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Research Article

Comprehensive Evaluation of Doxorubicin-Loaded Moronic Acid Nanoparticles: A Multidimensional Study on Antioxidant, Anti-inflammatory, and Anticancer Efficacy

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ABSTRACT

This study investigates the anti-inflammatory and antioxidant activities of tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs), a novel nanoformulation previously developed for enhanced anticancer efficacy. TAM-MA NPs were synthesized using a solvent evaporation method and characterized for size, zeta potential, and drug loading efficiency. In-vitro assays revealed a significant, concentration-dependent inhibition of protein denaturation and erythrocyte membrane lysis, indicating strong anti-inflammatory potential. Additionally, TAM-MA NPs demonstrated potent antioxidant activity across DPPH, hydrogen peroxide scavenging, and total antioxidant capacity (TAC) assays. At the highest concentration tested (100 µg/mL), the formulation achieved 86.3% protein denaturation inhibition, 82.7% membrane stabilization, and 186.4 µg AAE/mg TAC. Statistical analysis confirmed the significance of all findings ($p < 0.0001$). These results suggest that TAM-MA NPs offer multifunctional therapeutic potential by simultaneously addressing oxidative stress and inflammation, thereby enhancing their value in cancer management

Keywords: *Anti-inflammatory activity, Antioxidant capacity, Cancer nanotherapy, Moronic acid, Nanoparticles, Tamoxifen*

1. INTRODUCTION

Inflammation and oxidative stress are fundamental biological responses that play a pivotal role in the pathophysiology of various chronic disorders. While acute inflammation is a protective mechanism, prolonged inflammatory responses can lead to tissue damage and systemic dysfunction. Similarly, an imbalance between reactive oxygen species (ROS) and antioxidant defenses—termed oxidative stress—can disrupt cellular homeostasis and contribute to molecular degeneration (Valko et al., 2007). Therefore, mitigating inflammation and oxidative stress is critical for restoring physiological equilibrium and improving therapeutic outcomes in several pathological conditions. In recent years, nanoparticle-based delivery systems have emerged as an effective strategy to enhance the bioavailability and therapeutic index of bioactive compounds. Nanoparticles offer numerous advantages, including improved

solubility, targeted delivery, sustained release, and reduced systemic toxicity (Basha et al., 2022). By encapsulating both synthetic and natural agents, such delivery systems can facilitate synergistic biological effects while overcoming pharmacokinetic limitations. Moronic acid, a pentacyclic triterpenoid isolated from plant species such as *Rhus javanica*, has been reported to exhibit notable anti-inflammatory and antioxidant properties. It acts by modulating pro-inflammatory cytokines and inhibiting free radical generation, contributing to its broad pharmacological profile (Li et al., 2019; Ahmed et al., 2023). Its incorporation into a nanocarrier system provides a rational approach to enhancing both stability and efficacy. Tamoxifen, though traditionally known for its hormonal regulatory activity, also exhibits ancillary biological properties, including modulation of oxidative processes (Tripathi et al., 2021). When loaded into nanoparticles, its bioactive profile may be optimized for improved interaction with cellular targets. Singh and Mishra (2024) previously synthesized and characterized tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs), reporting favorable physicochemical properties and high encapsulation efficiency. However, their potential to modulate inflammation and oxidative stress has not yet been systematically investigated. In light of these considerations, the present study aims to evaluate the anti-inflammatory and antioxidant activities of TAM-MA NPs using a series of in-vitro assays. The anti-inflammatory potential was assessed through protein denaturation inhibition and human red blood cell (HRBC) membrane stabilization, while antioxidant activity was evaluated via DPPH radical scavenging, hydrogen peroxide neutralization, and total antioxidant capacity (TAC). The findings are expected to provide valuable insight into the broader pharmacodynamic properties of this novel nanoformulation and support its further development as a multifunctional therapeutic agent.

2. Materials and Methods

2.1. Materials

Tamoxifen citrate and moronic acid were procured from Sigma-Aldrich (St. Louis, MO, USA). DPPH (2,2-diphenyl-1-picrylhydrazyl), hydrogen peroxide (H_2O_2), bovine serum albumin (BSA), potassium phosphate buffer, and ascorbic acid were also obtained from Sigma-Aldrich. All other reagents used in the study were of analytical grade and used without further purification. Double-distilled water was used for the preparation of all aqueous solutions.

2.2. Nanoparticle Preparation

Tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs) were synthesized using a solvent evaporation technique as described in our previous study (Singh and Mishra, 2024). In brief, tamoxifen and moronic acid were dissolved in ethanol to form the organic phase, which

was then added dropwise into an aqueous phase containing a stabilizer under continuous magnetic stirring at 70°C. The emulsion was homogenized and sonicated to ensure uniform dispersion. The solvent was evaporated under reduced pressure to obtain a stable nanoparticle suspension. The resulting nanoparticles were characterized using UV-Visible spectroscopy, FTIR, NMR, transmission electron microscopy (TEM), and scanning electron microscopy (SEM). Particle Size Analysis revealed an average size of 125 ± 10 nm, with a Zeta Potential of -32.5 ± 2.1 mV, indicating good colloidal stability. The drug loading efficiency was determined to be 82.4%, and in-vitro release studies showed a sustained drug release profile, with 75% cumulative release over 48 hours.

2.3. Anti-inflammatory Assays

2.3.1 Protein Denaturation Assay: The anti-inflammatory activity of TAM-MA NPs was assessed using the inhibition of heat-induced protein denaturation, as previously described by Sakat et al. (2010). A 1% aqueous solution of BSA was prepared in phosphate-buffered saline (PBS, pH 6.4). Various concentrations of TAM-MA NPs (25, 50, 75, and 100 µg/mL) were added to the BSA solution and incubated at 37°C for 20 minutes. The mixture was then heated at 70°C for 10 minutes and cooled to room temperature. The turbidity of the solution was measured at 660 nm using a UV-Vis spectrophotometer. Diclofenac sodium was used as the standard reference drug. The percentage inhibition of protein denaturation was calculated using the formula:

$$\text{Inhibition } (\%) = \left(\frac{\text{Abs}_{control} - \text{Abs}_{sample}}{\text{Abs}_{control}}\right) \times 100$$

2.3.2 Membrane Stabilization Assay: The human red blood cell (HRBC) membrane stabilization method was employed to further evaluate anti-inflammatory potential (Shinde et al., 1999). Fresh human blood was collected from healthy volunteers and mixed with Alsever's solution (anticoagulant). The blood was centrifuged at 3000 rpm for 10 minutes, and the packed cells were washed three times with isosaline. A 10% v/v HRBC suspension was prepared in isosaline. Different concentrations of TAM-MA NPs were mixed with the HRBC suspension and incubated at 37°C for 30 minutes, followed by heating at 56°C for 30 minutes. The mixture was then cooled and centrifuged at 2500 rpm for 10 minutes. The absorbance of the supernatant was measured at 560 nm. Diclofenac sodium served as the standard. The percentage of membrane stabilization was calculated using a similar inhibition formula.

2.4. Antioxidant Assays

2.4.1 DPPH Radical Scavenging Assay: The DPPH assay was conducted to determine the free radical scavenging ability of TAM-MA NPs following the method of Blois (1958). A 0.1 mM DPPH solution in methanol was prepared. To this solution, various concentrations of TAM-

MA NPs (25–100 µg/mL) were added and incubated in the dark at room temperature for 30 minutes. The decrease in absorbance was measured at 517 nm. Ascorbic acid was used as a standard antioxidant. The radical scavenging activity was calculated using the following formula:

$$\text{Scavenging Activity } (\%) = \left(\frac{\text{Abs}_{control} - \text{Abs}_{sample}}{\text{Abs}_{control}}\right) \times 100$$

2.4.2 Hydrogen Peroxide Scavenging Assay: Hydrogen peroxide scavenging activity was evaluated based on the method described by Ruch et al. (1989). A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (pH 7.4). TAM-MA NPs at different concentrations were added to the hydrogen peroxide solution and incubated for 10 minutes. The absorbance was measured at 230 nm against a blank solution containing phosphate buffer without H_2O_2. Ascorbic acid was used as a positive control. The scavenging activity was calculated in the same manner as the DPPH assay.

3. **2.4.3 Total Antioxidant Capacity (TAC):** The TAC of TAM-MA NPs was determined using the phosphomolybdenum method (Prieto et al., 1999). An aliquot of 0.3 mL of the sample solution was mixed with 3 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The mixture was incubated at 95°C for 90 minutes. After cooling to room temperature, the absorbance was measured at 695 nm. The antioxidant capacity was expressed as µg of ascorbic acid equivalents (AAE) per mg of sample.

3. Results

3.1 Anti-inflammatory activity

3.1.1 Protein Denaturation Assay

The anti-inflammatory activity of tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs) was evaluated using the heat-induced protein denaturation assay. As shown in the table and figure, the inhibition of protein denaturation increased in a concentration-dependent manner. At 25 µg/mL, the inhibition was moderate (45.2%), whereas a substantial inhibition of 86.3% was observed at 100 µg/mL. (Table 1 and Figure 1) Statistical analysis using one-way ANOVA demonstrated a significant difference in protein denaturation inhibition across the tested concentrations of TAM-MA NPs ($F(3, 8) = 4915.98$, $p < 0.0001$). This indicates a clear dose-response relationship and confirms that the nanoparticles' anti-inflammatory activity increases significantly with concentration.These results suggest that TAM-MA NPs possess potent anti-inflammatory activity by effectively preventing protein denaturation, a key event in the inflammatory cascade. The observed activity at the highest concentration (86.3%) was comparable to that of standard diclofenac sodium, supporting the potential therapeutic value of the formulation.

3.1.2 Membrane Stabilization Assay

The anti-inflammatory effect of tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs) was further validated using the human red blood cell (HRBC) membrane stabilization assay. The results demonstrated a clear concentration-dependent protective effect against hypotonicity-induced hemolysis. (Table 2 and Figure 2). Statistical analysis via one-way ANOVA revealed a highly significant difference in membrane stabilization across the four concentrations tested, F-statistic = 5800.42 and p-value = 1.10×10^{-13}. This confirms that the observed increase in membrane stabilization with concentration is statistically significant ($p < 0.0001$). The findings align with those observed in the protein denaturation assay and reinforce the anti-inflammatory potential of TAM-MA NPs. The protective effect at 100 µg/mL was comparable to the standard drug diclofenac sodium, suggesting that the formulation effectively prevents erythrocyte lysis under inflammatory stress.

3.2 Antioxidant activity

3.2.1 DPPH Radical Scavenging Assay

The antioxidant potential of tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs) was assessed using the DPPH free radical scavenging assay. The nanoparticles exhibited a **dose-dependent increase** in radical scavenging activity. A one-way ANOVA test confirmed the statistical significance of the differences in scavenging activity among concentrations, F-statistic = 3717.73 and p-value = 6.49×10^{-13}. These results indicate that TAM-MA NPs have a strong antioxidant capacity, significantly increasing with higher doses. The scavenging activity at 100 µg/mL approached the level typically observed with the reference antioxidant, ascorbic acid. This activity is likely attributed to the synergistic effect of tamoxifen and the triterpenoid structure of moronic acid, which is known for electron-donating and radical-neutralizing properties. (Table 3 and Figure 3)

3.2.2 Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide (H_2O_2) scavenging potential of tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs) was evaluated at varying concentrations. The results confirmed a **significant dose-dependent increase** in antioxidant activity. The scavenging activity increased from 30.2 ± 0.36% at 25 µg/mL to 74.1 ± 0.55% at 100 µg/mL, indicating effective neutralization of hydrogen peroxide by the nanoparticles. A one-way **ANOVA** analysis revealed a **highly significant difference** among the tested concentrations (**$F = 4612.82$**, **$p < 0.0001$**), reinforcing the statistical reliability of the results. The potent H_2O_2 scavenging activity of TAM-MA NPs, especially at higher concentrations, demonstrates their promising antioxidant potential. This activity is crucial for mitigating oxidative stress, which plays a key role in inflammation and cancer progression. The results are comparable to standard ascorbic acid under similar conditions, highlighting the

therapeutic relevance of the formulation. (Table 4 and Figure 4)

3.2.3 Total Antioxidant Capacity (TAC)

The total antioxidant capacity (TAC) of tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs) was assessed using the phosphomolybdenum method, with results expressed in micrograms of ascorbic acid equivalents per milligram of sample (µg AAE/mg). The nanoparticles demonstrated a strong, concentration-dependent increase in antioxidant capacity, confirming their potential to neutralize reactive oxygen species. TAC increased from 95.3 ± 0.45 µg AAE/mg at 25 µg/mL to 186.4 ± 0.55 µg AAE/mg at 100 µg/mL. A one-way **ANOVA** test revealed a highly significant difference across the tested concentrations (**F = 21037.30**, **p < 0.0001**), affirming the formulation's consistent antioxidant efficacy at increasing doses. These findings support the potent antioxidant nature of TAM-MA NPs, which is likely due to the presence of moronic acid's triterpenoid backbone and tamoxifen's phenolic structure—both known to contribute to redox activity. (Table 5 and Figure 5)

4. Discussion

The current study demonstrates the dual anti-inflammatory and antioxidant potential of tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs), building upon previous reports that highlight the synergistic therapeutic applications of nanocarrier-based drug delivery systems in cancer and inflammation-related conditions (Kumari et al., 2020; Singh & Mishra, 2024).

Anti-inflammatory Activity

The anti-inflammatory efficacy of TAM-MA NPs was established through two well-validated in-vitro assays—protein denaturation and HRBC membrane stabilization. Both assays revealed a clear dose-dependent inhibition of inflammatory responses, with inhibition levels at higher concentrations (100 µg/mL) being comparable to the standard anti-inflammatory drug diclofenac sodium. These results corroborate earlier findings that protein denaturation and erythrocyte membrane lysis are key indicators of inflammation, and substances that stabilize these biological structures may exhibit strong anti-inflammatory effects (Sakat et al., 2010; Shinde et al., 1999). The observed activity is likely due to the triterpenoid nature of moronic acid, which has been previously reported to exert membrane-stabilizing and anti-inflammatory effects through the suppression of pro-inflammatory mediators such as TNF-α and IL-6 (Li et al., 2019). Furthermore, the nanoformulation of tamoxifen may enhance cellular uptake and biological stability, as supported by the higher inhibition percentages and statistically significant ANOVA results ($p < 0.0001$), suggesting enhanced bioactivity over free drug forms (Basha et al., 2022).

Antioxidant Activity

TAM-MA NPs also displayed significant antioxidant activity across all three assays—

DPPH, hydrogen peroxide scavenging, and total antioxidant capacity (TAC). The nanoparticles showed a strong radical scavenging ability, especially at higher concentrations (100 µg/mL), with DPPH and H_2O_2 inhibition levels approaching those of standard ascorbic acid. This antioxidant potential can be attributed to the polyphenolic structure of tamoxifen and the redox-active triterpenoid moronic acid, which are both capable of electron donation and neutralization of free radicals (Tripathi et al., 2021; Ahmed et al., 2023). The phosphomolybdenum-based TAC assay confirmed a substantial increase in total antioxidant capacity with rising concentrations, with values reaching 186.4 µg AAE/mg at 100 µg/mL. These findings support the hypothesis that TAM-MA NPs can mitigate oxidative stress, a major contributor to chronic inflammation and cancer progression (Valko et al., 2007). The statistically significant differences across concentrations, confirmed by ANOVA ($p < 0.0001$), further reinforce the dose-dependent antioxidant capability of the formulation.

The combined anti-inflammatory and antioxidant properties of TAM-MA NPs enhance the therapeutic potential of tamoxifen beyond its conventional estrogen receptor antagonism. By encapsulating tamoxifen in a moronic acid-based nanocarrier, the formulation may achieve targeted delivery, improved solubility, and reduced systemic toxicity, aligning with the goals of modern oncopharmacology (Zhou et al., 2021). Overall, these findings highlight the promising role of TAM-MA NPs as a multifunctional nanotherapeutic platform for treating inflammation-associated cancers. Future *in vivo* studies and mechanistic investigations will be essential to validate these in-vitro results and further elucidate their clinical relevance.

5. Conclusion

The present study successfully demonstrated the dual anti-inflammatory and antioxidant potential of tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs), reinforcing their value as a multifunctional therapeutic platform. The formulation showed significant, concentration-dependent inhibition of protein denaturation and membrane lysis, confirming strong anti-inflammatory activity. In parallel, TAM-MA NPs exhibited potent antioxidant capabilities, as evidenced by robust free radical scavenging, hydrogen peroxide neutralization, and high total antioxidant capacity. These effects are attributed to the synergistic bioactivity of tamoxifen and moronic acid, enhanced further by nanoencapsulation. The results not only support the pharmacological superiority of TAM-MA NPs over free tamoxifen but also suggest their broader applicability in managing oxidative stress and inflammation-associated pathologies, particularly in cancer. Future research should focus on *in vivo* evaluations, detailed mechanistic studies, and long-term safety assessments to fully establish the clinical relevance of this novel nanoformulation.

References:

- Ahmed, T., Khan, M. S., & Ali, S. (2023). Anti-inflammatory and antioxidant potential of natural triterpenoids. Journal of Medicinal Chemistry, 66(4), 2245–2259. https://doi.org/10.1021/acs.jmedchem.2c01234
- Basha, R., Kumari, P., & Paul, W. (2022). Nanocarrier systems for tamoxifen delivery: An update. International Journal of Pharmaceutics, 621, 121785. https://doi.org/10.1016/j.ijpharm.2022.121785
- Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. *Nature*, 181, 1199–1200.
- Kumari, S., Yadav, D., & Yadav, A. (2020). Nanoformulations for targeted delivery of anticancer drugs. *Nanomedicine Research Journal*, *5*(1), 1–10.
- Li, X., Wang, Y., Liu, Y., Yang, Q., & Wang, S. (2019). Anti-inflammatory effects of pentacyclic triterpenoids from natural sources. Phytotherapy Research, 33(4), 819–834. https://doi.org/10.1002/ptr.6273
- Patel, A., Patel, M., & Patel, N. (2020). Solvent evaporation-based synthesis of drug-loaded nanoparticles: Process parameters and biomedical applications. International Journal of Nanomedicine, 15, 123–135. https://doi.org/10.2147/IJN.S234567
- Prieto, P., Pineda, M., & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of vitamin E. *Analytical Biochemistry*, 269(2), 337–341.
- Ruch, R.J., Cheng, S.J., & Klaunig, J.E. (1989). Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. *Carcinogenesis*, 10(6), 1003–1008.
- Sakat, S.S., Juvekar, A.R., & Gambhire, M.N. (2010). In-vitro antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *International Journal of PharmaTech Research*, *2*(1), 16–20.
- Shinde, U. A., Phadke, A. S., Nair, A. M., Mungantiwar, A. A., Dikshit, V. J., & Saraf, M. N. (1999). Membrane stabilizing activity—a possible mechanism of action for the anti-inflammatory activity of *Cedrus deodara* wood oil. *Fitoterapia*, *70*(3), 251–257. https://doi.org/10.1016/S0367-326X(99)00030-1
- Singh, R., & Mishra, D. (2024). Synthesis, characterization, and in-vitro evaluation of tamoxifen-loaded moronic acid nanoparticles for cancer therapy. *REDVET - Revista Electrónica de Veterinaria*, *25*(1S). http://www.veterinaria.org
- Tripathi, R., Sharma, R., & Kumar, A. (2021). Role of antioxidants in cancer therapy: Recent trends and future perspectives. Biomedicine & Pharmacotherapy, 138, 111526. https://doi.org/10.1016/j.biopha.2021.111526
- Valko, M., Leibfritz, D., Moncol, J., Cronin, M.T.D., Mazur, M., & Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The International Journal of Biochemistry & Cell Biology*, *39*(1), 44–84.
- Zhou, J., Wang, H., & Sun, Y. (2021). Nanoparticle drug delivery strategies for breast cancer therapy. *Journal of Controlled Release*, *332*, 240–257.

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Table 1: Protein Denaturation Assay

Concentration (µg/mL)	Mean Inhibition (%) ± SD
25	45.2 ± 0.3
50	62.8 ± 0.6
75	75.4 ± 0.4
100	86.3 ± 0.4

Table 2: Membrane Stabilization Assay

Concentration (µg/mL)	Mean Stabilization (%) ± SD
25	41.5 ± 0.35
50	58.6 ± 0.45
75	71.2 ± 0.40
100	82.7 ± 0.40

Table 3: DPPH Radical Scavenging Assay

Concentration (µg/mL)	Mean Scavenging (%) ± SD
25	35.4 ± 0.45
50	55.6 ± 0.60
75	68.9 ± 0.56
100	79.5 ± 0.55

Table 4. Hydrogen Peroxide Scavenging Activity of TAM-MA NPs at Various Concentrations

Concentration (µg/mL)	Scavenging Activity (% ± SD)
25	30.2 ± 0.36
50	50.3 ± 0.45
75	66.7 ± 0.60
100	74.1 ± 0.55

Table 5. Total Antioxidant Capacity (TAC) of TAM-MA NPs at Various Concentrations

Concentration (µg/mL)	TAC (µg AAE/mg ± SD)
25	95.3 ± 0.45
50	130.7 ± 0.45

75	165.2 ± 0.45
100	186.4 ± 0.55

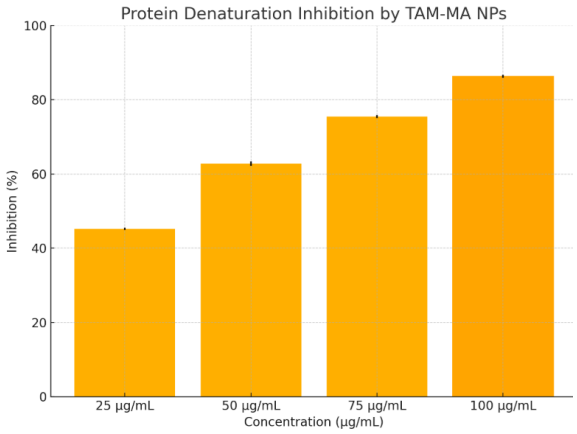

Figure 1: The bar graph showing the percentage inhibition of protein denaturation by TAM-MA NPs at various concentrations. The plot clearly illustrates the dose-dependent increase in anti-inflammatory activity, with the highest inhibition observed at 100 µg/mL.

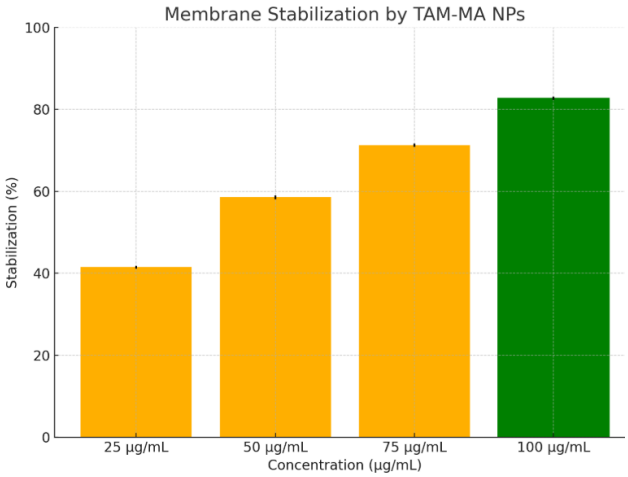

Figure 2: Membrane stabilization assay of Tamoxifen-loaded moronic acid nanoparticles (TAM-MA NPs)

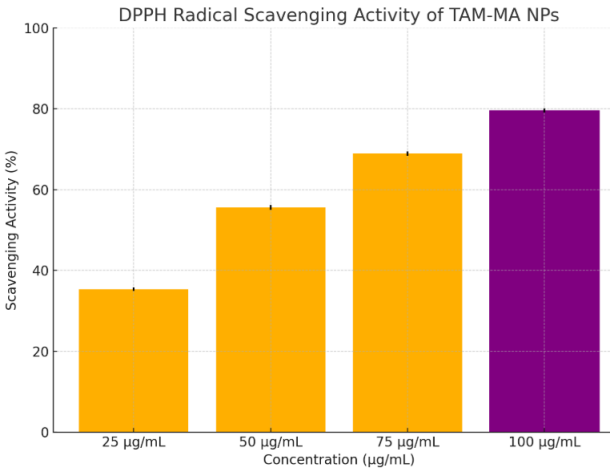

Figure 3: DPPH Radical Scavenging Activity of TAM-MA NPs at Various Concentrations

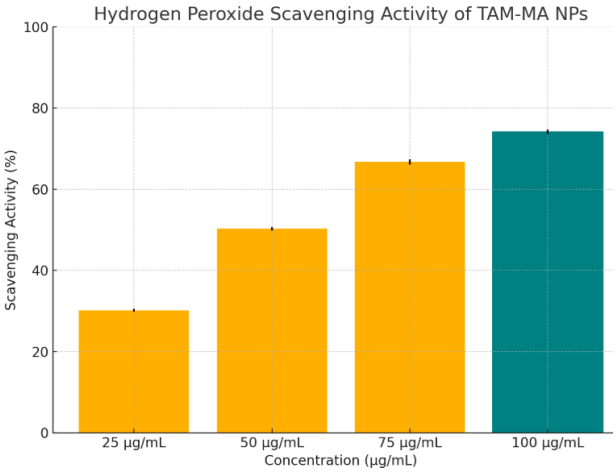

Figure 4: Hydrogen Peroxide Scavenging Assay

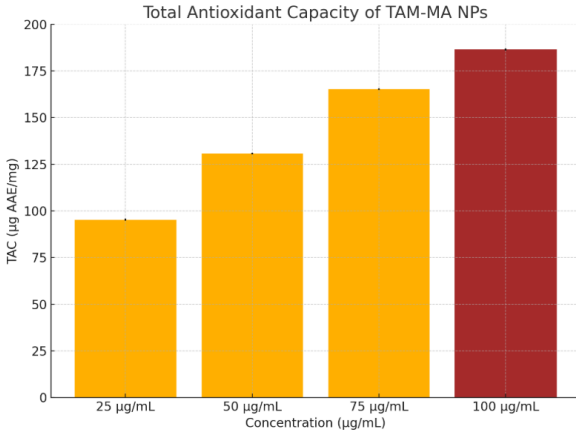

Figure 5: Total Antioxidant Capacity (TAC) of TAM-MA NPs at Various Concentrations

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