was performed at the Faculty of Sciences Sfax University, focusing on the Tunisian flora (Cuenod, 1954) and a specimen certification has been preserved in the National Research Institute of Rural Engineering, Water and Forestry, Tunisia (VS1-PL2009) (Mezni et al. 2018). Fruit characteristics and composition were analyzed for vegetable oil extraction, using the traditional cold pressing method. The extracted oil was then characterized, and its biological activities were assessed.

### Animals and treatments

The study used Wistar rats that are aged adult males (45 days old, 130 grams in weight) obtained from the Tunisian Central Pharmacy. Animals were confined to a regulated environment (20 °C; 55-60% humidity; 12-hour dark/light cycle) with unrestricted water and food access. The animals were given a typical laboratory diet (SICO, Sfax, Tunisia) and, they fasted overnight before collecting blood and tissue. Following the local Ethical Committee's Guidelines for the Care and Use of Laboratory Animals, laboratory animal handling procedures have been approved.

To induce hepatotoxicity, rats received a weekly intraperitoneal injection of a $CCl_4$ and olive oil mixture (1:3) at a dosage of 2 ml $kg^{-1}$ of body weight over one month. Five groups of eight rats each were randomly selected from among the rats:

- G 1: Negative, non-treated group.
- G 2: Positive, group treated with *P. lentiscus* oil (5 grams per kilogram of body weight).
- G 3: Exposed to $CCl_4$/olive oil but untreated with *P. lentiscus* oil.
- G 4: Treated with $CCl_4$/olive oil and *P. lentiscus* oil at 5 g $kg^{-1}$ body weight.
- G 5: Treated with $CCl_4$/olive oil and *P. lentiscus* oil at 10 g $kg^{-1}$ body weight.

Body weight was registered daily. When the experiment was finished, rats were anesthetized with ether and decapitated. Samples of arterial blood (~10 ml) were collected to measure plasma glutathione peroxidase (GPx) levels and standard serum liver function indices. For liver function assessment, blood samples were clotted, and centrifuged. An autoanalyzer (Hitachi, Type 7170A) was used to measure AST, ALP, ALT, and total bilirubin in the serum at Habib Bourguiba Hospital University Center (Sfax). To facilitate additional analysis, all samples were kept at -80 °C.

## Physico-chemical analyses

### Fruit characterization

A study was conducted on ten different fruits to assess their fruit and stone middleweight, as well as the pulp-to-stone yield. The fruits were dried at 105 °C to measure their dry matter (DM) and $H_2O$ content. Ash was evaluated by calcining dry matter at 550 °C for 6 h, while nitrogen (TN) was measured using the Kjeldahl method, a standard technique for analyzing nitrogen in organic materials. Additionally, the ash was mineralized with concentrated nitric acid for mineral analysis, and using atomic absorption (Hitachi Z-6100, Japon), both micro- and macro-elements were examined.

### Oil physicochemical properties

The oil density and viscosity were determined using a densitometer and a d'Ubbelholde capillary viscometer respectively. The oil refractive index was measured using an Abbe refractometer (EEC, 1991). The acidity of the oil was assessed by weighing free oleic acid, which was obtained through the chemical degradation of the oil. This process increased free acidity and intensified the rancid organoleptic characteristics as additional fatty acids were released from the glycerides. The iodine, peroxide, and saponification indices were determined following the AOAC (2005) guidelines, while the coefficients of specific extinction K232 and K270 were measured based on the standards established by the European Economic Community (EEC, 1991). Lastly, phenolic compounds were quantified spectrophotometrically by using the Folin Ciocalteu reagent method (Singleton and Rossi, 1965), by using standard gallic acid. The chlorophylls and the carotenoid levels were determined using a spectrophotometer Hitachi U200, based on the formulations expressed in mg $l^{-1}$ as follows (EEC, 1991):

- [Chlorophyll a] = 12.70 x $DO_{663}$ – 2.69 x $DO_{645}$
- [Chlorophyll b] = 22.90 x $DO_{645}$ – 4.68 x $DO_{663}$
- [Carotenoids] = 4.07 x $DO_{450}$ - 0.0435 Chlorophyll a – 0.367 Chlorophyll b

### Fatty acids analysis

A Hewlett-Packard gas chromatograph and an HP 5973 mass spectrometer (Agilent Technologies) were utilized to analyze the oil fatty acid. For this analysis, a capillary column (HP-5MS) with dimensions of 60 m in length, 0.25 mm in internal diameter, and 0.25 µm was employed. Helium served as the carrier gas, flowing at a rate of 1.5 ml $min^{-1}$. From 40 °C to 280 °C, the column oven was heated by increasing it at a rate of 5 degrees per minute until it reached 280 °C, which was kept for 5 minutes. A diluted sample of 1 µl was manually injected. The oil's constituent identification was achieved by matching the retention indices to homologous sequences of n-alkanes and interpreting the mass spectrometry data against the Wiley 275 Mass Spectral Library (http://eu.wiley.com/WileyCDA/WileyTittle/productCd-04-70047852.html).

### DPPH radical scavenging assay

The modified Kirby and Schmidt method (1997) was used to evaluate the activity of DPPH radical reducing in *Pistacia lentiscus* oil. In summary, 0.5 ml of oil solution with varying concentrations (0.4 to 10 mg $ml^{-1}$ in ethanol) was mixed with 125 µl of a solution (0.2 mM) of DPPH to produce free radicals. The reaction mixtures were kept in darkness for an hour at room temperature.

The scavenging capacity was assessed by measuring the absorbance at 517 nm. The smaller absorbance values are largely associated with radical scavenging. The results were expressed as a percentage of inhibition concerning the IC50 value. The formula used for calculating the percentage of DPPH radical scavenging was as follows:

DPPH radical scavenging activity (%) = [$A_{control}$ – $A_{sample}/A_{control}$] × 100, Here $A_{control}$ represents the reaction absorbance with all components excluding the sample, and $A_{sample}$ denotes the absorbance in the presence of *P. lentiscus* oil. The test was conducted in triplicate. The IC50 value, as defined by the needed concentration for a 50% reduction in DPPH activity, represents the potency of radical scavenging.

## Biological activities evaluation

### Lipid peroxidation evaluation

Thiobarbituric acid-reactive substances (TBARS) were used to quantify lipid peroxidation levels using the method established by Yagi in 1976. A 1 g liver sample was finely minced and mixed with 2 ml of Tris-buffer saline (TBS, pH 7.4), following sonication twice for 10 seconds each. The homogenate was centrifuged after that at 5000 g for 30 min at 4 °C, and the supernatant obtained was stored at −80 °C. For the TBARS measurement, a 125 µl aliquot of the supernatant was syndicated with 50 µl of TBS and 125 µl of TCA-BHT to precipitate the proteins. Then, the mixture was centrifuged for 10 min at 1000 g at 4 °C. 200 µl of the supernatant was mixed with 40 µl of HCl (0.6 M) and 160 µl of TBA, and then heated for 10 min at 80 °C. The absorbance was determined at 530 nm, and TBARS concentration was determined using a 156 $mM^{-1}$ $cm^{-1}$ extinction coefficient.

### Antioxidant enzyme activities

The activities of CAT, SOD, and GPx were measured in liver tissue. Catalase activity was evaluated using the Aebi (1984) method that used hydrogen peroxide ($H_2O_2$) as substrate. The reaction starts by adding $H_2O_2$, and its decomposition was observed by counting the decrease in absorbance at 240 nm over one minute. The extinction coefficient of 0.043 $mM^{-1}$ $cm^{-1}$ was used to calculate

enzyme activity. SOD activity was determined by assessing the ability to inhibit the photo-reduction of nitro-blue tetrazolium (NBT), following the protocol outlined by Asada et al. (1974). SOD One unit is established, as a necessary quantity achieving 50% inhibition of NBT photo-reduction. Glutathione peroxidase (GPx) levels were estimated using a modified version of Ellman's method (1959), which relies on the yellow color produced when dithiobisnitrobenzoic acid (DTNB) reacts with sulfhydryl-containing compounds. In this method, liver supernatant (0.2 ml) was homogenized with 4% sulfosalicylic acid solution (3 ml) and then centrifuged for 15 min at 2500 g. 0.2 ml of the supernatant was then mixed with 0.4 ml of DTNB (10 mM) and 1 ml of phosphate buffer (0.1 M, pH 7.4), and at 412 nm, the absorbance was determined. GPx activity is stated in µmoles of GSH per mg of protein.

### Liver function assessment

By centrifugation of rats' blood (at 1610 g for 15 min at 4 °C), plasma was obtained. Activities of serum ALT, AST, GT, PAL, and bilirubin were assessed using Biomerieux enzymatic test kits (Lyon, France) per the manufacturer's instructions, measured on an automatic analyzer (Hitachi 7170A, Japan).

### Statistical analysis

All results are expressed as mean ± standard deviation. Comparison between groups was undertaken using one-way ANOVA, and the Student's t-test, by using a statistical significance of $p \leq 0.05$. All analyses were conducted in triplicate.

## RESULTS AND DISCUSSION

### Fruit physicochemical characterization

*Pistacia lentiscus* fruit was characterized by relatively high humidity (42%) (Table 1) compared to *Pistacia vera* (9.6%) (Gamli and Hayoglu, 2007).

The studied fruit showed a mineral content three times as high as that of *Pistacia vera* (Gamli and Hayoglu, 2007). Magnesium was the most abundant element followed by calcium, potassium, and sodium. Furthermore, the *P. lentiscus* oil content extracted under cold conditions represented an important fraction in the fruit (Table 1) compared to that found by Charef et al. (2008) in Algerian *P. lentiscus* black fruit (32.8%) extracted by petroleum ether.The petroleum ether extraction method yielded an oil content of 42.54% when investigating the total lipid content accumulation in the fully ripe fruit of *P. lentiscus* that grows wild in Tunisia (Trabelsi et al. 2012). Using petroleum ether extraction, the oil content in fully ripe *Pistacia lentiscus* fruit from Tunisia was found to be 42.54%, similar to other species in the Anacardiaceae family (Trabelsi et al. 2012).

**Table 1: Physico-chemical characterization of *Pistacia lentiscus* fruit.**

| Parameters | |
|---|---|
| **Plant characterization** | |
| **Average weight (g)** | 0.066 ± 0.002 |
| **Report pulp/nucleus** | 1.04 ± 0.02 |
| **Dry matter (%)** | 58.09 ± 0.70 |
| **Oil (% DM)** | 38.19 ± 0.55 |
| **Total nitrogen (% DM)** | 0.86 ± 0.07 |
| **Total proteins (% DM)** | 3.23 ± 0.41 |
| **Ash (% DM)** | 2.24 ± 0.12 |
| **Mineral composition (mg/kg DM)** | |
| **Mg** | 1037.68 ± 0.15 |
| **Ca** | 150.40 ± 0.20 |
| **K** | 132.82 ± 0.43 |
| **Na** | 117.04 ± 0.13 |
| **Fe** | 62.80 ± 0.72 |
| **Zn** | 19.01 ± 0.54 |

Oil quality varies with soil, weather, fruit ripeness, and storage. Given its nutritional and economic value, *P. lentiscus* is considered a significant oleaginous plant.

### Oil characterization

### Physico-chemical properties and composition

*Pistacia lentiscus* oil was noted for its relatively higher density and viscosity, surpassing that of olive oil (Table 2). According to the International Olive Council (IOC), 2011, the specific extinction coefficient K232 conforms to the standard value of 2.55, while the K270 value exceeds the acceptable threshold of 0.22. The K232 coefficient reflects the oil's primary oxidation, indicating the polyunsaturated fatty conjugation acids. In contrast, K270 is associated with secondary oxidation products and serves as a marker for carboxylic compounds, including aldehydes and ketones found in the fruit (Atifi et al. 2017).

As a result, this oil showed resistance to oxidation at temperatures below 60 °C but becomes more susceptible to oxidation at elevated temperatures (IOC, 2001). Furthermore, the extinction values observed for *P. lentiscus* differ from those reported by other studies. Khedir et al. (2018) reported K232 and K270 values of 6.856 and 0.458, respectively. However, they were 0.093 and 0.133 respectively obtained by Brahmi et al. (2020). Nevertheless, the *Pistacia lentiscus* L. oil high saponification index is about the free fatty acids rate. Brahmi *et al.* (2020) revealed a high Algerian *Pistacia lentiscus* oil density (1.02) and acidity value (3.52). The same authors explained that this could indicate seeds' enzymatic hydrolysis during handling, oil harvesting, and bad preservation.

**Table 2: *Pistacia lentiscus* oil quality evaluation.**

| Characterization | Parameters | Oil |
|---|---|---|
| **General parameters** | Density (g/cm$^3$) | 0.919 ± 0.050 |
| | Viscosity (mPa.s) at 25 °C | 62.050 ± 0.120 |
| | Refractive index | 1.468 ± 0.002 |
| | Acidity | 1.200 ± 0.030 |
| | K232 index | 1.578 ± 0.070 |
| | K270 index | 0.310 ± 0.090 |
| **Indices** | Iodine (g $I_2$/100 g) | 32.081 ± 0.780 |
| | Peroxide (meq $O_2$/kg) | 3.200 ± 0.360 |
| | Saponification (mg KOH/g) | 215.000 ± 8.170 |
| **Pigments and polyphenols** | Chlorophyll a (mg/kg) Chlorophyll b (mg/kg) | 7.090 ± 0.170 10.490 ± 0.210 |
| | Carotenoids (mg/kg) | 19.652 ± 0.110 |
| | Polyphenols (mg gallic acid/kg) | 5.996 ± 0.280 |
| **Fatty acid (%)** | Oleic acid (C18:1) | 46 |
| | Palmitic acid (C16:0) | 27 |
| | Linoleic acid (C18:2) | 22 |
| | Palmitoleic acid (C16:1) | 2 |
| | Stearic acid (C18:0) | 2 |
| | Oleic/Linoleic | 2.1 |

The iodine index indicates the level of unsaturated fatty acids and ranges from 9.37 to 145 g $I_2$/100 g, which aligns with the values typically found in vegetable oils (Tan et al. 2002). Zahir et al. (2017) reported that the peroxide value assesses the level of peroxides in the oil, providing a crucial assessment of fats and oils stability. The peroxide value for *Pistacia lentiscus* oil was measured at 3.2, similar to that of corn oil (Tan et al. 2002) and less than the 7.2 meq $O_2$ kg$^{-1}$ noted by Khedir et al. (2018). The low peroxide content suggests that the oil was obtained shortly after harvesting, allowing it to maintain good quality during storage under appropriate conditions. This suggests that the oil is less prone to premature oxidation and will maintain its quality over time (Khedir et al. 2018). Additionally, *P. lentiscus* oil exhibited high levels of chlorophylls (a and b), ranging from 2 to 27 ppm (17.5 mg kg$^{-1}$), which are characteristic of virgin olive oil (IOC, 2001). The oil also contained a relatively high carotenoid content of 19.65 mg kg$^{-1}$, although these values were inferior to those found by Brahmi et al. (2020). As stated by Mezni et al. (2020), the carotenoid compounds identified in *P. lentiscus* oil included β-carotene, zeaxanthin, and lutein, with respective concentrations of 6.13; 1.35, and 2 mg kg$^{-1}$ of oil. The polyphenols content (6 mg kg$^{-1}$), a minor oil bio-molecules group with an important antioxidant activity, were at a considerable rate in the *P. lentiscus* oil. Consequently, the oil could be considered an abundant antioxidant natural source. According to Brahmi et al. (2020). At the onset of fruit ripening, the total polyphenol content in the oil reaches its peak before gradually decreasing until harvest, potentially leading to very low levels. This decline may, at least in part, explain the reduced total polyphenol content observed in the oils studied. The variation in pigment concentrations is influenced by several factors, including the extraction process, the level of maturity, and conditions of storage (Ramadan et al. 2003). Additionally, variations in growth conditions, fruit handling, soil characteristics, and geographical differences among the oils investigated can result in discrepancies in their physical and chemical parameters (Brahmi et al. 2020). In vegetable oils, the major pigments, chlorophylls a and b, carotenoids, and polyphenolic compounds are responsible for antioxidant activities. The chlorophyll pigment rates are important parameters in determining the oil color and its sensory quality. These pigments have pro-oxidizing roles in light and antioxidant activity in the darkness. In addition, the carotenoids are an oil color determinant and their rate exceeds slightly that of chlorophylls which explains the oil's intense yellow color. These pigments may establish powerful protective effects against photooxidation and may attract the oxygen singlet produced by the chlorophyllous pigments. β-Carotenes are biologically active compounds that play a vital role in neutralizing particular organic free radicals and quenching excited molecules, especially singlet oxygen (Hamidi et al. 2020).

Additionally, the fatty acid composition of the oil revealed five major acids, with oleic acid (C18:1) being the most prominent, followed by palmitic acid (C16:0) and linoleic acid (C18:2) (Table 2). However, palmitoleic (C16:1) and stearic (C18:0) acids were relatively at lower rates. Thus, lentisc oil can be classified as a monounsaturated oil, with its monounsaturated fatty acid content around 48%. Similar findings were observed in other *Pistacia lentiscus* oil samples collected from different locations in Tunisia (Khedir et al. 2018). The oleic/linoleic rate is an important factor in estimating oxidative oil stability. Indeed, the low oleic acid content and the high linoleic acid contribute to the decrease in oxidative stability (Pardo et al. 2007). This rate was equal to 2 exhibiting medium oxidative oil stability compared to that of olive oil exceeding the value of 3 (IOC, 2001). Elevated levels of oleic and linoleic acids protect against various metabolic disorders, making *P. lentiscus* oil nutritionally and industrially significant, particularly in the

pharmaceutical sector.

**Antiradical activity**

The DPPH, a commercial oxidizing radical, was used to study the antioxidant kinetics to quantify and compare different antioxidants' free radical scavenging capacities. The *P. lentiscus* oil DPPH radical scavenging activities increased with the oil concentration (Fig. 1a).

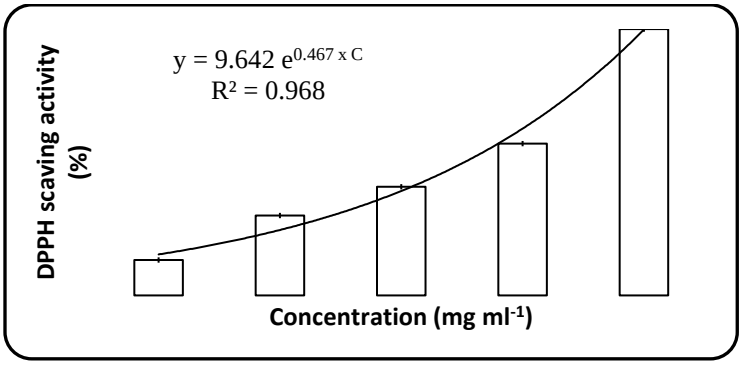
The inhibition percentage model fitted an exponential equation expressed as:

Inhibition percentage (%) = 9.64 $e^{0.47\ C}$ ($R^2$ = 0.968), where c is the oil concentration (mg $ml^{-1}$).

This model established the oil $IC_{50}$ that was equal to 3.5 mg $ml^{-1}$. Since the reduced $IC_{50}$ value indicates a strong antioxidant activity, the lipid fraction has a relatively considerable anti-radical activity compared to the $IC_{50}$ of *P. lentiscus* leaf extracts, ranging between 5.09 and 11.0 mg $l^{-1}$ (Gardeli et al. 2008). This value is lower than the IC50 of 1,2,3,4,6-pentagalloylglucose and gallic acid derived from *P. lentiscus* fruit, but it is nearly the same as that of vitamin E (Abdelwahed et al. 2007). Additionally, *P. lentiscus* oil from the same region (Tunisia) showed a notable anti-DPPH activity and reported an IC50 value of approximately 5.34 mg $ml^{-1}$ (Daoued et al. 2016). However, *P. lentiscus* oil harvested in Algeria exhibited a significantly higher IC50 of 20.62 mg $ml^{-1}$ (Belyagoubi-Benhammou et al. 2018) and an even higher value of 4170 mg $ml^{-1}$ (Brahmi et al. 2020). However, *P. lentiscus* oil from northern Morocco showed a strong DPPH• radical scavenging activity, yielding an IC50 of 37.38 µg $ml^{-1}$ (Bouyahya et al. 2018).

The *Pistacia lentiscus* L. ability to scavenge free radicals may stem from the beneficial action of its active components, including flavonoid content and phenolic acids.

**a)**




**b)**

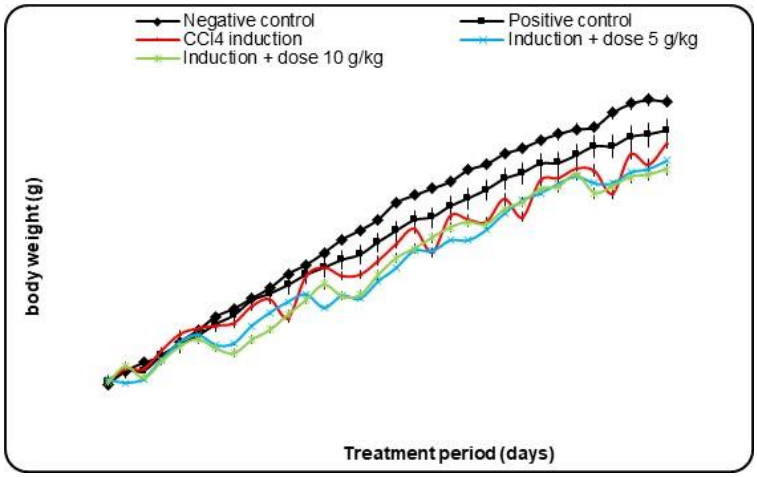



**Figure 1: DPPH radical scavenging effect of *Pistacia lentiscus L*. oil (a); The effect of daily oral dosing of 5 mg $kg^{-1}$ of *Pistacia lentiscus oil*, $CCl_4$ – treated rats added or not by 5 and 10 mg $kg^{-1}$ of oil on the rats body weights (b).**

The DPPH radical is capable of accepting either an electron or a hydrogen atom, resulting in steady diamagnetic compounds (Gardeli et al. 2008; Gonçalves et al. 2013; Can-Cauich et al. 2021). Researchers have revealed that *P. lentiscus* is abundant in phenolic acid, particularly gallic acid, and flavonoid compounds, such as myricetin derivatives and flavon-3-ol (Brahmi et al. 2020; Gonçalves et al. 2013; Trabelsi et al. 2012; Mezni et al. 2018). Additionally, Baratto et al. (2003) isolated galloyl derivatives from the *P. lentiscus* leaves, which were identified as effective free radical scavengers.

## Oil hepatoprotective activities

### Effect on body weight

The different rat groups subjected to various dietary treatments were weighed daily throughout the treatment period (Fig. 1b). After the treatment, the growth rates for the negative (G1) and the positive (G2) control groups were 44.8% and 42%, respectively. This could be explained by the *P. lentiscus* oil's particular effect on the rat's growth. The growth of the two groups fitted simple linear models with high regression coefficients confirming the model's validity as follows:

- Body weight (G1) = 3.68*t* + 127; $R^2$ = 0.987.
- Body weight (G2) = 3.25*t* + 127; $R^2$ = 0.992; where t is the treatment period expressed in days.

In group G3, where a hepatotoxic effect was induced, growth disturbances were evident, as indicated by the observed loss in growth

The equation model: Body weight (G3) = 2.85*t* + 128; $R^2$ = 0.976.

However, the induction association with rat food supply by *P. lentiscus* oil in two different doses (groups G4 and G5), showed a growth improvement. The rat growth of groups 4 and 5 fitted the same model expressed as

- Body weight = 2.88*t* + 123; $R^2$ = 0.983.

This model confirmed that the oil-dose protective effect does not influence the rat's body weight. The same rat growth effect was achieved by Ljubuncic et al. (2005) when using *P. lentiscus* leaf extract on rats with hepatic injury caused by thioacetamide.

Results indicated that body weight growth remained consistent across all groups throughout the treatment period.

### Determination of lipid peroxidation levels (TBARS)

The oxidative stress induced by the $CCl_4$/olive oil mixture significantly increased ($p$<0.05) the hepatic TBARS levels in group G3 by comparing with the negative control group (G1) (Fig. 2a). In addition, the injection of *P. lentiscus* oil at two different doses, 5 g $kg^{-1}$ (G4) and 10 g $kg^{-1}$ (G5), led to a reduction in TBARS concentrations ($p$ < 0.05). The higher dose in G5 proved to be more effective than the lower dose in G4, as it restored TBARS levels to those observed in G1. Notably, the weekly administration of *P. lentiscus* oil at 5 g $kg^{-1}$ body weight (G2) halved the normal TBARS levels from their initial value. This reduction could be attributed to the compounds found in *P. lentiscus* oil, like saturated fatty acids (C16 and C18), which are known to mitigate lipid peroxidation (Pardo et al. 2007). The damage caused by $CCl_4$ results from its conversion into a trichloromethyl free radical ($CCl_3^{\bullet}$) through the cytochrome P450 system. This radical is subsequently transformed into a highly reactive $CCl_3O_2^{\bullet}$, leading to lipid peroxidation and hepatocellular damage (Janakat and Al Merie, 2002). *In vivo*, *P. lentiscus* oil inhibited the metabolic activation of $CCl_4$, resulting in a decrease in covalent $CCl_3$ metabolites linked to hepatic lipids. Consequently, this reduction in the metabolic activation by cytochrome P450 diminished the initial biosynthesis of the trichloromethyl free radical, thereby decreasing the initiation of lipid peroxidation (Valko et al. 2016).

### Effect on enzymatic antioxidant activities

#### Catalase (CAT), superoxide dismutase (SOD), and glutathione peroxide (GPx) activities

In the induced group (G3), the catalase activity resembled thereto of the negative control group (G1) ($p$ > 0.05) (Fig. 2b), indicating that the stressing agent ($CCl_4$/olive oil) did not significantly affect catalase activity. In contrast, comparisons between the control (G2) and the treated (G4 and G5) groups revealed that *Pistacia lentiscus* oil hurt catalase activity ($p$ < 0.05). Particularly, catalase activity was reduced by approximately 30% in group G2, which received *P. lentiscus* oil, yet this did not affect superoxide dismutase (SOD) activity in comparison with the group (G1). Nevertheless, the $CCl_4$injection, which induced hepatotoxicity, significantly reduced hepatic SOD activity in group G3 ($p$ < 0.05) (Fig. 2c), confirming that oxidative stress has occurred. Notably, in both groups that received treatment (G4 and G5), a correction of the toxic effect was observed ($p$ < 0.05), as indicated by elevated enzyme expression levels. Additionally, a dose-dependent effect was noted.

Furthermore, GPx activity followed the same trend. The positive (G2) and the negative (G1) control groups ($p$ > 0.05) had the same activity rate as noticed for SOD activity, confirming the absence of *P. lentiscus* oil effect on the GPx (Fig. 2d). However, this enzymatic activity increased significantly in rats treated by the $CCl_4$ (group G3) when compared with the group G1 ($p$ < 0.05), confirming the GPx response to oxidant stress. For both groups G4 and G5, which received the oil at two different doses, the GPx activity was nearby to those in group G1 ($p$ > 0.05). However, a significant enzymatic variation activity was noted ($p$ < 0.05) with regard to the group G3. This fact explained the *P. lentiscus* oil corrective effect,

which was due to $H_2O_2$ trapping and the antioxidants included in the oil.

Zachara et al. (2006) noted that in aerobic organisms, SOD, catalase, and GPx serve important functions in detoxifying free radicals. SOD facilitates the dismutation of superoxide radicals ($O_2$•−) into molecular oxygen ($O_2$) and hydrogen peroxide ($H_2O_2$). Subsequently, either catalase or GPx is responsible for eliminating the produced hydrogen peroxide. In many tissues, the enzyme GPx primarily decomposes $H_2O_2$ by catalyzing the reduction of hydrogen peroxide and various organic hydroperoxides (ROOH) to produce $H_2O$ and alcohols (ROH), using glutathione (GSH) as a specific reducing agent. The enzymes involved in scavenging free radicals catalyze the following reactions (Zachara et al. 2006):

1) $O_2^{\bullet-} + O_2^{\bullet-} + 2\,H^+ \xrightarrow{SOD} H_2O_2 + O_2$

2) $H_2O_2 + H_2O_2 \xrightarrow{CAT} 2\,H_2O + O_2$

3) $ROOH(H_2O_2) + 2\,GSH \xrightarrow{GPx} ROH(H_2O) + H_2O + GSSG$

A primary mechanism of defense consists of antioxidant enzymes like SOD and GPx that transform reactive oxygen species into harmless substances that protect the liver. The oil's composition includes polyphenols and other compounds known for their high DPPH antioxidant activity *in vitro*, which act as scavengers of superoxide anions. Our findings showed notable enhancements in antioxidant activity and reduction in the levels of TBARS in the livers of rats treated with $CCl_4$. Thus, *P. lentiscus* oil may be regarded as a valuable addition to food engineering for health promotion .Notably, all observed disturbances in the antioxidant system were reversed with the administration of *P. lentiscus* oil, particularly in a dosage of 10 g $kg^{-1}$ of body weight. Consequently, the oil's hepatoprotective action in hepatotoxic animals may be attributed to an increase in the activity of the antioxidant enzymes.

This study highlighted the potential benefits of *P. lentiscus* oil in mitigating oxidative stress and boosting the antioxidant defenses in rats with toxicity of the liver. Notably, these biological effects exhibited a dose-dependent relationship, with oil administration being significantly more effective at 10 g $kg^{-1}$ of body weight compared to the lower dose of 5 g $kg^{-1}$ of body weight. These results may provide insights into one of the mechanisms by which polyphenols influence the regulation of liver metabolism. In conclusion, *P. lentiscus* oil appears to facilitate liver recovery by supporting the antioxidant defense system. Additionally, the health advantages of the oil are probably linked to its content of polyphenols and polyunsaturated fatty acids.

**Liver functional enzymes**

The liver function assessment was by measurement of levels of AST, ALP, GT, ALT, and bilirubin in serum. The results are presented in Table 3. The negative control group (G1) exhibited enzyme activities and bilirubin levels comparable to those observed in humans. Weekly dose of 5 mg $kg^{-1}$ of body weight of *P. lentiscus* oil (G2) did not significantly affect serum enzyme activities ($p > 0.05$) when compared with G1 (Table 3). This finding aligns with results from Ljubuncic et al. (2005), Who have not reported any significant changes in serum enzyme activities in thioacetamide-intoxicated rats given daily doses of *P. lentiscus* leaf extracts for five weeks. In contrast, the intoxicated group treated with $CCl_4$ (G3) displayed significant increases ($p < 0.05$) in all serum enzyme activities and bilirubin levels, which rose by 15-72%. The elevation in these serum parameters was attributed to significant hepatotoxicity caused by free radicals, leading to fibrosis, cell necrosis, and potentially cirrhosis. Increased ALP activity and bilirubin levels are related to jaundice, for which biliary tree obstruction could ultimately contribute to liver cancer metastasis. Elevated GT levels indicate liver affection, particularly in cholestatic conditions.

**Table 3: Effect of *Pistacia lentiscus* oil on liver function tests.**

| Enzymatic activities | Rats groups | | | | |
|---|---|---|---|---|---|
| | Controls | | Intoxicated | Intoxicated and treated | |
| | G1 (Normal) | G2 (Positive) | G3 (Untreated) | G4 (5 g/kg) | G5 (10 g/kg) |
| AST (IU/L) | 40.70 ± 0.11$^a$ | 40.10 ± 0.15$^a$ | 145.60 ± 0.56$^b$ | 90.18 ± 0.67$^c$ | 70.05 ± 0.58$^d$ |
| ALT (IU/L) | 9.00 ± 0.46$^a$ | 10.30 ± 0.33$^a$ | 15.90 ± 0.88$^b$ | 11.50 ± 0.94$^c$ | 10 ± 1.12$^d$ |
| ALP (IU/L) | 29.88 ± 0.72$^a$ | 30.12 ± 0.90$^a$ | 35.00 ± 1.11$^b$ | 30.65 ± 0.84$^c$ | 28.66 ± 1.44$^a$ |
| GT (IU/L) | 18.33 ± 0.47$^a$ | 20.43 ± 0.44$^a$ | 25.55 ± 0.89$^b$ | 20.75 ± 1.32$^c$ | 17.90 ± 0.91$^a$ |
| BRN (mg/L) | 4.47 ± 0.12$^a$ | 4.57 ± 0.35$^a$ | 5.82 ± 0.61$^b$ | 4.38 ± 0.47$^a$ | 4.32 ± 0.57$^a$ |

AST: Aspartate amino-transferase; ALT: Alanine aminotransferase; GT: Glutamyl transferase; BRN: Bilirubin; ALP: Alkaline phosphatase; n = 8; negative control, normal rats; positive control, rat fed with *Pistacia lentiscus* oil; Intoxicated rats, $CCl_4$-intoxicated rats. Data are presented as means ± SD. Means with different online letters (a-d) differed significantly ($p < 0.05$).

Janakat and Al Marie, (2002) mentioned that the increases in serum concentrations of hepatospecific enzymes (ALT, ALP, AST) correlate with their cytoplasmic localization, as their release into the bloodstream leads to damage in liver cellular. In this work, the elevated AST levels (145.60 IU/L) can be attributed to the high dose of $CCl_4$ administered in a mixture with olive oil (2 ml $kg^{-1}$ of body weight per week). Maarouf et al. (2008) also reported the ALP reduction and the AST and ALT rise in rabbits exposed to mercury. Furthermore, ingestion of *P. lentiscus* oil at both doses (G4 and G5) led to significant reductions in all liver function tests performed (Table 3), demonstrating hepatoprotective effects that appeared to be dose-dependent. This could be linked to the high levels of flavonoids, phenolic, and β-carotene compounds in *P. lentiscus* oil (Abdelwahed et al. 2007; Bampouli et al. 2015; Mezni et al. 2018). Comparable findings were observed by Janakat and Al Merie (2002), who administered both boiled and raw aqueous extracts of *P. lentiscus* to rats intoxicated with $CCl_4$. Hamed et al. (2022) also found that using pistachio external hull polysaccharides (PHP) restored hepatotoxicity biomarkers in $CCl_4$-treated rats, resulting in decreases of 23.23% and 98.97% in AST and ALT levels, respectively. The higher dose of *P. lentiscus* oil (G5) (Table 3) demonstrated significant antioxidant activities compared to the lower dose (G4) and helped restore serum parameters to normal levels. However, AST activity remained notably elevated. These antioxidants inhibit the formation of peroxyl radicals and help preserve plasma membrane integrity, thereby preventing the escape of cytosolic enzymes such as ALT, ALP, and AST (Maarouf et al. 2008; Abdallah et al. 2023).

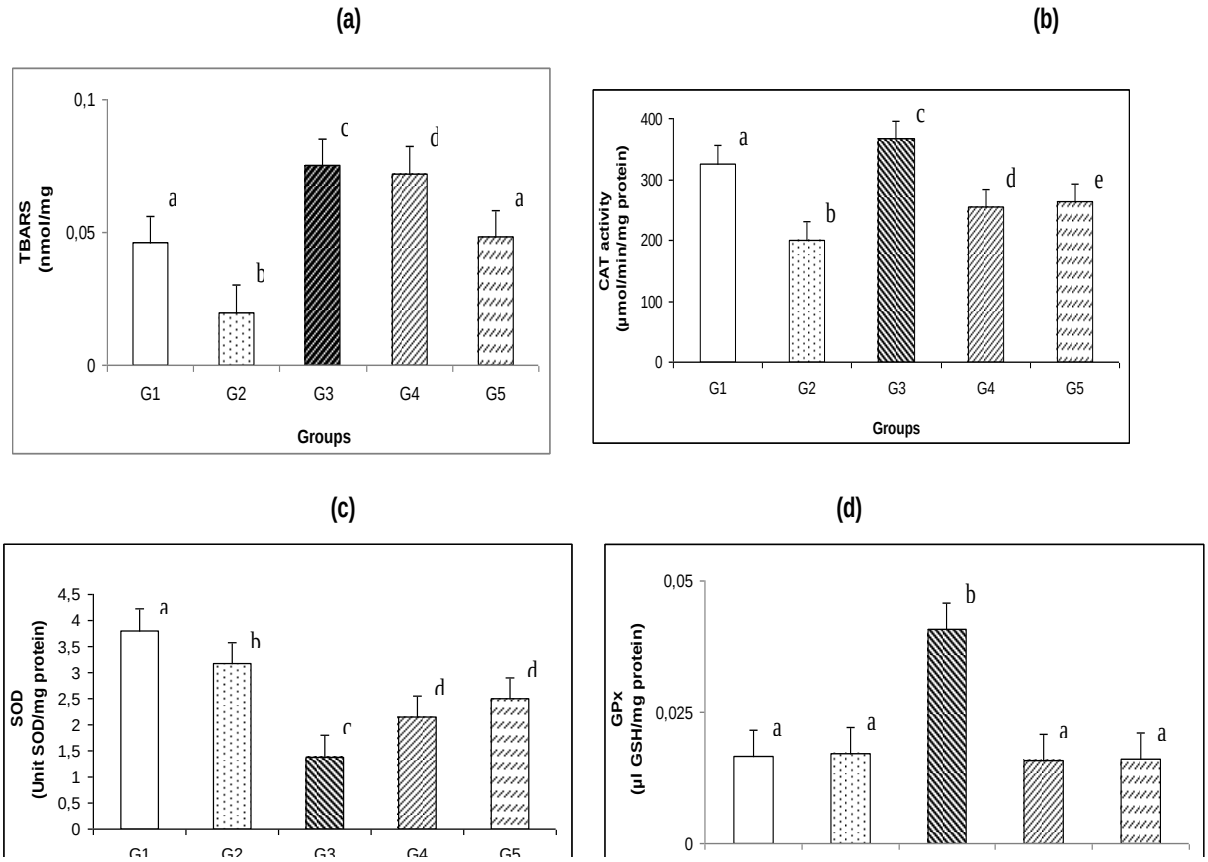



**Figure 2: Effect of *Pistacia lentiscus* oil on TBARS levels (a), CAT (b), SOD (c) and GPx (d) activities in liver; G1: negative control rats; G2: positive control rats which received *Pistacia lentiscus* oil at 5 g/kg of body weight; G3: $CCl_4$ intoxicated rats; G4: intoxicated rats treated with *P. lentiscus* oil at 5 g/kg body weight; G5: intoxicated rats treated with *P. lentiscus* oil at 10 g/kg body weight. Means with different online letters (a-e) differed significantly ($p < 0.05$). Each bar represents mean ± SD from 8 rats.**

## CONCLUSIONS

The present study proved that *Pistacia lentiscus* oil exhibited notable antioxidant activities, *in vitro* and *in vivo* experiments, at two distinct doses. These antioxidant effects were attributed to the oil's unique composition, particularly its important content of unsaturated fatty acids, carotenoids, and phenolic compounds. These components significantly decreased lipid peroxidation and strengthened the antioxidant defense system in a model of hepatotoxicity. These findings highlight *Pistacia lentiscus* oil as a valuable antioxidant source capable of mitigating metabolic diseases associated with oxidative stress, including hepatotoxicity. Consequently, *P. lentiscus* oil may represent a promising potential treatment for human liver diseases.

### Supplementary materials

All data supporting the findings of this study are included in the manuscript.

### Author contributions

Experimental procedure innovation, data interpretation, and manuscript writing: R.J., E.A.; Study supervisor, conception, design and experimental procedures facilitator: E.A. Experiments: R.N.; Animal feeding protocol: E.B., A.E.F.; Biochemical analyses and statistical analysis: M.N.; Plant collection and identification: M.D.; Biochemical analyses validation: F.A.; Physiological study support: A.E.F.
The authors have read and approved the manuscript.

### Funding statement

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

### Institutional Review Board Statement

The study was approved by the local Ethical Committee approved animal handling for the Care and Use of Laboratory Animals.

### Informed Consent Statement

Not applicable.

### Data Availability Statement

The data supporting this study are not publicly available due to privacy restrictions.

### Acknowledgments

The authors are grateful to Mrs Mounira Ben Mrad from Sfax, for her help with the English language.

### Conflict of interest

The authors declared that the present study was performed in the absence of any conflict of interest.



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