

The Effect of Enoxaparin on Inflammatory Marker, Apoptosis in Sw480 Colon Cancer Cell Line

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Abstract

Background and Objectives: The world's fourth-deadliest malignancy is colon cancer (CC). is becoming more prevalent. salt enoxaparin Heparin in its fractionated low molecular weight (LMWH) form prevents factor Xa from contributing to clotting. enoxaparin inhibit viability of sw480 colon cancer cell line. Additionally, enoxaparin improved apoptosis while having little effect on cell-cycle progression, which raised doxorubicin's effectiveness. pro-inflammatory cytokines secretion during inflammatory reactions, including TNF-, IL-1, IL-1, and IL-6. The most popular LMWH, enoxaparin, is known to prevent the release of several cytokines from T cells that are involved in inflammatory diseases like asthma, for example IL-4, IL-5, IL-13, and TNF.

Materials and Methods: SW480 colon cancer and VERO normal cell line used; these cells were treated with diverse concentrations of enoxaparin to assess the impact of enoxaparin on the viability. Cell viability determines by MTT assay. SW480 cells were treated with different concentrations of enoxaparin the to assess the impact of enoxaparin on inflammation and apoptosis. cell lines were taken for immunoassay by ELISA method using TNF-alpha and caspase3.

Results: enoxaparin has no significant effect on the viability of vero normal cell while significantly ($P < 0.050$) reduce in viability of sw480 colon cancer cell. enoxaparin significantly ($P < 0.050$) reduce in TNF- α and significant ($P < 0.050$) increase in caspase3.

Conclusion: Enoxaparin decrease viability of SW480 colon cancer cell line and have anti-inflammatory and apoptotic effect.

Keywords: Colon Cancer, Enoxaparin, Inflammation, Proinflammatory Cytokine.

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INTRODUCTION

Colorectal cancer which is the 4th malignancy worldwide, is becoming more common. About 10% of all malignant tumors in both genders. CC is greatly influenced by a variety of factors, including genetic, environmental, physical action, and age. Presently, operation and chemotherapy are two chief management choices for CC, rendering to cancer position and patient features (Toolabi *et al.*, 2021). colonic cancer SW480 cell line derives from the primary tumor of adenocarcinoma of the colon in a 50-year-old male (Shanehbandi *et al.*, 2021). Enoxaparin sodium The fractionated low molecular weight shape of heparin (LMWH), created in 1981 and accepted for therapeutic practice in 1993, is identified as enoxaparin and reduces factor Xa in the coagulation system. According to the World Health Organization, enoxaparin is one of the safest and most useful prescription drug (Shirazi *et al.*, 2021).

MAPK and PI3K were downregulated by enoxaparin, which also decreased MMP-2 expression and prevented the

migration of A549 cells. Furthermore, enoxaparin enhanced doxorubicin's effectiveness by improving apoptosis, while no result on cell-cycle development was noticed (Alturkistani *et al.*, 2019).

Inflammation is a vital immune process that takes place in reaction to tissue damage done by infection or injury. Inflammatory processes work to remove pathogenic debris and material from injured tissue areas and to start the healing process for wounds. Chronic inflammation that persists despite being a necessary defensive mechanism against invasive microorganisms might worsen tissue damage. This may cause cells to emit reactive oxygen and nitrogen species, and it may also cause greater genomic instability, which raises the risk of cancer. (Archer, Dogra, and Kyprianou, 2020). Cytokines have special effects on the action of many cells. However, their relevance in controlling the immune system makes them particularly significant. Animals were used to conduct extensive research on the role of cytokines in the progression of inflammatory illness brought on by bacterial infection or exposure to

lipopolysaccharide (LPS) (Abdulkhaleq *et al.*, 2018). Pro-inflammatory cytokines like TNF-, IL-1, IL-1, and IL-6 that are produced during inflammatory responses have been shown to contribute to the pathogenesis of illnesses like intervertebral disc degeneration, epilepsy, osteoarthritis, the start and progression of cancer, depression, and the up-regulation of chemokine secretion from macrophages (Asanka Sanjeeva *et al.*, 2020)

The most popular LMWH, enoxaparin, is known to prevent the release of several cytokines from T cells that are involved in inflammatory diseases like asthma, comprising IL-4, IL-5, IL-13, and TNF- α (Shastri *et al.*, 2015).

METHOD

1. Cytotoxicity Evaluation

A cancer research lab, college of medicine, and university of Babylon kindly provided the colon cancer SW480 cell line and vero cell, which were then cultivated in RPMI-1640 media with penicillin (100 U/ml), streptomycin (100 g/ml), and 5% fetal bovine serum at 37°C in 5% CO₂. SW480 cells were planted at a thickness of 5*10⁵ cells/ml into tissue culture 96-well plates prior 24 hours of enoxaparin treatments. cells exposed to 200 μ L with different concentrations of enoxaparin at serial dilutions(1000, 500, 250, 125, 62 31 μ g/ml) (four repeats were used for every concentration of both heparin and enoxaparin for each type of cells) along with two replicates as a control group for each cell type. Then the plate was covered with a self-plastic lid and incubated for 24 hours, at the end of the exposure period, the cell line growth was assessed by cytotoxicity assay by MT assay. The influence of these enoxaparin on the progress of the sw480 and vero cell line evaluated by MTT assay. MTT assay evaluates the cellular transformation of a tetrazolium salt into a formazan product (purple color). The number of live cells is directly related to the opacity of the purple color, this may be defined using

spectrophotometry and yields a relative approximate of cell growth.

2. Effect of Enoxaparin on TNF- α and caspase3 in SW480 colon cancer cell

Colon cancer (SW480) cells lines were seeded and labeled in 96 tissue culture plates. entirely cells were cured with diverse concentrations of drugs (heparin and enoxaparin) at serial dilutions ranging from 1000 to 31.25 μ g/ml (four repeats were used for every concentration of both heparin and enoxaparin for each type of cells) along with Two replicates as a control group for each cell type. Then the plate was enclosed with a self-elastic lid and incubated for 24 hours, at the finish of the coverage period, the cell lines were taken for immunoassay by ELISA method using TNF-alpha and caspase3. This ELISA kit contains the sandwich-ELISA concept where the slab is precoated with a specific antibodies to the human cytokine of interest. Specimens or standards are placed on the plate and mixed with the antibody. The biotinylated detection antibody and avidin-HRP conjugate are placed on the plate modifying the color to blue. The enzyme-substrate response is ended by the addition of solution provider and the color turns yellow. The optical density is determined using a wavelength of 450 nm. The concentration of cytokine of interest is determined by contrasting the OD of the specimen to the standard curve (Paulie and Perlmann, 2016).

RESULTS

1. Effect of Enoxaparin on the viability of Vero cell, SW480 colon cancer cell line

Results shown in figure 3.1 revealed that enoxaparin have no significant effect on the viability of vero cell when compared with control group.

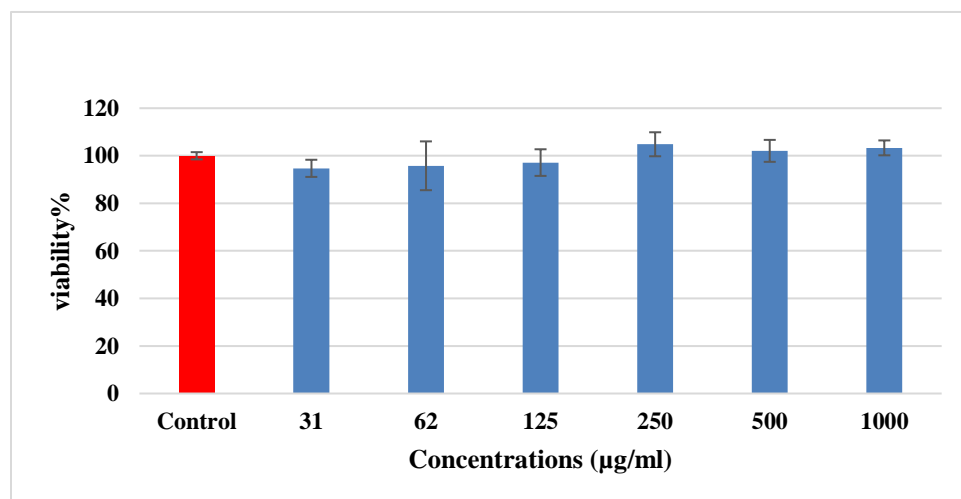


Figure 3.1 Impact of enoxaparin on the viability of vero cell line

For SW480 colon cancer cells, The result showed in figure 3.2 that enoxaparin causes at concentration (1000,500,125 μ g/ml) significantly ($P<0.050$) reduce in

viability of colon cancer cell when compared with the control group after incubation for 24hr.

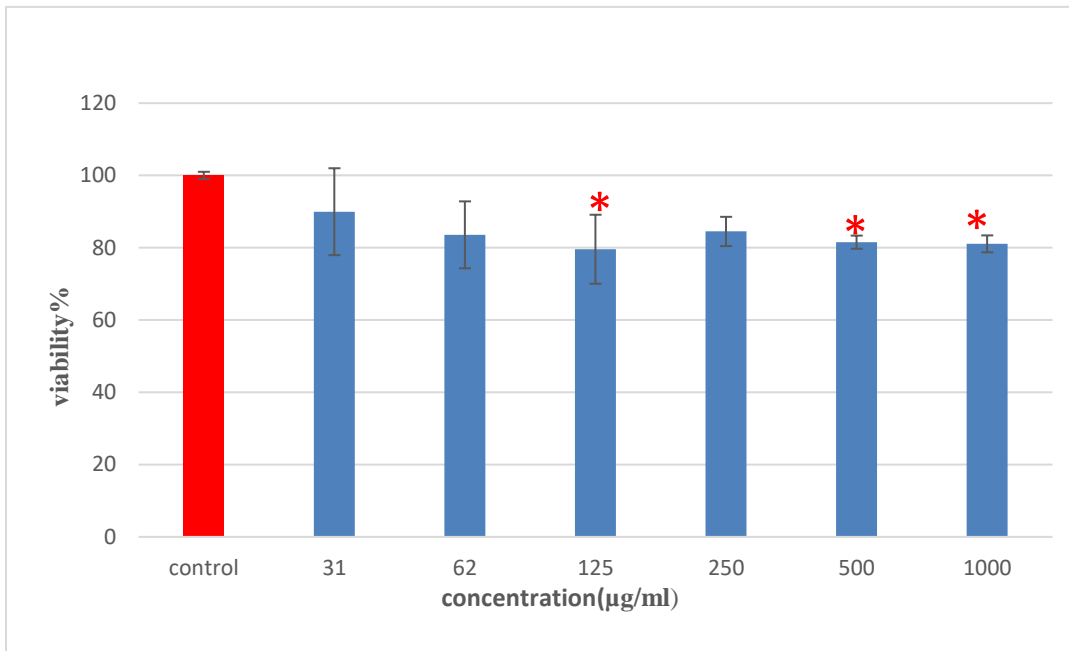


Figure 3.2 effect of enoxaparin on the viability of SW480 colon cancer cell line

2. Impact of enoxaparin on TNF- α in SW480 colon cancer cell

(1000,250,31 μ g/ml) significantly ($P<0.050$) reduce in level of TNF- α in colon cancer cell.

The result showed that enoxaparin causes at concentration

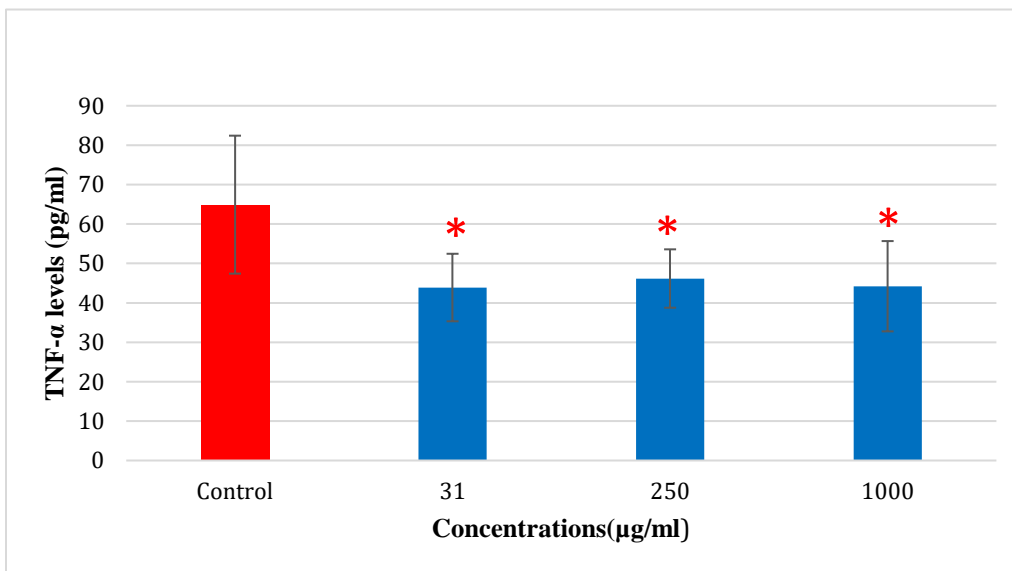


Figure 3.3 :Impact of enoxaparin on TNF- α level in SW480 colon cancer cell line.

3. Effect of enoxaparin on caspase 3 in SW480 colon cancer cell

increase in caspase3 level and in SW480 colon cancer cell when compared with the control group.

The result is shown in (figure 3.4) that enoxaparin at the concentration (1000,250,31 μ g/ml) significantly ($P<0.050$)

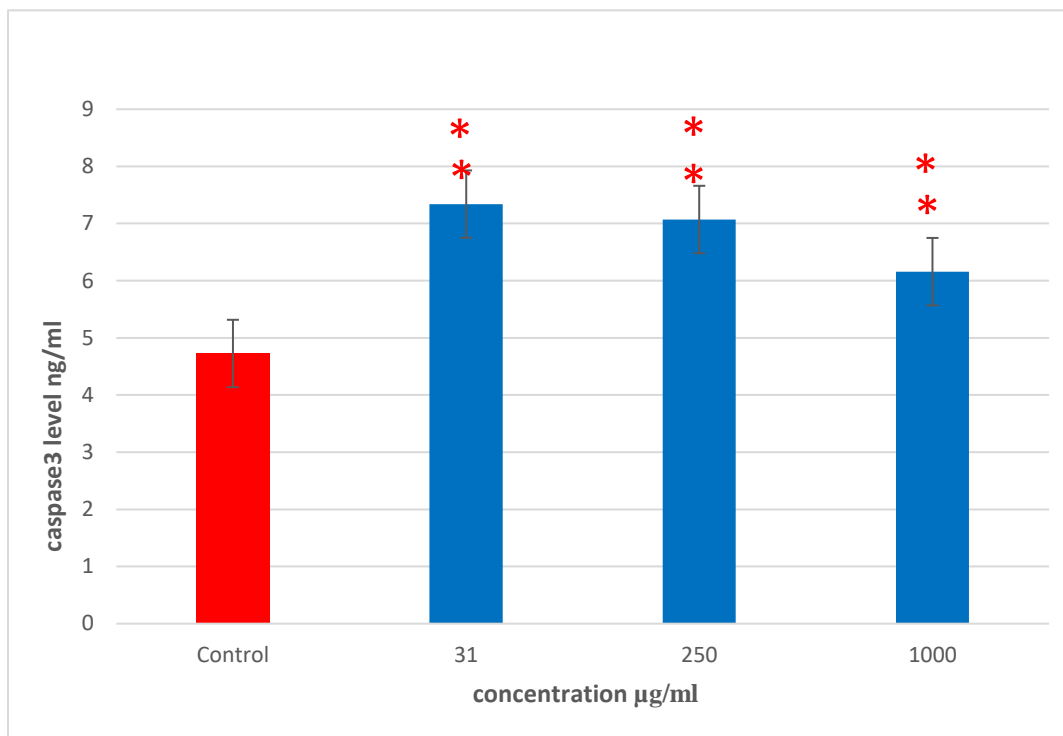


Figure 3.4 effect of enoxaparin on caspase3 in SW480 colon cancer cell line.

DISCUSSION

1. Effect of Enoxaparin on the viability of Vero cell, SW480 colon cancer cell line

For the Vero cell, The result of the current study revealed that enoxaparin has no significant effect on the viability of the vero cell (figure 3.1).

Heparin and enoxaparin, respectively, had no impact on the growth of endothelial cells Human primary osteoblasts and human pulmonary epithelial cells A-549 This is understandable, as heparin is an endogenous substance of the human body (Bittkau *et al.*, 2019). For the colon cancer cell line sw480, The current study's findings showed that enoxaparin significantly ($P < 0.050$) reduces the viability of SW480 colon cancer cells at concentrations of (1000, 500, and 125 $\mu\text{g/ml}$) (figure 3.2) Advanced tumor patients frequently have hypercoagulable blood, necessitating anticoagulant medication. According to clinical studies, cancer patients who get heparin or LMWH anticoagulant treatment live longer. Heparin has the ability to prevent tumor angiogenesis, which is the primary explanation. There is mounting proof that medications like LMWH may prevent cancer from spreading and growing through a number of different methods. Angiogenesis is also necessary for tumor development and metastasis. Therefore, preventing angiogenesis is a very effective method of treating malignancies. (Qiu *et al.*, 2021) Enoxaparin significantly decreased the growth of colon cancer metastases in the liver when related to the control group ($p = 0.001$). The mixture of cisplatin and enoxaparin sodium demonstrated a synergic result in decreasing cell capability and migration ability and increasing apoptosis in H357 human OSCC cells. This outcome was related with an

reducing of heparanase mRNA expression and protein manufacture both in vivo and in vitro. The current findings indicate that enoxaparin sodium may be helpful for OSCC patients receiving chemotherapy (Camacho-Alonso *et al.*, 2020).

2. Effect of Enoxaparin on TNF- α in SW480 colon cancer cell

Enoxaparin at concentration (1000,250,31 $\mu\text{g/ml}$) significantly ($P < 0.050$) reduce in level of TNF- α in colon cancer cell (figure3.3) The most popular LMWH, enoxaparin, is known to prevent the release of several cytokines from T cells, containing IL-4, IL-5, IL-13, and TNF-, which are linked to a variety of inflammatory diseases. The particular mechanism or mechanisms beyond the anti-inflammatory properties of LMWHs remain largely unexplored. But, it has been proposed that due to their significant negative charge, they can connect with and impact a variety of biological components, such as different immune cell types and cytokines that promote inflammation (Shastri *et al.*, 2015).

3. Effect of Enoxaparin on caspase 3 in SW480 colon cancer cell

Enoxaparin at the concentration (1000,250,31 $\mu\text{g/ml}$) causes a significant ($P < 0.050$) increase in caspase3 level and in SW480 colon cancer cell when compared with the control group (figure3.4) Combining cisplatin and enoxaparin sodium has a synergistic effect on H357 human OSCC cells, decreasing their capability to migrate and enhance apoptosis. The current findings imply that enoxaparin sodium may be helpful for OSCC patients receiving chemotherapy (Camacho-Alonso *et al.*, 2020).

CONCLUSION

Enoxaparin decrease viability of SW480 colon cancer cell line and have anti-inflammatory and apoptotic effect.

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