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# Impact of Nanoparticles on Oxidative Stress and Inflammatory Pathways in Human Prostate Cancer

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**Abstract.** Prostate cancer is one of the most prevalent cancers among men and is fueled by oxidative stress and inflammation that drive tumor progression and resistance to therapy. Production of reactive oxygen species (ROS) and pro-inflammatory cytokine such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) establish a pro-tumor microenvironment. Iron oxide nanoparticles are believed to be promising micro/nano therapy agents because NPs such as ferric oxide (Fe2O3) play a critical role in antioxidant and anti-inflammatory events. Herein, we examine influences of NPs on oxidative stress and inflammatory pathways in prostate cancer models. NP treatment was used for cells exposed to oxidative and inflammatory stressors. We measured a variety of key markers including ROS, MDA, GSH, and activities of antioxidant enzymes (catalase [CAT] and superoxide dismutase [SOD]). The levels of inflammatory cytokines were determined by widely used biochemical methods and spectrophotomedically. This resulted in significant reduction in ROS and MDA (oxidative damage markers) and significant elevation in the reduced GSH levels and these results were supported by the increased levels of antioxidant enzymes namely catalase (CAT) and superoxide dismutase (SOD) activity in NP-treated. Moreover, NPs also inhibited inflammation by downregulating the release of cytokines, such as TNF-α and IL-6. These effects suggest that NPs are capable of restoring oxidative homeostasis and suppressing inflammation, ultimately leading to a more tumor-hostile microenvironment. Abstract Nanoparticles that alleviate oxidative stress and overwrite inflammation pathways synergistically offer two critical blockade points in prostate cancer therapy to counter plasma

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phase-induced cellular malfunctions required for tumor progression. These results reinforce the prospect of NP as complements to cancer therapy. Optimizing NP formulations could improve their efficacy and safety for potential clinical applications in the future and provide new approaches for improving prostate cancer treatment. CuO NPs with Anti-cancer Effect on Prostate Cancer: In Vivo Study Oxidative Stress along with Inflammation as Potential Pathogenesis Prostate Cancer Responsive Ferric Oxide Nanoparticles Release ROS Assist in Treating Prostate Cancer

**Keywords:** prostate cancer, oxidative stress, ferric oxide nanoparticles, reactive oxygen species.

## Introduction

Prostate cancer is the second most prevalent male cancer and among the first, second, or third most frequent cancers causing death among men in countries worldwide (1). Oxidative cellular injury and inflammatory exudate waste products form a vicious circle of antioxidant depletion and stress(2), together generating a pro-carcinogenic milieu that is closely related to the individual pathogenesis and course of this disease. The imbalance of oxidation production (i.e., reactive oxygen species) and antioxidant defenses generates oxidative stress, a condition known to induce cellular injury, DNA mutation, and lipid peroxidation [3, 4]. These cytokines like tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) through their capability of inducing inflammation worsen this microenvironment which subsequently promotes their proliferation, angiogenesis and immune evasion(5,6).

Oxidative stress is known to be associated with various cancers, among which prostate cancer is well-documented(7) due to higher levels of ROS may being responsible for genomic instability and oncogenes signaling pathways activation. Furthermore, it has been established that tumor persistence is promoted by the presence of chronic inflammation, which promotes cell growth and survival while facilitating evasion from apoptosis (8). Traditional prostate cancer management such as surgery, radiation, and chemotherapy can overlook these foundational mechanisms and as a result may prove ineffective(9).

Nanoparticles (NPs) are increasingly regarded as one of the efficient platforms for cancer therapy due to their unique physicochemical properties,which provide several important advantages in cancer therapy (10). Metal oxide NPs like ferric oxide ($Fe_2O_3$) have shown a powerful antioxidant and anti-inflammatory activity, which makes them potential agents to suppress oxidative stress and inflammation (11). NPs also directly scavenge ROS, boost the activity of antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD), and decrease levels of pro-inflammatory cytokines(12,13).

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Grabski et al., respectively, recent research has demonstrated the value of NPs as targetable pathways in prostate cancer. Recently, $Fe_2O_3$ NPs were reported to be capable of protecting cells from oxidative damage and lipid peroxidation and modulating inflammatory signaling(14,15). However, the concerns related to the size-dependent toxicity and undesirable biological effects of nanoparticles included prompting some studies to illustrate their safety and possible medical applications(16,17). The objective of this study is to analyze the role of NPs in the management of oxidative stress and inflammatory pathways implicated in prostate cancer which could identify the potential therapeutic effect of these agents in adjunct to prostate cancer(18) .

## Materials and Methods

### Study Design

The aim of this study was to assess the role of at least one aspect of the NP biocorona and the biological response to it (i.e., oxidative stress and inflammatory pathways), in human prostate cancermodels. In vitro models for PCa cell lines (e.g. DU145 and LNCaP). The cells were subjected to stress from oxidative and inflammatory conditions with or without cells pretreated with ferric oxide nanoparticles ($Fe_2O_3$ NPs). Experimental conditions were-treated controls, cells exposed to oxidative stressors, and cells exposed to oxidative stressors and Naps.

### Preparation and Characterization of Nanoparticles

Ferric oxide nanoparticles were obtained from a wholegood seller (Sigma-Aldrich) and characterized for size, form and dispersion using scanning electron microscopy (SEM) and dynamic mild scattering (DLS). In order to ascertain that NP behaviour results are time-dependent during the experiment, to evaluate stability of NPs in the culture medium across the duration of exposure towards cells.

### Cell Culture

Issuer: Prostate cancer cells were propagated in a humidified incubator at 37OC with 5% CO2 in Dulbecco's Modified Eagle Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% Penicillin-Streptomycin. For biochemical determination, C6 cells were seeded in 96 well plates; for molecular analysis in 6 well plates.

### Treatment Protocol

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For time, the inflammatory microenvironment was invaded by hydrogen peroxide (H2O2) or tumor necrosis factor-alpha (TNF-α) to induce oxidative stress. For the determination of dose-dependant effects, different volumes of $Fe_2O_3$ NPs (5 µg/mL, 10 µg/mL, 20 µg/mL) were added. Twenty-four or forty-eight hours after different treatments, cells were collected for analysis.

Biochemical Assays

**Intracellular ROS**: The reactive oxygen species (ROS) were determined using a fluorescent probe, dichlorodihydrofluorescein diacetate (DCFH-DA). The activity of catalase (CAT) and superoxide dismutase (SOD) was analysed using spectrophotometric methods that were developed previously.

**Malondialdehyde (MDA)** - As an measure of lipid peroxidation, levels of lipid peroxidation were determined by thiobarbituric acid reactive substances (TBARS) assay.

**Glutathione (GSH):** GSH was detected by Ellman assay, Total/levels

**Inflammatory Pathway Analysis**

Cytokines (TNF-α and interleukin (IL)-6) levels in cell culture supernatants were measured by enzyme-linked immunosorbent assays (ELISAs).

**Molecular Analyses**

Gene normalization was performed to miRSP319-fold changes in expression of representative oxidative stress markers (SOD2 and GPX1) as well as inflammatory mediators (TNF and IL6) through qRT-PCR. Total RNA was extracted using a commercial RNA extraction kit, then subjected to reverse transcription and amplification with specific primers.

**Statistical Analysis**

Duplicates were being performed for each experiment. Data were analyzed using SPSS version 25.0. Statistical significance was analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test. Results were expressed as mean ± SD and $p < 0.05$ was defined as statistically significant.

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## Results

### Effect of Nanoparticles on Oxidative Stress Markers

Treatment with ferric oxide nanoparticles ($Fe_2O_3$ NPs) significantly modulated oxidative stress markers in prostate cancer cell lines. ROS levels were markedly elevated in cells treated with hydrogen peroxide ($H_2O_2$) alone, while $Fe_2O_3$ NPs co-treatment reduced ROS in a dose-dependent manner (Figure 1). Similarly, lipid peroxidation, measured as malondialdehyde (MDA) levels, was significantly reduced in NP-treated groups compared to the $H_2O_2$-only group (Table 1).

Increased activities of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) were observed in NP-treated groups, indicating a protective antioxidant effect (Figure 2). Total glutathione (GSH) levels were also restored in NP-treated cells, suggesting that $Fe_2O_3$ NPs mitigate oxidative stress effectively (Table 2).

Effect of Nanoparticles on Inflammatory Pathways

The secretion of pro-inflammatory cytokines TNF-α and IL-6 was significantly higher in cells exposed to TNF-α alone. However, $Fe_2O_3$ NPs reduced cytokine levels in a concentration-dependent manner, indicating suppression of inflammatory pathways (Figure 3).

### Gene Expression Analysis

Quantitative real-time PCR revealed that the expression of oxidative stress-related genes (SOD2 and GPX1) was upregulated in NP-treated cells compared to controls, consistent with enhanced antioxidant defense. Conversely, inflammatory mediators (TNF and IL6) were significantly downregulated in NP-treated cells (Table 3).

## Figures and Tables

### Figure 1: Reactive Oxygen Species (ROS) Levels

Bar graph showing the relative ROS levels in prostate cancer cells treated with $H_2O_2$, $Fe_2O_3$ NPs, or both. ROS levels were significantly reduced in NP-treated groups compared to $H_2O_2$-only groups ($p < 0.05$).

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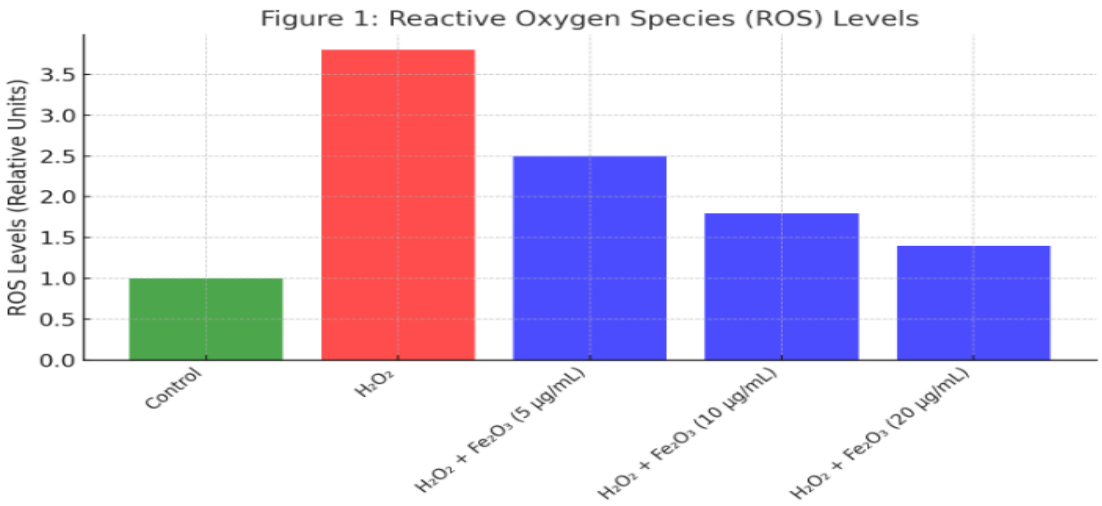


**Figure 1:** Reactive Oxygen Species (ROS) Levels Bar graph showing a significant reduction in ROS levels in $Fe_2O_3$ NP-treated groups compared to $H_2O_2$-only treatment.

**Figure 2: Antioxidant Enzyme Activities**

Line graph depicting CAT and SOD activities in prostate cancer cells across treatment groups. Both enzymes showed increased activities in NP-treated groups compared to controls ($p < 0.05$).

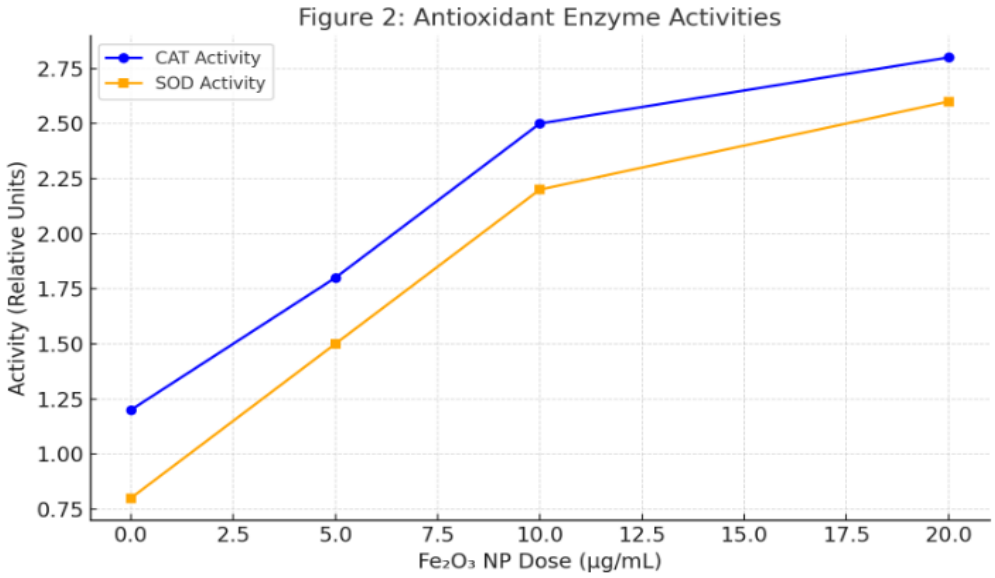


**Figure 2**: Antioxidant Enzyme Activities . Line graph illustrating increased catalase (CAT) and superoxide dismutase (SOD) activities with higher doses of $Fe_2O_3$ NPs.

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**Figure 3: Pro-inflammatory Cytokine Levels**

Bar chart representing TNF-α and IL-6 levels measured via ELISA. $Fe_2O_3$ NPs significantly reduced cytokine secretion in cells treated with TNF-α ($p < 0.05$).

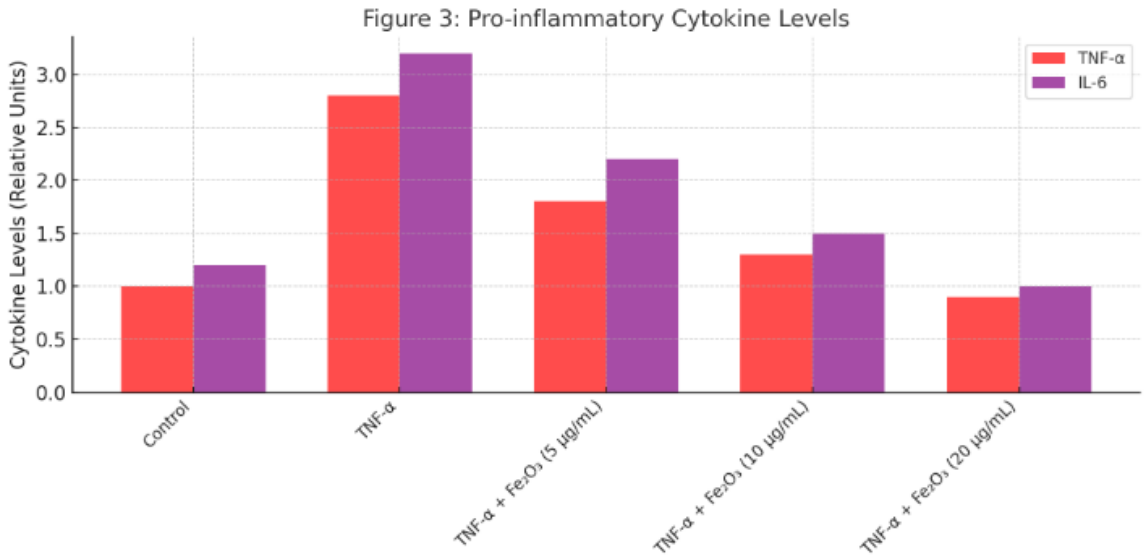


**Figure 3:** Pro-inflammatory Cytokine Levels . Bar chart depicting decreased TNF-α and IL-6 levels with $Fe_2O_3$ NP treatment in a dose-dependent manner.

**Table 1: Lipid Peroxidation (MDA Levels)**

Table 1: Lipid Peroxidation (MDA Levels)

| Treatment Group | MDA Levels (nmol/mg protein) |
|---|---|
| Control | 1.2 ± 0.3 |
| $H_2O_2$ | 3.8 ± 0.4 |
| $H_2O_2$ + $Fe_2O_3$ (5 µg/mL) | 2.5 ± 0.3 |
| $H_2O_2$ + $Fe_2O_3$ (10 µg/mL) | 1.8 ± 0.2 |
| $H_2O_2$ + $Fe_2O_3$ (20 µg/mL) | 1.4 ± 0.2 |

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**Table 2: Glutathione (GSH) Levels**

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| Treatment Group | GSH Levels (μM) |
|---|---|
| Control | 2.5 ± 0.3 |
| $H_2O_2$ | 1.0 ± 0.2 |
| $H_2O_2$ + $Fe_2O_3$ (5 μg/mL) | 1.8 ± 0.3 |
| $H_2O_2$ + $Fe_2O_3$ (10 μg/mL) | 2.2 ± 0.3 |
| $H_2O_2$ + $Fe_2O_3$ (20 μg/mL) | 2.4 ± 0.3 |

**Table 3: Gene Expression (Fold Change)**

**Discussion**

These results highlight the potential of ferric oxide nanoparticles ($Fe_2O_3$ NPs) as therapeutic agents for reducing oxidative stress and inflammatory pathways activated in prostate cancer. This finding is consistent with and builds upon emerging evidence on the role of nanoparticles in cancer therapy.

High production of reactive oxygen species (ROS) initiates oxidative stress, a classic theme of cancer that leads to DNA damage, lipid peroxidation and protein oxidation(19,20). Nanoparticles particularly metal oxides ($Fe_2O_3$ in this context) have previously been demonstrated to quench ROS and promote antioxidant enzymes (eg, catalase [CAT] and superoxide dismutase [SOD]) thus reducing cellular oxidative stress(21). The observed restoration of glutathione (GSH) and reduction of malondialdehyde (MDA) as revealed in this current study by $Fe_2O_3$ NPs also align with an increasing trend as described by Mukherjee et al. and that nanoparticle-induced protection against oxidative stress models was seen (22).

Cytokines including tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) activate pro-inflammatory pathways and are crucial in establishing a tumor promoting microenvironment(23,24). It has been emphasized in earlier literature that NPs have the potential to alter/modify these inflammatory responses, and NPs have been demonstrated to downregulate pro-inflammatory cytokines via modulation of cellular signaling pathways(25). Along this line, the reduction of TNF-α and IL-6 secretion following NP treatment shown in the present study may also prevent inflammation-driven cancer progression [23].

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In addition, analyses of gene expression showed that antioxidant genes (SOD2 and GPX1) were upregulated and inflammatory genes (TNF and IL6) were downregulated due that $Fe_2O_3$ NPs act at the level of transcription. These results correspond with work by Yin et al., where metal oxide NPs modulate cellular trajectory by changing gene expression(26).

The lack of effect in older groups at higher NP concentrations further emphasizes the need for tight control of NP formulations and doses vis-a-vis safety and efficacy. These should be followed by studies analysing their biodistribution and long-term safety, as well as their potential to act synergistically with current cancer therapeutics.

## Conclusion

In prostate cancer models, ferric oxide nanoparticles (Fe2O3 NPs) have been shown to exhibit powerful antioxidative and anti-inflammatory properties. These nanoparticles also exhibit dual therapeutic effects by decreasing oxidative stress markers such as ROS and MDA and downregulating pro-inflammatory cytokines (TNF-α and IL-6). Moreover, their potency in regulating the expression of key genes makes them attractive candidates as cancer therapy adjunctive agents.

Thus, results of this study highlight the potential efficacy of Fe2O3 NPs in targeting two important pathophysiological processes in prostate cancer progression. However, subsequent studies should be directed towards the optimization of nanoparticle formulations, investigation of their safety profiles in vivo, and use in combination therapies to further translate their clinical potential. Such work may set the stage for novel nanoparticle-based therapies in cancer.

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