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Original Research Article

Nanostructured lipid carriers loaded with capecitabine modulate apoptosis via IL-6 signaling: A novel approach to targeted cancer therapy

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Abstract

Purpose: *To examine the impact of capecitabine-loaded nanostructured lipid carriers (NLC) on cancer cell death mechanisms and apoptosis through interleukin-6 (IL-6) signaling system.*

Method: *In a triplicate MTT assay, cell viability was assessed using 100 µg/mL capecitabine-loaded nanostructured lipid carrier (NLC) formulation. Apoptotic DNA degradation was determined by DNA fragmentation analysis. The molecular processes behind cellular responses were clarified by gene expression profiling of IL-6 pathway components. The formulations were characterized using zeta potential, particle size, and polydispersity index (PDI). Furthermore, morphological analysis, MTT testing, DNA fragmentation assay, and qRT-PCR were used to examine gene expression of interleukin cytokines IL-6, IL-6R, gp130, Bcl-2, Bax, NF-kB, and JAK-STAT.*

Results: *Microscopic examination of cell lines revealed morphological alterations suggestive of cell death and apoptosis. Results from MTT assay showed that nano-formulation had a much lower IC50 (8 µg/mL) compared to pure drug (48 µg/mL). There were statistically significant ($p < 0.05$) differences between nano formulation and pure drug in each treatment concentration. Expression of inflammatory and apoptotic genes was significantly lower in pure and Nano-Cap treated cell lines compared to control ($p < 0.05$). However, treatment with Nano-Cap significantly reduced expression of inflammatory and apoptotic markers compared to pure drug ($p < 0.05$). Furthermore, level of Bax was significantly increased ($p < 0.001$) in Nano-Cap-treated cell lines compared to pure drug and control*

Conclusion: *Capecitabine-loaded nanostructured lipid carriers (NLC) are more effective than pure drug in treating colon cancer. The results may contribute to developing more effective, targeted cancer therapies and personalized treatment strategies by elucidating the interplay between nanoparticle drug delivery, chemotherapy agents, and intracellular signaling pathways.*

Keywords: *Capecitabine, NLC, Apoptosis, MTT, Interleukin, IL-6 signaling*

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INTRODUCTION

Globally, colorectal cancer (CRC) is one of the most frequent cancers and a leading cause of death and disability. In 2012, colorectal cancer (CRC) had a high morbidity rate of 9.7 % and a death rate of 8.5 %, making it one of the most common malignancies globally [1]. With an

estimated 1.9 million new cases of colorectal cancer in 2020, it claimed the lives of more than 930,000 individuals worldwide. Several risk factors for colorectal cancer (CRC) include an unhealthy lifestyle, a diet rich in fat and protein, exposure to carcinogenic environments, and chromosomal instability [2]. The complicated biology of colorectal cancer means that the survival rate is still poor, despite improvements in diagnostic and therapeutic methods.

Administration of pharmacological substances to kill tumor cells is the mainstay of chemotherapy, which is a cancer treatment approach. Clinical trials have demonstrated that oxaliplatin and capecitabine, alone or in combination, have tolerable effects on colorectal cancer (CRC). However, drug resistance and side effects make it difficult to assess novel treatments that mitigate these problems [3]. Although capecitabine (CAP) is a successful therapy for colon cancer, it does have unique adverse effects that are linked to dosage [4]. If the dose of CAP is reduced in any manner, the advantages of using it would also be greatly reduced. So, alternative approaches are required to mitigate the toxicity as well as improve the effectiveness of the current antineoplastic drugs. To achieve this, novel drug delivery systems, including nanodrug delivery systems, have been pursued. Among them, lipid nanocarriers, including nanostructured lipid carriers (NLC), have been widely investigated for anticancer treatment.

Nanostructured lipid carriers (NLCs) are beneficial in drug delivery systems and nanotechnology. These carriers are made up of a combination of liquid and solid lipids, which results in a matrix that facilitates controlled release and encapsulation of active pharmaceutical constituents. Compared to conventional drug delivery methods, improved bioavailability, reduced adverse effects, and the capacity to target specific tissues or cells are among the numerous benefits of NLCs. The lipid matrix of NLCs is capable of encapsulating poorly water-soluble drugs, protecting them from degradation. Furthermore, the small size and surface properties of NLCs facilitate enhanced penetration and absorption in biological membranes [5]. NLCs are a prospective tool for a variety of medical applications, such as treatment of chronic diseases, cancer therapy, and vaccination, due to their efficacy and versatility.

Cancer treatment relies on nanotechnology advancements, ultimately improving cancer patients' survival rates. Nanotechnology has a significant role in cancer diagnosis, integrative therapy, and treatment, as well as in improving the results of traditional methods. Size, shape, and structural morphology of nanocarriers play significant roles in cancer treatment due to their efficacy in cancer management. Cancer research has shown the use of nanocarriers to deliver some antineoplastic drugs [6]. Inflammatory cytokines (IL-6, IL-1β, TNF-α), apoptosis (Bcl-2 and BAX), and other proteins involved in cell signaling pathways (JAK-STAT) are all involved in the progression of cancer. Reducing the production of inflammatory cytokines and pathways involved in cancer development and metastasis is now a major focus for researchers. This all-encompassing strategy sought to comprehend how IL-6 signaling regulation affects cell fate and apoptosis in response to NLC-mediated Capecitabine administration.

EXPERIMENTAL

Materials

Capecitabine was purchased from Dr. Reddy's Laboratories Ltd., Unit VI, Andhra Pradesh, India. Stearic acid (SA; Himedia, Mumbai, India), Tween-80 (T80; Loba Chemie, Mumbai, India), PanReac AppliChem (Darmstadt, Germany). Sesame oil (Jazan province, Saudia Arabia). Macrogen Inc. (Seoul, Korea) designed the primers, and RNA extraction kits were from Bio-Rad (MA, USA). cDNA reverse transcription, and SYBER green were purchased from Applied Biosystem (USA), SDS and all other chemicals used were analytical-grade reagents purchased from Sigma (USA).

Preparation

The NLC, which includes CAP (Nano-Cap), was formed by blending the lipid phase with the aqueous phase containing surfactants under hot conditions. Capecitabine (CAP; 25 mg) was dissolved in a mixture of molten stearic acid (SA) weighing 500 mg and 200 µL of sesame oil (SO) in a beaker to generate the lipid phase. Thereafter, 25 mL of water with a varying weight ratio of Tween-80 (T80; 50 µL) and sodium dodecyl sulfate (SDS; 50 mg) was mixed to prepare the aqueous phase. Both phases were heated to a temperature of 70 °C. The aqueous phase was mixed with the lipid phase using a homogenizer (HG-15D, WiseTis, Germany) for 20 min at 6000 rpm. The preparation was filtered through Whatman filter paper (Sigma-Aldrich) and then stored for further investigations.

Particle size, zeta potential (ZP), and polydispersity index (PDI)

Size analysis, polydispersity index (PDI) of the Nano-Cap was conducted using a zeta-sizer (Malvern, Nano ZS90, UK). To avoid the occurrence of multi-scattering events, the Nano-Cap formulations were appropriately diluted with Millipore water before analysis. The zeta potential (ZP) of the surface charge of Nano-Cap was measured using the same zeta sizer. The sample (1 mL) was filled in a cuvette and placed inside the instrument. The result was analyzed using the Malvern software. The measurement was done in triplicate.

Encapsulation efficiency

The entrapment efficiency (EE) calculation for Nano-Cap formulations was performed using the ultrafiltration-centrifugation technique. This experiment used the Amicon Ultra-2 mL, 3K ultra-filter tube, produced by Millipore in Ireland. The Nano-Cap formulation was filled with 1 mL, containing approximately 2 mg of CAP. The sample was centrifuged at 4000 × g (6861 rpm) for 20 min at 25 °C.

The filtrate obtained was subsequently diluted with Millipore water and then subjected to spectrophotometric analysis. Measurement of unentrapped CAP in the filtrate was performed by analyzing the absorbance at 241 nm using a UV-visible spectrophotometer (Shimadzu, Kyoto, Japan). Percentage encapsulation efficiency (% EE) of CAP in NLC was calculated using Eq 1.

EE (%) = ((IA-FA)/IA)100 …… (1)

where, IA = Initial amount, FA = Final amount

Surface morphology

Morphology of the sample was investigated using a transmission electron microscope (Morgagni 268, FEI, Czech Republic) operating at an acceleration voltage of 80-kilo electron volts (keV). To obtain images, a small amount of the dispersed solution was applied onto TEM grids (400 mesh) that had been covered with a thin layer of carbon film and dried. The films were placed under the microscope for further analysis.

Cell culture

The HCT116 cell lines gifted by King Abdulaziz University, Jeddah (Saudi Arabia) were grown in Roswell Park Memorial Institute (RPMI) 1640 medium (Gibco, Grand Island, NY, USA) with 10 % fetal bovine serum (FBS; Gibco, Paisley, UK), 1 % streptomycin and penicillin antibiotics (Sigma Aldrich, St. Louis, MO, USA). The Cells were kept at pH 7.4, temperature at 37 °C in a CO_2 incubator (New Brunswick, Scotland).

Cell viability assay (MTT)

Cell viability was investigated using an MTT test [7]. Culture medium (10,000 cells/mL) was added to 96-well culture plates. A CO_2 incubator cultivated the cells for 24 h, allowing them to proliferate and adhere. After the first day, the plates were taken out of the incubator and fresh media and formulations were added to the culture medium. The cells were exposed to various formulation concentrations, with the highest dose reaching 200 µg/mL and incubated for a further 72 h. In each well, 20 µL of MTT dye (5 mg/mL; Invitrogen Corporation, San Diego, CA, USA) was added after the treatment ended and left to incubate for another 4 h.

Careful removal of the medium from the plates was performed after the incubation time had elapsed to avoid disturbing the formazan crystals that had formed at the bottom of the plates. Thereafter, 100 µL of dimethylsulphoxide (DMSO; Fischer Chemicals, UK) was added to every well to make the crystals dissolve more easily. A microplate reader (BMG LABTECH, SpectroStar Nano, Ortenberg, Germany) was used for further analysis of the violet-hued result at 570 nm. Determination was done in triplicate and percentage viability (V) was calculated using Eq 2.

V (%) = ((Ac-At)/At)100 …… (2)

Where Ac is the absorbance of control and At is the absorbance of sample.

DNA fragmentation

Genomic DNA was isolated from the cells following previous methods [8]. The cells (1×10^6 cells) were cultured and exposed to known amounts of both pure drugs (CAP) and nano-formulation (Nano-Cap). The treated cells were harvested using trypsin and then washed with Dulbecco's Phosphate Buffered Saline (DPBS).

The DNA was isolated using the Qiagen DNA isolation kit, following the directions provided by the manufacturer. The concentration of DNA was measured in a nanodrop at 260 nm. Afterward, electrophoretic DNA analysis was conducted using 0.2 agarose gels that contained 0.1 µg/mL ethidium bromide.

cDNA synthesis and RNA extraction

Extraction of mRNA was carried out in line with the instructions provided by the Bio-Rad AurumTM Mini Kit. mRNA was quantified using a nanodrop spectrophotometer (Thermo Scientific). Purity of the mRNA was evaluated by measuring the ratio of absorbance at 260 to 280 nm. To generate cDNA, extracted mRNA was employed in conjunction with a cDNA reverse transcription kit [9].

Real-time PCR

Primer sequences for IL-6, IL-6R, gp130, NF-kB, TNF-alpha, Bcl-2, Bax, JAK, and STAT were acquired from Macrogen Inc. (Korea), and reaction temperature and cycle time were followed according to manufacturer's instructions. Expression of GADPH is widely regarded as a benchmark, and estimation of gene expression is conducted using the 2^ΔΔ CT technique [10]. The cDNA templates were used for conducting quantitative real-time polymerase chain reaction (qRT-PCR) using the CFX96 instrument (Bio-Rad), in addition to the SYBR green master mix (Applied Biosystems).

Statistical analysis

Data was analysed using Statistical Packages for Social Sciences (SPSS 21.0, IBM, Armonk, NY, USA). Values were presented in mean ± standard deviation (SD) and compared using one-way analysis of variance (ANOVA). $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of NLC

The NLC was spherical (Figure 1 - left), particle size of 177.1 ± 56.52 d.nm (n = 3; Figure 1 - middle), polydispersity index (PDI) of 0.279 ± 0.021 (n = 3), zeta potential (ZP) of 48.87 ± 6.0 mV (n = 3; Figure 1 - right) and EE of 96.53 ± 0.91 % (n = 3). Differences in the result of DLS (zeta sizer) and TEM can be attributed to the differences in sample preparation and the principle of analysis.

Morphological features

Treated cells exhibited several morphological changes detectable at 20x magnification compared to untreated cells (Figure 2 A - D). Treated cells displayed characteristics such as cell membrane blebbing, greater inhibition of growth, and cell shrinkage, while the control group contained a higher proportion of healthy cells. On the other hand, cells that had not been treated continued to cling to one another during the entire incubation period.

Cell viability

Results from MTT assay showed that nano-formulation had a much lower IC_{50} (8 µg/mL) compared to the pure drug (48 µg/mL; Figure 3). There were statistically significant ($p < 0.05$) differences between nano formulation and the pure drug treatment in each treatment concentration.

DNA fragmentation

The agarose gel electrophoretic pattern of the pure drug (lane 2) and the Nano-Cap (lane 3) induced DNA degradation, is shown in Figure 4. Control cell displayed a compact DNA (lane 1), indicating that there was no fragmentation in the cancerous cell. The ladder-like (smear) pattern of the DNA was found in the Nano-Cap group, which is a typical marker of apoptosis as compared with the pure drug.

Real-time qRT-PCR data

Real-time qRT-PCR was performed to analyze inflammatory and apoptotic gene expression in control, pure drug, and Nano-Cap-treated cell lines (Figure 5). Expression of inflammatory and apoptotic genes was significantly lower in pure and Nano-Cap treated cell lines compared to control ($p < 0.05$). However, treatment with Nano-Cap significantly reduced expression of inflammatory and apoptotic markers compared to pure drug ($p < 0.05$). Furthermore, level of Bax was significantly increased ($p < 0.001$) in Nano-Cap-treated cell lines compared to pure drug and control (Figure 6).

DISCUSSION

Nanostructured lipid carriers (NLCs) with the observed particle size greatly improve drug delivery. Their increased surface area-to-volume ratio enhances the solubility and dissolution rates of drugs, resulting in enhanced bioavailability, more effective absorption and distribution throughout the body. Furthermore, these NLCs avoid being quickly eliminated by the reticuloendothelial system (RES), leading to longer period of circulation, continuous drug release and minimal side effects [11] which is an advantage in treating chronic diseases. Due to their moderate size, they may effectively target specific areas, such as tumors, which improves effectiveness of treatments while minimizing exposure to the rest of the body.

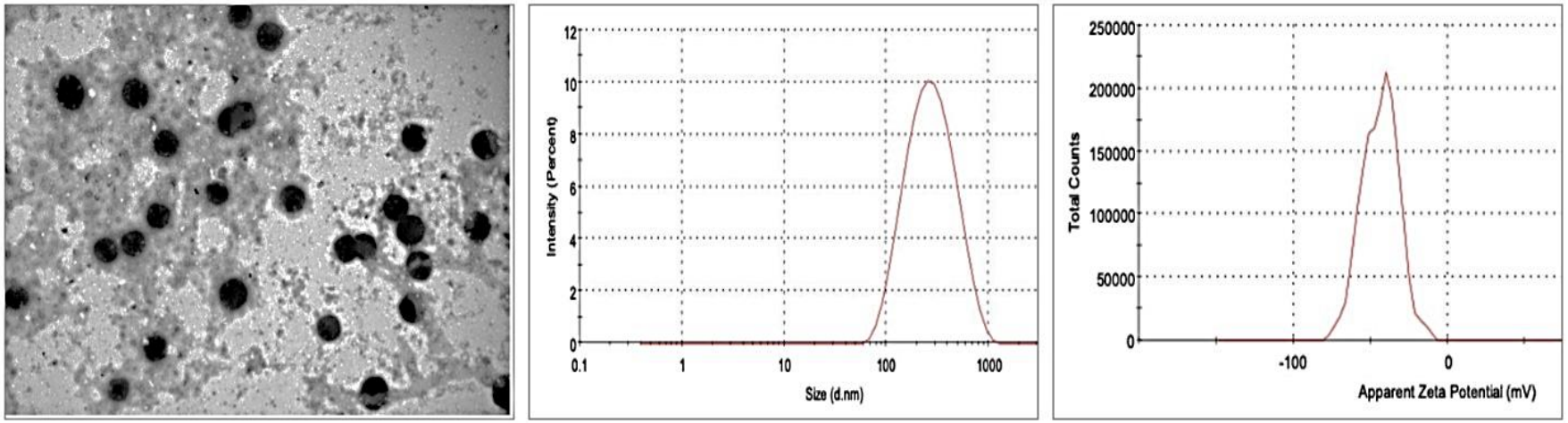

Figure 1: TEM image (left), size distribution of Nano-Cap (223.1 d.nm; n = 1; middle), zeta potential (-43.6 mV; right)

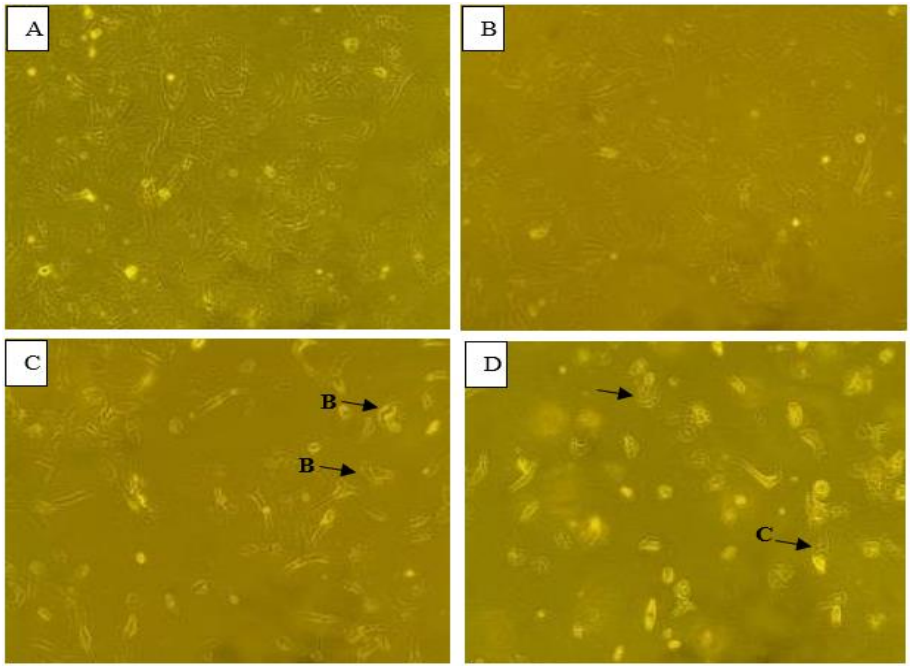

Figure 2: Phase contrast microscopical pictures of cell morphology after treatments (a) Control; (b) Pure drug (CAP) @ 100 μg/mL; (c) Nano formulation (Nano-Cap) @ IC_{50} dose, (12.5 μg/mL); (d) Nano formulation (Nano-Cap) at half of IC_{50} dose (6.25 μg/mL). BL: Blebbing of cells; CS: Cell shrinking

A polydispersity index (PDI) value of 0.27 for nanostructured lipid carriers (NLCs) signifies a narrow size distribution, indicating uniformity and stability, which reduces aggregation and maintains drug efficacy and safety.

The zeta potential of Nano-Cap indicated significant electrostatic repulsion, preventing particle aggregation and maintaining colloidal stability, which is crucial for consistent therapeutic effectiveness. This strong negative charge also increases circulation time in the bloodstream by limiting fast clearance by the reticuloendothelial system (RES), which is beneficial for chronic medical conditions that need continuous drug release [5]. An entrapment efficiency (EE) of 96 % is clinically significant as it guarantees efficient encapsulation, enhancing bioavailability, stability, and controlled release. This high EE leads to improved drug delivery and absorption, prolonged shelf-life, facilitates targeted delivery, more consistent therapeutic levels, and reduced side effects. Additionally, NLC formulations with high EE are designed for convenient dosing, improved patient compliance, reduced drug wastage and overall healthcare costs [12].

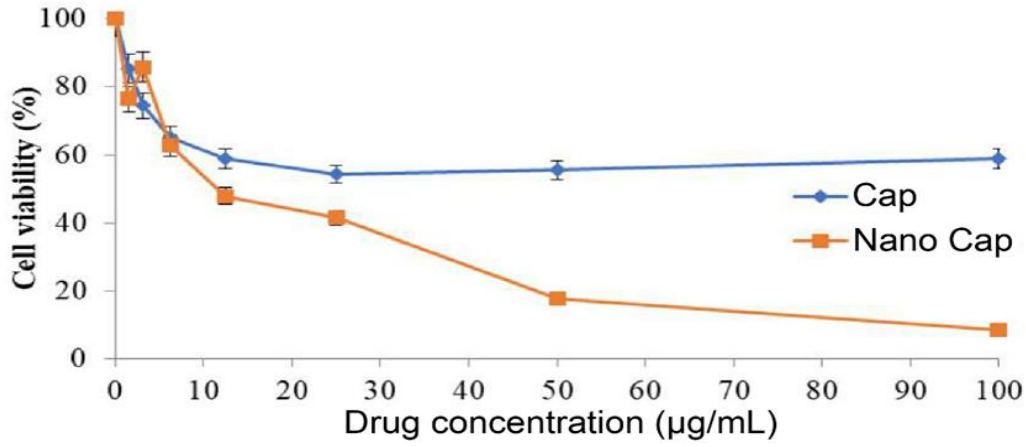

Figure 3: Cytotoxicity assay of CAP (blue colour) and Nano-CAP (orange colour). The average across three replicates was used to represent values

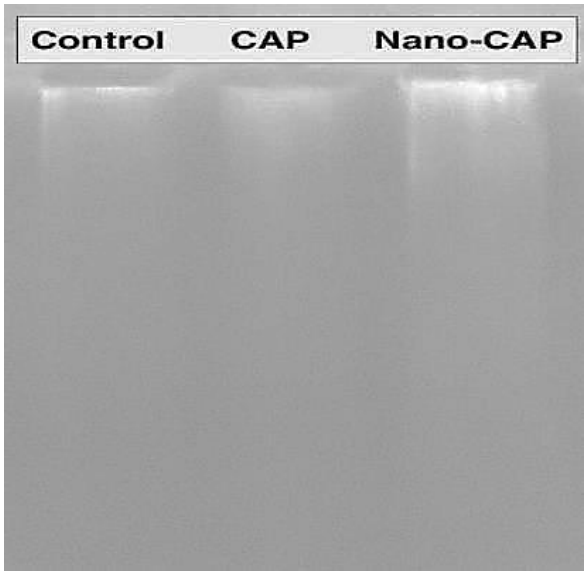

Figure 4: DNA fragmentation. The control lane represents an intact DNA band while the pure drug lane showed some fragmentation as a smear. The Nano-Cap Lane showed an extensive smear representing significant DNA fragmentation

Transmission electron microscopy (TEM) images provided critical insights into the morphology of the Nano-Cap formulation, revealing that the nanoparticles exhibited well-defined spherical shape, which is essential for maintaining uniformity in drug delivery. The spherical morphology is advantageous as it promotes stability and consistent dispersion within biological systems, facilitating efficient delivery of capecitabine to targeted cancer cells. Furthermore, TEM analysis confirmed that the particle size of Nano-Cap is within the nanometer range, typically below 200 nm, which is ideal for enhanced cellular uptake and improved penetration through biological barriers such as tumor vasculature [13]. These findings validate the successful formulation of Nano-Cap as a stable, uniformly shaped nanoparticle system suitable for effective cancer therapy.

The MTT assay was used to investigate the find out if Nano-Cap has anticancer properties, the cytotoxicity assay (MTT) was used. The enhanced cytotoxicity of Nano-Cap was compared to that of CAP alone. The results showed that Nano-Cap was 6 times more cytotoxic than CAP alone, which showed significant cytotoxicity with an IC_{50} of 48 ug. Also, the NLC blank did not show any cytotoxicity throughout the study. Morphological investigation also confirms the cytotoxicity demonstrated in the MTT experiment. Due to the significant dye absorption, Nano-Cap exhibits a similar level of cytotoxicity in cancer cells even at low doses. T-lymphocytes, fibroblasts, and monocytes are only a few of the typical cell types that generate interleukin (IL-6), a multifunctional cytokine. This cytokine acts via a membrane receptor complex made up of glycoprotein 130 (gp130) and IL-6 receptor a (IL-6Ra). To start the signal transduction process, IL-6 first attaches to IL-6Ra, which is unable to do so. This complex then draws gp130 molecules, which dimerize to provide the intracellular signal [14]. Numerous malignancies, including melanoma, renal cell carcinoma, Kaposi's sarcoma, ovarian carcinoma, lymphoma and leukemia, multiple myeloma, and prostatic carcinoma, have been demonstrated to be stimulated Nano-Cap.

IL-6-dependent STAT3 signaling is a fundamental promoter of CRC cell survival and proliferation. Activation of IL-6 receptors subsequently stimulates signal transduction pathways, including the JAK-STAT pathway. This study revealed that the expression of JAK-STAT proteins was reduced following treatment with Nano-Cap, consistent with findings from a previous study [15].

Meanwhile, activated STAT3 translocated inside the nucleus binds the STAT binding site on the promoter and starts the transcription of genes responsible for cytokines production. Increased inflammation in CRC has been demonstrated to promote tumor growth. Majority of cancer development processes, including tumor initiation, proliferation, migration, and angiogenesis, are influenced by IL-6 and TNF-α in CRC [16]. Patients with colorectal cancer showed elevated levels of TNF- α and IL-6 in tumor tissues and sera, and these cytokines were linked to worse overall survival, metastasis, and greater tumor burden. Patients with high levels of IL-6 had lower survival compared to those with low levels.

This study showed a significant reduction in mRNA expression of IL-6, IL-6R, and TNF-α following treatment with Nano-Cap compared to the pure drug. Similar results have also been reported in earlier studies [17]. Expression of Bcl-2 family gene products, which include the promoters (bax, bad, or bak) and inhibitors (bcl-2, mcl-1, or bcl-xl), plays a significant role in the control of apoptosis. Expression of Bcl-2 and its family proteins in human breast cancer has been discussed extensively. The primary determinant of apoptosis induction or inhibition appears to be the Bcl-2 to Bax ratio. Disruption of this balance is considered one of the key characteristics of cancer [18]. Findings from this present study revealed lower Bcl-2 expression and higher Bax levels following treatment with Nano-CAP.

CONCLUSION

Nano-Cap reduces the expression of IL-6, which in turn reduces the expression of all downstream pathways' proteins accountable for the genesis, development, and metastasis of cancer. Nano-Cap exerted antitumor effects by targeting IL-6, IL-6R, gp130, JAK, STAT3, and TNF-α in tumors. Nano-Cap may be a strategy in the treatment of CRC through modulation of the tumor microenvironment.

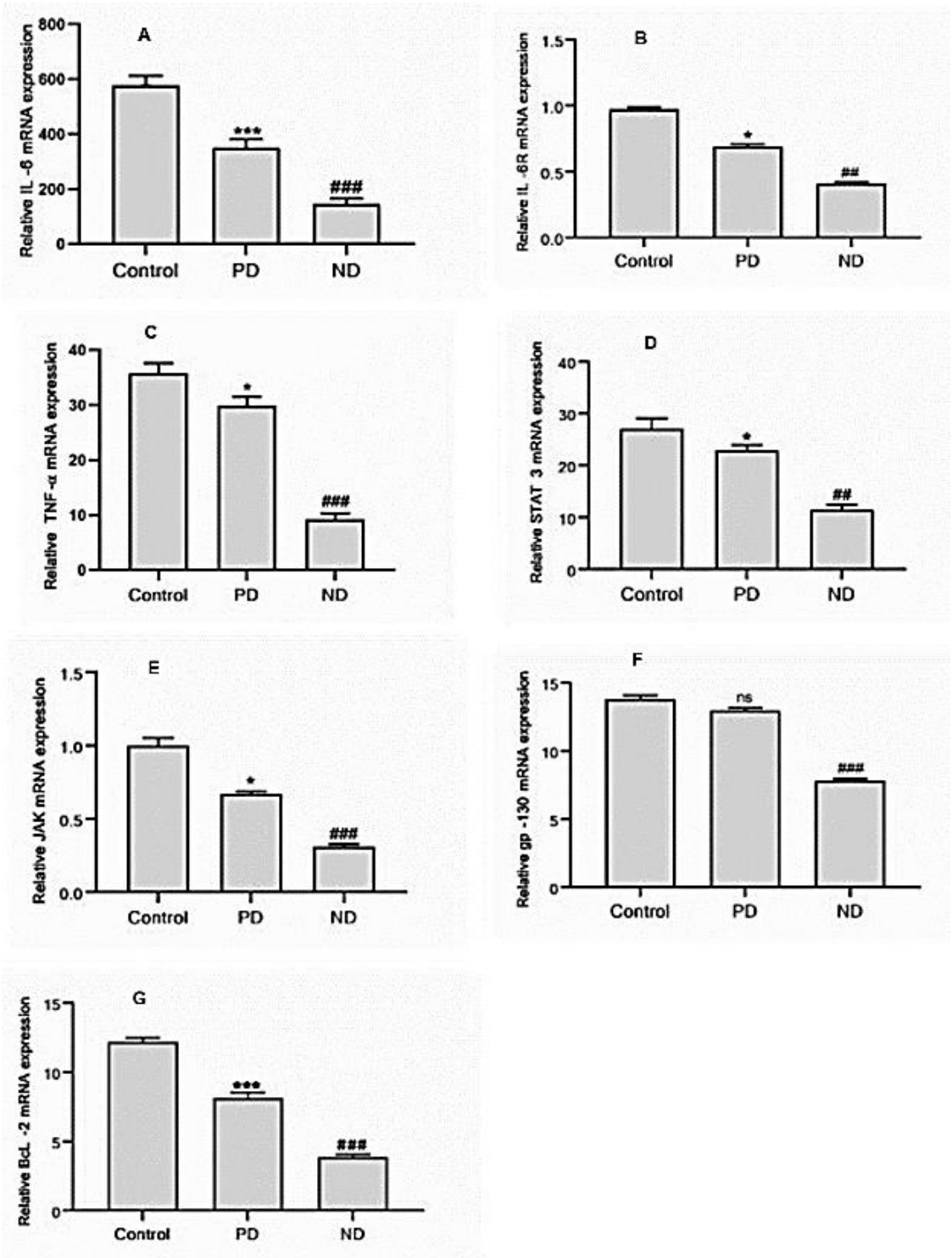

Figure 5: Relative mRNA Expression of IL-6 (A), IL-6r (B), TNF-α (C), STAT-3 (D), JAK (E), gp-130 (F), Bcl-2 (G). Data indicated as the mean ± SE. *P < 0.05, **p < 0.01, ***p < 0.001 vs control group. $^{\#\#}P$ < 0.01, $^{\#\#\#}p$ < 0.001 vs Nano-Cap treated cell lines

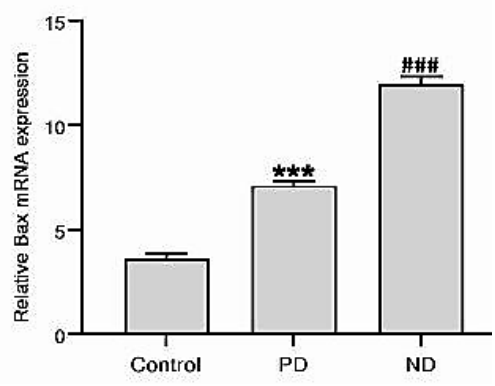

Figure 6: Relative mRNA Expression of Bax. Values represented as mean ± SE. ***P < 0.01 vs control group, $^{###}p$ < 0.001 to Nano-Cap treated cell lines

DECLARATIONS

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None.

Ethical approval

Not required.

Use of Artificial intelligence/Large language models

We declare that we did not use Generative artificial intelligence (AI) and AI-assisted technologies in writing the manuscript.

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the author(s) named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Conceptualization, validation, writing original draft by Sohail Hussain and Mohammad Intakhab Alam. Funding acquisition by Ahmad Salawi, formal analysis, project administration, writing, review and editing, visualization by Syam Mohan, Mohammad Ashafaq, Rahimullah Siddiqui. Methodology, data curation, investigation, software by Ahmad Salawi, Dalin A. Hassan, and Wedad Alhasan Mawkili. Validation, resources by Mohammad Intakhab Alam, Dalin A. Hassan, Wedad Alhasan Mawkili and Syam Mohan. All authors have read and agreed to the published version of the manuscript.

REFERENCES

1. *Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J, Jemal A. Global cancer statistics, 2012. CA A Cancer J Clin 2015; 65(2): 87–108.*
2. *Carethers JM, Doubeni CA. Causes of socioeconomic disparities in colorectal cancer and intervention framework and strategies. Gastroenterol 2020; 158(2): 354–367.*
3. *Hammond WA, Swaika A, Mody K. Pharmacologic resistance in colorectal cancer: a review. Ther Adv Med Oncol 2016; 8(1): 57–84.*
4. *Hartmann J, Haap M, Kopp HG, Lipp HP. Tyrosine kinase inhibitors – a review on pharmacology, metabolism and side effects. CDM 2009; 10(5): 470–481.*
5. *Makeen HA, Mohan S, Al-Kasim MA, Sultan MH, Albarraq AA, Ahmed RA, Alhazmi HA, Alam MI. Preparation, characterization, and anti-cancer activity of nanostructured lipid carriers containing imatinib. Pharmaceutics 2021; 13(7): 1086.*
6. *Cheng Z, Li M, Dey R, Chen Y. Nanomaterials for cancer therapy: current progress and perspectives. J Hematol Oncol 2021; 14(1): 85.*
7. *Syam S, Abdelwahab SI, Al-Mamary MA, Mohan S. Synthesis of Chalcones with Anticancer Activities. Molecule 2012; 17(6): 6179–6195.*
8. *Hussain S, Ashafaq M, Alshahrani S, Bokar IAM, Siddiqui R, Alam MI, Taha MME, Almoshari Y, Alqahtani SS, Ahmed RA, et al. Hepatoprotective effect of curcumin nano-lipid carrier against cypermethrin toxicity by countering the oxidative, inflammatory, and apoptotic changes in Wistar rats. Molecules 2023; 28(2): 881.*
9. *Hussain S, Jali AM, Alshahrani S, Khairat KHM, Siddiqui R, Alam MI, Ali R, Mohammed M, Khan A, Al Shahi H, et al. Hepatoprotective and antioxidant effects of nanopiperine against cypermethrin via mitigation of oxidative stress, inflammation and gene expression using qRT-PCR. Int J Mol Sci 2023; 24(20): 15361.*
10. *Ashafaq M, Hussain S, Alshahrani S, Siddiqui R, Alam MI, Elhassan Taha MM, Almoshari Y, Alqahtani SS, Jali AM, Aljohani HM. Neuroprotective effects of nano-curcumin against cypermethrin-associated oxidative stress and up-regulation of apoptotic and inflammatory gene expression in rat brains. Antioxidants (Basel) 2023; 12(3): 644.*
11. *Alam MI, Baboota S, Ahuja A, Ali M, Ali J, Sahni JK, Bhatnagar A. Pharmacoscintigraphic evaluation of potential of lipid nanocarriers for nose-to-brain delivery*

of antidepressant drug. Int J Pharm 2014; 470(1–2): 99–106.
12. Üner M, Karaman E, Aydoğmuş Z. Solid lipid nanoparticles and nanostructured lipid carriers of loratadine for topical application: physicochemical stability and drug penetration through rat skin. Trop J Pharm Res 2014; 13(5): 653.
13. Zhang W, Taheri-Ledari R, Ganjali F, Mirmohammadi SS, Qazi FS, Saeidirad M, KashtiArayb A, Zarei-Shokatb S, Tianc Y, Maleki A, et al. Effects of morphology and size of nanoscale drug carriers on cellular uptake and internalization process: a review. RSC Adv 2022; 13(1): 80–114.
14. Heinrich PC, Behrmann I, Müller-Newen G, Schaper F, Graeve L. Interleukin-6-type cytokine signalling through the gp130/Jak/STAT pathway. Biochem 1998; 334(2): 297–314.
15. Grivennikov S, Karin E, Terzic J, Mucida D, Yu GY, Vallabhapurapu S, Scheller J, Rose-John S, Cheroutre H, Eckmann L, et al. IL-6 and STAT3 are required for survival of intestinal epithelial cells and the development of colitis-associated cancer. Cancer Cell 2009; 15(2): 103–113.
16. West NR, McCuaig S, Franchini F, Powrie F. Emerging cytokine networks in colorectal cancer. Nat Rev Immunol 2015; 15(10): 615–29.
17. Lin MT, Juan CY, Chang KJ, Chen WJ, Kuo ML. IL-6 inhibits apoptosis and retains oxidative DNA lesions in human gastric cancer AGS cells through up-regulation of anti-apoptotic gene mcl-1. Carcinogen 2001; 22(12): 1947–1953.
18. Nathan B, Gusterson B, Jadayel D, O'Hare M, Anbazhagan R, Jayatilake H, Ebbs S, Micklem K, Price K, Gelber R, et al. Expression of BCL-2 in primary breast cancer and its correlation with tumour phenotype. Annals Oncol 1994; 5(5): 409–414.