

Enoxaparin induces apoptosis and autophagy, modulates inflammatory signaling, and reduces oxidative DNA damage in breast and liver cancer cells

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TITLE PAGE

Title: Enoxaparin Induces Apoptosis and Autophagy, Modulates Inflammatory Signaling, and Reduces Oxidative DNA Damage in Breast and Liver Cancer Cells

Running Title: Enoxaparin's Anticancer Mechanisms in Breast and Liver Cells

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Statements and Declarations**Author contributions**

S.C. and N.B. conceived of and designed the study. S.C., N.B., M.B., G.O., and D.G performed material preparation, data collection, and analysis. The first draft of the manuscript was written by S.C., N.B., G. O., D.G., and A.Y., who critically reviewed and edited the manuscript. A.Y. supervised the study and provided critical revisions. All authors have read and approved the final manuscript.

Data availability statement

Data supporting the findings of this study are available from the corresponding author upon reasonable request.

Competing Interests Statement

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

Not applicable. This study was conducted exclusively in vitro using established cell lines and did not involve human participants, human biological samples, or animals. Therefore, approval from the ethics committee and informed consent were not required.

ABSTRACT

Background

Cancer progression involves intricate interactions between inflammatory signaling, programmed cell death mechanisms, and oxidative stress. Although enoxaparin is widely used for managing cancer-associated thrombosis, its direct cellular effects on tumor biology remain insufficiently characterized. This study aimed to evaluate the impact of enoxaparin on apoptosis, autophagy, inflammatory mediators, and oxidative DNA damage in breast (MDA-MB-231) and liver (HepG2) cancer cell lines.

Methods

MDA-MB-231, HepG2, and non-cancerous HEK-293 cells were treated with varying concentrations (5, 10, 20, 40, and 80 mg/mL) of enoxaparin for 24 and 48 hours. Cell viability was assessed using the MTT assay, while apoptosis was quantified by TUNEL analysis. Immunofluorescence staining was employed to evaluate the expression of NF- κ B, IL-6, TNF- α , LC3, and p62. Oxidative DNA damage was determined by measuring extracellular 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels using a competitive ELISA. Statistical analyses were conducted to compare the treated and control groups.

Results

Enoxaparin significantly reduced cell viability in MDA-MB-231 and HepG2 cells without inducing cytotoxicity in HEK-293 cells. Apoptosis was markedly increased in both cancer cell lines following treatment. Enoxaparin differentially modulated inflammatory signaling; NF- κ B expression was significantly increased in MDA-MB-231 cells, accompanied by suppression of IL-6 and TNF- α , whereas no significant inflammatory changes were observed in HepG2 cells. Enoxaparin treatment was observed to increase LC3 and p62 expression in both MDA-MB-231 and HepG2 cells, triggering autophagy-related pathways. Moreover, enoxaparin significantly reduced extracellular 8-OHdG levels, suggesting a reduction in oxidative DNA damage.

Conclusion

Enoxaparin exhibits multifaceted anticancer effects by promoting apoptosis and autophagy, selectively modulating inflammatory pathways, and reducing oxidative DNA damage in breast and liver cancer cells.

Keywords: 8-OHdG; Oxidative DNA damage; Apoptosis; Autophagy; Inflammatory signaling

1. Introduction

Cancer is widely recognized as the second leading cause of mortality worldwide and remains a significant public health challenge. Although biomedical research has expanded rapidly and in great depth, the availability of anticancer therapies that significantly reduce the overall disease burden remains inadequate. This situation underscores the continuing need for treatment strategies that are both more effective and grounded in contemporary insights into cancer biology, supported by advanced technological approaches (1, 2). The core objective of anticancer therapy is to eliminate malignant cells as selectively as possible while minimizing injury to normal tissues. In practice, conventional chemotherapeutic agents do not sufficiently discriminate between malignant and healthy cells, resulting in limited selectivity that leads to unavoidable toxicity and side effects in noncancerous tissues (3). In patients with solid tumors, metastatic dissemination accounts for more than two-thirds of cancer-related deaths, and metastases are reported to contribute to approximately 66.7% of all deaths (4). Beyond their central role in hemostasis, platelets have been shown to engage with circulating tumor cells (CTCs), shielding them from hemodynamic shear forces, supporting immune evasion—particularly from natural killer (NK) cells—and enhancing metastatic colonization by increasing intravascular stalling (5-7). Consistent with this concept, experimental platelet depletion significantly reduced metastasis in mouse models, underscoring the importance of platelet–tumor cell interactions during metastatic progression. At the same time, it is well established that cytotoxic anticancer agents can disrupt essential cellular functions, including membrane integrity, metabolic activity, biosynthetic pathways, ion homeostasis, and cell division, thereby contributing to a broad spectrum of adverse effects (8).

Venous thromboembolism (VTE), which includes deep vein thrombosis and pulmonary embolism, is a common complication in patients with cancer; it is estimated that 20–30% of all VTE cases are associated with malignancy. Cancer patients exhibit a 3- to 9-fold increased risk of VTE compared to the general population (9). Current guidelines recommend anticoagulant therapy for at least 3–6 months in patients with cancer-associated thrombosis (CAT), with extended treatment in selected high-risk groups (10-13). However, anticoagulation carries an inherent risk of bleeding, particularly in oncology patients, further complicating disease management (11, 14, 15). These observations highlight the complex interplay between thrombosis and cancer biology, both of which contribute to poor prognosis and increased mortality (16-18).

Cancer progression is closely associated with dysregulation of key biological processes, including inflammatory signaling, programmed cell death pathways, and oxidative stress. Nuclear factor- κ B (NF- κ B) and pro-inflammatory cytokines such as TNF- α and IL-6 play central roles in tumor-associated inflammation and metastasis, while apoptosis and autophagy are critical determinants of cancer cell survival and therapeutic response (19, 20). In addition, oxidative DNA damage, commonly assessed by 8-hydroxy-2'-deoxyguanosine (8-OHdG), is a major contributor to genomic instability and tumor progression (21).

Enoxaparin, a widely used low-molecular-weight heparin, is routinely administered in the management of cancer-associated thrombosis (22). Beyond its anticoagulant properties, emerging evidence suggests that enoxaparin may exert additional biological effects, including modulation of tumor growth, inflammation, and cellular stress responses (23). However, its direct impact on key molecular pathways involved in apoptosis, autophagy, and oxidative stress in cancer cells remains insufficiently characterized.

Based on these considerations, we hypothesized that enoxaparin may exert cell type-specific regulatory effects on critical molecular pathways associated with cancer progression. To the best of our knowledge, no study has comprehensively evaluated the simultaneous effects of enoxaparin on apoptosis, autophagy, inflammatory mediators, and oxidative DNA damage in both MDA-MB-231 breast cancer and HepG2 hepatocellular carcinoma cell lines. Therefore, the aim of this study was to investigate the modulatory effects of enoxaparin on apoptotic signaling, autophagic activity, inflammatory pathways, and oxidative DNA damage in these cancer models, thereby providing insight into its potential biological roles beyond anticoagulation.

2. Materials and methods

2.1. Cell Culture

The human breast cancer cell line MDA-MB-231, the human hepatocellular carcinoma cell line HepG2, and the human embryonic kidney cell line HEK-293 were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). All three cell lines (MDA-MB-231, HepG2, and HEK-293) were maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Thermo Fisher Scientific, USA) supplemented with fetal bovine serum (10%, FBS, Sigma-Aldrich, USA), l-glutamine (1 %, Gibco, USA), penicillin (100 units/ml, Gibco, USA), and streptomycin (100 mg/mL, Gibco, USA). Cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂.

2.2. Cell Viability Assessment

For cell viability assessment, MDA-MB-231, HepG2, and HEK-293 cells were seeded into 96-well tissue culture plates at a density of 5×10^3 cells/mL 24 h before enoxaparin (Oksapar®, 6000 anti-Xa IU/0.6 mL, Koçak Farma, İstanbul, Türkiye) exposure. The cells were then treated with enoxaparin serially diluted in culture medium to final concentrations of 5, 10, 20, 40, and 80 mg/mL. For each concentration and cell type, four replicates were included, and incubation was performed for 24 and 48 h. Following the exposure period, 10 µL of MTT solution was added to each well, and the plates were incubated for 4 h at 37°C. Subsequently, the culture medium was removed using a syringe. Formazan crystals were solubilized by adding 100 µL dimethyl sulfoxide to each well. Absorbance was measured at 570 nm using a plate reader (24). Following absorbance measurements, cell viability was calculated as percentage relative to the corresponding time-matched control groups. The respective 24-h IC_{50} concentrations determined from the MTT assay, were used in all subsequent TUNEL, immunofluorescence, and 8-OHdG experiments.

2.3. Analysis of apoptosis by TUNEL

To confirm DNA fragmentation associated with enoxaparin-induced apoptosis, a Terminal Deoxynucleotidyl Transferase-mediated dUTP Nick End Labeling (TUNEL) assay was conducted. MDA-MB-231 and HepG2 cells (5×10^4 cells/mL) were seeded on round coverslips in sterile 12-well culture plates. After 24 hours of incubation, the cells were treated with enoxaparin for an additional 48 hours. DNA strand breaks were detected by enzymatic end labelling using a commercial kit (Millipore S7110, Sigma Aldrich, US) in accordance with the manufacturer's protocol, and the fluorescence signal was evaluated under a fluorescence microscope (Olympus BX74, Tokyo, Japan). TUNEL-positive cells were quantified from 10 randomly selected non-overlapping microscopic fields per sample using ImageJ software (ImageJ, Bethesda, USA) (25).

2.4. Immunofluorescence assay

Immunofluorescence staining was performed to evaluate the expression profiles of nuclear factor- κ B p65 (RelA) (NF- κ B) (1:50, clone F-6, Cat. No. sc-8008, Santa Cruz Biotechnology, Texas, USA), Tumor necrosis factor- α (TNF- α) (1:100, Novus Biologicals, Littleton, USA), Interleukin 6 (IL-6) (1:100, Bioss, Massachusetts, USA), LC3 (1:250, Cell Signaling Technology, Danvers, USA), and p62 (1:200, Novus Biologicals, Littleton, USA). For protein expression quantification, ten images were randomly captured from microscopic fields at 400x magnification using an Olympus BX74 fluorescence microscope (Tokyo, Japan). Immunoreactivity intensity was quantified using ImageJ software (ImageJ, Bethesda, USA) (26).

2.5. Oxidative DNA Damage Assay

Extracellular 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were determined to assess oxidative DNA damage. MDA-MB-231 and HepG2 cells were seeded at 1×10^6 cells/mL in 6-well plates and incubated for 24 h. Enoxaparin was then applied, and after 48 h of incubation, the cell culture supernatants were carefully collected to eliminate cellular debris. Extracellular 8-OHdG levels in the supernatant samples were measured using commercial kits based on the competitive chemiluminescence ELISA principle (Bioassay Technology Laboratory, Shanghai, China), strictly following the manufacturer's instructions. Measurements were recorded at 450 nm, and analyte concentrations were calculated from the standard curve provided by the kit using the obtained absorbance values (27).

2.6. Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS Statistics for Windows version 25.0; IBM Corp., Armonk, NY, USA). Quantitative data are expressed as mean $\pm$ standard deviation (SD). Before statistical comparisons, the normality of the data distribution was assessed using the Shapiro–Wilk test. For comparisons between two independent groups (control vs. enoxaparin-treated cells), the independent samples t-test was applied for normally distributed variables. In contrast, the Mann–Whitney U test was used for data that did not meet normality assumptions. For analyses involving multiple concentrations of enoxaparin, one-way analysis of variance (ANOVA) followed by Tukey's post hoc test was used for normally distributed datasets. Alternatively, the Kruskal–Wallis test followed by Dunn's multiple comparison test was used for non-normally distributed data. Cell viability assays were performed in triplicate, and IC_{50} values were calculated based on 48-hour dose–response curves using nonlinear regression analysis. Immunofluorescence intensity data (TUNEL, NF- κ B, IL-6, TNF- α , LC3, p62, and 8-OHdG) were quantified from at least five randomly selected microscopic fields per sample and averaged before statistical evaluation. All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant. The levels of statistical significance were denoted as follows: $p < 0.05$, $p < 0.01$, $p < 0.001$, and $p < 0.0001$.

3. Results

3.1. Effect of Enoxaparin on Cell Viability

Cell viability values were normalized to the corresponding time-matched control groups and expressed as percentage viability. Enoxaparin treatment resulted in concentration- and time-dependent alterations in cell

viability in both MDA-MB-231 and HepG2 cells. In MDA-MB-231 cells, cell viability remained relatively stable at lower concentrations, whereas significant reductions were observed at 40 mg/mL ($p < 0.01$) and 80 mg/mL ($p < 0.001$), particularly after 48 h exposure. Similarly, HepG2 cells exhibited a marked decrease in viability at higher concentrations of enoxaparin, with significant reductions detected at 40 mg/mL ($p < 0.01$) and 80 mg/mL ($p < 0.001$), with a more pronounced effect observed following 48 h treatment compared to 24 h. The IC_{50} values determined from the 24-hour dose–response curves were 44.5 mg/mL for MDA-MB-231 cells and 40 mg/mL for HepG2 cells, and these concentrations were used in the subsequent experiments. In contrast, HEK-293 cells did not show a statistically significant reduction in viability across most concentrations and time points. Minor fluctuations observed at lower concentrations may reflect normal biological variability in proliferative non-cancerous HEK-293 cells rather than treatment-related cytotoxicity. Overall, these findings suggest that enoxaparin selectively decreases cell viability in cancer cell lines while largely preserving the viability of non-cancerous cells.

3.2. Effect of Enoxaparin on apoptosis

The effect of enoxaparin on apoptosis in breast and liver cancer cell lines was evaluated using the TUNEL assay (Figure 2). Enoxaparin treatment significantly increased apoptosis in both the MDA-MB-231 and HepG2 cell lines. In MDA-MB-231 cells, the median TUNEL positivity increased from 0.27 in the control group to 0.50 following enoxaparin treatment. Similarly, in HepG2 cells, the median apoptotic index rose from 0.27 in the control group to 0.57 after treatment. These increases were statistically highly significant in both cell lines ($p < 0.0001$) (Table 1, Figure 3). Accordingly, comparisons between the control and enoxaparin-treated groups demonstrated statistically significant differences, indicating the pro-apoptotic effect of enoxaparin in both cell types.

3.3. Effect of Enoxaparin on expression of NF- κ B, TNF- α and IL-6

We investigated the impact of enoxaparin on the expression of NF- κ B, TNF- α , and IL-6 in MDA-MB-231 and HepG2 cells. Protein expression levels were quantified using immunofluorescence analysis. Alterations in NF- κ B, TNF- α , and IL-6 immunoreactivity were observed following 48-h enoxaparin treatment (Figure 4, Figure 5, Figure 6). In MDA-MB-231 cells, enoxaparin treatment resulted in a marked and statistically significant increase in NF- κ B expression, rising from 0.36 ± 0.02 in the control group to 2.19 ± 0.58 in the treated group ($p < 0.0001$). In contrast, no significant change in NF- κ B expression was observed in HepG2 cells, where levels remained

comparable between control (0.42 ± 0.07) and enoxaparin-treated groups (0.43 ± 0.04) ($p = 0.74$) (Table 2, Figure 7).

Regarding TNF- α expression, enoxaparin significantly reduced its levels in MDA-MB-231 cells, decreasing from 0.74 ± 0.23 in the control group to 0.45 ± 0.07 following treatment ($p = 0.003$). However, no statistically significant difference was observed in HepG2 cells, with TNF- α levels remaining similar between control (0.48 ± 0.08) and treated groups (0.46 ± 0.07) ($p = 0.55$) (Table 2, Figure 7).

Similarly, IL-6 expression was significantly decreased in MDA-MB-231 cells after enoxaparin exposure, declining from 2.50 ± 0.70 in the control group to 1.49 ± 0.33 in the treated group ($p = 0.001$). In contrast, HepG2 cells showed no significant change in IL-6 expression, with values of 0.35 ± 0.05 in the control group and 0.34 ± 0.03 in the treated group ($p = 0.41$) (Table 2, Figure 7). Overall, these findings indicate that enoxaparin selectively modulates inflammatory signaling in MDA-MB-231 cells, while exerting minimal effects on these markers in HepG2 cells.

3.4. Effect of Enoxaparin on Autophagy

Autophagy-related protein expression was analyzed by assessing LC3 and p62 levels in both MDA-MB-231 and HepG2 cells following enoxaparin treatment. Figures 8 and 9 illustrate LC3 and p62 expression profiles, respectively. In MDA-MB-231 cells, LC3 expression increased significantly from a median value of 1.01 in the control group to 1.51 following enoxaparin treatment ($p = 0.006$). Similarly, in HepG2 cells, LC3 levels showed a marked and statistically significant elevation, rising from 0.47 in the control group to 0.91 in the treated group ($p < 0.0001$) (Table 3, Figure 10).

A comparable pattern was observed for p62 expression. In MDA-MB-231 cells, p62 levels significantly increased from 2.73 in the control group to 3.60 following enoxaparin treatment ($p = 0.024$). Likewise, HepG2 cells exhibited a significant rise in p62 expression, from 0.58 in the control group to 0.93 after treatment ($p = 0.0002$) (Table 3, Figure 10). Overall, these findings indicate that enoxaparin significantly enhances the expression of autophagy-related markers LC3 and p62 in both breast and liver cancer cell lines.

3.5. Effect of Enoxaparin on 8-OHdG

Table 4 summarizes the statistical analysis of oxidative DNA damage in MDA-MB-231 and HepG2 cells. In MDA-MB-231 cells, 8-OHdG levels decreased significantly from 262.4 ± 2.73 in the control group to 108.0 ± 0.12 following enoxaparin treatment ($p < 0.0001$). Similarly, in HepG2 cells, a pronounced reduction was observed,

with 8-OHdG levels declining from 84.51 ± 1.22 in the control group to 2.27 ± 0.18 in the treated group ($p < 0.0001$) (Figure 11). Overall, these findings demonstrate that enoxaparin significantly reduces oxidative DNA damage in both breast and liver cancer cell lines.

4. Discussion

This study aimed to elucidate the interaction between enoxaparin and MDA-MB-231 and HepG2 cancer cells and to determine how this interaction influences key pathways involved in tumor progression. In the present study, enoxaparin did not exert cytotoxic effects on healthy cells but significantly promoted cell death in both MDA-MB-231 and HepG2 cancer cells through pro-inflammatory mediators, apoptosis and autophagy-related mechanisms. These findings highlight a selective anticancer effect, suggesting that enoxaparin may preferentially target malignant cells while sparing non-cancerous cells.

Low-molecular-weight heparins (LMWHs) have been extensively used in the management of cancer-associated thrombosis and have also been associated with potential antitumor effects. Previous studies have demonstrated that enoxaparin can inhibit tumor cell proliferation, migration, and metastasis in various experimental models (28-31). However, the clinical relevance of these findings remains uncertain, as many studies involve patients receiving concurrent chemotherapy (32, 33). In this context, our results provide additional mechanistic insight by demonstrating that enoxaparin directly modulates multiple cellular pathways in cancer cells, independent of its anticoagulant function, consistent with emerging evidence that anticancer strategies may act not only through direct cytotoxicity but also by modulating protein stability and stress-related cellular pathways, including protein aggregation and degradation mechanisms (34, 35). The concentrations used in the present study were higher than clinically achievable plasma levels of enoxaparin. However, in vitro studies often require higher concentrations to overcome the absence of systemic metabolism, protein binding, and tissue distribution and to achieve detectable cellular responses under controlled experimental conditions. In the present study, treatment concentrations were selected based on preliminary dose-response analyses and measurable biological effects in cancer cells, while no significant cytotoxicity was observed in non-cancerous HEK-293 cells (36, 37).

Apoptosis is a critical determinant of tumor progression and therapeutic response (38). In the present study, enoxaparin significantly increased apoptosis in both MDA-MB-231 and HepG2 cells, as evidenced by elevated TUNEL positivity. This consistent pro-apoptotic effect across two distinct cancer cell types suggests that enoxaparin may activate conserved cell death pathways that are not restricted to a specific tissue origin. These findings are consistent with previous reports showing variable apoptosis-related effects depending on cellular

context and concentration (36, 39). Moreover, the heterogeneous composition of enoxaparin, including pro- and antiproliferative oligosaccharides, may contribute to these differential responses(39).

Inflammation is closely linked to both thrombosis and cancer progression(40). IL-6 and TNF- α are key mediators of inflammatory signaling and immune regulation (41, 42), while NF- κ B represents a central pathway controlling inflammatory responses and tumor progression (43). Previous studies have shown that platelet–tumor interactions can enhance tumor invasion through NF- κ B activation (44, 45). Under basal conditions, NF- κ B is sequestered in the cytoplasm by I κ B proteins and translocates to the nucleus upon activation to regulate gene expression (46, 47). In the present study, enoxaparin exerted cell type–specific effects on inflammatory mediators. Notably, NF- κ B expression was significantly increased in MDA-MB-231 cells, whereas TNF- α and IL-6 levels were significantly reduced. This pattern suggests that enoxaparin may regulate inflammatory signaling through NF- κ B–independent mechanisms or through post-transcriptional regulation of cytokine expression. In contrast, the absence of significant changes in HepG2 cells supports the notion that enoxaparin’s immunomodulatory effects are dependent on tumor-specific signaling contexts. These findings emphasize the complexity of the thrombo-inflammatory axis and its modulation by LMWHs (48-50). Also, in MDA-MB-231 cells, the observed increase in cytosolic NF- κ B expression compared to the control suggests a robust induction of the pathway, though the protein remains primarily localized in the cytoplasm at this time point. In contrast, the marked nuclear localization in HepG2 cells indicates a more rapid or efficient translocation, potentially reflecting earlier activation kinetics in this cell line. These results highlight that Enoxaparin modulates NF- κ B signaling through cell-type-specific kinetic profiles.

Autophagy is a fundamental cellular process involved in maintaining homeostasis and regulating tumor progression (51-54). Depending on the cellular context, autophagy may either promote tumor survival or contribute to programmed cell death (55, 56). It has also been implicated in tumor growth, metastasis, stemness, and chemoresistance (57-64). In the present study, enoxaparin significantly increased LC3 and p62 expression in both MDA-MB-231 and HepG2 cells, indicating activation of autophagy-related pathways. It has been reported that p62 levels can increase during prolonged stress despite active autophagy, due to its transcriptional upregulation via the Nrf2 pathway (65). Therefore, the simultaneous increase in LC3 and p62 in our 48-hour samples may reflect a high rate of autophagosome formation that temporarily exceeds the degradative capacity, or a compensatory transcriptional increase in p62 expression. This consistent response suggests that enoxaparin induces a conserved cellular stress mechanism across different tumor types.

Oxidative stress plays a crucial role in carcinogenesis by inducing DNA damage and genomic instability (66-68). 8-hydroxy-2'-deoxyguanosine (8-OHdG) is a well-established biomarker of oxidative DNA damage and has been associated with cancer prognosis and progression (68-70). Elevated levels of 8-OHdG have been reported in various tumor types, including breast, bladder, kidney, and other cancers (71-74). In the present study, enoxaparin significantly reduced 8-OHdG levels in both MDA-MB-231 and HepG2 cells, indicating a protective effect against oxidative DNA damage. This finding suggests that enoxaparin may contribute to the regulation of redox homeostasis. The simultaneous modulation of oxidative stress, apoptosis, and autophagy further supports the concept that enoxaparin induces a coordinated cellular response involving multiple interconnected pathways.

Limitations. This study has several limitations that should be acknowledged. First, the enoxaparin concentrations used exceeded clinically achievable plasma levels; although this is common in *in vitro* pharmacological studies, it limits direct extrapolation to clinical dosing. Second, the analysis was restricted to two cancer cell lines (MDA-MB-231 and HepG2) without validation in additional cell lines or *in vivo* tumor models, which limits the generalizability of the findings. Third, the mechanistic pathways identified here are based on correlative expression changes rather than direct causal manipulation, such as pathway inhibition or gene silencing; future studies incorporating such approaches are needed to establish causality. Finally, functional endpoints relevant to tumor progression, including migration, invasion, and *in vivo* tumor growth, were not assessed in the present study.

5. Conclusion

In conclusion, the present study provides *in vitro* evidence that enoxaparin produces multifaceted anticancer effects in MDA-MB-231 and HepG2 cells through coordinated modulation of apoptosis, autophagy, inflammatory signaling, and oxidative DNA damage. Taken together, these findings indicate that enoxaparin may have biological relevance beyond its role in thromboprophylaxis. Although the data were generated under *in vitro* conditions, they provide a mechanistic rationale for further investigation of enoxaparin's potential relevance to oncologic treatment strategies. Future studies, including *in vivo* and clinical investigations, are needed to clarify whether these cellular effects translate into improved therapeutic responses, reduced metastatic potential, and better clinical outcomes in cancer patients.

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Figure Legends

Figure 1. Impact of enoxaparin on cell viability in (A) MDA-MB-231, (B) HepG2, and (C) HEK-293 cell lines after 24 and 48 h of treatment, as assessed by the MTT assay. Cell viability values were normalized to the corresponding time-matched control groups and expressed as percentage viability. Data are presented as mean $\pm$ SD. Statistical analysis was performed using two-way ANOVA. Statistical analyses were performed separately for the 24 h and 48 h groups. **p < 0.01, ***p < 0.001.

Figure 2. TUNEL staining of MDA-MB-231 and HepG2 cells after 48 h enoxaparin treatment. Apoptotic cells appear as green fluorescence ($\times 40$). Data are presented as median (quarter1-quarter3) (n = 10) and analyzed using Mann–Whitney U test.

Figure 3. Effects of enoxaparin on the intensity of TUNEL immunoreactivity in MDA-MB-231 and HepG2 cells. Cells were treated with enoxaparin for 48 h. Fluorescence intensity was quantified using ImageJ software. ***p < 0.0001.

Figure 4. Representative immunofluorescence images of NF- κ B expression in MDA-MB-231 and HepG2 cells after 48 h enoxaparin treatment. NF- κ B is visualized as green fluorescence ($\times 40$). Data are presented as mean $\pm$ SD (n = 10) and analyzed using an independent samples t-test.

Figure 5. Representative immunofluorescence images of TNF- α expression in MDA-MB-231 and HepG2 cells after 48 h enoxaparin treatment. TNF- α is visualized as green fluorescence ($\times 40$). Data are presented as mean $\pm$ SD (n = 10) and analyzed using an independent samples t-test.

Figure 6. IL-6 expressions of the MDA-MB-231 and HepG2 cell lines treated with 48 h enoxaparin. Green immunofluorescence is reflective of IL-6 expressions under the fluorescent microscope (X40). Data are presented as mean $\pm$ SD (n = 10) and analyzed using an independent samples t-test.

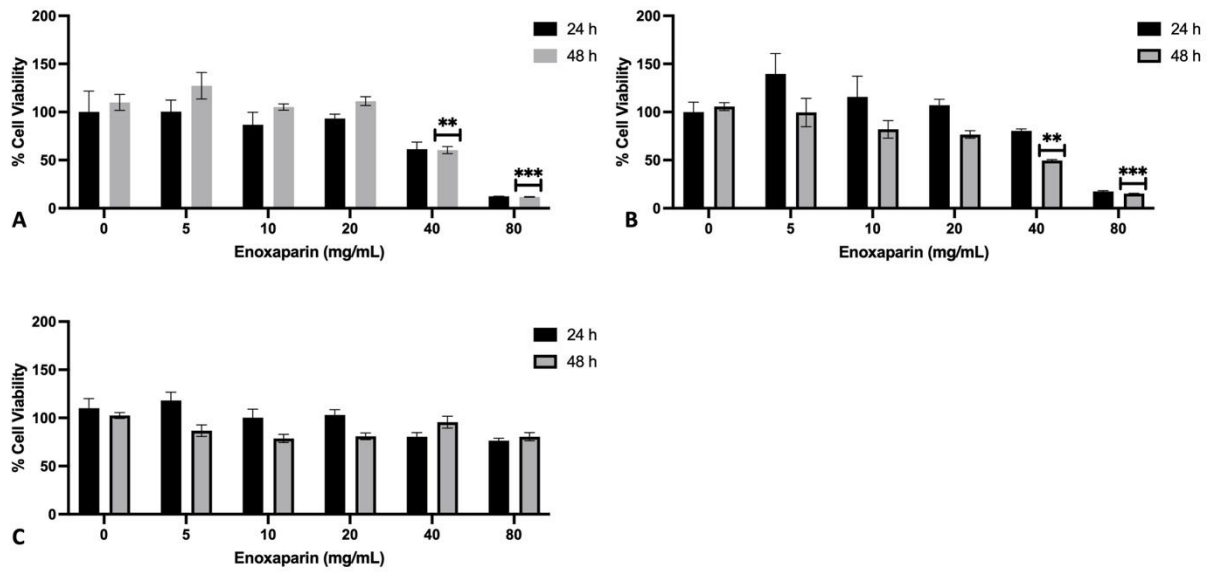
Figure 7. Immunoreactivity intensity of NF- κ B, TNF- α , and IL-6 in MDA-MB-231 and HepG2 cells treated with enoxaparin for 48 h. Data are presented as mean $\pm$ SD (n = 10). Statistical analysis was performed using an unpaired t-test. **p < 0.01, ***p < 0.0001, ns: not significant.

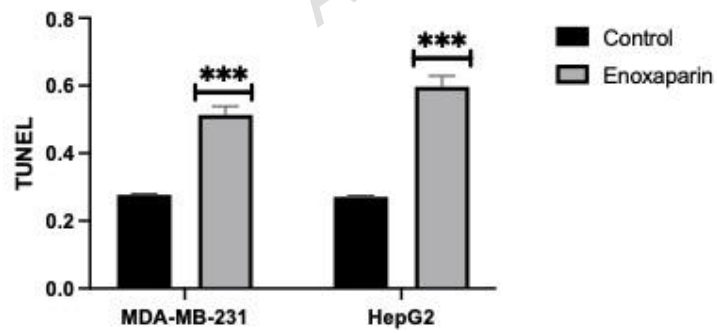
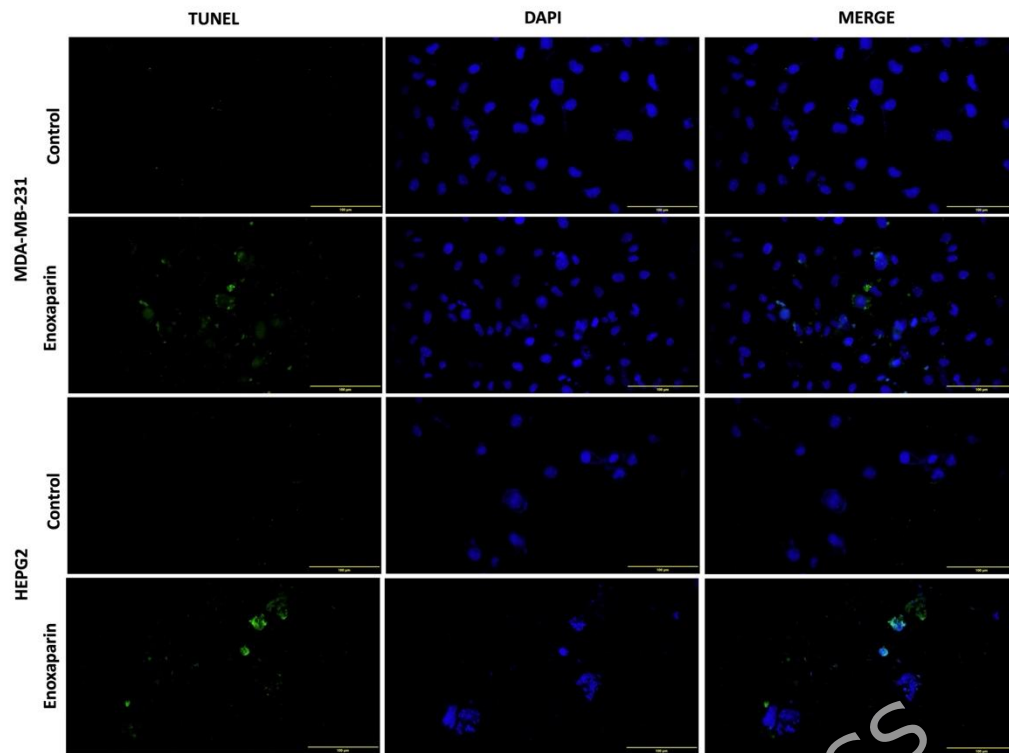
Figure 8. Representative immunofluorescence images of LC3 expression in MDA-MB-231 and HepG2 cells after 48 h enoxaparin treatment. LC3 is visualized as green fluorescence ($\times 40$). Data are presented as median (quarter1-quarter3) (n = 10) and analyzed using a Mann–Whitney U test.

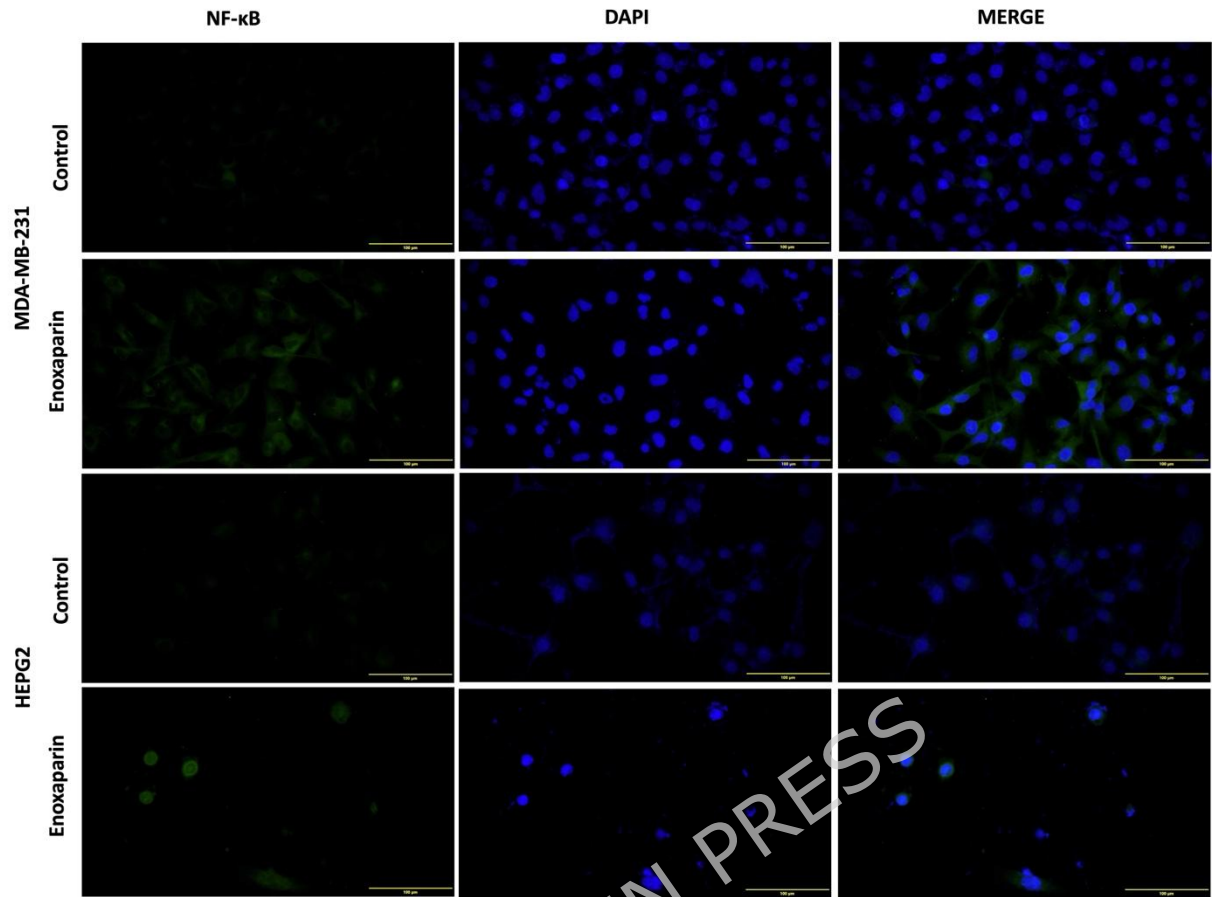
Figure 9. p62 expressions of the MDA-MB-231 and HepG2 cell lines treated with enoxaparin. Red immunofluorescence is reflective of p62 expressions under the fluorescent microscope (X40). p62 is visualized as red fluorescence ($\times 40$). Data are presented as median (quarter1-quarter3) (n = 10) and analyzed using a Mann–Whitney U test.

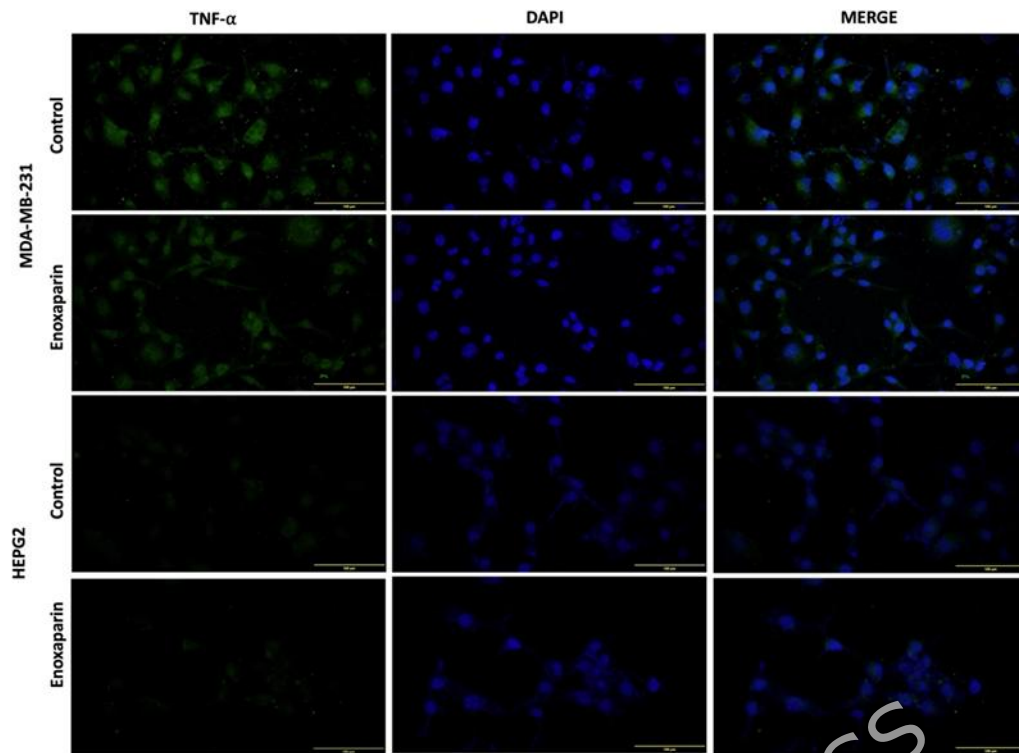
Figure 10. Immunoreactivity intensity of LC3, and p62 in MDA-MB-231 and HepG2 cells treated with enoxaparin for 48 h. Data are presented as median (Q1–Q3) (n = 10). Statistical analysis was performed using the Mann–Whitney U test. *p < 0.05, **p < 0.01, ***p < 0.0001.

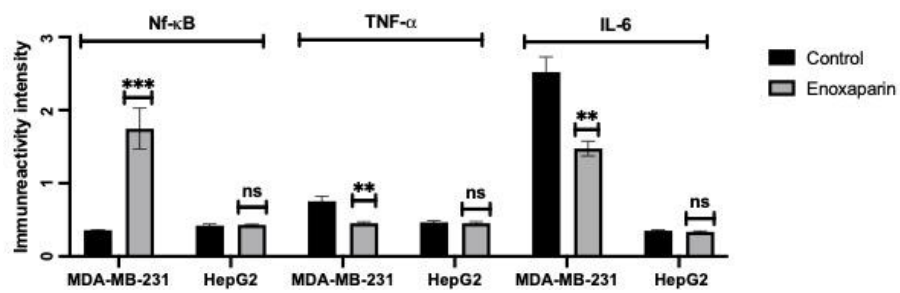
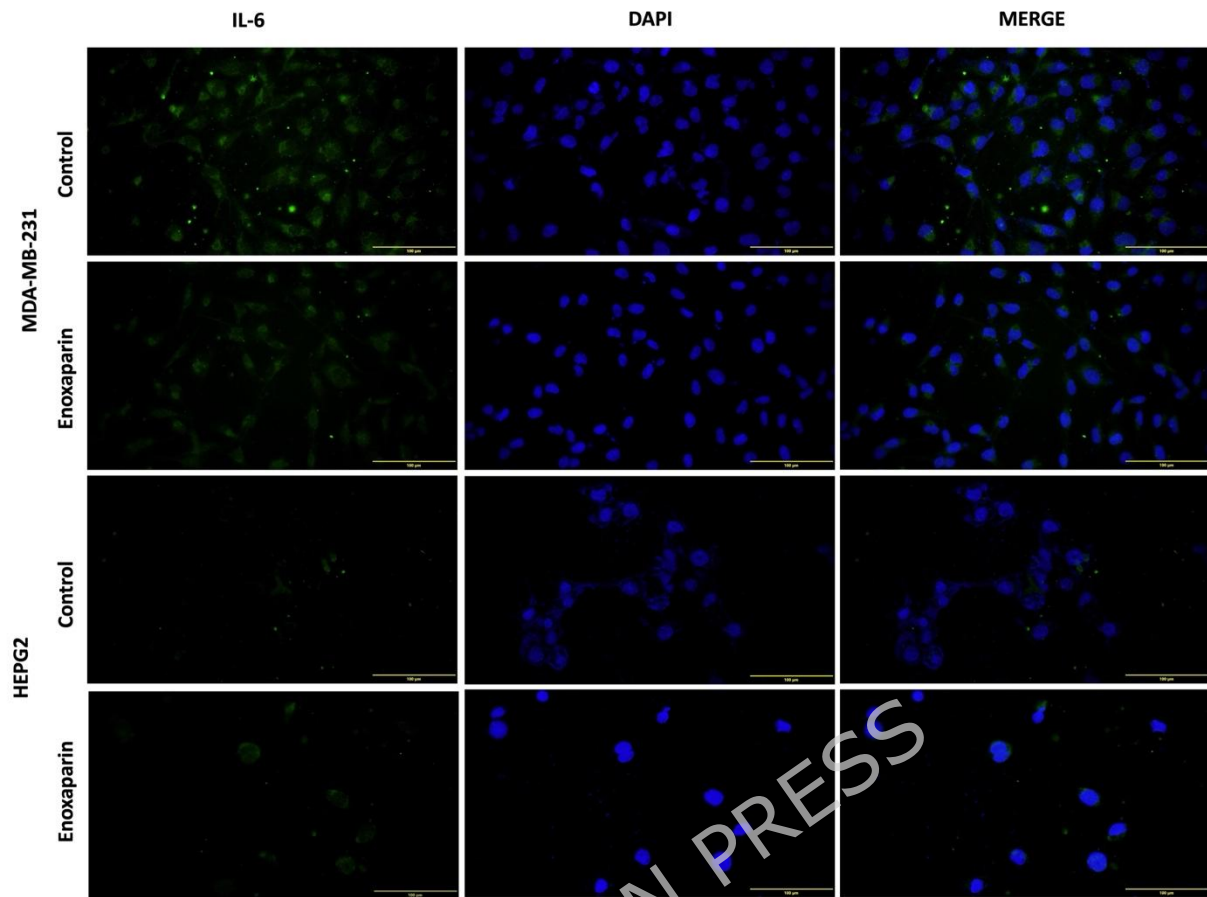
Figure 11. Bar graph showing mean 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels in MDA-MB-231 and HepG2 cells following enoxaparin treatment. Group comparisons were performed using an independent samples t-test. Data are presented as mean $\pm$ SD, ***p < 0.0001.

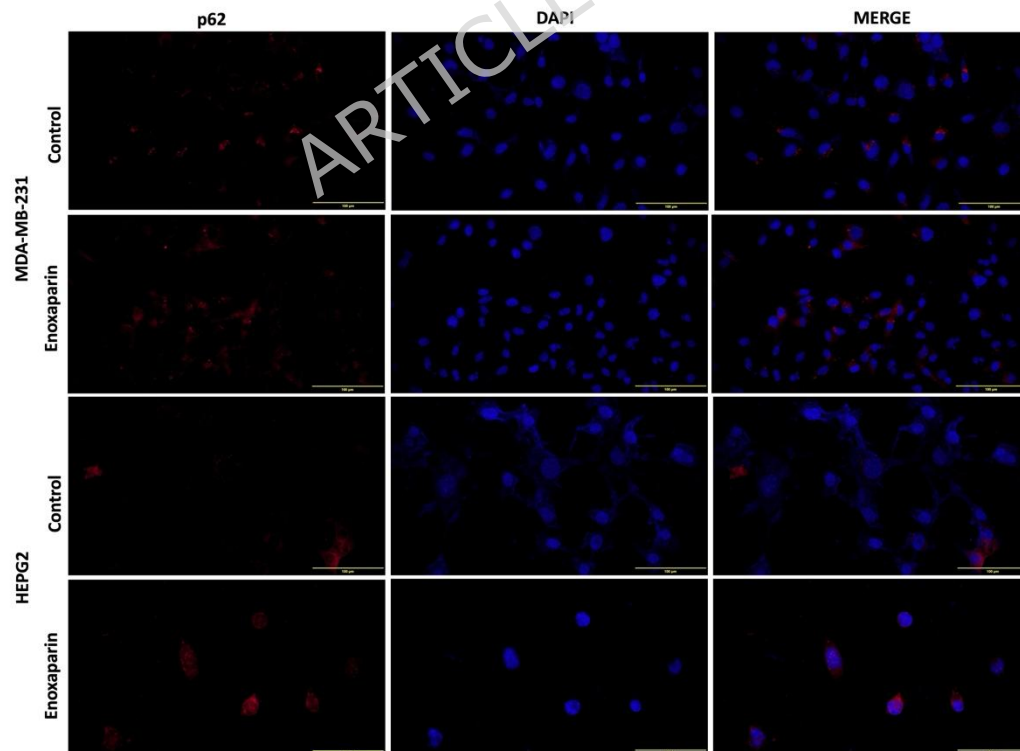
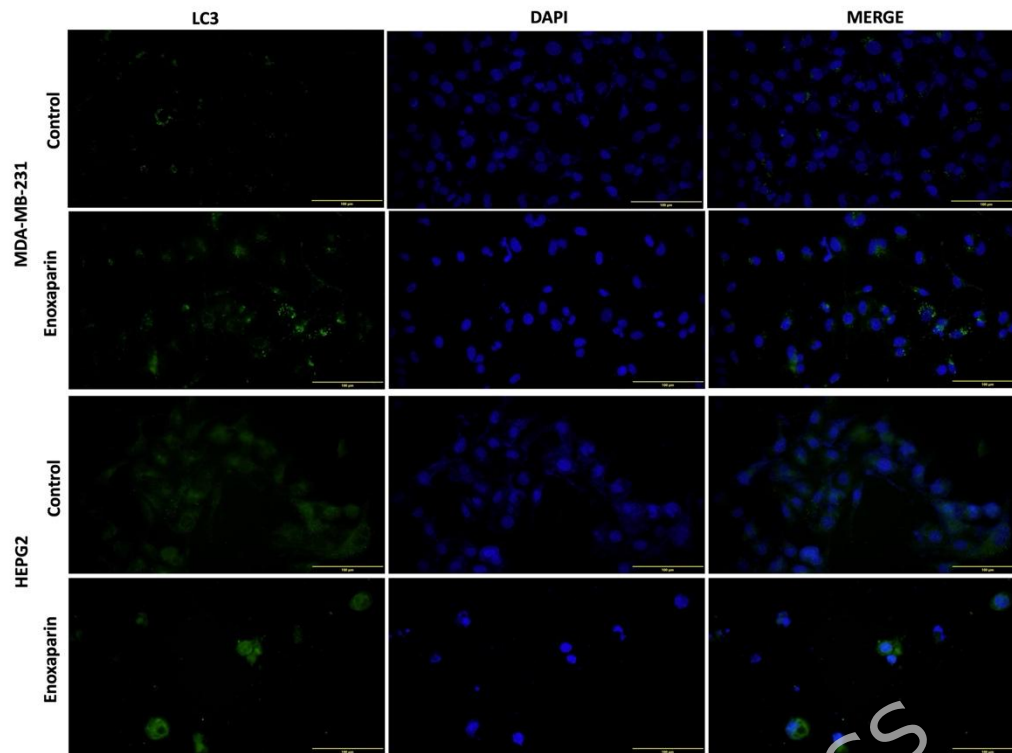












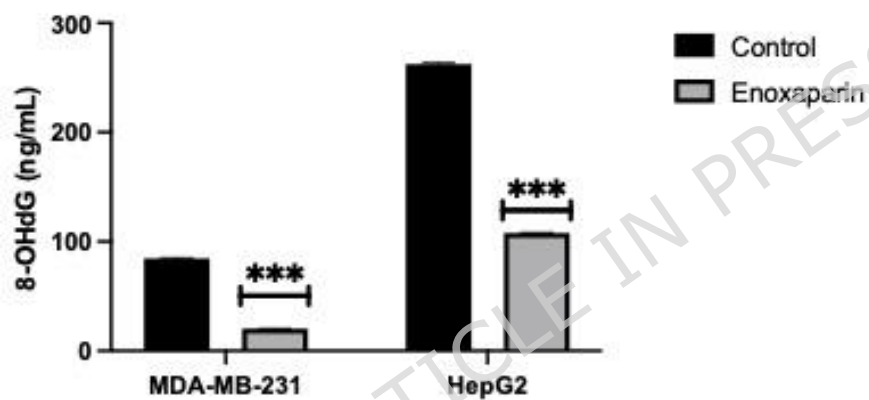
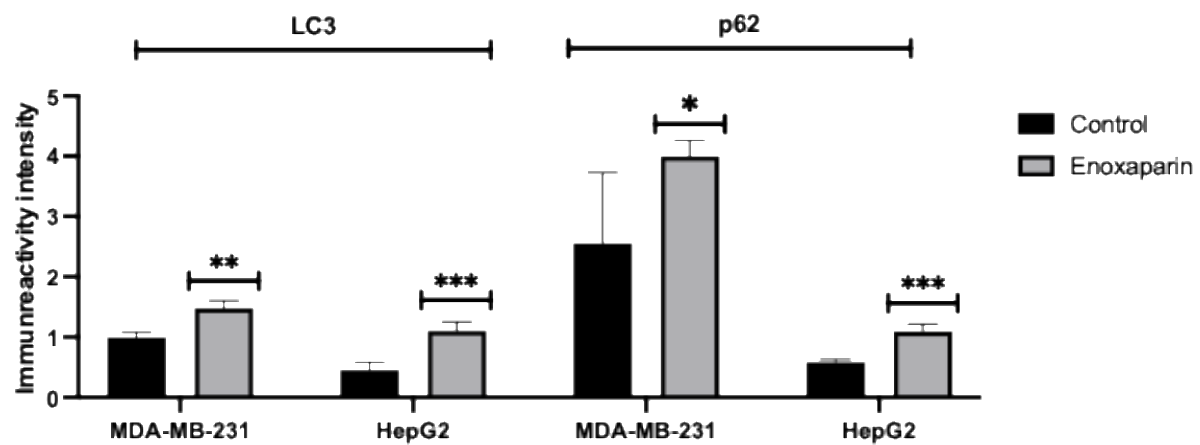


Table 1. Statistical analysis results of TUNEL of MDA-MB-231 and HepG2 cancer cell line at 48 h.

		Control	Enoxaparin	p
NF- κ B	MDA-MB-231	0.36 $\pm$ 0.02	2.19 $\pm$ 0.58	<0.0001
	MDA-MB-231	0.27(0.27-0.29)	0.50(0.45-0.58)	<0.0001
	HEPG2	0.42 $\pm$ 0.07	0.43 $\pm$ 0.04	0.74
	HEPG2	0.27(0.26-0.27)	0.57(0.53-0.64)	<0.0001
TNF- α	MDA-MB-231	0.74 $\pm$ 0.23	0.45 $\pm$ 0.07	0.003
	HEPG2	0.48 $\pm$ 0.08	0.46 $\pm$ 0.07	0.55
IL-6	MDA-MB-231	2.5 $\pm$ 0.70	1.49 $\pm$ 0.33	0.001
	HEPG2	0.35 $\pm$ 0.05	0.34 $\pm$ 0.03	0.41

Data are expressed as median (quarter1-quarter3). *p*: refers to the significance of the difference between the groups. *p* value of <0.05 was used for significance.

Table 2. Statistical analysis results of NF- κ B, TNF- α , and IL-6 expressions of MDA-MB-231 and HepG2 cancer cell line at 48 h.

Data are expressed as mean $\pm$ standard deviation. *p*: refers to the significance of the difference between the groups. *p* value of <0.05 was used for significance.

Table 3. Statistical analysis results of LC3 and p62 expressions of MDA-MB-231 and HepG2 cancer cell line at 48 h.

		Control	Enoxaparin	p
LC3	MDA-MB-231	1.01(0.84-1.08)	1.51(1.06-1.82)	0.006
	HEPG2	0.47(0.42-0.62)	0.91(0.71-1.68)	<0.0001
p62	MDA-MB-231	2.73(2.14-3.78)	3.60(3.46-4.72)	0.024
	HEPG2	0.58(0.46-0.68)	0.93(0.76-1.57)	0.0002

Data are expressed as median (quarter1-quarter3). *p*: refers to the significance of the difference between the groups. *p* value of <0.05 was used for significance.

Table 4. Statistical analysis results of 8-OHdG (ng/mL) in MDA-MB-231 and HepG2 cancer cell line at 48 h.

	Control	Enoxaparin	p
MDA-MB-231	262.4±2.73	108.0±0.12	<0.0001
HEPG2	84.51±1.22	2.27±0.18	<0.0001

Since the data showed normal distribution, data are expressed as mean±standard deviation. *p*: refers to the significance of the difference between the groups. *p* value of <0.05 was used for significance.