

Original Article

Comprehensive intervention strategies in N-Methyl N-Nitrosourea-testosterone-induced prostate cancer: Exploring the multifaceted impact of dutasteride and docetaxel nanostructured lipid carriers on hormonal, oxidative, immunological and histological parameters

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ABSTRACT

Objectives: This study investigates the impact of NMU-testosterone-induced prostate cancer on serum hormone levels, oxidative stress markers, cytokine profiles, and histological changes in the prostate gland. Additionally, it evaluates the therapeutic potential of Dutasteride Nanostructured Lipid Carriers (Dutasteride NLC) and Docetaxel Nanostructured Lipid Carriers (Docetaxel NLC) in modulating these pathological alterations.

Materials and Methods: Male rats were divided into control and experimental groups, with prostate cancer induced using N-methyl-N-nitrosourea (NMU) and testosterone. Serum hormone levels, including testosterone, dihydrotestosterone (DHT), and prostate-specific antigen (PSA), were analysed. Antioxidant enzyme activities (SOD, catalase, GR, GPx, and reduced glutathione) and lipid peroxidation levels were assessed to determine oxidative stress. Cytokine profiles were evaluated to examine immune dysregulation. Histopathological examination of prostate tissues was conducted to assess morphological alterations. Treatment groups received Dutasteride NLC, Docetaxel NLC, or a combination of both to determine their therapeutic effects.

Results: Prostate cancer induction resulted in significant elevations in testosterone, DHT, and PSA levels, indicating hormonal imbalances. Oxidative stress markers showed increased lipid peroxidation and depletion of antioxidant enzymes in the PCA-induced group. Inflammatory cytokine levels were dysregulated, reflecting immune imbalances. Histological analysis revealed high-grade neoplasia, secretory cell proliferation, and stromal hyperplasia in the prostate tissue of cancer-induced rats. Treatment with Dutasteride NLC and Docetaxel NLC reduced hormone levels, restored antioxidant enzyme activity, and normalized cytokine levels. Histopathological examination showed improvements, with reduced neoplasia and stromal cell hyperplasia. Co-administration of Dutasteride NLC and Docetaxel NLC demonstrated superior therapeutic effects, indicating a synergistic action in mitigating cancer-related alterations.

Conclusion: This study highlights the potential of Dutasteride NLC and Docetaxel NLC in counteracting prostate cancer-associated hormonal imbalances, oxidative stress, immune dysregulation, and histopathological alterations. The findings suggest a promising role for these nanocarrier-based therapies in prostate cancer management. Further research and clinical investigations are warranted to validate their efficacy in translational applications.

Keywords: Antioxidant enzymes, Dutasteride and docetaxel, Hormonal imbalance, Immunomodulation, Inflammatory, N-methyl-N-nitrosourea-testosterone, Oxidative stress, Prostate cancer, Superoxide dismutase

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INTRODUCTION

In 2023, it was estimated that approximately 288,300 new cases of prostate cancer (PCa) of the prostate were diagnosed in the U.S., with 34,700 resulting in fatalities.^[1]

PCa was more detected amongst black men than in white men, the exact reason remains unexplored.^[2]

Males are primarily affected by PCa between the age group of 45 and 60 and rank as first of the leading causes of cancer-related deaths in men worldwide.^[3] It stands as the second most common cancer in men. PCa diagnosis typically involves health screening, prostate biopsy analysis, prostate-specific antigen (PSA) testing, magnetic resonance imaging and digital rectal examination. The disease's heterogeneity factors such as genetics, age, obesity, ethnicity and environmental conditions contribute to its varied epidemiology across different locations and racial groups.^[4-6]

Detection of PCa often presents no clear symptoms, although symptoms include nocturia, difficulty initiating nocturia, difficulty initiating or maintaining urine flow, haematuria and dysuria frequent urination. Advanced stages of the disease can manifest symptoms such as bone pain, urinary and faecal incontinence, tingling, leg weakness and fatigue due to low red blood cell levels.^[7,8]

Treatment for PCa includes chemotherapy radiation therapy, radical prostatectomy, high-intensity focused ultrasound, chemotherapy (including oral chemotherapeutic drugs like Temozolomide), cryosurgery, hormonal therapy or a combination of these modalities. Palliative care is also crucial for managing symptoms.^[9]

Chemotherapeutic drugs such as docetaxel (Dxt) and cabazitaxel, administered intravenously on a 3-week cycle, inhibit tubulin depolymerisation, a process critical for mitotic cell division. However, traditional cytotoxic anticancer drugs pose challenges due to poor specificity, high toxicity and pharmaceutical resistance. Nanostructured lipid carriers (NLCs) offer a promising alternative for drug delivery, leveraging the enhanced permeability and retention effect to target tumours passively. Surface-engineering techniques further enhance NLC's ability to precisely deliver drugs to tumour tissues, potentially improving therapeutic efficacy and minimising side effects.^[10,11]

Research efforts have explored the effects of nanostructured lipid carriers containing dutasteride (Dut) and Dxt in experimentally induced PCa in rats, suggesting the potential for developing strategies that deliver multiple drugs or target various sites simultaneously.^[12-17]

MATERIALS AND METHODS

Materials

Samples of Docetaxel (DXT) and dutasteride (DUT) were generously provided as a gift by Mac-Chem Labs Pvt. Ltd, based in Mumbai. The procurement of stearic acid was facilitated through Sigma-Aldrich Pvt. Ltd. (India). Oleic acid was sourced from Sun Pharmaceuticals Ltd, headquartered in Mumbai; The glyceryl monostearate utilized in this study was acquired directly from Croda Chemicals Pvt. Ltd (India); Poloxamer 407, an essential ingredient in the formulation procedure, was generously supplied as a complimentary sample by BASF India Ltd., with its headquarters situated in Mumbai, India. Tween 20, a fundamental element in the formulation, was procured from Central Drug House (Pvt) Ltd., based in New Delhi, India.

Method of NLC preparation

In this study, NLCs loaded with Dxt-NLC and Dut-NLC were developed to enhance therapeutic outcomes for PCa treatment. By selecting biocompatible lipids, and appropriate surfactants and using a hot homogenisation technique followed by ultrasonication, stable NLCs with high drug-loading capacity and nanoscale particle size (average diameter of 103.1 nm) were achieved. Comparative analysis of therapeutic dosages showed that NLCs allow significantly lower doses – 120 mg/kg body weight for Dxt-NLC and 0.5 mg/kg for Dut-NLC – while maintaining or enhancing efficacy, attributed to their improved bioavailability. These reduced dosages minimise systemic toxicity and side effects, potentially improving patient compliance. This study underscores the clinical advantage of NLCs in PCa treatment by providing an effective and safer alternative to traditional formulations.

PCa induction

The experimental protocol involved administering cyproterone acetate to rats at a dosage of 50 mg/kg for 21 consecutive days, dissolved in corn oil. On the 23rd day, the rats were given intraperitoneal injections of testosterone propionate at 100 mg/kg in corn oil for the subsequent 3 days. On the 27th day, a single intraperitoneal injection of N-methyl-N-nitrosourea (NMU) was administered at a 50 mg/kg concentration. Following a 1-week interval from NMU administration, rats were given daily intraperitoneal injections of testosterone propionate at 2 mg/kg in corn oil for the next 125 days.

The rats were divided into five groups, each consisting of 10 animals. Group I acted as the control, while PCa was induced in Group II using a modified version of the method described by Liao *et al.* (2002). The treatment groups (III: Dxt-NLC at 120 mg/kg body weight, IV: Dut-NLC at 0.5 mg/kg body weight and V: Dxt-NLC at 120 mg/kg body weight + Dut-NLC

at 0.5 mg/kg body weight) received their respective treatments alongside a promoting dose of testosterone propionate (2 mg/kg body weight intraperitoneally in corn oil) from the 35th day to the 160th day. Animals were sacrificed with ketamine 50 mg/kg of the prostate gland and blood was removed for further estimation.^[18-22] The study was conducted following the guidelines provided by the Institutional Ethical Committee Protocol No: IAEC/KIMS/2018/2.

Tissue processing

The processing of prostate tissue began with the removal of the prostate, which was then washed with ice-cold saline. Ventral prostate tissues were prepared at a 10% w/v concentration by homogenising them in chilled phosphate buffer (0.1 M, pH 7.4) using a homogeniser. The homogenate was filtered through muslin cloth and centrifuged at $3000 \times g$ for 10 min at 4°C using a Remi Cooling Centrifuge (C-24 DL) to eliminate nuclear debris. The resulting supernatant was then subjected to a second centrifugation at $12000 \times g$ for 20 min at 4°C to obtain the post-mitochondrial supernatant (PMS) used for various enzyme assays.

Preparation and estimation of parameters from PMS

The PMS obtained from the above centrifugation steps was utilised for the estimation of various biochemical parameters, including lipid peroxidation (LPO), reduced glutathione (GSH), catalase (CAT) activity, glutathione peroxidase (GPx) activity, glutathione reductase (GR) activity and superoxide dismutase (SOD) activity. Each assay was performed according to established protocols as detailed below.^[23-29]

Estimation of hormone levels in serum

Serum levels of dihydrotestosterone (DHT) and testosterone were assessed using specialised enzyme-linked immunosorbent assay (ELISA) kits, following the guidelines provided by the manufacturer. To ensure the precision and consistency of the results, the assays were conducted in triplicate.^[30]

Estimation of PSA levels in serum

Serum PSA levels were measured using a quantitative sandwich enzyme immunoassay technique. The PSA levels were determined according to the kit manufacturer's protocol, and the results were expressed as ng/mL of serum.^[31]

Measurement of LPO

LPO in the prostate tissues was assessed by measuring the concentration of malondialdehyde, a by-product of LPO. The tissues were homogenised in phosphate buffer (0.1 M, pH 7.4) at 10% w/v, followed by centrifugation as described earlier. The PMS was mixed with thiobarbituric acid and

heated at 95°C for 1 h. The absorbance of the resulting pink colour was measured at 532 nm.^[32]

Estimation of reduced GSH level

GSH levels in the prostate tissues were determined using the method of Jollow *et al.*, with modifications. 1 mL of PMS and 1 mL of 4% sulphosalicylic acid were incubated at 4°C for 1 h. After centrifugation at $1200 \times g$ for 15 min at 4°C, the supernatant was collected, and 0.4 mL was added to 2.2 mL of phosphate buffer (0.1 M, pH 7.4) and 0.4 mL of 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB). The yellow colour developed was measured at 412 nm, and GSH content was calculated as nmol DTNB conjugate formed per gram of tissue.^[33]

Estimation of CAT

CAT activity in prostate tissues was measured according to Claiborne's (1985) method. The reaction mixture consisted of 1.95 mL of 0.1 M phosphate buffer (pH 7.4), 1.0 mL of 0.019 M hydrogen peroxide and 0.05 mL of PMS. The decrease in absorbance at 240 nm due to the breakdown of hydrogen peroxide was monitored, and CAT activity was calculated as nmol H₂O₂ consumed per minute per milligram of protein.^[34]

Estimation of GPx

GPx activity was assessed using the method described by Mohandas *et al.* (1984). The reaction mixture contained 1.44 ml of phosphate buffer (0.1 M, pH 7.4), 0.1 mL of 1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 mL of 1.0 mM sodium azide, 0.05 mL of 1 eu/mL GR, 0.05 mL of 1.0 mM reduced GSH, 0.1 mL of 0.2 mM nicotinamide adenine dinucleotide phosphate (NADPH), 0.01 mL of 0.25 mM hydrogen peroxide (H₂O₂) and 0.1 mL of PMS. The reduction of NADPH was monitored at 340 nm, and GPx activity was expressed as nmol NADPH.^[35]

Estimation of GR activity

GR activity was measured using Carlberg and Mannervik's method (1975). The reaction mixture included 1.65 mL of phosphate buffer (0.1 M, pH 7.6), 0.1 mL of 0.5 mM EDTA, 0.05 mL of 1.0 mM oxidised GSH, 0.1 mL of 0.1 mM NADPH and 0.1 mL of PMS. The decrease in absorbance due to NADPH oxidation was monitored at 340 nm, and GR activity was calculated as nmol NADPH oxidised per minute per milligram of protein using an extinction coefficient of $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$.^[36]

Estimation of SOD activity

SOD activity was determined following the method of Marklund and Marklund (1974). The reaction mixture comprised 2.875 mL of Tris- Hydrochloric acid (HCl) buffer (50 mM, pH 8.5), 100 µL of PMS and pyrogallol (24 mM in

10 mM HCl) to a total volume of 3 mL. The auto-oxidation of pyrogallol was measured at 420 nm, and one unit of SOD activity was defined as the amount that inhibits the auto-oxidation by 50%.^[37]

Estimation of cytokines by ELISA

Cytokine levels in the prostate tissues were quantified using ELISA kits. Tumour necrosis factor alpha (TNF- α) levels were measured with an e-Bioscience sandwich ELISA kit, while interleukin-1 β (IL-1 β), interleukin-6 (IL-6), interferon-gamma (IFN- γ) and interleukin-10 (IL-10) levels were determined using ELISA kits from RAY Biotech, according to the manufacturer's instructions.^[38]

Histological investigation

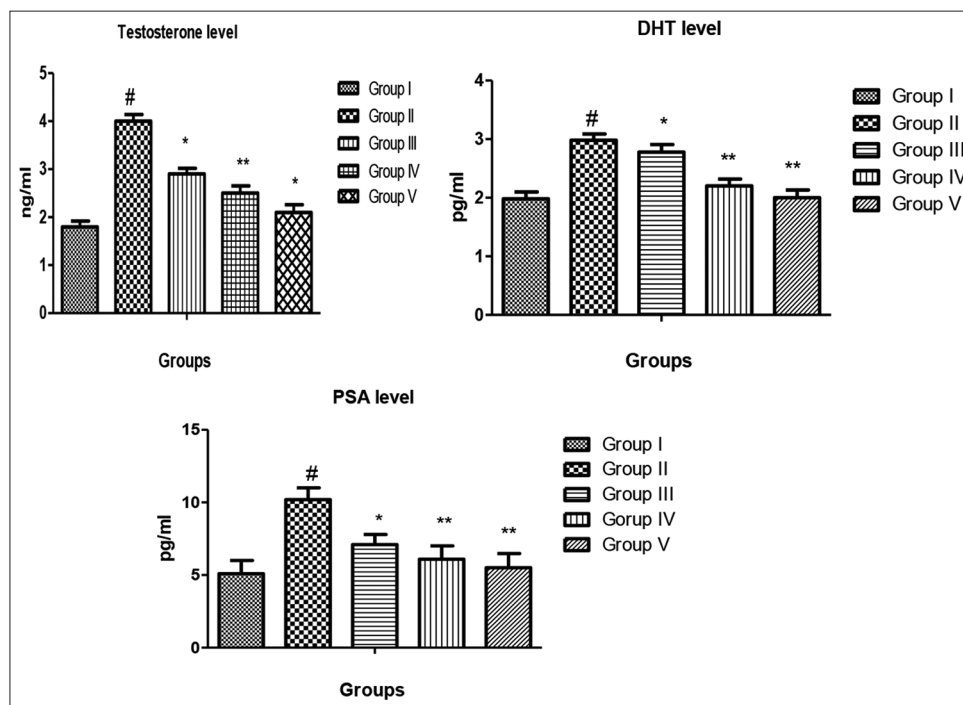
For histological analysis, prostate tissues were fixed in freshly prepared 10% neutral buffered formalin at 4°C, followed by embedding in paraffin wax. Vertical sections (5 μ m thick) were prepared and rehydrated through a graded series of ethyl alcohol, cleared in xylene and re-embedded in paraffin wax. The sections were then deparaffinised, stained with haematoxylin and eosin (H&E) and mounted in DPX. The histological sections were examined under a microscope

(Olympus BX 51) for the assessment of morphological changes, including prostatic intraepithelial neoplasia (PIN), secretory cell proliferation and stromal hyperplasia.^[39,40]

RESULTS

Impact of Dut and Dxt nanostructured lipid carriers on serum hormones

In the PCa-induced group (Group II), there was a statistically significant increase in testosterone levels compared to the control group (Group I) ($P < 0.01$). Treatment with Dxt NLC in Group III, Dut NLC in Group IV and a combination of Dxt and Dut in Group V resulted in a statistically significant, dose-dependent decrease in testosterone levels ($P < 0.05$, $P < 0.01$ and $P < 0.001$, respectively). Similarly, for DHT serum levels, the PCa-induced group (Group II) showed elevated levels compared to the control group ($P < 0.01$). Administration of Dxt NLC (Group III), Dut NLC (Group IV) and combination therapy (Group V) resulted in a dose-dependent reduction in DHT levels, with the combination therapy showing the most significant effect ($P < 0.001$). Elevated serum PSA levels were also observed in the PCa-induced group (Group II) compared to the control ($P < 0.01$). Treatment with Dxt NLC (Group III), Dut NLC (Group IV) and their combination (Group V) led



Graph 1: Dutasteride and docetaxel nanostructured on impact serum hormone levels. Values are expressed in mean \pm standard error of the mean. statistical analysis is done by one-way analysis of variance followed by Bonferroni's multiple comparison test. [#] $P < 0.001$ when compare with Group I, ^{*} $P < 0.01$, ^{**} $P < 0.001$ when compared with Group II. DHT: Dihydrotestosterone level, PSA: Prostate-specific antigen level

to substantial and dose-dependent reductions in serum PSA levels, with statistical significance ($P < 0.001$ for the combination therapy). Confidence intervals for the reductions in testosterone, DHT and PSA levels were calculated, demonstrating robust effects across the treatment groups [Graph 1].

Impact of Dut and Dxt nanostructured lipid carriers on antioxidants

The PCa-induced group (Group II) exhibited a significant decline in antioxidant enzyme activities, including GR, GPx and CAT, as well as reduced GSH levels, alongside an increase in LPO ($P < 0.01$ for all parameters compared to the control group). Administration of Dut and Dxt NLCs in Groups III, IV and V resulted in a statistically significant, dose-dependent restoration of these antioxidant levels to near-normal values ($P < 0.05$ for GR, GPx and CAT; $P < 0.001$ for GSH levels and reduction in LPO). Bonferroni's multiple comparison analysis using confirmed the significance of differences between treatment groups and the PCa-induced group [Table 1].

Impact of Dut and Dxt nanostructured lipid carriers on cytokines and anti-inflammatory cytokine (IL-10) levels

In the PCa-induced group (Group II), there was a significant elevation in pro-inflammatory cytokines, including TNF- α , IL-1 β , IL-6 and IFN- γ , accompanied by a decrease in the anti-inflammatory cytokine IL-10 ($P < 0.01$ for all compared to the control group). Treatment with Dut and Dxt NLCs in Groups III and IV significantly and dose-dependently restored cytokine levels to near-normal values, with a notable increase in IL-10 levels ($P < 0.05$ for cytokines; $P < 0.001$ for IL-10). Co-administration of Dut and Dxt NLCs in Group V also exhibited significant restoration of cytokine levels to normal ($P < 0.001$ for IL-10 restoration) [Graph 2].

Impact Dut and Dxt nanostructured lipid carriers on histopathological changes

Histological examination of prostate tissue using H&E staining revealed high-grade PIN with significant secretory cell proliferation and stromal hyperplasia in the PCa-induced group (Group II), compared to the control group. Administration of Dut NLC in Group IV showed low-grade PIN with stromal cell hyperplasia. In contrast, Dxt NLC in Group III demonstrated low-grade PIN with prostate atrophy and acinar degenerative changes. The combination of Dut NLC and Dxt NLC in Group V resulted in the most significant histological improvements, including low-grade PIN, reduced secretory cell proliferation and notable atrophy with degenerative changes in the prostate acini ($P < 0.001$ for all histopathological changes compared to the PCa-induced group) [Figure 1].

DISCUSSION

This study explored the impact of Dut and Dxt nanostructured lipid carriers (NLCs) on various biochemical parameters, including serum hormones, antioxidants, cytokines and histopathological changes in a PCa model. Our findings provide significant insights into the therapeutic potential of these NLCs in managing PCa and its associated pathological alterations. To better contextualise our results, we compare our findings with existing literature, analyse potential clinical implications and acknowledge the limitations of our study.

Our results indicate that PCa induction led to a significant increase in testosterone and DHT levels in the PCa-induced group (Group II), consistent with the established role of androgens in promoting PCa progression.^[41] This observation aligns with previous studies that underscore the significance of androgen regulation in PCa pathogenesis.^[42] The administration of Dut and Dxt NLCs in Groups III, IV and V resulted in a dose-dependent decrease in both testosterone and DHT levels, with the combination of Dut and Dxt in Group V being particularly effective. This finding suggests that the NLC formulation enhances the therapeutic efficacy of these drugs, likely due to improved bioavailability and targeted delivery, as demonstrated in similar studies using NLCs in cancer treatment.^[43]

The reduction in serum PSA levels further corroborates the therapeutic impact of these NLCs, reinforcing their potential to suppress PCa progression.^[44] Notably, the ability to achieve these effects at lower therapeutic dosages underscores the clinical relevance of our approach, as highlighted in our comparative analysis of therapeutic dosages.

The PCa -induced group exhibited a significant decline in key antioxidant enzymes (GR, GPx and CAT) and reduced GSH levels, alongside an increase in LPO, indicative of heightened oxidative stress. This oxidative stress is a well-documented contributor to cancer progression and resistance to therapy.^[45] Treatment with Dut and Dxt NLCs effectively restored antioxidant levels in a dose-dependent manner, aligning with other studies showing the antioxidative benefits of NLCs in cancer models.^[46] The restoration of antioxidant levels is crucial, as it suggests that these NLCs not only counteract oxidative stress but also potentially reverse oxidative damage, thereby enhancing the overall therapeutic outcome.

Our study also demonstrated significant elevations in pro-inflammatory cytokines and a decrease in the anti-inflammatory cytokine IL-10 in the PCa-induced group, reflecting the inflammatory milieu typical of cancer progression.^[47] Treatment with Dut and Dxt NLCs normalised cytokine levels and restored IL-10 levels, underscoring the potential of these NLCs to modulate the immune response by reducing inflammation and promoting anti-inflammatory pathways. This immunomodulatory effect is consistent with

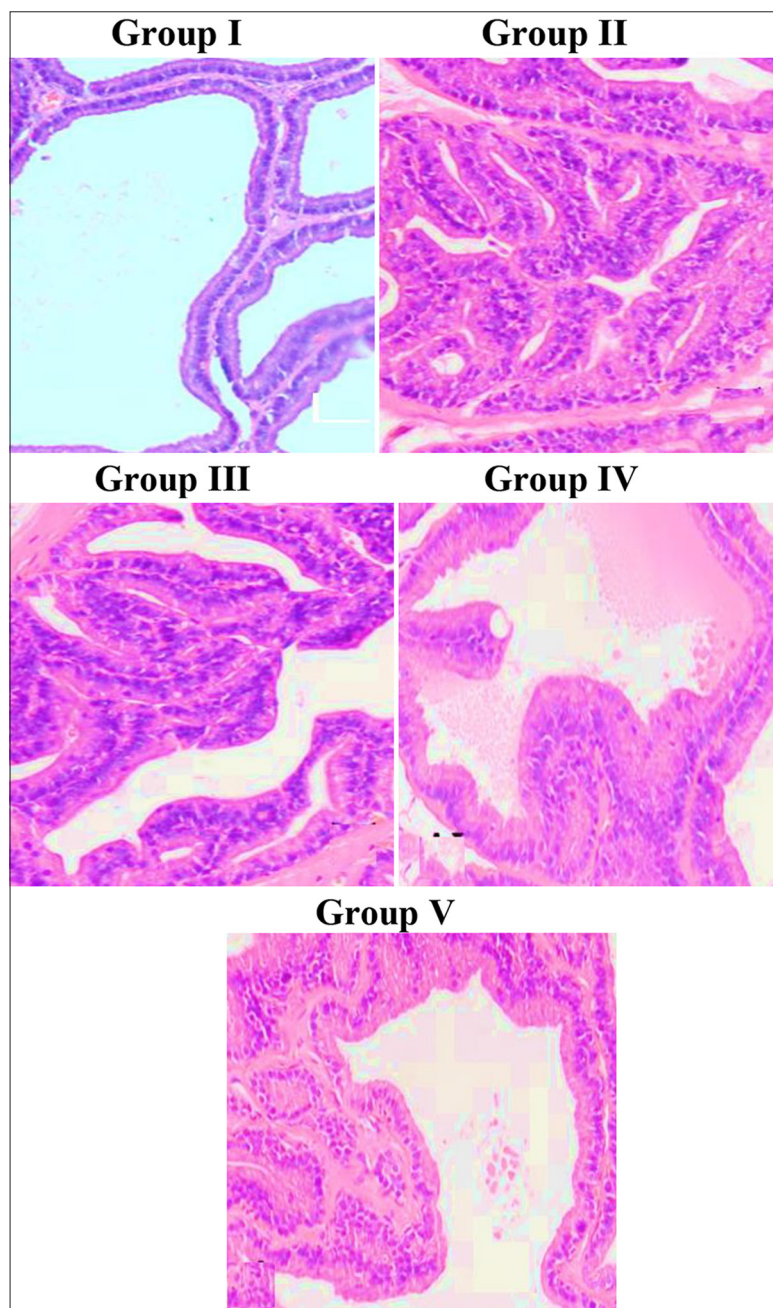
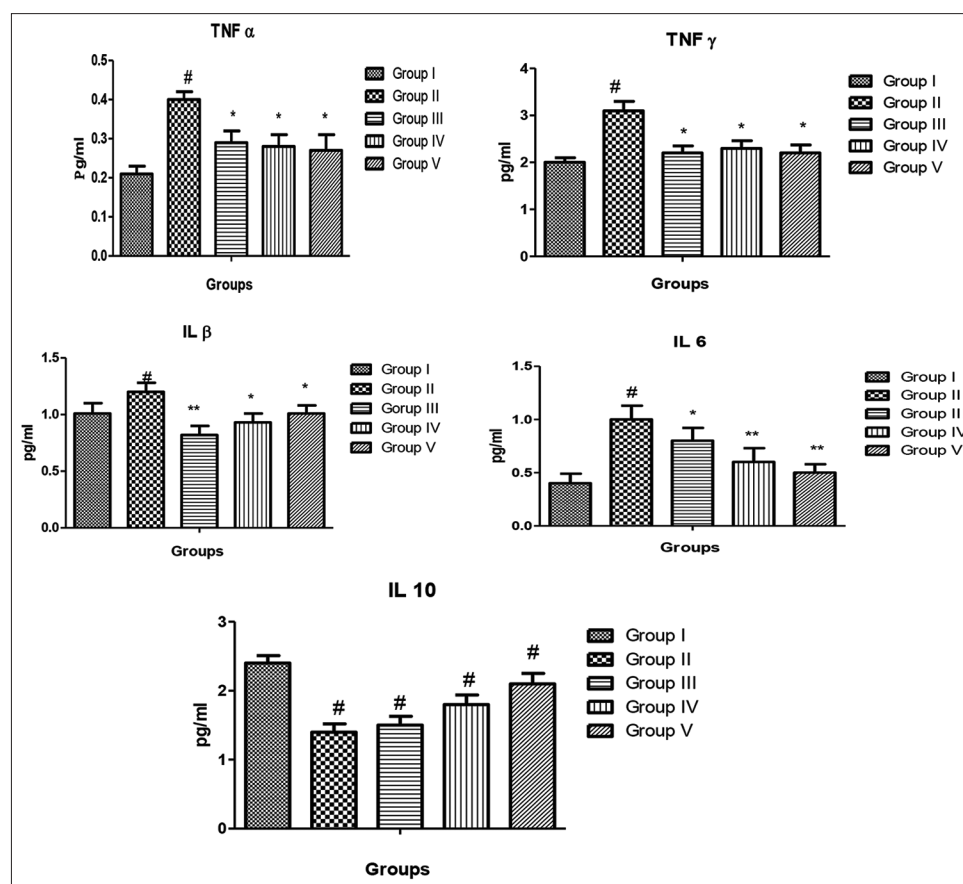


Figure 1: Effect of dutasteride and docetaxel nanostructured lipid carriers on histopathology changes. Group I (Control): Normal histoarchitecture of the prostate gland. Well-organized glandular structures with intact epithelial lining. No evidence of neoplasia, cellular proliferation, or inflammatory infiltration. Normal stromal composition without hyperplasia or fibrotic changes. Group II (PCa-Induced Group): High-grade Prostatic Intraepithelial Neoplasia (PIN) observed. Increased secretory cell proliferation with loss of normal glandular architecture. Stromal hyperplasia with an increase in fibroblasts and extracellular matrix deposition. Presence of nuclear pleomorphism, increased mitotic activity, and hyperchromasia. Evidence of inflammatory infiltration and disrupted basement membrane. Group III (Dxt-NLC Treatment): Low-grade PIN with reduced glandular proliferation. Moderate reduction in stromal hyperplasia, indicating partial recovery. Atrophy of prostate acini, with degenerative changes in epithelial cells. Fewer mitotic figures and reduced nuclear pleomorphism compared to Group II. Group IV (Dut-NLC Treatment): Presence of low-grade PIN, but with less stromal hyperplasia than Group III. Significant reduction in secretory cell proliferation, showing therapeutic impact. Stromal components show moderate fibrosis, but no severe abnormalities. Improved cellular morphology with less nuclear pleomorphism and mitotic activity. Group V (Dxt-NLC + Dut-NLC Co-administration): Marked improvement in histoarchitecture with near-normal glandular structures. Minimal PIN and reduced secretory cell proliferation. Lower stromal hyperplasia compared to other treatment groups. Atrophic and degenerative changes in acinar cells significantly reduced. Enhanced restoration of normal prostate tissue, indicating a synergistic therapeutic effect. Dut-NLC: Dutasteride nanostructured lipid carriers, Dxt-NLC: Docetaxel nanostructured lipid carriers. [haematoxylin and Eosin (H&E) 400x].

Table 1: Dutasteride and docetaxel nanostructured lipid carriers impact the antioxidant.

Groups → Antioxidant Enzyme ↓	Group I	Group II	Group III	Group IV	Group V
GR nmol	299.59±2.3*	40.43±0.63 [#]	192.08±1.4*	209±3.2*	251.81±1.9*
GPx nmol	103.98±0.4*	14.04±0.2	60.10±1.96*	78.79±1.3*	90.95±0.8*
GSH nmol	535.70±1.4*	216.41±0.5 [#]	237.84±0.1*	272.12±0.3*	304.93±0.2*
SOD U/mg	11.16±0.4*	1.94±0.10 [#]	2.65±0.2*	5.5±0.2*	8.86±0.1*
CAT	142±0.2*	39.62±0.2 [#]	100.10±0.3*	102.98±0.5*	152.81±0.1*
LPO nmol	042.21±0.6*	89.92±0.9 [#]	72.38±0.8*	49.41±0.6*	50.89±0.6*

Values are expressed in mean±standard error of the mean. Statistical analysis is done by one-way analysis of variance followed by Bonferroni's multiple comparison test. **P*<0.001 when compared with Group I **P*<0.01 when compared with Group II. GSH: Glutathione, GR: Glutathione reductase, GPx: Glutathione peroxidase, SOD: Superoxide dismutase, CAT: Catalase, LPO: Lipid peroxidation

**Graph 2:** Dutasteride and docetaxel nanostructured lipid carriers impact on serum cytokine levels. Values are expressed in mean \pm standard error of the mean. Statistical analysis is done by one-way analysis of variance followed by Bonferroni's multiple comparison test. **P*<0.001 when compared with Group I, **P*<0.01, ***P*<0.001 when compared with Group II. TNF: Tumor necrosis factor, IL: Interleukin

previous research that highlights the anti-inflammatory potential of NLCs in cancer therapy.^[48] The ability to modulate the immune environment further enhances the therapeutic potential of these NLCs in inhibiting tumour

growth and managing cancer-associated inflammation.

Histopathological analysis revealed that PCa induction resulted in high-grade PIN with significant secretory cell

proliferation and stromal hyperplasia. Treatment with Dut and Dxt NLCs led to notable histological improvements, with Dut NLC treatment associated with low-grade PIN and reduced stromal cell hyperplasia, and Dxt NLC treatment resulting in low-grade PIN with prostate atrophy and acinar degenerative changes. The combination of Dut and Dxt NLCs in Group V further enhanced these effects, significantly reducing secretory cell proliferation, atrophy and degenerative changes in the prostate acini. These histological findings align with the biochemical data, supporting that combining Dut and Dxt NLCs exerts a synergistic effect in mitigating PCa progression.^[49] This synergistic effect is also supported by other studies investigating the combination of NLCs with different therapeutic agents in cancer treatment.^[50]

The findings from this study suggest that Dut and Dxt NLCs could offer a more effective treatment option for PCa by enhancing drug delivery, improving therapeutic efficacy and potentially reducing side effects due to lower required dosages. However, it is important to acknowledge the limitations of our study, particularly the need for further *in vivo* studies and clinical trials to validate these findings in a clinical setting. Future research should also explore the long-term effects of these NLCs and their impact on patient outcomes.

CONCLUSION

The study highlights the potential therapeutic benefits of Dut and Dxt NLC in modulating hormonal, oxidative and inflammatory responses associated with PCa. In addition, Dut and Dxt, NLCs when administered alone or in combination, demonstrated efficacy in ameliorating histological alteration in the prostate gland. Further research and clinical trials are warranted to explore the full therapeutic potential of these interventions in the management of PCa.

Ethical approval: This study was conducted in accordance with the guidelines set by the Institutional Ethical Committee, and approval was obtained along with Animal House clearance under Protocol No. 1ARC/KIMS/2018/2.

Declaration of patient consent: Patient's consent is not required as there are no patients in this study.

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Conflicts of interest: There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript and no images were manipulated using AI.

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