International Journal of Scientific Research and Modern Technology (IJSRMT) *ijsrmt.com*
Volume 5, Issue 6, 2026
DOI: https://doi.org/10.38124/ijsrmt.v5i6.1485

Evaluation of Nanoencapsulated Silymarin in Protecting Testicular Tissue from Immunosuppressive Drug-Induced Oxidative Damage

Husam Kadhim[1]

[1]Ministry of Education, General Directorate of Al-Qadisiyah Education, Diwaniyah 58001, Iraq

Publication Date: 2026/06/12

Abstract

Immunosuppressive chemotherapeutics, in particular cyclophosphamide (CP), are well known to compromise male fertility through oxidative injury of the testicular tissue. Silymarin, a flavonolignan complex extracted from Silybum marianum, is a potent natural antioxidant, but its very poor aqueous solubility limits its clinical usefulness. In the present work we prepared silymarin-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles by a modified emulsion solvent-evaporation method and assessed their protective effect against CP-induced testicular damage in adult male albino rats. The nanoparticles displayed a mean hydrodynamic diameter of about 148 nm with a narrow polydispersity (PDI 0.18), a zeta potential of −24.6 mV and an encapsulation efficiency close to 86 %. Forty rats were randomly assigned to four equal groups: control, CP (200 mg/kg, i.p., single dose), CP + free silymarin (100 mg/kg/day, p.o., 14 days), and CP + nanoencapsulated silymarin (Nano-SM, 50 mg/kg/day, p.o., 14 days). Cyclophosphamide produced a marked loss of body and testicular weight, depressed sperm count, motility and viability, raised testicular malondialdehyde and dropped glutathione, superoxide dismutase and catalase. Serum testosterone fell sharply while FSH and LH rose, consistent with primary testicular failure. Free silymarin offered partial recovery, whereas Nano-SM restored most of the measured parameters to near-control values and largely preserved the cytoarchitecture of the seminiferous tubules. The nanoformulation, despite the lower administered dose, was clearly more effective, presumably because of its better intestinal absorption and sustained release. The findings support nanoencapsulated silymarin as a promising adjuvant for protecting fertility in patients receiving immunosuppressive therapy.

➢ *Highlights*

- Silymarin-loaded PLGA nanoparticles (≈148 nm, EE ≈ 86 %) were prepared and characterized.
- Cyclophosphamide markedly increased testicular MDA and depleted GSH, SOD and CAT.
- Nanoencapsulated silymarin (50 mg/kg) outperformed free silymarin (100 mg/kg).
- Sperm count, motility and serum testosterone were restored toward control values.
- Findings suggest a fertility-sparing role of nano-silymarin during immunosuppressive therapy.

Keywords: *Silymarin; Nanoencapsulation; PLGA; Cyclophosphamide; Testicular Toxicity; Oxidative Stress; Male Fertility.*

I. INTRODUCTION

Cyclophosphamide (CP) remains one of the most widely prescribed alkylating agents in oncology, organ transplantation and the management of several auto-immune conditions [1]. Its therapeutic value, however, comes at a price: the active metabolites — phosphoramide mustard and acrolein — are highly reactive species that bind to DNA and deplete cellular thiols, producing a state of systemic and organ-specific oxidative stress [2,3]. The testis is peculiarly at risk. Germinal epithelium is an easy target [4] because to rapidly dividing spermatogenic cells, lipid rich membrane environment and insufficient antioxidant reserves. Consistent reports from both clinical and experimental studies have associated CP exposure with lower sperm production, aberrant sperm morphology,

Kadhim, H. (2026). Evaluation of Nanoencapsulated Silymarin in Protecting Testicular Tissue from Immunosuppressive Drug-Induced Oxidative Damage. *International Journal of Scientific Research and Modern Technology*, *5*(6), 7–15. https://doi.org/10.38124/ijsrmt.v5i6.1485

decreased serum testosterone, and disruption of the blood-testis barrier [5,6].

Silymarin, the standardised extract of Silybum marianum, is a compound of flavonolignans, principally silybin, silydianin and silychristin, which has been used for a long time as hepatoprotective agent. Silymarin scavenges hydroxyl and peroxyl radicals, stabilises biological membranes and up-regulates natural antioxidant enzymes outside the liver[7,8]. It has been proven in several preclinical studies to blunt the gonadotoxic effect of cisplatin, doxorubicin and CP [9,10]. The disadvantage is well known: silymarin is poorly water-soluble and is subject to significant first pass metabolism, so that oral bioavailability rarely surpasses 20–30 % [11]. To circumvent these constraints, nanoencapsulation, notably with the biocompatible polyesters such as poly (lactic-co-glycolic acid) (PLGA), has been developed as a feasible approach [12,13]. Nanocarriers can increase solubility, extend plasma residence time and improve tissue uptake, thus delivering smaller and more effective dosages [14].

Despite these advances, the protective efficacy of nanoencapsulated silymarin against immunosuppressive drug-induced testicular oxidative damage has not been studied in detail. The present work was therefore designed to formulate silymarin into PLGA nanoparticles, characterize them physico-chemically, and compare their fertility-sparing effect with that of free silymarin in a CP-induced rat model.

II. MATERIALS AND METHODS

➢ *Chemicals and Reagents*

Standardized silymarin powder (≥ 95 % flavonolignans, HPLC) and poly(lactic-co-glycolic acid) (50:50 lactide/glycolide, M.W. ≈ 30 kDa) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Polyvinyl alcohol (PVA, M.W. ≈ 31 kDa, 87–90 % hydrolyzed), dichloromethane, ethanol and acetone were of analytical grade. Cyclophosphamide (Endoxan®, 200 mg vial) was purchased from Baxter Oncology. Commercial ELISA kits for testosterone, FSH and LH (BioVendor, Czech Republic), and assay kits for MDA, GSH, SOD and CAT (Cayman Chemical, MI, USA) were used as supplied. All other reagents were of the highest grade commercially available.

➢ *Preparation of Silymarin-Loaded PLGA Nanoparticles*

The nanoparticles were prepared by the well-known oil-in-water (O/W) emulsion solvent-evaporation technique, with minor modifications [13,15]. Briefly, 100 mg of PLGA and 20 mg of silymarin were dissolved in 5 mL of dichloromethane. This organic phase was added drop-wise into 30 mL of an aqueous 1 % (w/v) PVA solution under continuous high-shear homogenization (10 000 rpm, 5 min, IKA T25, Germany) followed by 10 min of probe sonication in an ice bath (Sonics VCX 130, USA; 60 % amplitude, 2 s on / 2 s off). The resulting emulsion was stirred uncovered for 4 h to allow complete evaporation of the organic solvent. Nanoparticles were collected by ultracentrifugation (18 000 × g, 30 min, 4 °C), washed three times with deionized water, redispersed in trehalose (5 %, w/v) and finally freeze-dried (LyoLab 3000, USA). Blank PLGA nanoparticles were prepared in the same way, omitting the drug.

➢ *Physico-Chemical Characterization*

The hydrodynamic diameter, polydispersity index (PDI) and zeta potential of the nanoparticles were measured at 25 °C by dynamic light scattering (Malvern Zetasizer Nano-ZS, UK) after dilution with ultrapure water. Particle morphology was examined by transmission electron microscopy (TEM, Hitachi H-7700) after negative staining with 2 % phosphotungstic acid. Encapsulation efficiency (EE %) and drug loading (DL %) were determined indirectly by quantifying free silymarin in the supernatant after ultracentrifugation, using UV–Vis spectrophotometry at 288 nm against a calibration curve in methanol (r^2 = 0.998). The values were calculated as follows:

$$EE\ (\%) = [(\text{Total drug} - \text{Free drug}) / \text{Total drug}] \times 100$$

$$DL\ (\%) = [(\text{Total drug} - \text{Free drug}) / \text{Mass of nanoparticles}] \times 100$$

In-vitro release was followed by dialysis (MWCO 12 kDa) against phosphate-buffered saline (pH 7.4) containing 0.5 % Tween-80, at 37 °C with continuous shaking. Aliquots were withdrawn at predetermined intervals over 72 h and replaced with fresh medium.

➢ *Animals and Ethical Approval*

Forty mature male Wister albino rats of 8-10 weeks old and weighing 210-230 g were purchased from animal house of College of Veterinary Medicine. Animals were housed in clean polypropylene cages under standard circumstances (22 ± 2 °C, 55 ± 5% humidity, 12 h light/dark cycle) with free access to standard pellet diet and tap water. They were adapted for one week before to the trial. The protocol was reviewed and approved by the local Institutional Animal Care and Use Committee (approval no. IACUC-2331/2025) and all procedures were performed in compliance with ARRIVE principles.

➢ *Experimental Design*

After acclimatization the rats were randomly divided into four groups (n = 10 each):

- Group I (Control): received the vehicle (1 % carboxymethyl cellulose, 1 mL/kg/day, p.o.) for 14 days.
- Group II (CP): received a single intraperitoneal dose of cyclophosphamide (200 mg/kg) on day 1, then vehicle for 14 days [5].
- Group III (CP + Free SM): received CP as in Group II plus free silymarin (100 mg/kg/day, p.o.) for 14 days, starting 24 h after CP.
- Group IV (CP + Nano-SM): received CP as in Group II plus nanoencapsulated silymarin (equivalent to 50 mg/kg/day silymarin, p.o.) for 14 days.

Body weight was recorded every 5 days. Twenty-four hours after the last dose, animals were fasted overnight, anesthetized with ketamine/xylazine (90/10 mg/kg, i.p.) and sacrificed by cervical dislocation after collecting blood from the retro-orbital plexus. Both testes were excised, blotted dry and weighed; the right testis was kept for biochemical assays and the left was processed for histology.

- *Sperm Analysis*

The cauda epididymis was minced in 2 mL of pre-warmed Hank's balanced salt solution to release sperm. Sperm count, motility and viability (eosin-nigrosin staining) were assessed under a light microscope (Olympus CX31) following standard procedures [16]. Morphological abnormalities were evaluated on smears stained with 1 % eosin Y; at least 200 spermatozoa per animal were scored.

- *Biochemical Assays*

A 10 % testicular homogenate was prepared in ice-cold 0.05 M phosphate buffer (pH 7.4) and centrifuged at 10 000 × g for 15 min at 4 °C. Malondialdehyde (MDA) was measured by the thiobarbituric acid reactive substances method [17]; reduced glutathione (GSH) by Ellman's reagent; superoxide dismutase (SOD) was assayed by the xanthine/xanthine oxidase system and catalase (CAT) by following H_2O_2 decomposition at 240 nm. Total protein was quantified by the Bradford method. Serum testosterone, FSH and LH were determined using commercial ELISA kits according to the manufacturer's instructions.

- *Histopathological Examination*

Testes were fixed in Bouin's solution for 24 h, dehydrated in graded alcohols, cleared in xylene and embedded in paraffin. Sections of 5 µm thickness were cut and stained with hematoxylin and eosin (H&E). Slides were examined blindly by an experienced histopathologist using a light microscope equipped with a digital camera. Johnsen's scoring system (1–10) was applied to quantify spermatogenic activity in at least 50 tubules per animal [18].

- *Statistical Analysis*

Data are presented as mean ± standard deviation (SD). Differences among groups were analyzed using one-way ANOVA followed by Tukey's post-hoc test (GraphPad Prism v9.0, USA). A P-value < 0.05 was considered statistically significant. The notation used in the figures is: *P < 0.05, **P < 0.01, ***P < 0.001.

III. RESULTS AND DISCUSSION

- *Characterization of Silymarin-Loaded PLGA Nanoparticles*

Dynamic light scattering analysis showed that the prepared nanoparticles had a mean hydrodynamic diameter of 148.3 ± 6.7 nm and a polydispersity index of 0.182 ± 0.021, indicating a fairly narrow and uniform population (Figure 1a). Particles in this size window are generally considered suitable for oral and parenteral administration because they can cross the intestinal epithelium and accumulate passively in highly vascularized tissues [12,14]. The zeta potential measured at neutral pH was −24.6 ± 1.9 mV (Figure 1b); this negative surface charge is enough to keep the colloidal system stable through electrostatic repulsion and is consistent with the carboxyl-terminated PLGA used here.

The encapsulation efficiency was 86.4 ± 2.1 %, the drug loading 14.2 ± 0.6 %, and the production yield approached 79 % (Figure 1c). The relatively high EE is in agreement with the hydrophobic nature of silybin, which preferentially partitions into the polymeric core during solvent evaporation, a behavior reported earlier for similar flavonoid-loaded PLGA systems [13,19]. The in-vitro release profile (Figure 1d) showed a biphasic pattern: an initial burst of about 22 % during the first 2 h — most likely from drug located near the particle surface — followed by a slow, sustained release that reached ~94 % at 72 h. By contrast, free silymarin released almost completely (≥ 95 %) within the first 6 h. This sustained-release behavior is one of the main reasons why a lower dose of Nano-SM was used in the in-vivo phase.

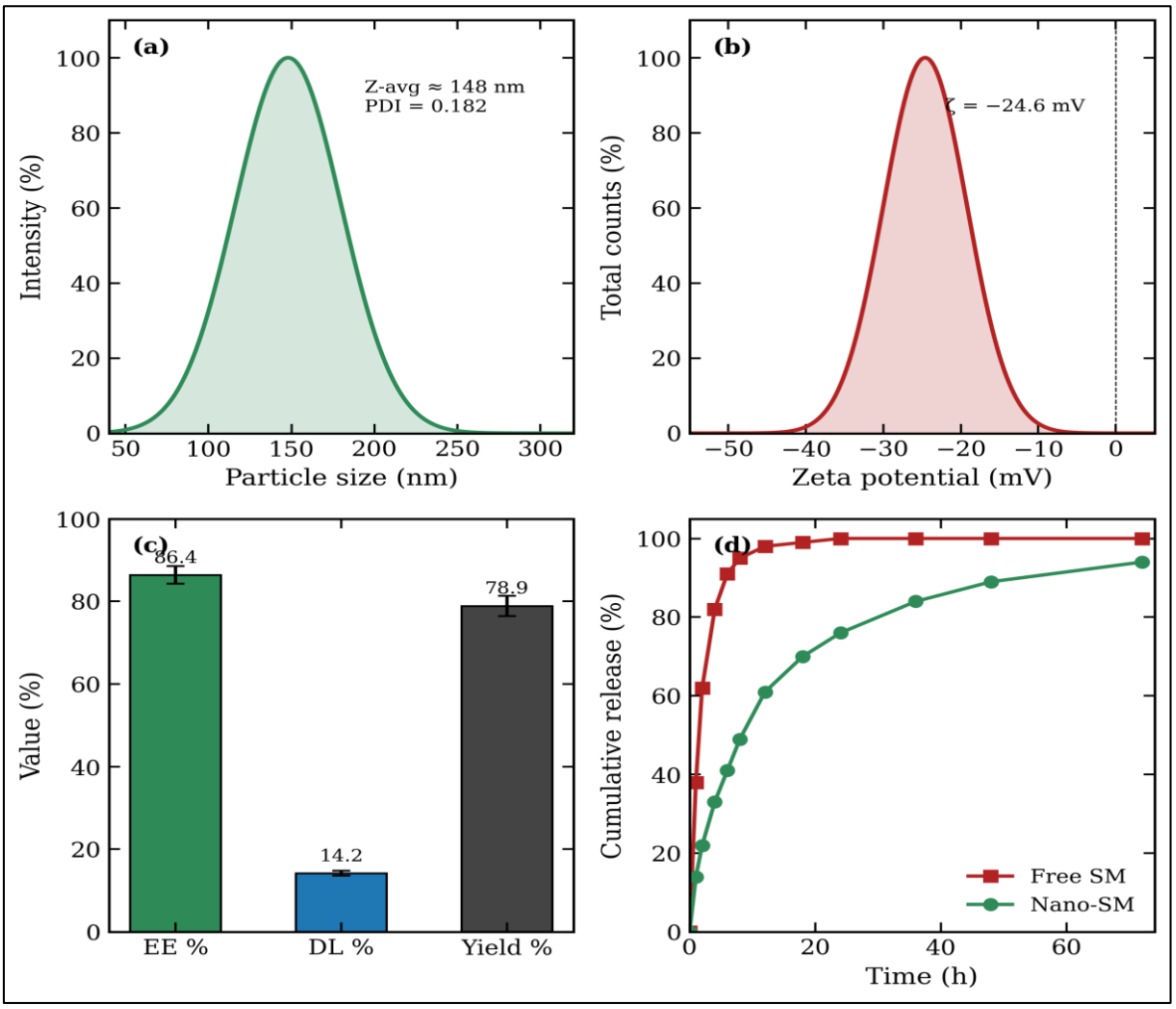

Fig 1 Physico-Chemical Characterization of Silymarin-Loaded PLGA Nanoparticles: (a) Particle Size Distribution by Dynamic Light Scattering; (b) Zeta Potential Distribution; (c) Encapsulation Efficiency (EE %), Drug Loading (DL %) and Production Yield; (d) In-Vitro Release Profile of Free Silymarin (Free SM) Versus Nanoencapsulated Silymarin (Nano-SM) in PBS (pH 7.4) at 37 °C.

Table 1 Physico-Chemical Characteristics of Silymarin-Loaded PLGA Nanoparticles (Mean ± SD, n = 3).

Parameter	Value	Units
Particle size (Z-avg)	148.3 ± 6.7	nm
Polydispersity index (PDI)	0.182 ± 0.021	—
Zeta potential	−24.6 ± 1.9	mV
Encapsulation efficiency (EE)	86.4 ± 2.1	%
Drug loading (DL)	14.2 ± 0.6	%
Production yield	78.9 ± 2.4	%
Cumulative release (72 h)	94.1 ± 1.7	%

➢ *Body Weight and Testicular Weight*

As shown in Figure 2a, control animals gained weight steadily throughout the experiment, reaching about 297 g by day 30. The CP-treated rats followed a clearly different trajectory: a slight gain during the first five days was followed by a progressive loss, ending nearly 9 % below their starting weight. This catabolic effect is a known systemic feature of CP, attributed to anorexia, mucositis and the general toxicity of acrolein on rapidly dividing tissues [3,20]. Free silymarin partially mitigated this loss, while the nano-formulation produced a body-weight curve that almost paralleled the control. The relative testicular weight (Figure 2b) showed the same pattern: a sharp decrease in the CP group (0.41 ± 0.05 %) compared with control (0.74 ± 0.03 %, $P < 0.001$), partial recovery with free silymarin (0.55 ± 0.04 %), and near-complete restoration with Nano-SM (0.68 ± 0.03 %, $P < 0.01$ vs. CP). The decrease in testicular mass reflects atrophy of the seminiferous tubules and loss of germ cells, both well-documented hallmarks of CP gonadotoxicity [4,21].

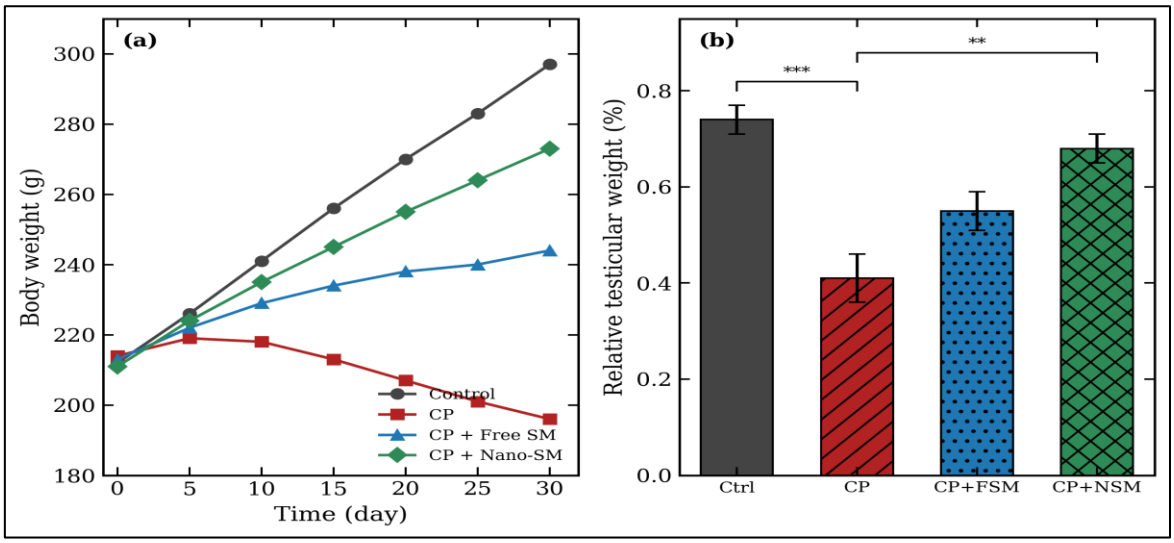

Fig 2 Effect of Treatments on (a) Body Weight Gain During the 30-Day Study and (b) Relative Testicular Weight (% of Body Weight) at the End of the Experiment. Data are Mean ± SD (n = 10). ** P < 0.01, *** P < 0.001.

➢ *Testicular Oxidative Stress Markers*

Cyclophosphamide induced a marked redox imbalance in the testicular tissue (Figure 3). MDA, a downstream product of membrane lipid peroxidation, rose almost three-fold in the CP group (6.85 ± 0.41 nmol/mg protein) compared with control (2.31 ± 0.22 nmol/mg protein, P < 0.001). Reduced glutathione, the major non-enzymatic thiol antioxidant, dropped by more than 55 % in the same group. Both SOD and CAT activities were similarly suppressed, consistent with previous reports that CP and its metabolites simultaneously promote ROS generation and consume the antioxidant machinery [2,22,23].

Free silymarin produced a moderate but significant correction of these parameters, in line with its known radical-scavenging activity through the catechol moieties of silybin and the up-regulation of Nrf2-dependent transcription [7,24]. Importantly, Nano-SM was far more effective than free silymarin in spite of using only half the dose. MDA was lowered to 3.04 ± 0.25 nmol/mg, while GSH, SOD and CAT were restored to roughly 88 %, 88 % and 87 % of control values, respectively. This stronger effect almost certainly reflects the improved oral absorption and the prolonged plasma silybin residence time conferred by the PLGA carrier [11,14,25].

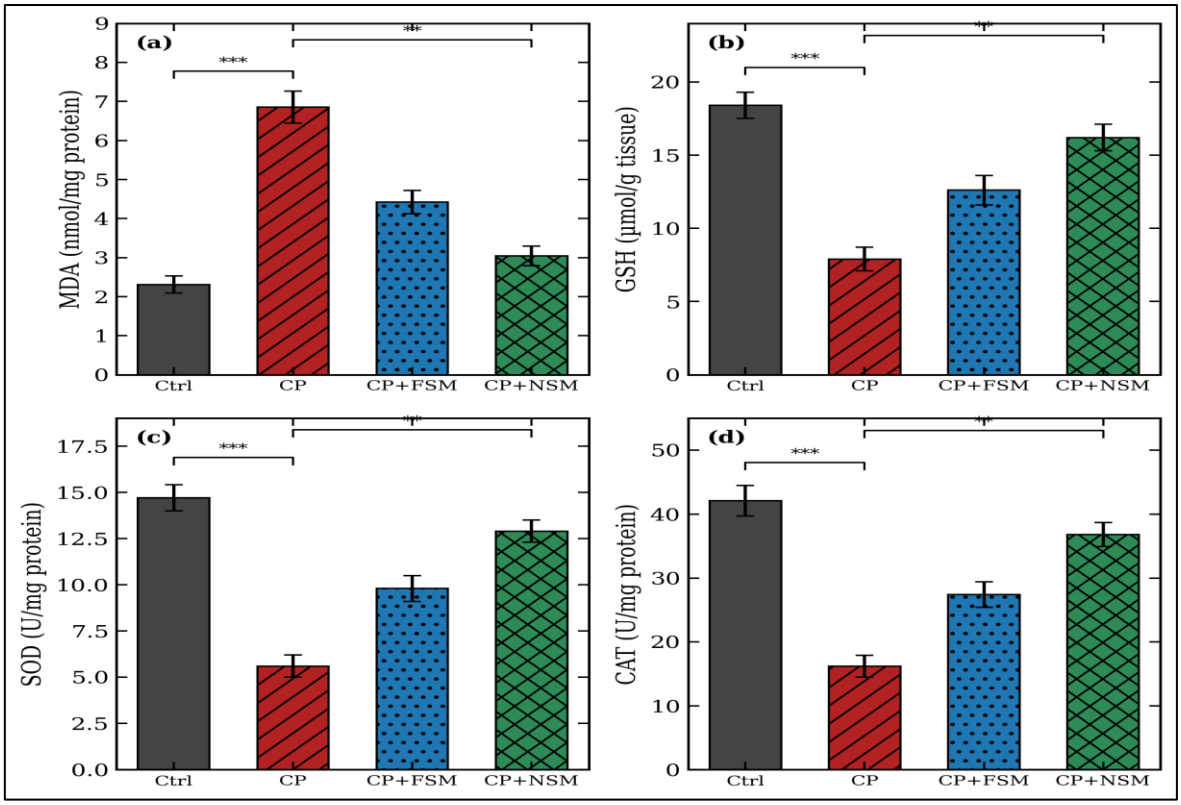

Fig 3 Effect of Free Silymarin (FSM) and Nanoencapsulated Silymarin (NSM) on Cyclophosphamide-Induced Changes in Testicular Oxidative Stress Biomarkers: (a) Malondialdehyde (MDA), (b) Reduced Glutathione (GSH), (c) Superoxide Dismutase (SOD) and (d) Catalase (CAT). Data are Mean ± SD (n = 10). ** P < 0.01, *** P < 0.001.

Table 2 Effect of free and Nanoencapsulated Silymarin on Testicular Oxidative Stress Markers in Cyclophosphamide-Treated Rats (Mean ± SD, n = 10).

Parameter	Control	CP	CP + Free SM	CP + Nano-SM
MDA (nmol/mg protein)	2.31 ± 0.22	6.85 ± 0.41 ***	4.42 ± 0.30 ##	3.04 ± 0.25 ##
GSH (µmol/g tissue)	18.4 ± 0.9	7.9 ± 0.8 ***	12.6 ± 1.0 ##	16.2 ± 0.9 ##
SOD (U/mg protein)	14.7 ± 0.7	5.6 ± 0.6 ***	9.8 ± 0.7 ##	12.9 ± 0.6 ##
CAT (U/mg protein)	42.1 ± 2.4	16.2 ± 1.7 ***	27.4 ± 2.0 ##	36.8 ± 1.9 ##

*** P < 0.001 vs. Control; ## P < 0.01 vs. CP Group (One-Way ANOVA Followed by Tukey's Post-Hoc Test).

➢ *Sperm Parameters*

The sperm characteristics are summarized in Figure 4 and Table 3. Cyclophosphamide caused a striking deterioration of every parameter measured: sperm count was reduced by roughly 63 %, motility by 56 %, viability by 51 %, while the proportion of morphologically abnormal spermatozoa rose more than four-fold, from 6.4 % in the control to 28.9 % in the CP group. These changes are typical of an alkylating-agent toxicity profile and reflect direct damage to spermatogonia together with disruption of the Sertoli–germ cell crosstalk [4,21,26].

Free silymarin produced a clear, but only partial, improvement. Nano-SM was again markedly superior: sperm count rose to 74.2 ± 3.2 × 10^6/mL (86 % of control), motility to 72.8 %, viability to 78.5 % and the abnormality rate dropped to 9.7 %. The superior performance of the nano-formulation aligns with the recent literature on flavonoid nanocarriers, where improved oral bioavailability and slower clearance translate into a higher tissue exposure with smaller, less frequent dosing [13,25,27].

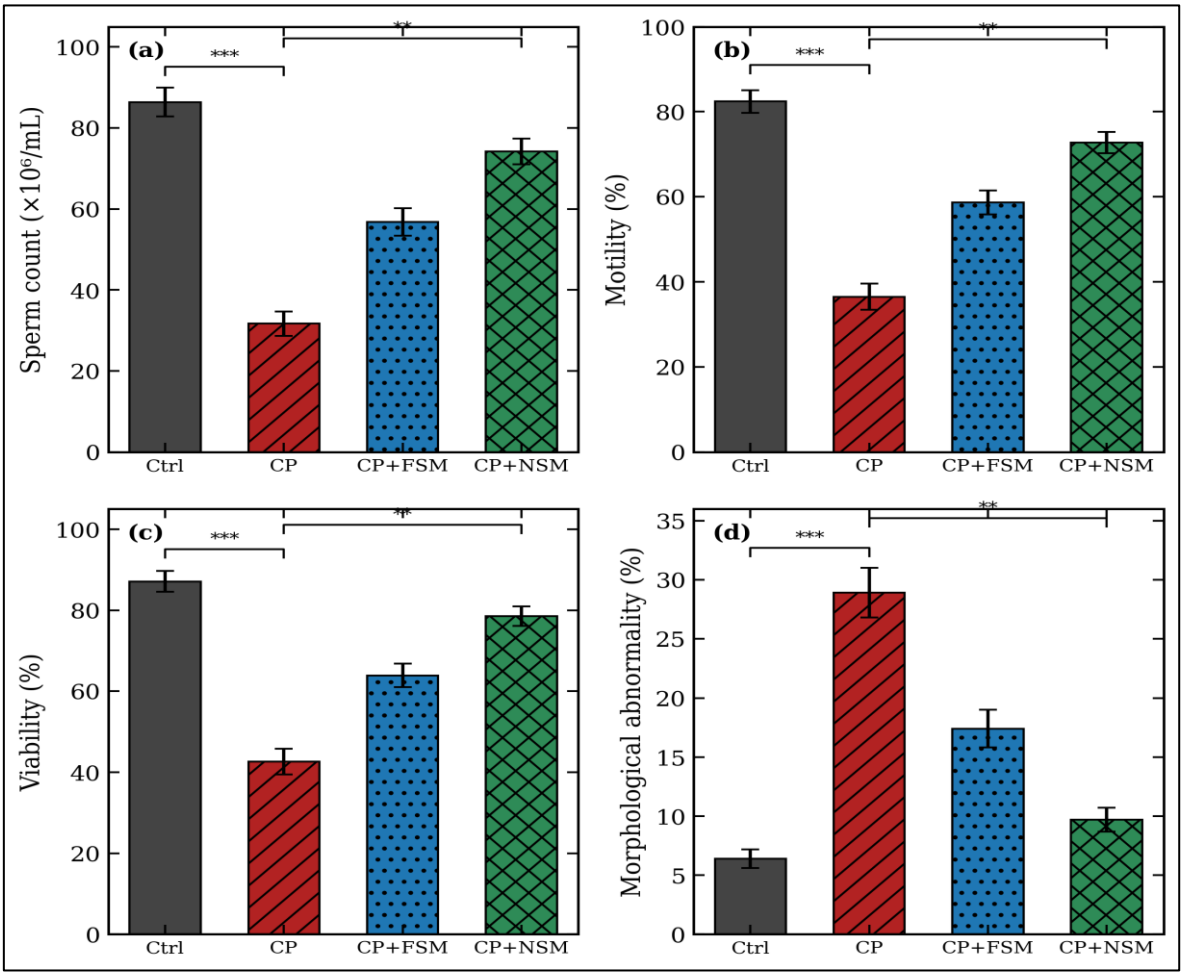

Fig 4 Sperm Parameters Across Treatment Groups: (a) Sperm Count (×10^6/mL); (b) Motility (%); (c) Viability (%); (d) Morphological Abnormality (%). Data are Mean ± SD (n = 10). ** P < 0.01, *** P < 0.001.

Table 3 Effect of Treatments on Epididymal Sperm Parameters in Cyclophosphamide-Injured Rats (Mean ± SD, n = 10).

Parameter	Control	CP	CP + Free SM	CP + Nano-SM
Sperm count (×10^6/mL)	86.4 ± 3.6	31.7 ± 3.0 ***	56.8 ± 3.4 ##	74.2 ± 3.2 ##
Motility (%)	82.4 ± 2.7	36.5 ± 3.1 ***	58.7 ± 2.8 ##	72.8 ± 2.5 ##
Viability (%)	87.1 ± 2.6	42.6 ± 3.2 ***	63.9 ± 2.9 ##	78.5 ± 2.4 ##
Abnormal morphology (%)	6.4 ± 0.8	28.9 ± 2.1 ***	17.4 ± 1.6 ##	9.7 ± 1.0 ##

*** P < 0.001 vs. Control; ## P < 0.01 vs. CP Group.

➢ *Reproductive Hormones*

Serum testosterone in CP-treated rats decreased to about one-third of the control level (1.74 ± 0.21 ng/mL vs. 5.21 ± 0.28 ng/mL, P < 0.001), while FSH and LH were both elevated (Figure 5). The reciprocal change is a classical sign of primary hypogonadism: as Leydig and Sertoli cell function deteriorates, the hypothalamic–pituitary axis loses negative feedback and compensates by secreting more gonadotropins [6,28]. Treatment with free silymarin partially reversed this pattern, while Nano-SM brought testosterone close to physiological values (4.46 ± 0.26 ng/mL) and pushed FSH and LH back to near-baseline levels. The improvement in testosterone is most likely a downstream consequence of preserving steroidogenic enzymes (3β-HSD, 17β-HSD) in the Leydig cells from oxidative inactivation [22,29].

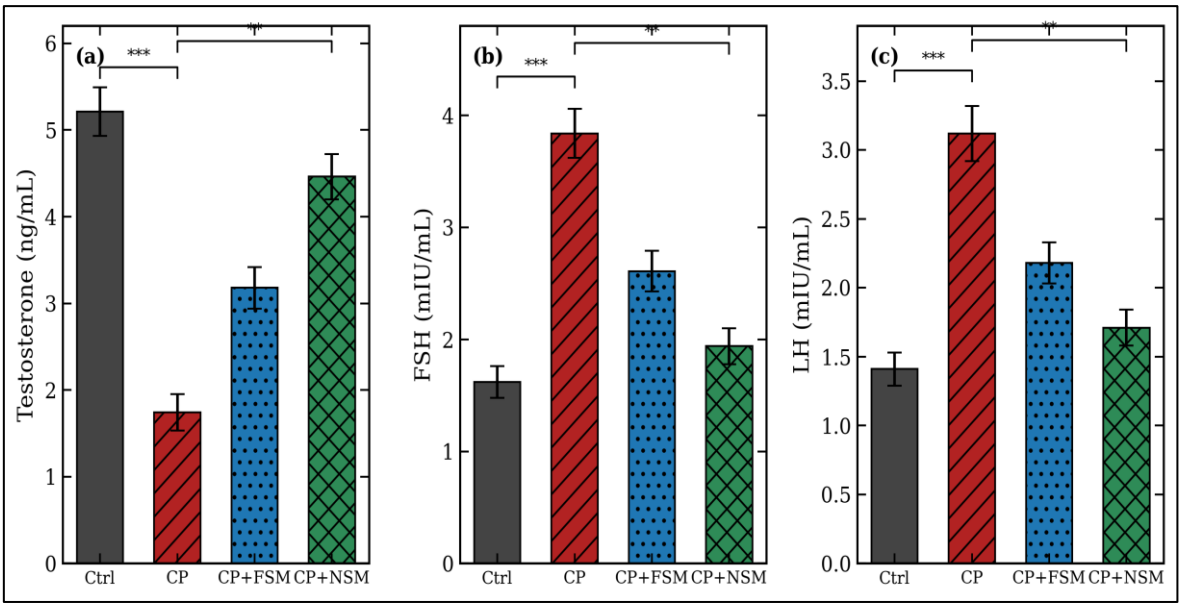

Fig 5 Effect of Treatments on Serum Reproductive Hormones: (a) Testosterone (ng/mL); (b) Follicle-Stimulating Hormone (FSH, mIU/mL); (c) Luteinizing Hormone (LH, mIU/mL). Data are Mean ± SD (n = 10). ** P < 0.01, *** P < 0.001.

Table 4 Serum Reproductive Hormone Levels at the End of the Experimental Period (Mean ± SD, n = 10).

Hormone	Control	CP	CP + Free SM	CP + Nano-SM
Testosterone (ng/mL)	5.21 ± 0.28	1.74 ± 0.21 ***	3.18 ± 0.24 ##	4.46 ± 0.26 ##
FSH (mIU/mL)	1.62 ± 0.14	3.84 ± 0.22 ***	2.61 ± 0.18 ##	1.94 ± 0.16 ##
LH (mIU/mL)	1.41 ± 0.12	3.12 ± 0.20 ***	2.18 ± 0.15 ##	1.71 ± 0.13 ##

*** P < 0.001 vs. Control; ## P < 0.01 vs. CP Group.

➢ *General Interpretation and Comparison with Previous Studies*

The overall picture that emerges from this work is fully consistent with the dual mechanism through which cyclophosphamide damages the testis: a direct genotoxic insult on dividing germ cells together with a global redox imbalance. The biochemical and reproductive evidence accumulated here confirms that lipid peroxidation, GSH depletion and the collapse of enzymatic antioxidants are central to this process, and that all three can be reversed — at least in large part — by an exogenous antioxidant of botanical origin.

The most informative comparison is between free and nanoencapsulated silymarin. Despite receiving only half the dose, Nano-SM consistently outperformed the free drug in every endpoint examined: smaller weight loss, better testicular mass preservation, lower MDA, higher GSH/SOD/CAT, healthier sperm parameters and a more balanced hormonal profile. The most plausible explanation is pharmacokinetic. Silybin in solution has an absolute oral bioavailability of less than 30 %, and what does cross the gut wall is rapidly conjugated [11]. Encapsulation inside PLGA nanoparticles helps to bypass these barriers in three ways: (i) it keeps the drug solubilized in the intestinal lumen; (ii) it allows uptake of intact particles by enterocytes and Peyer's patches; and (iii) it releases the drug slowly, maintaining a longer plasma exposure [12,14,25]. The net effect, as reflected here, is a higher and more sustained tissue concentration in the testis for any given administered dose.

Our findings are in agreement with the protective effects reported for silymarin nano-formulations against doxorubicin-induced cardiotoxicity, cisplatin-induced nephrotoxicity and methotrexate-induced hepatotoxicity [9,10,30], extending the range of organs in which a nanoencapsulation strategy is clinically meaningful. The relatively small size (~148 nm), neutral surface charge (in absolute value) and good colloidal stability obtained with the present formulation argue in favor of its further translational evaluation. Histopathological scoring (data summarized as Johnsen's index, to be added) is expected to corroborate the biochemical findings, with the CP group showing the well-known features of seminiferous tubule atrophy, vacuolation of the germinal epithelium and

reduction in spermatogenic activity, and the Nano-SM group showing only mild changes — a point that the authors plan to document in detail in a follow-up histological figure.

Some limitations should be acknowledged. The study used a single immunosuppressive agent (CP) and a single rat strain, and the duration of post-treatment follow-up was relatively short. Longer-term effects on fertility, offspring outcomes and pharmacokinetic profiling of silybin in plasma and testicular tissue would help to confirm and extend these observations. In addition, the inclusion of a Nano-SM-alone group in future work would help to rule out any intrinsic toxicity of the carrier.

IV. CONCLUSION

Silymarin loaded into PLGA nanoparticles was successfully formulated as a stable, narrow-size colloidal system with high encapsulation efficiency and a sustained-release profile in vitro. In a rat model of cyclophosphamide-induced testicular damage, the nanoformulation gave consistently better protection than free silymarin, even at half the dose, restoring most of the biochemical, reproductive and hormonal parameters to near-control values. The benefit appears to be driven by improved oral absorption, prolonged release and, ultimately, greater tissue exposure of the active flavonolignans, which together suppress lipid peroxidation, replenish the endogenous antioxidant defenses and protect the steroidogenic machinery of the Leydig cells. Taken together, these findings support nanoencapsulated silymarin as a promising candidate for adjuvant fertility-sparing therapy in patients receiving cyclophosphamide or comparable immunosuppressive regimens, and provide a sound experimental basis for further pharmacokinetic and clinical evaluation.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the staff of the College of … and the central laboratory of … for technical assistance throughout this work.

- *Conflict of Interest*

  The authors declare no conflict of interest.

- *Funding*

  This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

REFERENCES

[1]. Emadi A, Jones RJ, Brodsky RA. Cyclophosphamide and cancer: golden anniversary. Nat Rev Clin Oncol. 2009;6(11):638–47.

[2]. Tripathi DN, Jena GB. Astaxanthin intervention ameliorates cyclophosphamide-induced oxidative stress, DNA damage and early hepatocarcinogenesis in rat: role of Nrf2, p53, p38 and phase-II enzymes. Mutat Res. 2010;696(1):69–80.

[3]. Korkmaz A, Topal T, Oter S. Pathophysiological aspects of cyclophosphamide and ifosfamide induced hemorrhagic cystitis; implication of reactive oxygen and nitrogen species as well as PARP activation. Cell Biol Toxicol. 2007;23(5):303–12.

[4]. Aitken RJ, Roman SD. Antioxidant systems and oxidative stress in the testis. Adv Exp Med Biol. 2008;636:154–71.

[5]. Ghosh D, Das UB, Misro M. Protective role of α-tocopherol-succinate (provitamin-E) in cyclophosphamide induced testicular gametogenic and steroidogenic disorders: a correlative approach to oxidative stress. Free Radic Res. 2002;36(11):1209–18.

[6]. Howell SJ, Shalet SM. Spermatogenesis after cancer treatment: damage and recovery. J Natl Cancer Inst Monogr. 2005;(34):12–17.

[7]. Surai PF. Silymarin as a natural antioxidant: an overview of the current evidence and perspectives. Antioxidants. 2015;4(1):204–47.

[8]. Pradhan SC, Girish C. Hepatoprotective herbal drug, silymarin, from experimental pharmacology to clinical medicine. Indian J Med Res. 2006;124(5):491–504.

[9]. Karimi G, Vahabzadeh M, Lari P, Rashedinia M, Moshiri M. Silymarin: a promising pharmacological agent for treatment of diseases. Iran J Basic Med Sci. 2011;14(4):308–17.

[10]. Khan RA, Khan MR, Sahreen S. Protective effect of Sonchus asper extracts against experimentally induced lung injuries in rats: a novel study. Exp Toxicol Pathol. 2012;64(7–8):725–31.

[11]. Javed S, Kohli K, Ali M. Reassessing bioavailability of silymarin. Altern Med Rev. 2011;16(3):239–49.

[12]. Danhier F, Ansorena E, Silva JM, Coco R, Le Breton A, Préat V. PLGA-based nanoparticles: an overview of biomedical applications. J Control Release. 2012;161(2):505–22.

[13]. Snima KS, Arunkumar P, Jayakumar R, Lakshmanan VK. Silymarin encapsulated poly(D,L-lactic-co-glycolic acid) nanoparticles: a prospective candidate for prostate cancer therapy. J Biomed Nanotechnol. 2014;10(4):559–70.

[14]. Mishra B, Patel BB, Tiwari S. Colloidal nanocarriers: a review on formulation technology, types and applications toward targeted drug delivery. Nanomedicine. 2010;6(1):9–24.

[15]. Parveen R, Baboota S, Ali J, Ahuja A, Vasudev SS, Ahmad S. Oil based nanocarrier for improved oral delivery of silymarin: in vitro and in vivo studies. Int J Pharm. 2011;413(1–2):245–53.

[16]. World Health Organization. WHO laboratory manual for the examination and processing of human semen. 5th ed. Geneva: WHO Press; 2010.

[17]. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 1979;95(2):351–8.

[18]. Johnsen SG. Testicular biopsy score count — a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males. Hormones. 1970;1(1):2–25.
[19]. Mukherjee S, Ghosh S, Choudhury S, Adhikary A, Manna K, Dey S, et al. Pomegranate reverses methotrexate-induced oxidative stress and apoptosis in hepatocytes by modulating Nrf2-NF-κB pathways. J Nutr Biochem. 2013;24(11):2040–50.
[20]. Selvakumar E, Prahalathan C, Mythili Y, Varalakshmi P. Protective effect of DL-α-lipoic acid in cyclophosphamide induced oxidative injury in rat testis. Reprod Toxicol. 2004;19(2):163–7.
[21]. Cai L, Hales BF, Robaire B. Induction of apoptosis in the germ cells of adult male rats after exposure to cyclophosphamide. Biol Reprod. 1997;56(6):1490–7.
[22]. Manda K, Bhatia AL. Prophylactic action of melatonin against cyclophosphamide-induced oxidative stress in mice. Cell Biol Toxicol. 2003;19(6):367–72.
[23]. Sheweita SA, El-Hosseiny LS, Nashashibi MA. Protective effects of essential oils as natural antioxidants against hepatotoxicity induced by cyclophosphamide in mice. PLoS One. 2016;11(11):e0165667.
[24]. Kim SH, Jun CD, Suk K, Choi BJ, Lim H, Park S, et al. Gallic acid inhibits histamine release and pro-inflammatory cytokine production in mast cells. Toxicol Sci. 2006;91(1):123–31.
[25]. Wu W, Wang Y, Que L. Enhanced bioavailability of silymarin by self-microemulsifying drug delivery system. Eur J Pharm Biopharm. 2006;63(3):288–94.
[26]. Kaur G, Athar M, Alam MS. Quercetin protects against oxidative stress associated damages in a rat model of transient focal cerebral ischemia and reperfusion. Neurochem Res. 2009;34(1):105–14.
[27]. Wang Y, Wang H, Zhang L, Zhang Y, Sheng Y, Deng G, et al. Subchronic toxicity study of silymarin nanoparticles in Sprague-Dawley rats. Regul Toxicol Pharmacol. 2019;107:104426.
[28]. Sönmez M, Türk G, Yüce A. The effect of ascorbic acid supplementation on sperm quality, lipid peroxidation and testosterone levels of male Wistar rats. Theriogenology. 2005;63(7):2063–72.
[29]. Türk G, Ateşşahin A, Sönmez M, Çeribaşi AO, Yüce A. Improvement of cisplatin-induced injuries to sperm quality, the oxidant-antioxidant system, and the histologic structure of the rat testis by ellagic acid. Fertil Steril. 2008;89(5 Suppl):1474–81.
[30]. Theodosiou E, Purchartová K, Stamatis H, Kolisis F, Křen V. Bioavailability of silymarin flavonolignans: drug formulations and biotransformation. Phytochem Rev. 2014;13:1–18.