Acta Scientific NUTRITIONAL HEALTH (ISSN:2582-1423)

Volume 9 Issue 8 August 2025

Research Article

Can *Pistacia lentiscus* Mitigate Male Reproductive Toxicity Induced by Antihormonal Therapy for Breast Cancer?

Omayma Abidi[1,2],*, Ameni Smaoui[3] and Houcine Selmi[4]

[1]*Faculty of Sciences of Tunis, University Tunis El Manar, Tunisia*

[2]*Laboratory of Physiology and Pharmacology, National School of Veterinary Medicine, University of Manouba, Sidi Thabet Ariana, Tunisia*

[3]*Laboratory of Plant Productivity and Environmental Constraints, Department of Biological Sciences, Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis, Tunisia*

[4]*Laboratory of Sylvo-Pastorales Ressources, Sylvo-Pastoral Institut of Tabarka, University of Jendouba, Tabarka, Tunisia*

***Corresponding Author:** Omayma Abidi, Faculty of Sciences of Tunis, University Tunis El Manar and Laboratory of Physiology and Pharmacology, National School of Veterinary Medicine, University of Manouba, Sidi Thabet Ariana, Tunisia.

Received: June 20, 2025

Published: July 07, 2025

Abstract

Antihormonal therapy with tamoxifen (TAM), although standard in breast cancer management, is increasingly implicated in male reproductive toxicity through oxidative stress driven mechanisms. The Mediterranean shrub *Pistacia lentiscus* is rich in phenolic antioxidants with well documented free radical scavenging activity, yet its capacity to counter TAM induced gonadotoxicity has not been systematically explored.

To determine whether a methanolic extract of *P. lentiscus* (PL ME) can mitigate functional and biochemical indices of reproductive damage elicited by subacute TAM exposure in male mice. Adult Swiss albino males (8–10 weeks, 25–30 g) were randomized (n = 10/group) to four regimens for 20 days: (i) Control, (ii) TAM (30 mg kg^{-1} p.o.), (iii) PL ME (1g kg^{-1} p.o.), and (iv) TAM + PL ME (co treatment). Antiradical potency of PL ME was first confirmed by DPPH assay (IC_{50} = 107 µg mL^{-1}). Post treatment, semen quality (concentration, motility, viability, morphology) was analyzed, together with pro oxidant markers: malondialdehyde (MDA), hydrogen peroxide (H_2O_2), and protein thiols (SH) and also antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in testis and epididymis.

TAM produced a marked decline in sperm concentration (40 %), motility (42 %), and viability (31%) while elevating head (+12 %) and tail (+20 %) deformities ($p < 0.05$). Concomitantly, testicular/epididymal MDA and H_2O_2 rose 2.3 to 2.8 fold, with a 48 % drop in SH. Antioxidant enzymes were paradoxically up regulated (SOD +46 %, CAT +72 %, GPx +88 %), indicating a compensatory yet insufficient response. Co administration of PL ME normalized seminal parameters (all $p > 0.05$ vs. control) and restored oxidative balance: MDA, H_2O_2, and SH returned to baseline, while SOD, CAT, and GPx activities were realigned within physiological ranges.

Pistacia lentiscus methanolic extract confers substantive protection against TAM induced male reprotoxicity by quenching lipid peroxidation, lowering peroxide burden, preserving thiol groups, and recalibrating endogenous antioxidant defenses. These findings highlight PL ME as a promising phytotherapeutic adjunct for safeguarding male fertility during antihormonal breast cancer therapy.

Keywords: Bio-Compounds; *Pistacia lentiscus*; Antioxidant; Repro-Toxicity; Hormonal Therapy of Breast Cancer, Male Fertility

Abbreviations

g: Gramme; GPx : Glutathione Peroxidase*;* GSH: Glutathione; H_2O_2: Hydrogen Peroxide; MDA: Malondialdehyde; M.E: Methanolic Extract; mg: Milligram; mM: Millimole; nm: Nannomètre; Q.E: Quercetine Equivalent; *SOD:* Superoxide Dismutase; TAM: Tamoxifen; SH: Sulfhydryl Groups

Introduction

Men can develop Breast cancer (BC) in a relatively rare condition, but it does occur due to the presence of breast tissue in their bodies. Although the disease is more common in women (24,2 % newly diagnosed/year), men have a smaller amount of breast tissue and a lower lifetime risk of developing BC (about 0.5 to 1% of BC) [1]. Moreover, if a man is diagnosed with BC, treatment options are similar to those for women and may include surgery, radiation therapy, chemotherapy, targeted therapy, and hormone therapy. Tamoxifen (TAM) antiestrogen treatment is widely used in the hormone therapy of BC in both men and woman. It belongs to a class of drugs called selective estrogen receptor modulators (SERMs) [2].

The overall risk and severity of these side effects are typically outweighed by the benefits of TAM in treating male breast cancer. While there are no specific adjuvant that can completely eliminate the side effects of TAM, there are some natural antioxidant substances that may help alleviate certain symptoms or support overall well-being such as: curcumin, green tea, omega-3 and vitamin D [3,4]. *Pistacia lentiscus* L. is a Mediterranean plant that has been studied for its various potential health benefits. It contains bioactive compounds that contribute to its antioxidant effect [5-7]. Research has shown that extracts of *Pistacia lentiscus* have anti-inflammatory and antioxidant properties, which may contribute to the protection of reproductive tissues. However, more research is needed to fully understand the potential benefits of *P. lentiscus* in male reproductive health. As far as it could be ascertained, the aim of the present study was to evaluate the protective effect of *Pistacia lentiscus* decoction on seed quality alteration induced by TAM in male mice as well as investigate it's protective effect on TAM induced oxidative perturbation in testis and epididymis. Also, assess the the free radical scavenging capacity of the prepared decoction.

Materials and Methods

Free radical scavenging capacity of the extract

At room temperature, the synthetic radical 1,1-diphenyl-2-picrylhydrazyl (DPPH) exhibits a deep violet coloration in solution, which fades upon interaction with a proton-donating substance according to the following reaction:

DPPH• + AH → DPPH-H + A

The decrease in color intensity can thus be quantitatively monitored by spectrophotometry. This decolorization reflects the antioxidant capacity of a sample through its ability to scavenge free radicals.

The assay is performed via serial binary dilutions. For a total volume of 400 µL, 50 µL of the extract is mixed with 350 µL of methanol to obtain solution C1. From this C1 solution, 200 µL is taken and combined with 200 µL of methanol to yield solution C2. This dilution step is repeated sequentially to obtain solutions up to C5. From each dilution, 50 µL is transferred using a micropipette into a test tube, to which 1.95 mL of freshly prepared DPPH solution is added. A control tube containing DPPH but no extract is prepared in the same manner. All tubes are incubated in the dark for 30 minutes. Each measurement is performed in duplicate. Absorbance is recorded at 517 nm.

To prepare the DPPH solution, 0.0024 g of DPPH is dissolved in 100 mL of methanol, yielding an initial absorbance (Do) between 0.6 and 0.7.

Animals and experimental design

Adult male Swiss albino mice (8–10 weeks old, weighing 25–30 g) were randomly assigned to four experimental groups (n = 10 per group) and maintained under standard laboratory conditions (22 ± 2 °C, 12-hour light/dark cycle) with unrestricted access to food and water. The experimental framework was established to

investigate the potential neuroprotective and/or synergistic properties of a methanolic extract of *Pistacia lentiscus* (ME) in the context of tamoxifen (TAM)-induced neurotoxicity.

The animals were distributed into the following treatment groups:

- **Group I – TAM**: received tamoxifen (30 mg/kg), solubilized in an appropriate vehicle.
- **Group II – TAM + ME**: received tamoxifen (30 mg/kg, orally) in conjunction with the methanolic extract of *Pistacia lentiscus* (1 g/10 ml distilled water, orally), at a dose previously validated through phytochemical profiling and toxicological screening;
- **Group III – ME**: received only the methanolic extract, administered as described above;
- **Group IV – Control**: received an equivalent volume of distilled water, serving as vehicle control.

Treatments were administered once daily over a period determined based on the pharmacodynamic profile of TAM and prior literature regarding the bioactivity of the plant extract. Throughout the study, animals were monitored daily for general health status, body weight changes, and behavioral alterations. Upon completion of the treatment regimen, all animals were humanely euthanized under appropriate anesthesia, and tissues were harvested for subsequent biochemical and histopathological analyses

Effects of *pistacia lentiscus* decoction on sperm quality in mice

Semen analysis and seed quality

Sperm was assessed according to the World Health Organisation (WHO) laboratory manual [8]. Semen analysis should be performed no later than 1 hour after collection of sperm from the epididymis, to prevent dehydration or temperature changes from affecting sperm quality.

Sperm count

Sperm count was performed according to standard method [9]. The right epididymis was minced, and the sperm was diluted in a formalin (40%)-sodium bicarbonate solution (50g/10mL). Malassez cell was used under light microscope for counting spermatozoa in the squares (right and top sides are counted included).

Sperm viability

Sperm viability was examined by the eosin-nigrosin staining solution. Sperm (50µL) was diluted in the prepared solution (v/v) and let stand for 30 seconds at room temperature and coated a microscopic slide to be evaluated at 100 magnifications under light microscope. The dead spermatozoa whose cell membrane was damaged, absorbed the red color of eosin while the living cell with an intact membrane did not. Nigrosine provides white contrast for the unstained living cells. Therefore, white spermatozoa were classified as "alive" while pink spermatozoa were classified as "dead". The sperm viability was expressed as a percentage.

Sperm motility and morphology

The sperm mobility was expressed as a percentage.

Oxidative stress parameters

SOD activity according to a standard method [10]. SOD activity was measured by changes in absorbance every 60 seconds for 5 minutes at 480 nm and expressed as Unity U SOD/mg of protein. CAT was detected [10] and expressed as µmol H_2O_2 /min/mg of protein. The determination of enzymatic antioxidant activities was accomplished by detection of the glutathione peroxidase activity (GPx) performed according to standard method [11]. Results were expressed as UI/mg of protein.

Statistical analysis

Data was analyzed using GraphPad Prism 8.4.2 Software (La Jolla, CA, USA). Data were determined by one-way ANOVA, followed by Tukey's post hoc test and was expressed as the mean ± standard error (SE) $P < 0.05$ was considered significant.

Results and Discussion

Free radical scavenging capacity

We used the DPPH assay to evaluate the antioxidant capacity of the *Pistacia lentiscus* extract. Ascorbic acid was employed as the reference antioxidant compound. Our results clearly demonstrate that the radical-scavenging activity of both the plant extract (IC 50 = 105 ug/mL) and the reference molecule (IC 50 = 70 ug/mL) increased progressively in a dose-dependent manner (Figure 1).

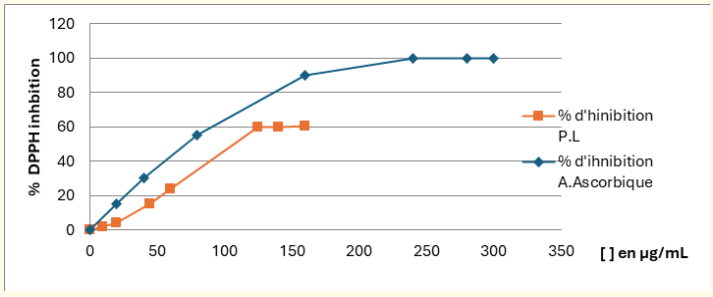

Figure 1: Dose-response of the antioxidant capacity of *Pistacia lentiscus* fixed oils against the DPPH radical.

Effects of *pistacia lentiscus* decoction on sperm quality in mice

Following subacute treatment with TAM, a marked decline was observed in several key sperm parameters: sperm concentration decreased to 5.6 × 10^6/mL, motility dropped to 60%, and viability to 42%. A significant increase in morphological abnormalities was also noted, with 18% of sperm displaying head defects and 22% exhibiting tail anomalies, compared to the control group. Notably, co-administration of TAM with the methanolic extract of *Pistacia lentiscus* demonstrated a corrective effect, substantially restoring these parameters toward normal levels when compared to the group receiving TAM alone.

	I	II	III	IV
Spermic concentration (10^6/mL)	5,6 ± 1*	6,9 ± 1.9#	7, 46 ± 1.9#	7,32 ± 2
Motility (%)	60 ± 5*	66 ± 6#	76 ± 7#	71 ± 5
Viability (%)	42 ± 6*	65 ± 6#	82 ± 7#	76 ± 5
Morphology (%)	60 ± 3*	76 ± 5,38#	86,3 ± 3,79#	82,2 ± 4,52
Head abnormality (%)	18 ± 2,1*	8,64 ± 1,1#	5,2 ± 0,81#	6,6 ± 0,96
Tail abnormality (%)	22 ± 4,2*	16,32 ± 1,9*#	6,7 ± 1*#	9,2 ± 1,41

Table 1: Effect of Subacute Treatment with *Pistacia lentiscus* Decoction on Sperm Quality Alterations Induced by Tamoxifen Exposure.

I : group TAM ; II : group TAM + E.M ; III : group E.M ; IV : control group (*: p< 0,05 vs Témoin et # : p < 0,05 vs Tamoxifen).

Oxidative stress parameters

Pro-oxidant

Our results showed a significant increase in malondialdehyde (MDA) levels in both the testis and epididymis following TAM administration. Subacute co-treatment with the methanolic extract of *Pistacia lentiscus* exerted a protective effect against TAM-induced lipid peroxidation, restoring MDA levels toward normal values (Figure 2).

Subacute administration of tamoxifen (TAM) for 28 days led to a depletion of thiol group levels in both the testis and epididymis. Co-treatment with the methanolic extract of *Pistacia lentiscus* provided protection against TAM-induced protein oxidation, restoring thiol levels toward normal values (Figure 3).

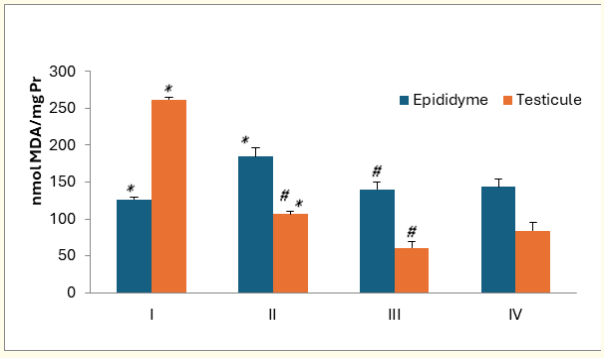

Figure 2: Effects of Subacute Treatment with *Pistacia lentiscus* and Tamoxifen on Testicular and Epididymal Malondialdehyde (MDA) Concentrations in mouse. I : group TAM ; II : group TAM + E.M ; III : group E.M ; IV : control group (*: p< 0,05 vs control and # : p < 0,05 vs Tamoxifen). (n = 10). (* : p < 0,05 vs Témoin et # : p < 0,05 vs Tamoxifène).

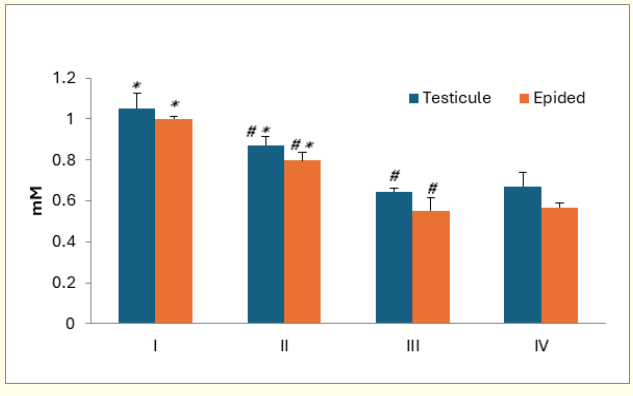

Figure 3: Effects of Subacute Treatment with *Pistacia lentiscus* and Tamoxifen on Testicular and Epididymal thiol groups Concentrations in mouse. I : group TAM ; II : group TAM + E.M ; III : group E.M ; IV : control group (*: p < 0,05 vs control and # : p < 0,05 vs Tamoxifen). (n = 10). (* : p < 0,05 vs Témoin et # : p < 0,05 vs Tamoxifène).

Subacute administration of TAM for 28 days resulted in increased hydrogen peroxide (H_2O_2) concentrations in both the epididymis and testis. Co-treatment with the methanolic extract of *Pistacia lentiscus* conferred protection against TAM-induced H_2O_2 overproduction, restoring the levels toward normal values (Figure 4).

Anti-oxydant

In the present study, we measured the activity of several key antioxidant enzymes, including catalase, superoxide dismutase (SOD), and glutathione peroxidases (GPx).

Our results show that subacute administration of tamoxifen (TAM) leads to an increase in catalase activity in both the testis and epididymis. Co-treatment with the methanolic extract of *Pistacia lentiscus* provided protection against TAM-induced moderate oxidative stress, restoring catalase activity toward normal levels (Figure 5).

In the testis, superoxide dismutase (SOD) activity increased significantly in the tamoxifen-treated group. Specifically, SOD activity in the control group was approximately 1.097 U/min/mg of protein, compared to 6.178 U/min/mg of protein in the TAM-treated

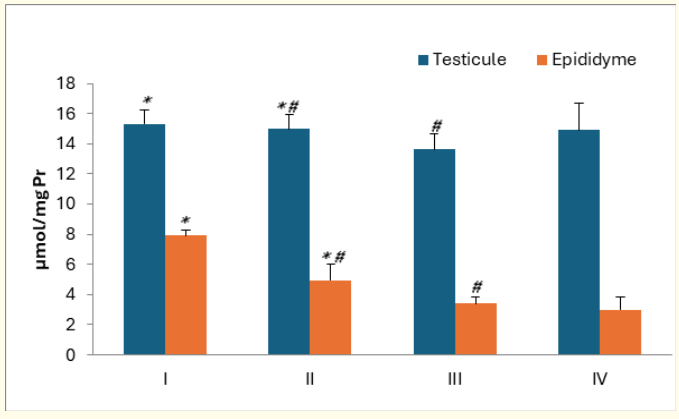

Figure 4: Effects of Subacute Treatment with *Pistacia lentiscus* and Tamoxifen on Testicular and Epididymal hydrogen peroxide levels in mouse. I : group TAM ; II : group TAM + E.M ; III : group E.M ; IV : control group (*: p < 0,05 vs control and # : p < 0,05 vs Tamoxifen). (n = 10). (* : p < 0,05 vs Témoin et # : p < 0,05 vs Tamoxifène).

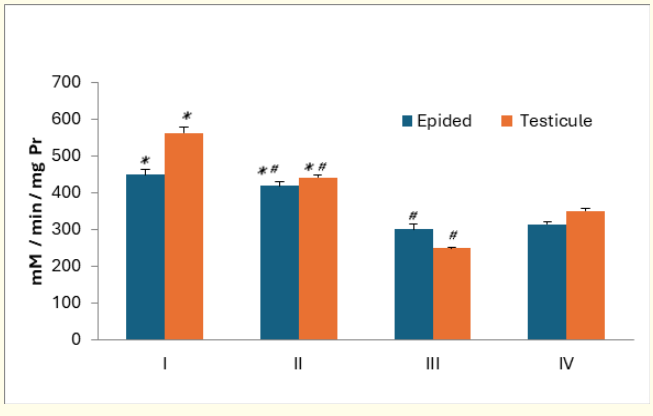

Figure 5: Effects of Subacute Treatment with *Pistacia lentiscus* and Tamoxifen on Testicular and Epididymal catalase activity in mouse. I : group TAM ; II : group TAM + E.M ; III : group E.M ; IV : control group (*: p< 0,05 vs control and # : p < 0,05 vs Tamoxifen). (n = 10). (* : p < 0,05 vs Témoin et # : p < 0,05 vs Tamoxifène).

group. Co-administration of *Pistacia lentiscus* with tamoxifen effectively restored this parameter to near-normal levels. Furthermore, administration of the methanolic extract of *Pistacia lentiscus* alone had no noticeable effect on hepatic SOD activity in the absence of tamoxifen co-treatment (Figure 6).

Our results indicate that subacute administration of TAM for 28 days significantly increases GPx activity in both the liver and nervous system. Co-treatment with the methanolic extract of *Pistacia lentiscus* exerted a protective effect against TAM-induced dysregulation, restoring GPx activity to near-normal levels (Figure 7).

In the present study TAM has been shown to increase oxidative stress markers in the testicles, which can lead to impaired sperm production and function. It may also affect the epididymis, potentially leading to abnormalities in sperm quality and motility. The epididymis is a coiled tube that connects to testicles and plays a crucial role in sperm motility and storage. TAM can affect epididymis by reducing sperm motility and altering the composition of the epididymal fluid, which can influence sperm function. It's interference with estrogen receptors can disrupt the hormonal signaling involved in sperm production (spermatogenesis). It may result in reduced sperm count, impaired sperm maturation, and changes in sperm morphology (shape) and motility. These effects can lead

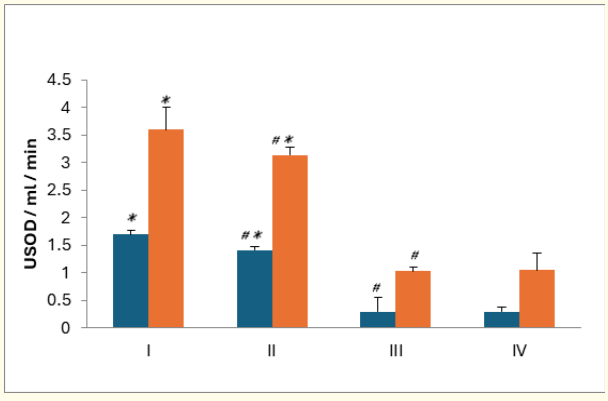

Figure 6: Effects of Subacute Treatment with *Pistacia lentiscus* and Tamoxifen on Testicular and Epididymal superoxide dismutase activity in mouse. I : group TAM ; II : group TAM + E.M ; III : group E.M ; IV : control group (*: $p < 0,05$ vs control and # : $p < 0,05$ vs Tamoxifen). ($n = 10$). (* : $p < 0,05$ vs Témoin et # : $p < 0,05$ vs Tamoxifène).

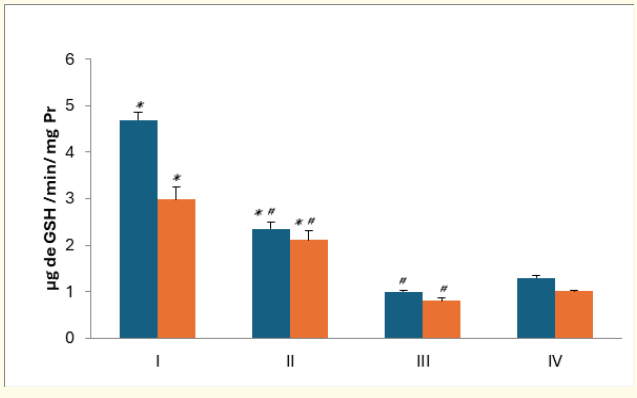

Figure 7: Effects of Subacute Treatment with *Pistacia lentiscus* and Tamoxifen on Testicular and Epididymal glutathione peroxidase activity in mouse. I : group TAM ; II : group TAM + E.M ; III : group E.M ; IV : control group (*: $p< 0,05$ vs control and # : $p < 0,05$ vs Tamoxifen). ($n = 10$).

to decreased fertility or even temporary infertility. Moreover, the impact of TAM on male fertility is variable. Several research have proved that TAM long term treatment leads to pathology in the endocrine and reproductive systems. TAM treatment (0.25mg/j for 14weeks) in albinos male mice induced decrease in spermatozoa motility, sperm count and sexe hormonal levels of FSH, LH, and testosterone. These modifications were associated to several histopathological changes in tunica albuginea, deformation in some seminiferous tubules and increased interstitial space, vacuolated cytoplasm of spermatogonia. Some epididymal duct lost its normal shape and other with destructed ducts [12]. It was proven that TAM induced endocrinal perturbation. Neonatal mice exposed to TAM exprimed an atrophy in the ovaries, vaginal thickening and abolition. TAM have an oestrogen-like effect in the hypothalamus, which decrease the expression ARNm kisspeptine [13]. Some studies suggest that TAM treatment can cause reversible infertility, with sperm production returning to normal levels after discontinuing the medication. It's important to note that TAM 's effects on male

reproductive parameters are typically reversible, and fertility can be restored after discontinuing the medication. Additionally, alternative fertility preservation options may be explored before starting TAM therapy if fertility preservation is a concern.

The sub-acute co-treatment with *Pistacia lentiscus* protects against the increase in oxidative stress damage induced by TAM and brings the values back to near normal. The co-treatment protects against TAM-induced protein oxidation (-SH), lipid peroxidation (MDA) and restore values to near normal in the testis and epididymis.

Conclusion

This study demonstrates that subacute exposure to tamoxifen induces significant reproductive toxicity in male mice, characterized by impaired sperm quality and oxidative damage in testicular and epididymal tissues. The observed increase in pro-oxidant markers (MDA, H_2O_2, and decreased thiol levels) alongside altered antioxidant enzyme activity (SOD, CAT, GPx) confirms that oxidative stress is a central mechanism underlying tamoxifen-induced gonado-toxicity.

Co-treatment with the methanolic extract of *Pistacia lentiscus* effectively counteracted these alterations, restoring sperm parameters and redox balance to near-normal levels. These protective effects are likely attributed to the strong free-radical-scavenging capacity of the extract, as demonstrated by its DPPH activity, and its ability to modulate endogenous antioxidant defense systems.

Altogether, our findings suggest that *Pistacia lentiscus* holds promise as a natural adjunctive agent to mitigate male reproductive toxicity associated with antihormonal therapies such as tamoxifen. Further investigations, including mechanistic studies and clinical validation, are warranted to support its integration into supportive fertility-preserving strategies.

Conflict of Interest

No conflict of interest exists.

Bibliography

1. WHO. Breast Cancer (2021).
2. I.A.R.C.-MONOGRAPHS-100A, IARC monographs on the evaluation of carcinogenic risks to humans. World Health Organization and the International Agency for Research on Cancer (2012): 131-162.
3. Baek SKJ., *et al*. "Camphene Attenuates Skeletal Muscle Atrophy by Regulating Oxidative Stress and Lipid Metabolism in Rats". *Nutrients* 12.12 (2021): 3731.
4. Wen KFX., *et al*. "Recent Research on Flavonoids and their Biomedical applications". *Current Medicinal Chemistry* 28.5 (2021): 1042-1066.
5. Beghlal DEBK., *et al*. "Phytochemical, organoleptic and ferric reducing properties of essential oil and ethanolic extract from Pistacia lentiscus (L.)". *Asian Pacific Journal of Tropical Medicine* 6.4 (2016): 305-310.
6. Klibet FBA., *et al*. "Oxidative stress-related liver dysfunction by sodium arsenite: Alleviation by Pistacia lentiscus oil". *Journal of Pharmaceutical and Biological Sciences* 54.2 (2016): 354-363.
7. Belyagoubi-Benhammou NBL., *et al*. "Fatty acid composition and antioxidant activity of Pistacia lentiscus L. fruit fatty oil from Algeria". *Journal of Food Measurement and Characterization* 12.2 (2018): 1408-1412.
8. WHO Edition F. "Examination and processing of human semen". Geneva: World Health (2010).
9. Gopalakrishnan K., *et al*. "Agglutination of human spermatozoa with antibodies to inhibin". *The Indian Journal of Experimental Biology* (1987).
10. Kakkar P., *et al*. "A modified spectrophotometric assay of superoxide dismutase" (1984).
11. Jt R., *et al*. "Selenium: biochemical role as a component of glutathione peroxidase". *Science* 179.4073 (1973): 588-590.

12. Taha S., *et al*. "Hormonal and histopathological changes in male reproductive system of albino mice after Tamoxifen administration". *Journal of Genetic and Environment Conservation* 4.1 (2016): 51-56.

13. Parandin R., *et al*. "Oestrogenic action of neonatal tamoxifen on the hypothalamus and reproductive system in female mice". *Reproduction, Fertility and Development* 29.5 (2017): 1012-1020.