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Hematology

The in vitro cytotoxic, genotoxic, oxidative damage potential of enoxaparin sodium in human peripheral blood mononuclear cells

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ABSTRACT

Objectives: Enoxaparin sodium, low-molecular weight heparin (LMWH) indicated for the prophylaxis deep vein thrombosis. As far as we know, its cytotoxic, genotoxic and oxidative effects have never been studied on any cell lines. The purpose of the present study is to evaluate the in vitro cytotoxic, genotoxic damage potential and antioxidant/oxidant activity of enoxaparin sodium on primary human whole blood cultures.

Methods: After exposure to different doses (from 0.5 to 100 mg/L) of enoxaparin sodium, cell viability was assessed by the cytotoxicity tests including MTT (3, (4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide) and lactate dehydrogenase (LDH) release assays. The antioxidant activity was measured by the total antioxidant capacity (TAC) and total oxidative stress (TOS) parameters. To determine the genotoxic damage potential, the rate of chromosomal aberrations (CAs) and 8-oxo-2'-deoxyguanosine (8-oxo-dG) levels were also assessed.

Results: Cytotoxicity assays showed that treatment with enoxaparin sodium caused significant decreases in the cellular viability in a clear dose-dependent manner. Also, it was found that enoxaparin sodium did not alter the TAC and TOS levels. The genotoxicity assay showed that the formation of CAs was not observed in the lymphocytes. Likewise, the levels of 8-oxo-dG did not change in treated cultures as compared to control values.

Conclusions: Enoxaparin sodium appeared to exhibit cytotoxic but not oxidative and genotoxic damage potentials in cultured human blood cells.

Keywords: Enoxaparin sodium, low-molecular-weight heparin, cytotoxicity, oxidative stress, DNA damage, human peripheral blood mononuclear cells

Venous thromboembolism (VTE), one of the most causes of cardiovascular diseases, is the leading preventable cause of mortality and morbidity in inpatients, the incidence of which is estimated to double in the future [1]. Heparins are commonly used drugs for the prophylaxis and treatment of VTE [2]. Low-Molecular-Weight Heparins (LMWHs) exert more predictable effects and require less coagulation level monitoring than that of unfractionated heparin (UFH) [2]. Enoxaparin sodium, a type of LMWHs, is often preferred due to its high bioavailability in the subcutaneous form [3, 4].

Anticoagulants have wide usage for preventing and treating VTE. Surveys have shown that the most widely used agent in therapeutic anticoagulation is LMWHs [5-9]. LMWHs are an important class of antithrombotic medications and derived from UFH by depolymerization procedure [10]. LMWHs have wide

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usage in comparison with UFH because of its advantages as pharmacokinetic profile and ease of use [11, 12]. The mean molecular weight of LMWHs is 4.0-6.0 kD. It varies by manufacturing process, thus it was influenced by its in vivo properties including pharmacokinetics, bioavailability, and plasma half-life [13, 14].

Among the LMWHs, enoxaparin sodium is one of the most widely prescribed agents and has been used since 1993 [5, 7, 15]. Enoxaparin sodium indicated for the prophylaxis deep vein thrombosis. It is used commonly in orthopedic clinical practice. Interestingly, the development of biosimilar versions of enoxaparin increased medical concerns about their efficacy and safety [16]. At this point, there is not sufficient data for elucidating the toxicity potentials of these bio-similar versions of the anticoagulants.

The enoxaparin sodium which is the most used agent in the orthopedic clinic approach. This agent is used by orthopedic patients during the long-time and sometimes a few times a day. Therefore, this study was aimed to research the potentials of the enoxaparin sodium on the human peripheral blood mononuclear cells (PBMCs) via determining cytotoxic, genotoxic and oxidative damage potentials for the first time.

METHODS

Chemicals and Reagents

Enoxaparin Sodium (Oksapar 6000, IU/0.6 mL; Koçak Farma, Turkey) was supplied in prefilled glass syringes from the manufacturer and diluted with the cell culture medium. All measurements by devices were performed according to the protocol of the manufacturer.

Mononuclear Cell Isolation

Whole blood samples were obtained from 5 donors (healthy, 22-25 aged, non-smoking, with no genotoxic agent history, n=5). The equal volume of Phosphate-buffered Saline (PBS) was used to dilute the blood sample. Then, the blood samples were layered on Ficoll-Hypaque-Plus (GE Healthcare Biosciences Corp., Piscataway, NJ, USA) and centrifuged at 400×g for 30 minutes at 18-24°C.

The PBMC layer was carefully removed, transferred, and washed with three volumes of PBS for twice and re-suspended in RPMI-1640 media (GIBCO, USA) including 10%, v/v Fetal Bovine Serum (FBS) (Sigma, USA) and 1% penicillin/streptomycin (Sigma, USA). PBMCs were counted using haemo-cytometer and 105 cells incubated each wheel during the experiment. In all cell viability, oxidative and genotoxic damage assays, the cultures without enoxaparin sodium were chosen as negative control groups [control (-)]. The cell cultures treated with Triton X-100 (1%, Sigma-Aldrich) were chosen as a positive control group [control (+)] for MTT and LDH release assays. In addition, ascorbic acid (10 mg/L, Sigma-Aldrich) and hydrogen peroxide (25 mg/L, Sigma-Aldrich) treated cells were chosen as the positive control groups in the analyses as Total Oxidant Status (TOS) and Total Antioxidant Capacity (TAC). Mitomycin C (10 mg/L, Sigma-Aldrich, USA) treated cells was also chosen as a positive control group in chromosomal aberrations (CAs) and 8-OH-dG assays [17].

MTT Assay

MTT assay is one of the most used colorimetric assays for detecting cell viability. The cells were placed in 96-well plates to perform the assay. They were incubated in carbon dioxide incubator (37 °C, 5% CO2) and treated with different concentrations of enoxaparin sodium (as 0.5, 1, 2.5, 5, 10, 25, 50 and 100 mg/L) for 72 h. MTT Cell Growth Assay Kit (Merck Millipore, USA) was used to perform MTT assay. 10 µl of MTT solution was added to well to incubate for 4h. 100 µl of acid-isopropanol (isopropanol with 0.04 N HCl) was added for the dissolving procedure of the formazan crystals. Then, a microplate reader (Synergy-HT; BioTek Winooski, VT, USA) was used to detect the optical density at 570 nm. The cell viability was expressed in percentages of viable cells.

LDH Release Assay

LDH assay is based on the measurement of the amount of released LDH from cells is to assess cell death [18]. The CytoSelect® LDH cytotoxicity assay kit was used to perform the assay. The cells were placed in 96-well plates and they were incubated in carbon dioxide incubator (37 °C, 5% CO2). They treated with enoxaparin sodium at selected concentrations for 72 h. 96-well plate was centrifuged for 5 minutes at 400×g. 90 µl of the supernatant with negative

control, positive control, and test groups was transferred to new plates. 10 µl of LDH as cytotoxicity assay reagent was added into well. They were incubated for 30 minutes at 37 °C. A microplate reader (Synergy-HT; BioTek Winooski, VT, USA) was used to measure the absorbance at 450 nm.

Total Antioxidant Capacity (TAC) Assay

TAC assay enables measurement of the capacity of all types of antioxidants in experimental samples. In brief, in this assay, the existing antioxidants in the test sample reduce 2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid cation radical (ABTS) which is dark blue/green colored to the reduced form of colorless ABTS. Due to this reduction, the alteration of the absorbance values at 660 nm has a relationship with the total antioxidant amount of the sample [19, 20]. The plasma samples, obtained from the whole blood cultures after 72h incubation with enoxaparin sodium at different concentrations, were examined using the TAC assay kit (Rel Assay Diagnostics®, Turkey). 30 µl of sample and 500 µl of reagent 1 were mixed. After 30 seconds, the absorbance was measured at 660 nm by using a spectrophotometer (Synergy-HT; BioTek Winooski, VT, USA). 75 µl reagent 2 was added and after 10 minutes incubation at room temperature condition, the absorbance was measured at 660 nm by using a spectrophotometer (Synergy-HT; BioTek Winooski, VT, USA). The same procedure was carried out using deionized water and standard as well as examples. Trolox equivalent (1 mmol / L), a vitamin E analog, was used as standard. Results were expressed in mmol / L (Erel 2004).

Total Oxidant Status (TOS) Assays

TOS assay provides the measurement of oxidants existing in test samples which oxidize the complex containing ferrous ion-chelator to ferric ion. The ferric ion procures colorful complex by chromogen in the medium at acidic conditions. The color intensity determined as spectrophotometrically has a relationship with the total amount of oxidants [21]. The plasma samples, obtained from the whole blood cell cultures after 72h incubation with enoxaparin sodium at different concentrations, were examined using the TOS assay kit (Rel Assay Diagnostics®, Gaziantep, Turkey). 75 µl of sample and 500 µl of reagent 1 were mixed. After 30 seconds, absorbance at 530 nm was measured using a spectrophotometer (Synergy-HT; BioTek Winooski, VT, USA). 25 µl of reagent 2 was added and incubated for 10 minutes at room temperature condition. A second absorbance measurement was performed at 530 nm by using spectrophotometer (Synergy-HT; BioTek Winooski, VT, USA). Hydrogen peroxide (20 mmol / L) was used as a standard. Results were expressed in µmol H2O2 Equiv/L [21, 22].

Chromosomal Aberrations (CA) Assay

Whole human blood samples were treated with enoxaparin sodium at selected concentrations. They were cultured for 72h. Before two hours of harvesting, 0.02 µg/ml of Colchicine (Sigma, USA) was given into the culture. Hypotonic treatment (0.075 M KCl/37.4°C) was performed. The fixation (methanol plus acetic acid) three times, cells were harvested by centrifugation. The slides were prepared from each fixed-cell suspension and they were air-dried. Then, in phosphate buffer (pH 6.8), all slides were stained with Giemsa. To score for each treatment, 30 well-spread metaphases were analyzed for chromosome aberration. All the aberrations (as chromatid/chromosome gap or/and chromatid/chromosome break) were determined and classified according to the criteria of Environmental Health Criteria 46 for Environmental Monitoring of Human Populations [23, 24].

Determination of 8-OH-dG Level

DNA oxidation was determined by detecting the amount of 8-OH-dG adducts. DNA was digested in the incubation period with DNase I, alkaline phosphatase, and endonuclease. The high-performance liquid chromatography (HPLC) was used to measure the amount of 8-OH-dG with electrochemical detection as described in the literature [25]. Waters S-3 4.6×150 mm column [with 5% methanol/95% 100 mM sodium acetate buffer (pH 5.2)] at a flow rate of 1.0 mL/min were used to separate the compound. The four electrochemical detector channels (at -100, 250, 475, and 875 mV) were set up.

Statistical Analysis

SPSS software (version 20.0, SPSS, Chicago, IL, USA) was used to perform statistical analysis. The Kruskall Wallis test was used for the statistical analysis of values. Statistical decisions were given with significance levels of 0.05 and 0.005.

RESULTS

LDH and MTT release assays were used to determine the cell viability of human PBMCs against enoxaparin sodium. The results for cytotoxicity measured by MTT assay were shown in Fig. 1. The cultured human PBMCs exposed to relatively low concentrations of enoxaparin sodium (as 0.5, 1, 2.5, and 5 mg/L) did not exhibit any important changes for cell viability over 72h ($p > 0.05$). However, 10 and 25 mg/L concentrations of enoxaparin sodium caused a weak cytotoxic effect on human PBMCs and there is no statistical significance ($p > 0.05$). Enoxaparin sodium leads to a decrease in the proliferation of human PBMCs, at higher concentrations than 25 mg/L (50 and 100 mg/mL) when compared to the control group ($p < 0.05$). The cytotoxicity of enoxaparin sodium on cultured human PBMCs by measuring the amount of intracellular LDH release was assessed in Fig. 2. LDH levels were not affected by low doses of enoxaparin sodium, only 50 and 100 mg/L of enoxaparin sodium caused to the significant increase of LDH level ($p < 0.05$). IC20 and IC50 values of enoxaparin sodium in PBMCs were determined according to the results of

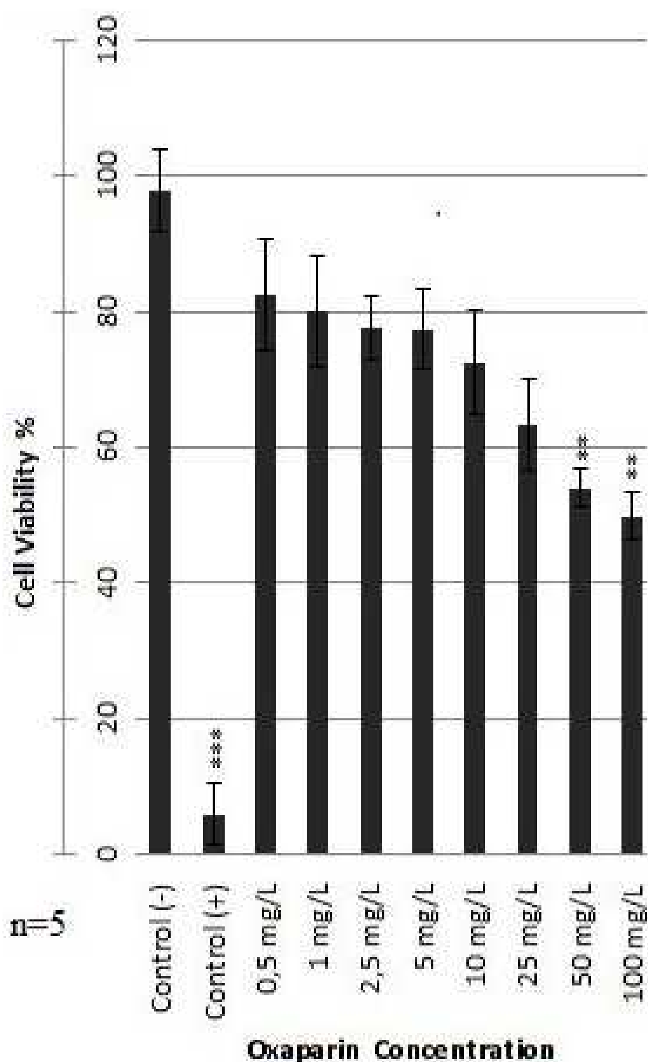

Fig. 1. **Cell viability rates of human PBMCs after exposure to various enoxaparin sodium concentrations for 72h. The cells grown in media without enoxaparin sodium was used as control (-) group. The cell cultures treated with Triton-X (1%) was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Statistical comparisons were made with control (-) group at levels of **p < 0.05 and ***p < 0.001 (n = 5).**

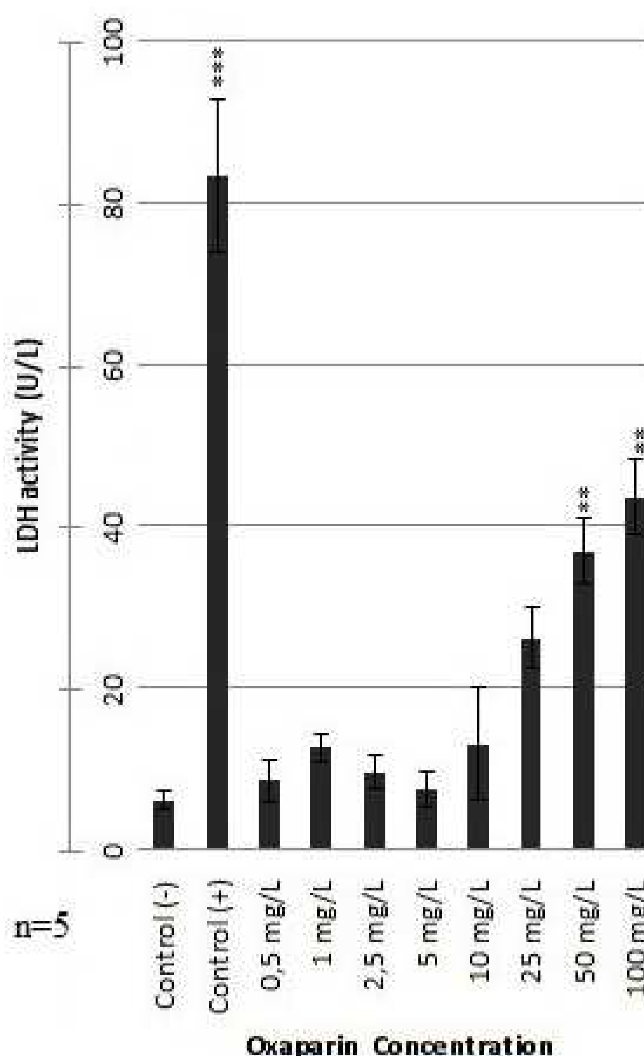

Fig. 2. **LDH levels of human PBMCs treated with different concentrations of enoxaparin sodium for 72 h. The cells grown in media without enoxaparin sodium was used as control (-) group. The cell cultures treated with Triton-X (1%) was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Statistical comparisons were made with control (-) group at levels of **p < 0.05 and ***p < 0.001 (n = 5)**

MTT assay and calculated as 25,647 and 93,416 mg/L, respectively.

The oxidative status effects of various enoxaparin sodium concentrations in human PBMCs cultures were evaluated by using TAC and TOS analysis. 0.5, 1, 2.5, and 5 mg/L concentrations of enoxaparin sodium exposure did not change TAC levels; but 10, 25, 50, and 100 mg/L concentrations of enoxaparin sodium treatment caused to a slight decrease of TAC levels, as shown in Fig. 3. Enoxaparin sodium did not cause to increase of TOS levels in cultured human PBMCs at all tested concentrations. Also, it did not change significantly both TAC and TOS levels when compared to the negative control groups ($p > 0.05$). The genotoxic effects of enoxaparin sodium were analyzed by CA assay in human PBMCs and it is shown in Fig. 5. All tested concentrations of enoxaparin sodium did not cause significant increases in the number of observed CAs ($p > 0.05$). Similarly, enoxaparin sodium concentrations did not show an increase in the levels of 8-OH-dG when compared to the control group as seen in Table 1.

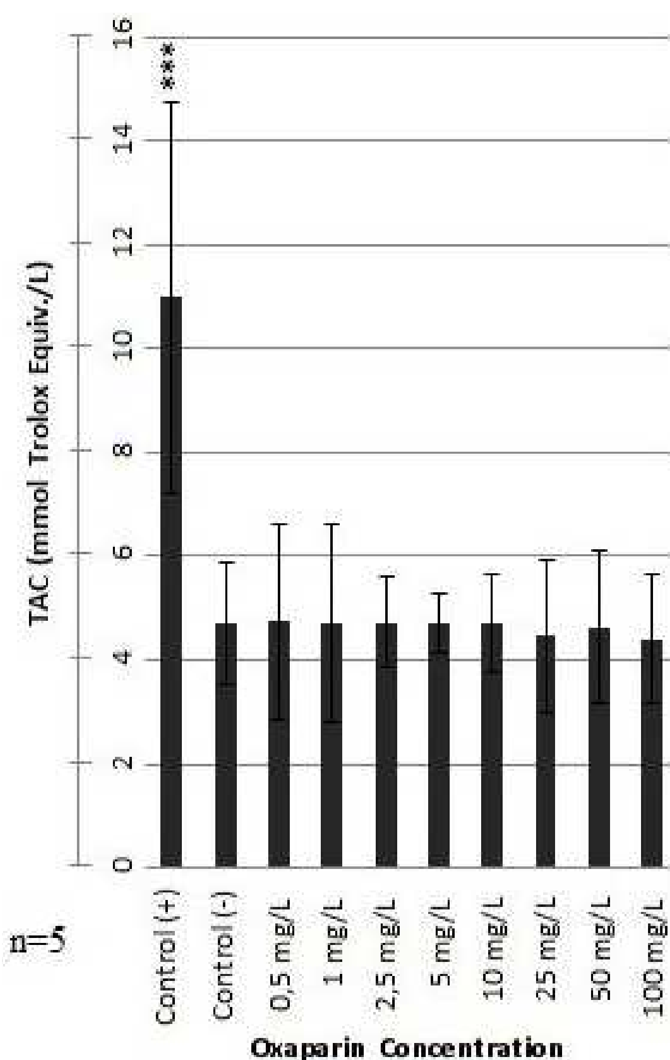

Fig. 3. **The levels of TAC in cultured human PBMCs exposed to enoxaparin sodium for 72 h. The cells grown in media without enoxaparin sodium was used as control (-) group. Ascorbic acid (10 mg/L) treated cell culture was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Statistical comparisons were made with control (-) group at levels of **$p < 0.05$ and ***$p < 0.005$ (n = 5).**

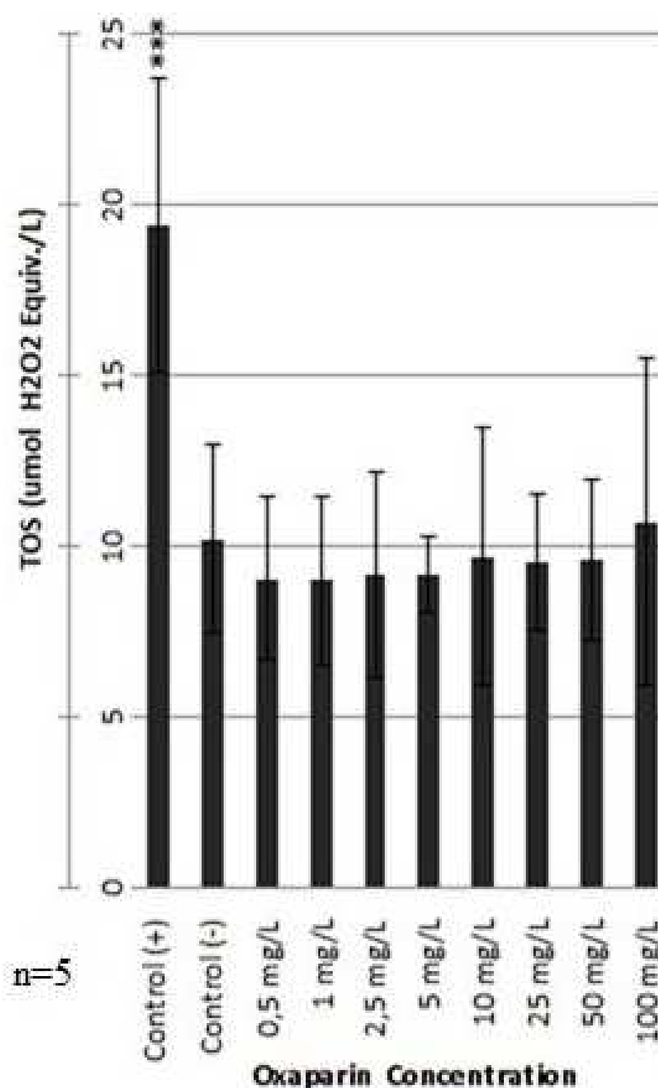

Fig. 4. **The levels of TOS in cultured human PBMCs exposed to enoxaparin sodium for 72 h. The cells grown in media without enoxaparin sodium was used as control (-) group. PBMCs treated with hydrogen peroxide (25 mg/L) was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Statistical comparisons were made with control (-) group at levels of **$p < 0.05$ and ***$p < 0.005$ (n = 5).**

Fig. 5. Representative images of chromosomal aberrations observed in cultured human PBMCs exposed to different concentrations of enoxaparin sodium for 72h. a) Control (-): undamaged chromosomes are seen, b) treatment with IC50 concentration of enoxaparin sodium, c) treatment with MMC, chromosomal aberrations indicated by arrows (n = 5).

Table 1. The 8-OH-dG levels in cultured PBMCs after exposure to enoxaparine sodium concentrations for 72h

Groups	CAs/cell	8-oxo-dG level (pmol/micro g DNA)
Control (-)	0.20 ± 0.03	0.81 ± 0.08
Control (+)	2.59 ± 0.18*	4.22 ± 0.35*
0.5 mg/L	0.20 ± 0.02	0.73 ± 0.06
1 mg/L	0.22 ± 0.03	0.76 ± 0.09
2.5 mg/L	0.16 ± 0.02	0.75 ± 0.08
5 mg/L	0.18 ± 0.03	0.72 ± 0.10
10 mg/L	0.20 ± 0.04	0.79 ± 0.07
25 mg/L	0.24 ± 0.03	0.81 ± 0.12
50 mg/L	0.26 ± 0.04	0.83 ± 0.07
100 mg/L	0.28 ± 0.03	0.88 ± 0.10

*Symbol presents significant statistical difference from the control (-) group. The cells grown in media without enoxaparin sodium was used as control (-) group. Mitomycin C (10 mg/L) treated cell group was used as control (+) group. The results are given as the means ± SD from five independent repetitions. Enoxaparin sodium concentrations did not show an increase in the levels of 8-oxo-dG on cultured human lymphocytes when compared with the control group

DISCUSSION

In this study, enoxaparin sodium was subjected to an in vitro toxicity evaluation in order to reveal its safety profile on human PBMCs. The performed MTT assay in this investigation showed that higher concentrations of enoxaparin sodium lead to a decrease in the viability of PBMCs. LDH release assay gave similar results with MTT assay. In fact, LDH activity reached the highest level at the concentrations as 25, 50, and 100 mg/L of enoxaparin sodium. In accordance with our findings, it was reported that enoxaparin sodium (lower concentrations than 0,024 mg/ml or 2.4 IU/mL) caused inhibition of proliferation whereas higher concentrations impaired cell growth in the dose-dependent manner [26]. Also, it was found that heparin and its low molecular-weight fragments could inhibit the proliferation of vascular smooth muscle cells (SMCs) both in vivo and in vitro conditions [27-31]. On the contrary, it was previously executed that enoxaparin did not show any significant effect on the proliferation of PBMC [32, 33].

In this study, TOS and TAC assays were used to detect the oxidative status of enoxaparin sodium. Results obtained from the assays demonstrated that enoxaparin sodium caused a slight decrease in the

TAC level but no significant changes in the TOS level. There is no study about the oxidative potential of enoxaparin sodium. It has been reported that LMWH reduced oxidative stress in hemodialysis patients [34]. Additionally, it was propounded the presence of protective effects by LMWH on oxidative injuries. Again, LMWHs led to decreases in lipid peroxidation in cardiac and hepatic tissues [35].

Our study also evaluated the genotoxicity of enoxaparin sodium on human PBMCs using CA and 8-OH-dG level assays. These assays revealed that enoxaparin sodium did not lead to increasing of 8-OH-dG levels and CA frequency. The results indicated that enoxaparin sodium might not be genotoxic on human PBMCs. On the contrary, the genotoxic damage potentials of heparin, dalteparin, enoxaparin, and nadroparin were reported using micronucleus (MN) assay. Moreover, all these tested agents caused a significant increase in the rates of MN in a dose-dependent manner for rat embryonic blood cells as well as inducing morphologic abnormalities [36].

CONCLUSION

The results of this study demonstrate that enoxaparin sodium does not cause genotoxicity but high concentrations (25-100 mg/L) of enoxaparin sodium exhibit cytotoxic action on PBMCs. Thus, it is suggested that the dose management should be reconsidered due to possible cytotoxic effects of enoxaparin sodium. Also, further in vivo investigations are required in order to evaluate the safety of enoxaparin sodium.

Authors' Contribution

Study Conception: KY; Study Design: KY; Supervision: KY; Funding: ŞEÖ, ŞE; Materials: KY; Data Collection and/or Processing: KY; Statistical Analysis and/or Data Interpretation: KY; Literature Review: KY; Manuscript Preparation: KY and Critical Review: KY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Parakh RS, Sabath DE. Venous thromboembolism: role of the clinical laboratory in diagnosis and management. J Appl Lab Med 2019;3:870-82.
2. Akhtar F, Wan X, Wu G, Kesse S, Wang S, He S. Low-molecular-weight heparins: reduced size particulate systems for improved therapeutic outcomes. Molecules 2018;23:1757.
3. Nadi S, Vreugdenburg TD, Atukorale Y, Ma N, Maddern G, Rovers M. Safety and effectiveness of aspirin and enoxaparin for venous thromboembolism prophylaxis after total hip and knee arthroplasty: a systematic review. ANZ J Surg 2019;89:1204-10.
4. Jupalli A, Iqbal AM. Enoxaparin. In StatPearls [Internet]. StatPearls Publishing. 2020. https://www.ncbi.nlm.nih.gov/books/NBK539865/.
5. Caprini JA, Arcelus J, Sehgal LR, Cohen EB, Reyna JJ. The use of low molecular weight heparins for the prevention of postoperative venous thromboembolism in general surgery. a survey of practice in the United States. Int Angiol 2002;21:78-85.
6. Tapson VF, Hyers TM, Waldo AL, Ballard DJ, Becker RC, Caprini J, et al. Antithrombotic therapy practices in US hospitals in an era of practice guidelines. Arch Intern Med 2005;165:1458-64.
7. MacDougall DA, Feliu AL, Boccuzzi SJ, Lin J. Economic burden of deep-vein thrombosis, pulmonary embolism, and postthrombotic syndrome. Am J Health Syst Pharm 2006;63:5-15.
8. Tapson VF, Decousus H, Pin M, Chong BH, Froehlich JB, Monreal M, et al. Venous thromboembolism prophylaxis in acutely ill hospitalized medical patients: findings from the International Medical Prevention Registry on Venous Thromboembolism. Chest 2007;132:936-45.
9. Vats V, Nutescu EA, Theobald JC, Wojtynek JE, Schumock GT. Survey of hospitals for guidelines, policies, and protocols for anticoagulants. Am J Heal Pharm 2007;64:1203-8.
10. Haines ST, DiPiro JT, Talbert RL, Yee GC, Matzke GR, Wells BG, et al. TED Pharmacotherapy. 5th Edition. Vol. 1. Chapter 26. Venous Thromboembolism. McGraw-Hill Global Education Holdings. LLC., 2002: pp. 373-413.
11. Fareed J, Leong W, Hoppensteadt D. Generic low-molecular-weight heparins: some practical considerations. Semin Thromb Hemost 2004;30:703-13.
12. Garcia DA, Baglin TP, Weitz JI, Samama MM. Parenteral anticoagulants: Anthithrombotic Therapy and Prevention of Thrombosis, 9th ed: American College of Chest Physicians Evidence-Based Clinical Practice Guidelines. Chest 2012;141(2

Suppl):e24S-e43S.
13. Fareed J, Fu K, Yang LH, Hoppensteadt DA. Pharmacokinetics of low molecular weight heparins in animal models. Semin Thromb Hemost 1999;25:51-5.
14. Merli GJ, Vanscoy GJ, Rihn TL, Groce JB 3rd, McCormick W. Applying scientific criteria to therapeutic interchange: A balanced analysis of low-molecular-weight heparins. J Thromb Thrombolysis 2001;11:247-59.
15. Mourier PAJ, Agut C, Souaifi-Amara H, Herman F, Viskov C. Analytical and statistical comparability of generic enoxaparins from the US market with the originator product. J Pharm Biomed Anal 2015;115:431-42.
16. Drouet L. Low molecular weight heparin biosimilars: how much similarity for how much clinical benefit? Target Oncol 2012;7:35-42.
17. Turkez H, Tozlu OO, Lima TC, de Brito AEM, de Sousa DP. A comparative evaluation of the cytotoxic and antioxidant activity of mentha crispa essential oil, its major constituent rotundifolone, and analogues on human glioblastoma. Oxid Med Cell Longev 2018:2083923.
18. Handschin AE, Trentz OA, Hoerstrup SP, Kock HJ, Wanner GA, Trentz O. Effect of low molecular weight heparin (dalteparin) and fondaparinux (Arixtra®) on human osteoblasts in vitro. Br J Surg 2005;92:177-83.
19. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277-8.
20. Turkez H, Aydin E, Aslan A. Xanthoria elegans (Link) (lichen) extract counteracts DNA damage and oxidative stress of mitomycin C in human lymphocytes. Cytotechnol 2012;64:679-86.
21. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103-11.
22. Aydin E, Türkez H, Taşdemir S. Anticancer and antioxidant properties of terpinolene in rat brain cells. Arh Hig Rada Toksikol 2013;64:415-24.
23. IPCS 1985. International Program on Chemical Safety Environmental Health Criteria 46. Guidelines for the Study of Genetic Effects in Human Populations. Geneva, Switzerland: WHO, 1985: pp. 45-54.
24. Geyikoglu F, Turkez H. Protective effect of sodium selenite on genotoxicity to human whole blood cultures induced by aflatoxin B-1. Brazil Arch Biol Technol 2005;48:905-10.
25. Floyd RA, Watson JJ, Wong PK, Altmiller DH, Rickard RC. Hydroxyl free radical adduct of deoxyguanosine: sensitive detection and mechanisms of formation. Free Radic Res Commun 1986;1:163-72.
26. Hemeda H, Kalz J, Walenda G, Lohmann M, Wagner W. Heparin concentration is critical for cell culture with human platelet lysate. Cytotherapy 2013;15:1174-81.
27. Castellot JJ, Cochran DL, Karnovsky MJ. Effect of heparin on vascular smooth muscle cells. I. Cell metabolism. J Cell Physiol 1985;124:21-8.
28. Castellot JJ Jr, Wong K, Herman B. Binding and internalization of heparin by vascular smooth muscle cells. J Cell Physiol 1985;124:13-20.
29. Clowes AW, Reidy MA, Clowes MM. Kinetics of cellular proliferation after arterial injury. I. Smooth muscle growth in the absence of endothelium. Lab Invest 1983;49:327-33.
30. Clowes AW, Clowes MM, Reidy MA. Kinetics of cellular proliferation after arterial injury. III. Endothelial and smooth muscle growth in chronically denuded vessels. Lab Invest 1986;54:295-303.
31. Karnovsky MJ, Ch B, Sc D. Regulation of vascular smooth muscle cell growth by heparin and heparan sulfates. Semin Thromb 1987;13:489-503.
32. Shastri MD, Stewart N, Eapen M, Peterson GM, Zaidi STR, Gueven N, et al. Opposing effects of low molecular weight heparins on the release of inflammatory cytokines from peripheral blood mononuclear cells of asthmatics. PLoS One 2015;10:e0118798.
33. Shastri MD, Stewart N, Horne J, Zaidi ST, Sohal SS, Peterson GM, et al. Non-anticoagulant fractions of enoxaparin suppress inflammatory cytokine release from peripheral blood mononuclear cells of allergic asthmatic individuals. Plos One 2015;10:e0128803.
34. Poyrazoglu OK, Dogukan A, Yalniz M, Seckin D, Gunal AL. Acute effect of standard heparin versus low molecular weight heparin on oxidative stress and inflammation in hemodialysis patients. Ren Fail 2006;28:723-7.
35. Deepa PR, Varalakshmi P. Protective effect of low molecular weight heparin on oxidative injury and cellular abnormalities in adriamycin-induced cardiac and hepatic toxicity. Chem Biol Interact 2003;146:201-10.
36. Uysal II, Karabulut AK, Ozdemir K, Aksoy M, Altunkeser BB, Acar H. Investigation of direct toxic and teratogenic effects of anticoagulants on rat embryonic development using in vitro culture method and genotoxicity assay. Anat Histol Embryol 2006;35:84-92.

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