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Research Article

From Leaves to Neurons: Neuroprotective Effects of a Pistacia lentiscus Decoction in Oxidative Stress Models

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Abstract

Oxidative stress is a central driver of neurodegeneration, yet safe, food derived antioxidants capable of penetrating the brain remain scarce. *Pistacia lentiscus* L., (lentisk) a Mediterranean shrub long celebrated in folk medicine, is unusually rich in redox active phenolics. We investigated whether a simple water decoction of *Pistacia . lentiscus* leaves confers neuroprotection in murine models of oxidative injury and characterized its bioactive metabolite profile.

Secondary metabolites were quantified (polyphenols, tannins, flavonoids, soluble sugars) by Folin–Ciocalteu, vanillin–HCl, $AlCl_3$ and phenol–sulfuric assays, respectively. Total *in vitro* antioxidant capacity was assessed by ABTS. Adult male Swiss mice (n = 10 per group) received tamoxifen (30 mg kg^{-1}), tamoxifen + decoction (1 g kg^{-1}), decoction alone, or vehicle for 28 days. Cerebral and cerebellar oxidative status were gauged by malondialdehyde (MDA), reduced thiols (SH) and hydroperoxides (H_2O_2), while endogenous defenses were probed via superoxide dismutase (SOD) and glutathione peroxidase (GPx) activities.

The decoction delivered 192 ± 5 mg gallic acid eq g^{-1} DW of total polyphenols, 33 ± 2 mg catechin eq g^{-1} DW of flavonoids, 11 ± 1 mg quercetin eq g^{-1} DW of condensed tannins and 20 ± 0.1 % soluble sugars. ABTS•$^{+}$ assays revealed potent antioxidant (30,0 mg E.A.G / g MS). *In vivo*, tamoxifen elevated cerebral MDA (+82 %) and hydroperoxides (+67 %), depleted SH (–40 %), and suppressed SOD/GPx activities (–34 %/–29 %). Co administration of the decoction normalized lipid peroxidation (MDA –44 %; H2O2 –38 %), restored thiol groups (+37 %), and up regulated SOD (+42 %) and GPx (+46 %) versus tamoxifen alone ($p < 0.01$). Decoction monotherapy produced comparable antioxidant reinforcement without signs of toxicity.

Aqueous metanolic leaf decoction of *Pistacia lentiscus* is a phenolic dense, low sugar preparation that potently scavenges radicals *in vitro* and fortifies endogenous antioxidant networks *in vivo*, thereby shielding brain and cerebellum from tamoxifen induced oxidative stress. These findings position lentisk decoction as a promising neuroprotective nutraceutical adjuvant against redox driven neural damage.

Keywords: Adjuvant Therapy; Antioxidant; Neuroprotective Effect; Phenolic Compounds; Oxidative Stress

Abbreviations

$AlCl_3$: Aluminum Chloride; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; C.E: Catechin Equivalent; CE: Cerebellum; g: Gramme; G.A.E: Gallic Acid Equivalent; GSH: Glutathione; GPx : Glutathione Peroxidase; H_2O_2: Hydrogen Peroxide; H_2SO_4: Sulfuric Acid; MDA: Malondialdehyde; M.E: Methanolic Extract;b mg: Milligram; mM: Millimole; NaH_2PO_4·: Sodium Phosphate; NaNO2: Sodium Nitrite; Na2CO3: Sodium Carbonate; $(NH_4)_6Mo_7O_{24}$: Ammonium Heptamolybdate; nm: Nannomètre; O_2: Dioxygen; OH·: Hydrogen Monoxide; PBS: Phosphate-Buffered Saline; Q.E: Quercetine Equivalent; SN : Brain; *SOD: Superoxide Dismutase;* TAM: Tamoxifen; TBARS: Thiobarbituric Acid Reactive Substances; -SH: Sulfhydryl Groups

Introduction

Oxidative stress, resulting from an imbalance between prooxidant species and antioxidant defenses, is now recognized as a pivotal trigger in the pathogenesis of neurodegenerative disorders, cancer, and metabolic disease [1]. Classical dietary antioxidants such as βcarotene (provitamin A), ascorbic acid (vitamin C) and tocopherols (vitamin E) have long dominated the landscape of redox biology. Yet, over the last two decades, phenolic compounds have emerged as equally powerful, multifunctional redox modulators owing to the presence of hydroxylated aromatic rings capable of scavenging hydroxyl (•OH) and superoxide (O_2•$^-$) radicals or chelating transition metals [2, 3]. Beyond their antioxidant credentials, polyphenols display antiinflammatory, antiallergic, antithrombotic, antidiabetic and phytooestrogenic properties, thereby representing a molecular pleiotropy unmatched by most synthetic drugs [4-6].

Among Mediterranean nutraceuticals, *Pistacia lentiscus* L. (Anacardiaceae), locally known as lentisk warrants special attention. Ethnobotanical surveys across NorthWestern Tunisia and the broader Mediterranean basin consistently cite its leaves, bark and resin for the management of hypertension, eczema, gastrointestinal distress, respiratory ailments and oral infections [7]. Contemporary phytochemical investigations corroborate these traditional claims [8]: lentisk leaf extracts exhibit antibacterial, antifungal, antiinflammatory, hepatoprotective and woundhealing activities, while the fixed oil is reported to attenuate mercury toxicity, stabilize serum cholesterol and protect the gastric mucosa [9, 10].

Tamoxifen (TAM), the firstline antioestrogen for hormoneresponsive breast cancer, exemplifies the therapeutic dilemma posed by redoxactive drugs [11]. While TAMinduced oxidative damage enhances tumor cytotoxicity, it simultaneously provokes compensatory antioxidant responses that can culminate in hepatotoxicity, thromboembolic events and cognitive dysfunction once the drug crosses the blood–brain barrie [12]. Evidence of TAMrelated lipid peroxidation, cerebral venous thrombosis and memory impairment highlights the urgent need for adjunctive strategies capable of quenching collateral oxidative injury without blunting TAM's antitumoral efficacy [11,13].

To the best of our knowledge, the neuroprotective potential of *Pistacia lentiscus* in the context of TAMinduced oxidative stress has never been explored *in vivo*. The present study therefore investigated, for the first time, whether phenolicrich decoction of *Pistacia lentiscus* can (i) attenuate systemic and cerebral oxidative stress, and (ii) preserve performance in adult male Swiss mice undergoing shortterm tamoxifen therapy. By integrating biochemical endpoints, our work seeks to position lentisk phenolics as a promising dietary adjuvant capable of safeguarding neural integrity during endocrine treatment, thereby broadening the clinical scope of a welldocumented Mediterranean medicinal plant.

Materials and Methods

Preparation of the extract

Pistacia lentiscus leaves (Anacardiaceae family) were harvested before flowering, from the Tabarka region (Tunisia). The leaves were washed in distilled water and placed in the shade to dry with ventilation and protection of light at room temperature. Then the dried material was finely powdered. The sieved powder was macerated with methanol and kept on a shaker with magnetic stirrer for 24 h. The mixture was filtered through the filter paper (Ash free paper). The filtrate underwent gentle evaporation using a rotary evaporator (rotavap), a device designed for the efficient and delicate removal of solvents through evaporation. The resulting methanolic extract was collected and used for experimental procedures.

Phenolic compounds extraction

Extraction of phenolic compounds was carried out using their reducing capacity of the Folin–Ciocalteu reagent, producing a blue tungsten and molybdenum oxide coloration in alkaline medium. The depth of this blue coloration is proportional to the amount of polyphenols in the sample. The assay is carried out by mixing 125 µl of the extract with 500 µl of distilled water and 125 µl of Folin–Ciocalteu reagent (a combination of phosphotungstic and phosphomolybdic acids). The combination is shaken well and rested for 3 min. One then adds 1,250 µl of 7% sodium carbonate (Na-2CO3) solution and the volume is adjusted to 3 ml with distilled water, following the method [14]. The mixture is then incubated for 90 minutes in the dark at room temperature. Absorbance is measured at 760 nm. A calibration curve is prepared using gallic acid at concentrations of 100, 200, 500, and 700 mg·L^{-1}. Results are expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

Extraction of flavonoids

Flavonoid content is determined using a colorimetric method [15]. A volume of 250 µl of ten-fold diluted ethanolic extract is mixed with 75 µl of 5% sodium nitrite ($NaNO_2$) solution. After a brief agitation, 150 µl of freshly prepared aluminum chloride ($AlCl_3$, 10%) solution are added. Following a 6-minute incubation at room temperature, the absorbance is measured at 510 nm against an appropriate blank. Results are expressed as milligrams of catechin equivalents per gram of dry weight (mg CE/g DW).

Extraction of tanins

The total tannin content is determined according to the method of Broadhurst and Jones (1978), [16]. To 50 µl of each sample, 3 ml of vanillin solution (4% in ethanol) and 1.5 ml of concentrated sulfuric acid (H_2SO_4, 70%) are added. After 15 minutes of incubation, the absorbance is read at 500 nm. Results are expressed as micrograms of catechin equivalents per milligram of extract (µg CE/mg extract).

Total soluble sugars

To extract soluble sugars, 100 mg of dry plant powder are mixed with 10 ml of 80% ethanol and heated in a water bath at 70 °C for 30 minutes, with agitation every 10 minutes. The tubes are then placed on ice for 5 minutes and centrifuged (10 min, 6,000 rpm, 4 °C). The supernatant constitutes the sugar-rich extract. For quantification, 2.45 ml of 80% ethanol, 5 ml of anthrone reagent (10 mg anthrone dissolved in 5 ml H_2SO_4), and 50 µl of the extract are added to a test tube. The blank contains 2.5 ml of ethanol and 5 ml of anthrone solution. Tubes are incubated at 100 °C for 10 minutes, and absorbance is measured at 640 nm.

In vitro antioxidant activity

This assay evaluates the total antioxidant capacity based on the phosphomolybdenum method. It involves the addition of 0.2 ml of a reagent solution containing sulfuric acid (H_2SO_4, 0.6 M), sodium phosphate ($NaH_2PO_4 \cdot H_2O$, 28 ml), and ammonium heptamolybdate ($(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, 4 mM) under acidic conditions. The reaction mixture is incubated in a water bath at 95°C for 90 minutes. After cooling to room temperature, absorbance is measured at 695 nm. The total antioxidant activity is expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE/g DW).

In vivo neuroprotective potential

Experimental Protocol

Adult male Swiss albino mice (8–10 weeks old, weighing 25–30 g) were randomly allocated into four experimental groups (n = 10 per group), housed under standard laboratory conditions (22 ± 2°C, 12 h light/dark cycle, ad libitum access to food and water). The experimental protocol was designed to evaluate the potential protective and/or synergistic effects of a methanolic extract of *Pistacia lentiscus* (M.E) against tamoxifen (TAM)-induced neurotoxicity.

The animals were assigned as follows:

- **Group I – TAM**: received tamoxifen (30 mg/kg, orally) dissolved in an appropriate vehicle;
- **Group II – TAM + M.E**: received tamoxifen (30 mg/kg, orally) in combination with the methanolic extract of *Pistacia lentiscus* (1 g/10 ml distilled water, administered orally at a dose determined in preliminary phytochemical and toxicological assessments);
- **Group III – M.E**: received only the methanolic extract (same dose and administration route as in Group II);
- **Group IV – Control**: received an equivalent volume of distilled water (vehicle control).

All treatments were administered once daily for a period determined based on the pharmacodynamic window of TAM and previous reports on plant extract bioactivity. Animals were monitored daily for general health, body weight, and behavioral changes. At the end of the treatment period, animals were sacrificed under appropriate anesthesia for sample collection and biochemical analyses.

Anthropometric parameters

Body weight, as well as food and water intake, were regularly monitored every two days throughout the duration of the study, starting from the first day of treatment until euthanasia on day 28.

Plasma parameters

Blood samples were collected in heparinized tubes for the assessment of liver and kidney function markers. Samples were centrifuged at 3,000 g for 10 minutes at 4°C, and plasma was subsequently separated and collected. Hepatic and renal function parameters, including aspartate aminotransferase (ASAT) and alanine aminotransferase (ALAT), were measured using a standard method [17] with commercial diagnostic kits (BioSystems S.A., Costa Brava 30, 08030 Barcelona, Spain; certified ISO 13485 and ISO 9001).

Oxidative stress

Animals were sacrificed by decapitation. The nervous system tissues specifically the brain and cerebellum were rapidly excised, rinsed in 0.9% NaCl, and blotted on ash-free filter paper before weighing. Preparation of tissue homogenates and protein estimation: Approximately 0.4 g of each tissue was homogenized in 4 ml of phosphate-buffered saline (PBS, 50 mM, pH 7.8) and centrifuged at 9,000 g for 15 minutes at 4°C. The supernatants were collected and stored at −80°C for subsequent assessment of oxidative stress biomarkers and enzymatic activity. Total protein content in the homogenates was determined using the biuret method, with serum albumin as the standard. Briefly, proteins in the supernatant formed a colored complex with copper, the absorbance of which was measured at 546 nm and compared to a blank. Results were expressed as milligrams of protein

Lipid peroxidation (TBARS Assay)

The malondialdehyde (MDA) concentration in liver and kidney supernatants was assessed as an index of lipid peroxidation using the thiobarbituric acid reactive substances (TBARS) method with a double-heating protocol [17]. A BHT-TCA solution (1% butylated hydroxytoluene in 20% trichloroacetic acid) was added to the supernatant. After centrifugation at 1,000 g for 5 minutes at 4°C, the homogenate was mixed with HCl (0.5 N) and TBA-Tris solution (120 mM thiobarbituric acid in 26 mM Tris), then incubated at 80°C for 10 minutes. Immediately afterward, the tubes were placed on ice to halt the chromophore reaction. MDA levels were determined using the extinction coefficient of $1.56 \times 10^5\ M^{-1}{\cdot}cm^{-1}$ for the MDA-TBA complex and were expressed as nmol/mg of protein.

Sulfhydryl groups (-SH)

The concentration of sulfhydryl (-SH) groups was determined using the Ellman method [18]. Liver and kidney homogenates were each mixed with 100 µL of EDTA (20 mM, pH 8.2) and vortexed. Absorbance was recorded at 412 nm (A1). Then, 100 µL of freshly prepared DTNB (10 mM) was added, and the mixture was incubated for 15 minutes before recording the absorbance again at 412 nm (A2). The concentration was calculated using the formula: (A2 – A1 – B) × 1.57 mM, where B is the blank. Results were expressed as nmol/mg of protein.

Hydrogen peroxide (H_2O_2)

Hydrogen peroxide levels were measured using a standard colorimetric method described by Kakinuma et al. (1979), using a commercial diagnostic kit (BioSystems S.A., Costa Brava 30, 08030 Barcelona, Spain; ISO 13485 and ISO 9001 certified). Briefly, H_2O_2 reacts with 4-aminoantipyrine and phenol to form a quinone-imine chromophore, producing a red color. The absorbance was measured at 505 nm. Results were calculated based on a calibration curve and expressed as nmol/mg of protein.

In Vivo antioxidant effect

Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase activity was determined using the method [19]. Briefly, 0.2 mL of liver or kidney tissue homogenate was mixed with 0.2 mL of phosphate buffer (0.1 M, pH 7.4), 0.2 mL of reduced glutathione (GSH, 4 mM), and 0.4 mL of H_2O_2 (5 mM). The reaction mixture was incubated at 37°C for 1 minute. After centrifugation at 1,500 g for 5 minutes, 0.5 mL of 5% TCA was added to stop the reaction. Then, 0.2 mL of the supernatant was mixed with 0.5 mL of DTNB (10 mM) and phosphate buffer. GPx activity was measured at 412 nm and compared to a blank. Results were expressed as units per milligram of protein (U/mg protein) (Rotruck, Pope et al., 1980).

Superoxide Dismutase (SOD) Activity

Superoxide dismutase (SOD) activity was measured according to the method of Kakkar [19]. In brief, 10 µL of tissue homogenates (SN and CE) were added to a reaction mixture containing 20 µL of bovine catalase (0.4 U/mL), 2.93 mL of sodium carbonate/bicarbonate buffer (50 mM; pH 10.2), and 40 µL of epinephrine (0.1 M), giving a final volume of 4 mL. SOD activity was monitored by measuring absorbance changes at 480 nm every 60 seconds for 5 minutes. Results were expressed as SOD units per milligram of protein (U SOD/mg protein).

Statistical Analysis

All data were expressed as means ± standard error of the mean (m ± SEM). Statistical significance between experimental groups was assessed using analysis of variance (ANOVA) followed by Student's t-test where applicable.

Results and Discussion

Phenolic compounds extraction

The phytochemical profiling of the decoction revealed a remarkable richness in bioactive compounds (Figure 1). The total polyphenol content was quantified at 192 mg of gallic acid equivalents per gram of dry weight (mg GAE/g DW), indicating a substantial antioxidant potential [1]. Concurrently, the flavonoid content reached 33 mg catechin equivalents per gram of dry weight (mg CE/g DW), further supporting the plant's capacity to scavenge free radicals [20]. The extract also demonstrated a moderate presence of condensed tannins, estimated at 11 mg quercetin equivalents per gram of dry weight (mg QE/g DW), which may contribute to its anti-inflammatory and neuroprotective properties. Additionally [4, 5]. The total soluble sugar content was measured at 20 mg/g DW, suggesting a relatively low sugar load that may enhance its therapeutic value without compromising metabolic stability.

In vitro antioxidant activity

The antioxidant activity of the fresh material (FM) was assessed through the total antioxidant capacity, which reached **30 mg GAE/g DM** (Table 1). This level of activity reflects a considerable capacity of the extract to neutralize free radicals and counteract oxidative stress, which is consistent with its polyphenolic richness (Figure 1). Such a TAC value positions the fresh material among botanicals with promising antioxidant potential, particularly when compared to similar Mediterranean medicinal plants [1]. This finding not only reinforces the traditional use of *Pistacia lentiscus* in folk medicine but also suggests its relevance in modern phytotherapy as a natural source of redox-modulating agents.

In vivo neuroprotective potential

Anthropometric Parameters

This study demonstrated a significant increase in water intake and a less pronounced increase in food intake in the group treated with tamoxifen (TAM) compared to the control group. An excellent improvement was observed in the group treated with the methanolic extract. The study also revealed a slight increase in water intake accompanied by a slight decrease in food intake in the group co-treated with the extract and TAM (Table 2).

A relative decrease in the absolute weight of the nervous system was noted in the TAM-treated group. However, the absolute organ weights were nearly normalized in both groups treated with TAM in combination with the ME of *Pistacia lentiscus*, as well as in the group receiving the extract alone. This observation strongly sug-

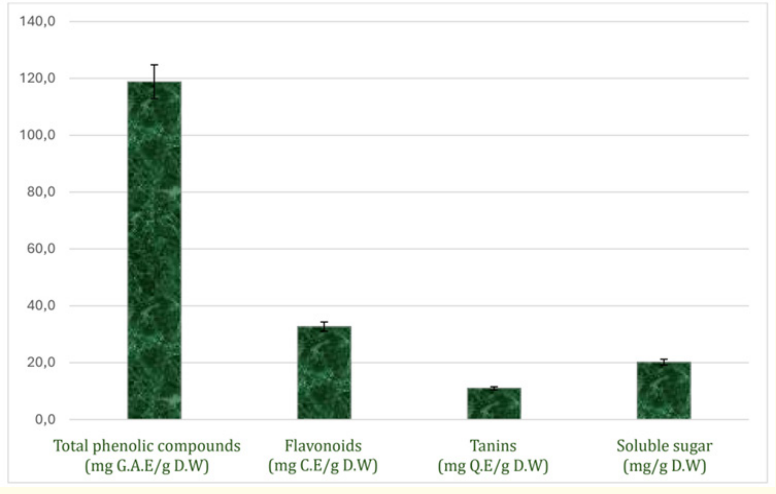

Figure 1: Phenolic Compound and Soluble Sugar Content in Pistacia lentiscus Leaves.

Activité antioxydante totale
30,0 mg E.A.G / g MS

Table 1: Total antioxidant activity of the fresh material of *Pistacia lentiscus* leaves.

Results are expressed as mean ± standard deviation (n = 3), ($p < 0,05$).

gests a corrective effect of *Pistacia lentiscus* in mitigating oxidative stress and counteracting tamoxifen-induced hepatotoxicity in our experimental model.

	I	II	III	IV
Water intake (ml)	28.6 ± 1*	20 ± 2,3#	24.9 ± 4,7#	25,8 ± 2,9
Food intake (g)	10 ± 1,6*	11± 2,7#	12 ± 2,6#	11,62 ± 3,8
Relative weight of the SN (g)	7.346 ± 0,192*	8.354 ± 0,25#	9.335 ± 0,216#	8.775 ± 0,342
Relative weight of the CE (g)	0.809 ± 0,0.021*	1.211 ± 0,041#	1.2257 ± 0,029#	1.249 ± 0,031

Table 2: Mean values of food and water intake, and organ weights across the different experimental groups.

I : TAM group ; II : TAM + M.E group ; III : M.E group ; IV : control group ; SN: Brain; CE: Cerebellum of swiss mouse (* : $p < 0,05$ vs Témoin et # : $p < 0,05$ vs Tamoxifene) ; n = 10.

Plasma parameters

Oral administration of TAM at a dose of 30 mg/kg body weight per day induced hepatic dysfunction in rats, as evidenced by a significant increase in plasma transaminase activities (ASAT and ALAT). Treatment with the methanolic extract of *Pistacia lentiscus* at 1 g/kg body weight effectively protected against TAM-induced hepatic dysfunction by restoring plasma transaminase levels to baseline values (Table 3).

Oxidative stress parameters in the nervous system

Our results showed a significant increase in MDA levels in the studied organs of the TAM-treated group. Co-treatment with the methanolic extract of *Pistacia lentiscus* provided protection against the TAM-induced lipid peroxidation increase and restored the values close to normal levels (Figure 2).

	I	II	III	IV
ASAT (U/L)	480 ± 14.9*	416 ± 4.75*#	325 ± 4.749*#	349 ± 2.2
ALAT (U/L)	152.5 ± 2.63*	115.2 ± 2.57#	97.5 ± 2.76#	101.5 ± 1.5

Table 3: Effects of sub-acute treatment with *Pistacia* fixed oil and tamoxifen on plasma biomarkers in mice. Results are expressed as mean ± SEM (n = 10).

I : TAM group ; II : TAM + M.E group ; III : M.E group ; IV : control group ; (* : $p < 0,05$ vs control and # : $p < 0,05$ vs Tamoxifene) ; n = 10.

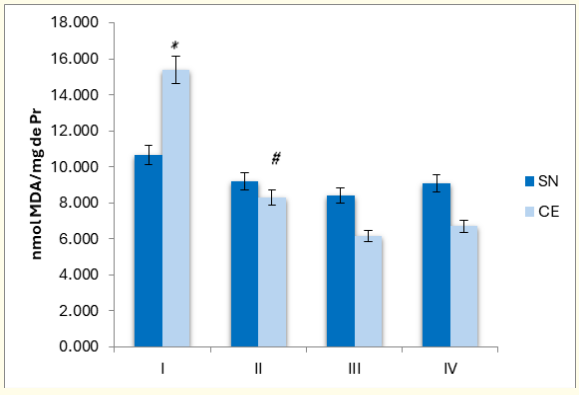

Figure 2: Effects of Sub-acute Treatment with the Methanolic Extract of Pistacia lentiscus and Tamoxifen on Malondialdehyde (MDA) Concentrations in the Brain and Cerebellum of swiss mouse. Results are expressed as mean ± SEM (n = 10). I : TAM group ; II : TAM + M.E group ; III : M.E group ; IV : control group ; (* : $p < 0,05$ vs Control and : $p < 0,05$ vs Tamoxifene) ; n = 10.

Subacute administration of TAM for 20 days led to a depletion of thiol group levels in the studied organs. Subacute co-treatment with the methanolic extract of *Pistacia lentiscus* protected against TAM-induced protein oxidation and restored the values close to normal levels (Figure 3).

Subacute administration of TAM for 20 days resulted in an increased concentration of hydrogen peroxide (H_2O_2) in the studied organs. Subacute co-treatment with the methanolic extract of *Pistacia lentiscus* protected against TAM-induced hydrogen peroxide production and restored the levels close to normal values (Figure 4).

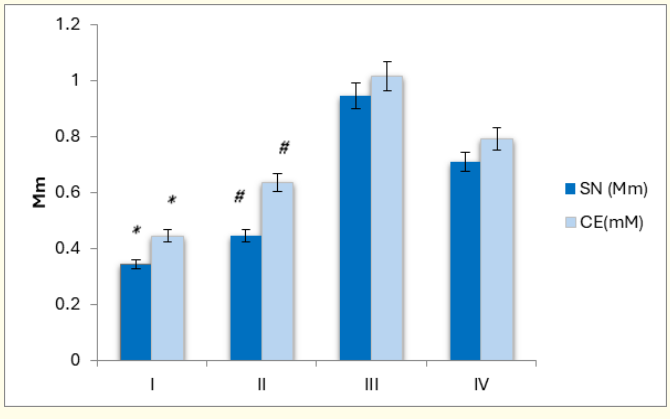

Figure 3: Effects of Subacute Treatment with the Methanolic Extract of Pistacia lentiscus and Tamoxifen on Thiol Group Levels in the Brain and Cerebellum of swiss mouse. Results are expressed as mean ± SEM (n = 10). I : TAM group ; II : TAM + M.E group ; III : M.E group ; IV : control group ; (* : $p < 0,05$ vs Control and # : $p < 0,05$ vs Tamoxifene) ; n = 10.

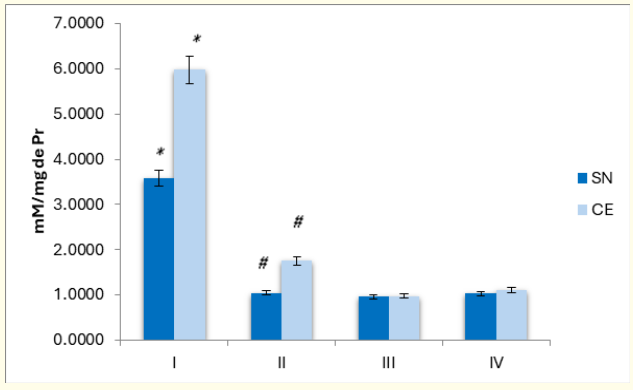

Figure 4: Effects of Subacute Treatment with the Methanolic Extract of *Pistacia lentiscus* and Tamoxifen on H_2O_2 concentration in the Brain and Cerebellum of swiss mouse. Results are expressed as mean ± SEM (n = 10). I : TAM group ; II : TAM + M.E group ; III : M.E group ; IV : control group ; (* : $p < 0,05$ vs Control and # : $p < 0,05$ vs Tamoxifene) ; n = 10.

It can be concluded that TAM induces a state of oxidative stress, as evidenced by increased levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2), along with a depletion of thiol (-SH) group levels in the studied organs of the TAM-treated group ($p<0,05$). In contrast, co-treatment with the methanolic extract of *Pistacia lentiscus* provides protection against oxidative stress and prevents the depletion of thiol groups induced by TAM administration.

Antioxidant parameters in the nervous system

In the present study, we measured the activity of key antioxidant enzymes, including CAT, SOD and GPx.

Our results show that subacute administration of TAM leads to an increase in catalase activity in the liver and nervous system. Co-treatment with the methanolic extract of *Pistacia lentiscus* provides protection against the moderate stress induced by TAM and restores the values close to normal levels ($p < 0,05$) (Figure 5).

SOD activity increased significantly in the group treated with TAM. In the control group, SOD activity was approximately 5.101 UI/min/mg of protein, compared to 21.996 UI/min/mg of protein in the TAM-treated group ($p < 0,05$). Moreover, co-administration of the methanolic extract of *Pistacia lentiscus* with TAM led to the normalization of this parameter. In contrast, administration of the methanolic extract of *Pistacia lentiscus* alone had no effect on SOD activity in the nervous system (without TAM co-treatment) (Figure 6).

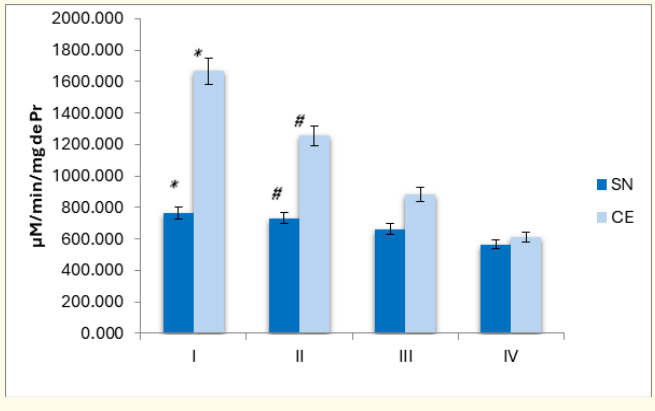

Figure 5: Effects of Subacute Treatment with the Methanolic Extract of *Pistacia lentiscus* and *Tamoxifen* on Catalase Activity in the Brain and Cerebellum of swiss mouse. Results are expressed as mean ± SEM (n = 10). I : TAM group ; II : TAM + M.E group ; III : M.E group ; IV : control group ; (* : $p < 0,05$ vs Control and # : $p < 0,05$ vs Tamoxifene) ; n = 10.

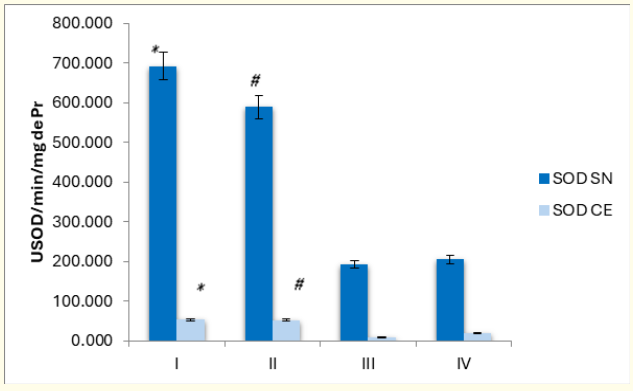

Figure 6: Effects of Subacute Treatment with the Methanolic Extract of *Pistacia lentiscus* and Tamoxifen on Superoxide Dismutase (SOD) Activity in the Brain and Cerebellum of swiss mouse. Results are expressed as mean ± SEM (n = 10). I : TAM group ; II : TAM + M.E group ; III : M.E group ; IV : control group ; (* : $p < 0,05$ vs Control and : $p < 0,05$ vs Tamoxifene) ; n = 10.

Research conducted on mice demonstrated that tamoxifen reduces tumor growth in estrogen receptor-alpha (ERα)-negative breast cancer through the upregulation of key antioxidant genes, including NQO1 (NAD(P)H quinone dehydrogenase 1), *HMOX1* (heme oxygenase 1), and *SOD1* (superoxide dismutase 1). These findings confirm that tamoxifen induces oxidative damage within mammary tumors in vivo and highlight the critical role of the oxidative response of cancer cells to tamoxifen. On one hand, such oxidative damage exerts a beneficial therapeutic effect by promoting cancer cell death. On the other hand, it simultaneously triggers a compensatory antioxidant response that may lead to structural and functional alterations within the nervous system [21,22]. This duality underscores the importance of nutritional supplementation and adjuvant therapies involving natural bioactive compounds rich in phenolic constituents [23].

Among these, *Pistacia lentiscus* and its phenolic compounds have long been utilized in both traditional and modern medicine for their potent antioxidant properties [20]. Due to their high redox potential, polyphenols function as reducing agents and hydrogen donors, effectively neutralizing free radicals and chelating transition metal ions [20,24].

Given the well-established link between oxidative stress and the pathophysiology of neurotoxicity and neurodegenerative diseases, cancer [23,25], and metabolic disorders , the observed antioxidant activity (Table 1; Figure 5 and 6) may underlie, at least in part, the therapeutic efficacy attributed to this plant.

Conclusion

In summary, our work demonstrates that a readily prepared aqueous decoction of *Pistacia lentiscus* leaves constitutes a phenolicrich, lowsugar matrix endowed with robust radicalscavenging power in vitro and remarkable neuroprotective efficacy in vivo. By curbing lipid peroxidation, replenishing endogenous thiols, and reactivating the SOD and GPx defense axes, the decoction effectively neutralized tamoxifeninduced oxidative insults in the brain and cerebellum of Swiss mice. These findings not only validate the longstanding ethnomedicinal reputation of lentisk but also highlight its promise as an affordable nutraceutical adjuvant for mitigating redoxdriven neuronal damage associated with endocrine therapy and other oxidative challenges. Future studies should dissect the individual and synergistic contributions of the identified phenolic subclasses, define pharmacokinetic parameters, and evaluate translational potential in clinical cohorts. Collectively, our data position *P. lentiscus* decoction as a compelling candidate for integrative strategies aimed at preserving neural integrity in the face of oxidative stress.

Conflict of Interest

The authors declare that they have no conflicts of interest.

Bibliography

1. Feng XH., *et al.* "The tumor suppressor Smad4/DPC4 and transcriptional adaptor CBP/p300 are coactivators for Smad3 in TGF-β-induced transcriptional activation". *Genes and Development* 12.14 (1998): 2153-2163.

2. Valko M., *et al.* "Free radicals, metals and antioxidants in oxidative stress-induced cancer". *Chemico-Biological Interactions* 160.1 (2006): 1-40.

3. Tiku AR. "Antimicrobial compounds (phytoanticipins and phytoalexins) and their role in plant defense". *Co-Evolution of Secondary Metabolites* (2020): 845-868.

4. Panikar S., *et al.* "Essential oils as an effective alternative for the treatment of COVID-19: Molecular interaction analysis of protease (Mpro) with pharmacokinetics and toxicological properties". *Journal of Infection and Public Health* 14.5 (2021): 601-610.

5. Sun Y and J Qian. "Botanical drug clinical trial: Common issues and future options". *Acta Pharmaceutica Sinica B* 11.1 (2021): 300-303.

6. Sain S., *et al.* "Beta caryophyllene and caryophyllene oxide, isolated from Aegle marmelos, as the potent anti-inflammatory agents against lymphoma and neuroblastoma cells". *Anti-Inflammatory and Anti-Allergy Agents in Medicinal Chemistryrrent Medicinal Chemistry-Anti-Inflammatory and Anti-Allergy Agents)* 13.1 (2014): 45-55.

7. Bagheri S., *et al.* "Effects of Pistacia atlantica on oxidative stress markers and antioxidant enzymes expression in diabetic rats". *Journal of the American College of Nutrition* 38.3 (2019): 267-274.

8. Kim KE., *et al.* "Artemisia annua leaf extract attenuates hepatic steatosis and inflammation in high-fat diet-fed mice". *Journal of Medicinal Food* 19.3 (2016): 290-299.

9. Yisak H., *et al.* "Total phenolics and antioxidant capacity of the white and brown teff [Eragrostic tef (Zuccagni) Trotter] varieties cultivated in different parts of Ethiopia". *Bulletin of the Chemical Society of Ethiopia* 36.4 (2022): 749-766.

10. Hagos M., *et al.* "Determination of total phenolic, total flavonoid, ascorbic acid contents and antioxidant activity of pumpkin flesh, peel and seeds". *Bulletin of the Chemical Society of Ethiopia* 37.5 (2023): 1093-1108.

11. Auerbach L. "Endokrine antihormonelle Therapie in der Prämenopause". *Journal für Gynäkologische Endokrinologie/Österreich* 27.4 (2017): 147-152.

12. Du J., *et al.* "Dual-targeting topotecan liposomes modified with tamoxifen and wheat germ agglutinin significantly improve drug transport across the blood– brain barrier and survival of brain tumor-bearing animals". *Molecular pharmaceutics* 6.3 (2009): 905-917.

13. Ferguson MW and S O'Kane. "Scar–free healing: from embryonic mechanisms to adult therapeutic intervention". *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 359.1445 (2004): 839-850.

14. Fatiha B., *et al.* "Phenolic composition, *in vitro* antioxidant effects and tyrosinase inhibitory activity of three Algerian Mentha species: M. spicata (L.), M. pulegium (L.) and M. rotundifolia (L.) Huds (Lamiaceae)". *Industrial Crops and Products* 74 (2015): 722-730.

15. Brahmi F HD., *et al.* "Phenolic composition, *in vitro* antioxidant effects and tyrosinase inhibitory activity of three Algerian *Mentha* species: *M. spicata* (L.), M. *pulegium* (L.) and *M. rotundifolia* (L.) *Huds* (Lamiaceae)". *Industrial Crops and Products* 74 (2015): 722-730.

16. Julkunen-Tiitto R. "Phenolic constituents in the leaves of northern willows: methods for the analysis of certain phenolics". *Journal of Agricultural and Food Chemistry* 33.2 (1985): 213-217.

17. Reitman, S., Liver enzymes (AST and ALT); Reitman and Frankel calorimetric method. *Am J Uni Path*, 1957. 28(1): 56-63

18. Draper HH and M Hadley. "Malondialdehyde determination as index of lipid Peroxidation". *Methods Enzymol* 186 (1990): 421-431.

19. Ellman GL. "Tissue sulfhydryl groups". *Archives of Biochemistry and Biophysics* 82.1 (1959): 70-77.

20. Kakkar P., *et al.* "A modified spectrophotometric assay of superoxide dismutase". *IJBB* (1984).

21. Beghlal D., *et al.* "Phytochemical, organoleptic and ferric reducing properties of essential oil and ethanolic extract from *Pistacia lentiscus* (L.)". *Asian Pacific Journal of Tropical Disease* 6.4 (2016): 305-310.

22. Lagadec C., *et al.* "Tamoxifen and TRAIL synergistically induce apoptosis in breast cancer cells". *Oncogene* 27.10 (2008): 1472-1477.

23. Tomková V., *et al.* "Mitochondrial fragmentation, elevated mitochondrial superoxide and respiratory supercomplexes disassembly is connected with the tamoxifen-resistant phenotype of breast cancer cells". *Free Radical Biology and Medicine* 143 (2019): 510-521.

24. Abidi O., *et al.* "*Pistacia lentiscus* L. revealed in vitro anti-proliferative activity on MCF-7 breast cancer cells and *in vivo* anti-mammary cancer effect on C57BL/6 mice through necrosis, anti-inflammatory and antioxidant enhancements". *Plos One* 19.4 (2024): e0301524.

25. Abidi O., *et al.* "Phytochemical analysis and biological activities of two oil-bearing extracts from fresh Pistacia lentiscus". *Bulletin of the Chemical Society of Ethiopia* 37.6 (2023): 1487-1501.

26. Catalani S., *et al.* "Oxidative stress and apoptosis induction in human thyroid carcinoma cells exposed to the essential oil from Pistacia lentiscus aerial parts". *PloS One* 12.2 (2017): e0172138.