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The dose-dependent antiangiogenic potential of apixaban: an experimental outlook

Apıxaban'ın Doza Bağlı Antianjiyojenik Potansiyeli: Deneysel Bir Bakış

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ABSTRACT

Aim: Direct oral anticoagulants (DOACs) are good alternatives to conventional medical regimens for the treatment and prevention of thromboembolism. Apixaban is one of the more popular variations of these newly developed drugs. Aside from its anticoagulant potential, possible cellular effects remain a topic for future studies. The object of this study was to investigate the possible antiangiogenic effects of apixaban in the chorioallantoic membrane (CAM) model.
Method: Drug pellets were prepared at 10-4, 10-5, and 10-6 M concentrations of apixaban and were placed in the chorioallantoic membrane on the fourth day of egg incubation. On the eighth day, all vascular densities of the membranes were compared with a 10-6 M concentration of bevacizumab, which is a known monoclonal, humanized, vascular endothelial growth-factor inhibitor.
Results: We find that a 10-4 M concentration of apixaban has strong antiangiogenic potential similar to that of bevacizumab. However, there was moderate antiangiogenic potential at a lower dose of apixaban (10-5 M, 10-6 M). A comparison of the higher doses of antiangiogenic potential (10-4 M concentration) with lower doses of apixaban (10-5 M, 10-6 M) revealed significant statistical differences ($p < 0.05$).
Conclusion: Our results indicate that a high dose of apixaban has strong antiangiogenic potential. The exact mechanism of this effect remains unknown. These pilot results should be confirmed with further studies to obtain an updated look at DOACs.

Keywords: angiogenesis, anticoagulation, apixaban, DOACs

ÖZ

Amaç: Direkt oral antikoagülanlar (DOAK'lar), tromboembolizmin tedavisi ve önlenmesi için geleneksel tıbbi rejimlere iyi alternatiflerdir. Apixaban, bu yeni geliştirilen ilaçların daha popüler varyasyonlarından biridir. Antikoagülan potansiyelinin yanı sıra, olası hücresel etkiler gelecekteki çalışmaların konusu olmaya devam etmektedir. Bu çalışmanın amacı, korioallantoik membran (CAM) modelinde apiksaban'ın olası **antianjiyogenik etkilerini araştırmaktı.**
Yöntem: Apixaban'ın 10-4, 10-5 ve 10-6 M konsantrasyonlarında ilaç peletleri hazırlandı ve yumurta inkübasyonunun dördüncü gününde korioallantoik membrana yerleştirildi. Sekizinci günde, membranların tüm vasküler yoğunlukları, bilinen bir monoklonal, insanlaştırılmış, vasküler endotelyal büyüme faktörü inhibitörü olan 10-6 M'lik bir bevacizumab konsantrasyonu ile karşılaştırıldı.
Bulgular: 10-4 M apiksaban konsantrasyonunun, bevacizumabınkine benzer güçlü bir antianjiyogenik potansiyele sahip olduğunu bulduk. Bununla birlikte, daha düşük bir apiksaban dozunda (10-5 M, 10-6 M) orta düzeyde antianjiyogenik potansiyel vardı. Daha yüksek antianjiyogenik potansiyel dozlarının (10-4 M konsantrasyon) daha düşük dozlarda apiksaban (10-5 M, 10-6 M) ile karşılaştırılması, önemli istatistiksel farklılıklar ortaya çıkardı ($p <0.05$).
Sonuç: Sonuçlarımız, yüksek doz apiksaban'ın güçlü antianjiyogenik potansiyele sahip olduğunu göstermektedir. Bu etkinin kesin mekanizması bilinmemektedir. Bu pilot sonuçlar, DOAK'lara yeni bir bakış elde etmek için daha ileri çalışmalarla doğrulanmalıdır.

Anahtar Kelimeler: anjiyogenez, antikoagülasyon, apiksaban, DOAK'lar

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INTRODUCTION

Anticoagulation is an important step in the treatment of prothrombotic disorders. Heparin and its derivatives are good solutions that can be administered via an invasive route (e.g., subcutaneous or intravenous) [1,2]. However, oral anticoagulants are better suited for daily use by patients. Warfarin, the most widely used oral anticoagulant in the world [3], requires routine dose management with blood tests to calculate the international normalized ratio (INR); additionally, interactions with various types of drugs and foods limit the use of this oral medication [4,5]. The side effects associated with the use of once-popular anticoagulant drugs have prompted recent investigations, resulting in the development of more safe and effective anticoagulant drugs. Thus, new anticoagulants have been developed that can be administered orally and without the need for routine blood tests to determine a dose range [3,6]. Apixaban, one of the recently developed oral anticoagulants, produces an anticoagulant effect by inhibiting the factor Xa directly, in reversible, competitive, and selective ways. Apixaban is metabolized in the liver and contraindicated for combined used with CYP3A4 inhibitors [7,8].

According to previous reports, the antiangiogenic properties of factor Xa inhibitors depend on the dose administered. Initially, some studies claimed that the prolonged survival of cancer patients who were administered unfractionated heparin could be associated with the stimulation of antiangiogenic mechanisms [9-11]. Also, recent reports have suggested that apixaban is superior to low molecular weight heparin (LMWH) in protecting against cancer-related thrombotic events and other prothrombotic events [12,13]. In the current study, we investigated the antiangiogenic properties of apixaban in the chorioallantoic membrane (CAM) model and completed a review of the relevant literature.

MATERIAL AND METHODS

The model used in this study was designed in accordance with previous studies that have evaluated the antiangiogenic properties of drugs in the CAM model [9-11,14,15]. Ethical approval was not required due to the in-vitro design of study.

Group Creation

In the negative control group (used to evaluate drug-free pellets on chick embryos alone), drug-free pellets were administered to twenty eggs.

In the positive control group (used to determine a standard antiangiogenic base via the use of a well-known antiangiogenic drug), bevacizumab-embedded pellets were administered to twenty eggs.

The study groups (used to compare the antiangiogenic potential of apixaban having different concentrations) consisted of three apixaban-embedded pellet groups having different concentrations (10-4 M, 10-5 M, 10-6 M), and twenty eggs were used to examine the evolution of each concentration (in total, sixty eggs were used in the study groups).

Preparation of Pellets

The preparation of pellets was implemented as described in previous studies [9-11,14,15]. Agarose with a 2.5% weight of total volume was composed by mixing agarose (Merck, Darmstadt, Germany) and distilled water; the solution was then heated in an autoclave for sterilization and placed in sterile containers for cooling, after which the selected drugs were added. A single pellet group embedded with bevacizumab (Avastin™; Roche, Grenzach, Germany), used to create the positive control group, was prepared at a 10-6 M concentration, and three pellet groups embedded with apixaban (Eliquis™; Bristol-Myers Squibb, New York, USA), used to create the study groups, were prepared at concentrations of 10-4 M, 10-5 M, and 10-6 M. Primarily, 10 IU concentrations of drug pellets were prepared with the addition of 1 mL of the selected drug to the cooled agarose, and consecutive serial 1 IU/10 µl dilutions were applied to reach each targeted molar (M) concentration. After reaching the expected concentrations, 10 µl of drops were prepared with micropipettes for application to each egg.

Preparation of the chorioallantoic membrane (CAM) model

The CAM model was designed in accordance with procedures described in the literature [9-11,14,15]. The shells of fertile eggs (Ross 308)

were cleaned with alcohol-soaked gauze and placed inside incubators for embryonic growth at a controlled humidity (~80%) and temperature (~37.5 °C). Up to the fifth day of incubation, eggs were rotated periodically to achieve central placement of the embryos; rotation was stopped before starting the study protocol (Image 1.A, B). The eggs were cleaned with alcohol again, and a syringe was used to draw 5 ml of whole-egg liquid from the oval tip of each egg (Figure 1.C); the hole was created at the sharp tip of each egg with micro forceps (Figure 1.D), and the development of the chick embryos was evaluated via the egg holes (Figure 1.E1, E2). Immature eggs were excluded from the study. Eggs determined as mature were then separated into groups (twenty eggs in each group), and sterile pellets were inserted through the egg holes. The hole of each egg was sealed with a sterile surgical drape and again placed inside the egg incubator. The eggs were controlled on the eighth day of incubation, and angiogenesis was scored (Figure 1.F) as described in previous reports.

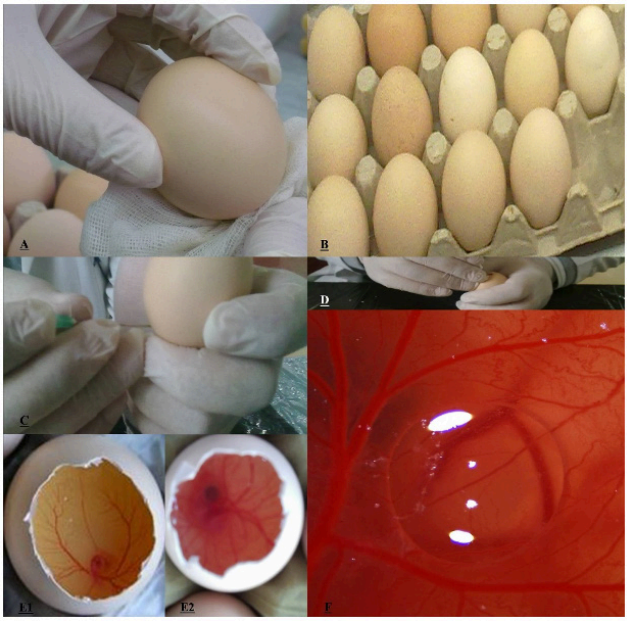

Figure 1. The steps in study protocols: A. Cleaning eggs with alcohol, B. Placement of each egg, C. Withdrawing albumin with syringe, D. Opening a hole with micro forceps, E.1-E.2. Evaluation of maturity in each egg via the egg hole, F. Scoring of anti-angiogenesis (score x2)

The pellets placed in chorioallantoic membranes were scored under light microscopy as described in previous reports on the surrounding capillary density of pellets (Figure 1.F). The degree of inhibition was recorded as follows [16,17]:

A normal surrounding capillary bed indicates a score of 0; slight (indistinct) changes in the capillary bed indicates a score of 0.5; decreased surrounding capillary bed density (less than twice of the pellet diameter) or a small, avascular free zone that surrounds the pellet indicates a score of 1; an extensive avascular free zone around the pellet (greater than twice the pellet diameter) indicates a score of 2.

After recording each score for each egg, an average score was calculated for each group as follows: twice the “score 2” number of eggs was added to the total “score 1” number of eggs egg number and divided into the total number of eggs in each group. An average score of 0.5 or lower indicates no antiangiogenic effect, an average score between 0.5 and 1 indicates a mild antiangiogenic effect, and an average score of >1 indicates a strong antiangiogenic effect [16,17].

Statistical Analysis

The statistical data of the scores were obtained with the Mann-Whitney U test and the Kruskal-Wallis one-way analysis. The antiangiogenic property was considered to be statistically significant for a p-value < 0.05.

RESULTS

The average antiangiogenic scores are summarized in Table 1. A comparison between the study groups and the positive control group (bevacizumab, 10-6 M concentration) revealed similar antiangiogenic effects in the 10-4 M concentration of apixaban (p = 0.367). However, there were statistical differences between the positive control group and the 10-5 M concentration apixaban (p = 0.017) and 10-6 M concentration apixaban (p = 0.001) groups.

The apixaban groups (10-4 M, 10-5 M, and 10-6 M concentrations) were statistically similar when compared each other (p = 0.053). However, there was a significant difference between the 10-4 M concentration apixaban group and the 10-6 M concentration apixaban group (p = 0.019). The distribution of antiangiogenic scores is demonstrated as a scatter graphic which is presented in Figure 2.

Table 1. Average antiangiogenic scores of drug groups

Groups	Number of eggs (with 0, 0.5, 1, and 2 scores)	Average Scores
Bevacizumab 10^{-6} M (n:20)	1 with "0.5" score	1.4 (strong antiangiogenic effect)
	10 with "1" score	
	9 with "2" score	
Apixaban 10^{-4} M (n:20)	3 with "0.5" score	1.2 (strong antiangiogenic effect)
	10 with "1" score	
	7 with "2" score	
Apixaban10^{-5} M (n:20)	5 with "0.5" score	0.9 (mild antiangiogenic effect)
	12 with "1" score	
	3 with "2" score	
Apixaban 10^{-6} M (n:20)	1 with "0" score	0.6 (mild antiangiogenic effect)
	6 with "0.5" score	
	12 with "1" score	
	1 with "2" score	

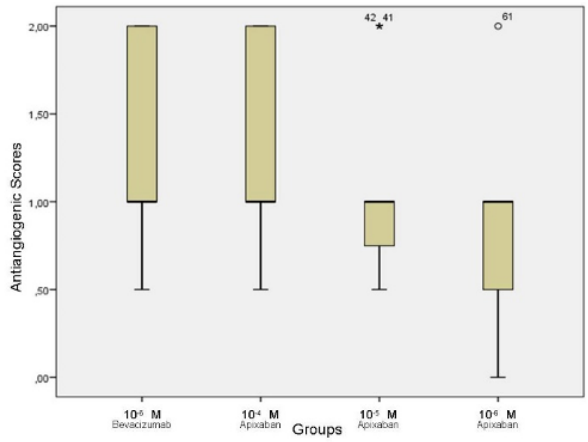

Figure 2. Comparison of antiangiogenic scores in bevacizumab and apixaban groups

DISCUSSION

We found that bevacizumab at a concentration of 10-6 M has similar antiangiogenic effects (strong antiangiogenic effects) to apixaban at a concentration of 10-4 M. On the other hand, apixaban has mild antiangiogenic effects at lower concentrations (10-5 M and 10-6 M). Based on our current knowledge, the present study is unique in that it used an experimental approach to investigate the antiangiogenic effects of different molar (M) concentrations of apixaban.

Anticoagulant drugs are important for the protection and treatment of cardiovascular thrombotic events. Conventional unfractionated heparin, low molecular weight heparin (LMWH) and vitamin K antagonists (e.g., Coumadin), which require blood-clotting test monitoring for determining sufficient or optimal dosages relative to body weight, are used in the management of these kinds of disorders. On the other hand, invasively applied heparin and LMWH (i.e., subcutaneous and intravenous), are negatively affecting patients' compliance and comfort [1-5].

The above-mentioned issues have led to the development of new strategies in the treatment or prophylaxis of thrombotic disorders. Therefore, new anticoagulants have been developed that are administered orally and do not require blood-clot test monitoring [6,7]. Rivaroxaban, dabigatran, apixaban and edoxaban are the most common examples of these types of newly developed drugs. Numerous studies have compared the anticoagulant effects of these drugs with conventional medical therapies, and most have reported favourable results with direct oral anticoagulants (DOACs) [6,7]. However, other systemic effects remain an important topic for future study to gain an understanding of the long-term efficacy and safety of these new drugs [18].

The most recent studies have focused on the angiogenic and vasculogenic effects of anticoagulants. Bevacizumab, which is a type of immune globulin G1 (IgG1), has been recently used to compare the antiangiogenic effects of drugs. It is well-known that Bevacizumab inhibits the activity of the vascular endothelial growth factor-A (VEGF-A) by binds with VEGF-A (forming large molecule) and preventing the binding of their receptors. Thus, bevacizumab shows a marked antiangiogenic effect and is indicated for use as an antitumor agent [19]. Previously published studies have reported that unfractionated heparin and its derivatives exerted significant antiangiogenic effects in the CAM model. These studies have compared the antiangiogenic effects of heparin and LMWH with bevacizumab in the CAM model. For instance, Katrancioglu et al. claimed that heparin showed strong antiangiogenic effects while the antiangiogenic effects of LMWH have been reported as dosage-dependent [10]. In contrast, Rema et al. suggested that heparin has pro-stimulant effects on angiogenesis in the CAM model [20]. In studies investigating the underlying mechanism of the anti-angiogenesis potential of anticoagulants, it was claimed that the possible

antiangiogenic effects of heparin derivatives can be dependent on the inhibition of VEGF expression via the binding of the tissue factor pathway inhibitor, which is a type of factor Xa inhibitor [21]. Rivaroxaban was also investigated in relation to angiogenesis in experimental models. It was claimed that rivaroxaban might promote neovascularization in hyperglycemic conditions in an animal model [22]. In contrast, in another study using the CAM model, it was found that rivaroxaban has dose-dependent antiangiogenic activity [11]. A potential action mechanism for the inhibition of angiogenesis was proposed by Liu et al., who stated that "Rivaroxaban (factor Xa inhibitor) might suppress coagulation-induced angiogenesis, which is related with ischemia during cellular growth in cancer cells or thrombosis [18]. Apixaban, also a type of factor Xa inhibitor, has similar effects to rivaroxaban [23]. In this context, Guasti et al. investigated the in vitro effects of apixaban in cancer cell lines and found that apixaban-treated cancer cells exerted a reduced migration capacity; additionally, it was shown that apixaban had dose-dependent antiproliferative effects [24]. Guasti et al. claimed that their study was the first study on high doses of this direct FXa inhibitor treatment on cancer cell lines. The authors suggested that this potential activity could be related to increased apoptosis in cell lines [25]. Taken together, Guasti et al.'s results could partially explain the results of the present study, and it is reasonable to assume that the antiangiogenic potential of apixaban could be related to dosage-dependent apoptotic and antiproliferative effects. Interestingly, we also have found that apixaban showed great antiangiogenic potential as many VEGF inhibitor agents in high doses. The other possible mechanism for the antiangiogenic potential of apixaban could relate to suppressed coagulation-induced angiogenesis, including the inhibition of factor Xa. From another of point of view, apixaban could also inhibit the VEGF expression via the binding of the tissue factor pathway inhibitor in the growth of embryos which has properties similar to a tumor growth.

Limitations of the study: There are several limitations of the present study. First, the CAM model is used to evaluate the vascular density without explaining cellular mechanism. Second, CAM is accepted as an in vitro model although it is a part of embryogenesis. Relatedly, current results are reflective of rudimentary findings indicating to the weakness of the study model.

CONCLUSION

Our results suggest that apixaban exerts dose-dependent antiangiogenic potential. However, the literature contains conflicting results about other factor Xa inhibitors, including their exact mechanism of action. Therefore, these results should be confirmed via further investigations and the potential mechanism of action needs to be clarified with cell studies.

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REFERENCES

1. Alquwaizani M, Buckley L, Adams C, Fanikos J. Anticoagulants: A Review of the Pharmacology, Dosing, and Complications Curr Emerg Hosp Med Rep. 2013;1(2):83–97. doi: 10.1007/s40138-013-0014-6.
2. Nowak-Göttl U, Bidlingmaier C, Krümpel A, Göttl L, Kenet G. Pharmacokinetics, efficacy, and safety of LMWHs in venous thrombosis and stroke in neonates, infants and children Br J Pharmacol. 2008;153(6):1120–7. doi: 10.1038/sj.bjp.0707447.
3. Barnes GD, Lucas E, Alexander GC, Goldberger ZD. National Trends in Ambulatory Oral Anticoagulant Use. Am J Med. 2015;128(12):1300-5.e2. doi: 10.1016/j.amjmed.2015.05.044.
4. Hansen PW, Sehested TSG, Fosbøl EL, Torp-Pedersen C, Køber L, Andersson C, et al. Trends in warfarin use and its associations with thromboembolic and bleeding rates in a population with atrial fibrillation between 1996 and 2011 PLoS One. 2018;13(3):e0194295. doi: 10.1371/journal.pone.0194295.
5. Kamuren Z, Kigen G, Keter A, Maritim A. Characteristics of patients with thromboembolic disorders on warfarin therapy in resource limited settings. BMC Health Serv Res. 2018; 18: 723. doi: 10.1186/s12913-018-3537-4.
6. Loo SY, Dell'Aniello S, Huiart L, Renoux C. Trends in the prescription of novel oral anticoagulants in UK primary care. Br J Clin Pharmacol. 2017;83(9):2096–106. doi: 10.1111/bcp.13299.
7. Mekaj YH, Mekaj AY, Duci SB, Miftari EI. New oral anticoagulants: their advantages and disadvantages compared with vitamin K antagonists in the prevention and treatment of patients with thromboembolic events. Ther Clin Risk Manag. 2015;11:967-77. doi: 10.2147/TCRM.S84210.
8. Zalpour A, Oo TH. Clinical utility of apixaban in the prevention and treatment of venous thromboembolism: current evidence. Drug Des Devel Ther. 2014;8:2181–91. doi: 10.2147/DDDT.S51006.
9. Dogan OT, Polat ZA, Karahan O, Epozturk K, Altun A, Akkurt I, Cetin A. Antiangiogenic activities of bemiparin sodium, enoxaparin sodium, nadroparin calcium and tinzaparin sodium. Thromb Res. 2011;128(4):e29-32. doi: 10.1016/j.thromres.2011.05.005.
10. Katrancioglu N, Karahan O, Kilic AT, Altun A, Katrancioglu O, Polat ZA. Comparison of the antiangiogenic effects of heparin sodium, enoxaparin sodium, and tinzaparin sodium by using chorioallantoic membrane assay. Blood Coagul Fibri-

nolysis. 2012;23(3):218-21. doi: 10.1097/MBC.0b013e3283504132.

11. Yavuz C, Caliskan A, Karahan O, Yazici S, Guclu O, Demirtas S, et al. Investigation of the antiangiogenic behaviors of rivaroxaban and low molecular weight heparins. Blood Coagul Fibrinolysis. 2014;25(4):303-8. doi: 10.1097/MBC.0000000000000019.
12. Browne C, Lanitis T, Hamilton M, Li X, Horbyluk R, Mardekian J, et al. Impact of apixaban vs low molecular weight heparin/vitamin k antagonist on hospital resource use in patients with venous thromboembolism. J Med Econ. 2017;20(1):98-106. doi: 10.1080/13696998.2016.1258365.
13. Gómez-Outes A, Suárez-Gea ML, Lecumberri R, Terleira-Fernández AI, Vargas-Castrillón E, Rocha E. Potential role of new anticoagulants for prevention and treatment of venous thromboembolism in cancer patients. Vasc Health Risk Manag. 2013;9:207-28. doi: 10.2147/VHRM.S35843.
14. Karahan O, Yavuz C, Demirtas S, Caliskan A, Atahan E. The Investigation of the Antiangiogenic Potential of Amiodarone HCl in the Chick Embryo Chorioallantoic Membrane Model. Biomedical Research 2013;24(1):131-4
15. Katrancioglu N, Karahan O, Kilic AT, Altun A, Katrancioglu O, Polat ZA. The antiangiogenic effects of levosimendan in a CAM assay. Microvasc Res. 2012;83(3):263-6. doi: 10.1016/j.mvr.2012.01.002.
16. Bürgermeister J, Paper DH, Vogl H, Linhardt RJ, Franz G. LaPSvS1, a (1-->3)-beta-galactan sulfate and its effect on angiogenesis in vivo and in vitro. Carbohydr Res. 2002;337(16):1459-66. doi: 10.1016/s0008-6215(02)00163-5.
17. Demirci B, Dadandi MY, Paper DH, Franz G, Baser KH. Chemical composition of the essential oil of Phlomis linearis Boiss.&Bal., and biological effects on the CAM assay: a safety evaluation. Z Naturforsch C. 2003;58(11–12):826–9. doi: 10.1515/znc-2003-11-1214.
18. Liu FD, Zhao R, Feng XY, Shi YH, Wu YL, Shen XL, et al. Rivaroxaban does not influence hemorrhagic transformation in a diabetes ischemic stroke and endovascular thrombectomy model. Sci Rep. 2018;8(1):7408. doi: 10.1038/s41598-018-25820-y.
19. Pavlidis ET, Pavlidis TE. Role of bevacizumab in colorectal cancer growth and its adverse effects: a review World J Gastroenterol. 2013;19(31):5051-60. doi: 10.3748/wjg.v19.i31.5051.
20. Rema RB, Rajendran K, Ragunathan M. Angiogenic efficacy of Heparin on chick chorioallantoic membrane. Vasc Cell. 2012;4(1):8. doi: 10.1186/2045-824X-4-8.
21. Hwang HH, Lee DY. Antiangiogenic Actions of Heparin Derivatives for Cancer Therapy. Macromol Res. 2016;24(9):767-72. doi: 10.1007/s13233-016-4111-8.
22. Wu TC, Chan JS, Lee CY, Leu HB, Huang PH, Chen JS, et al. Rivaroxaban, a factor Xa inhibitor, improves neovascularization in the ischemic hindlimb of streptozotocin-induced diabetic mice. Cardiovasc Diabetol. 2015;14:81. doi: 10.1186/s12933-015-0243-y.
23. Cohen AT, Hamilton M, Mitchell SA, Phatak H, Liu X, Bird A, Tushabe D, et al. Comparison of the Novel Oral Anticoagulants Apixaban, Dabigatran, Edoxaban, and Rivaroxaban in the Initial and Long-Term Treatment and Prevention of Venous Thromboembolism: Systematic Review and Network Meta-Analysis. PLoS One. 2015;10(12):e0144856. doi: 10.1371/journal.pone.0144856.
24. Kubat E, Gurpinar OA, Karasoy D, Onur MA. A link between cytotoxicity in cell culture and gastrointestinal side effects of oral anticoagulants: bench-to-bedside. Bratisl Lek Listy. 2018;119(11):706-12. doi: 10.4149/BLL_2018_126.
25. Guasti L, Squizzato A, Moretto P, Vigetti D, Ageno W, Dentali F, et al. In vitro effects of Apixaban on 5 different cancer cell lines. PLoS One. 2017;12(10):e0185035. doi: 10.1371/journal.pone.0185035.