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Antithrombotic Effects Of Dextran Sulfate And Starch Sulfate Derivatives On Thrombosis Model Induced By Thromboplastin

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ABSTRACT
This article demonstrates that dextran sulfate (D-38) and starch sulfate (K-39) derivatives are more effective in influencing the coagulation-platelet hemostasis system compared to heparin preparations in inducing thromboplastinemia in rats in vivo. This effect helps prevent the formation of thrombi in blood vessels.

Keywords: Thrombosis, Dextran sulfate derivative (D-38), Starch sulfate derivative (K-39), Anticoagulant, Antiplatelet, Thrombosan A2, Thromboplastin, Platelets, Heparin, TT, PTT

INTRODUCTION

Cardiovascular diseases are currently the leading cause of death [25]. The number of serious diseases such as thrombosis in blood vessels, pulmonary embolism (PE), myocardial infarction (MI), ischemia of organs and tissues is increasing. To prevent and treat such diseases, new antithrombotic agents are being investigated in experimental thrombosis models in rats [26]. Today, the search for new drugs for the prevention and treatment of thrombosis is an urgent problem in medicine and pharmacy.

Research Aim. To determine the antithrombotic effect of dextran sulfate (D-38) and starch sulfate (K-39) derivatives compared to heparin in an experimental thrombosis model in rats.

MATERIALS AND METHODS

Model experiments were conducted in vivo on 20 white non-albino rats weighing 180-200 g [8]. Thromboplastin (HUMAN Diagnostics Worldwide) at a dose of 25 mg/kg was used as a thrombotic agent, dissolved in 0.9% sodium chloride solution and administered in a volume of 0.02 ml into the tail vein of the animal. D-38 and K-39 compounds at a dose of 5 mg/kg were administered intravenously in the tail vein one day prior, and heparin (Sigma 5000 IU/ml) at a dose of 130 IU/kg (1 mg/kg) was administered intraperitoneally 10 minutes before the administration of the thrombotic agent. Ten minutes after the administration of thromboplastin, blood was collected from the rat's vein (3.8% sodium citrate solution) in a 9:1 ratio and centrifuged at 2000 g for 10 minutes (OPN-8 centrifuge, rotor RU180 L, 2000 g) [1-2-3-4]. The separated plasma was evaluated for coagulation tests: partial thromboplastin time (PTT), thrombin time (TT), and prothrombin time (PT) using an optical-mechanical coagulometer (CYANCoag, Belgium.CY003, SN: 5400439).

To study platelet aggregation, blood was collected from the vein of the model and control rats (3.8% sodium citrate solution) in a 9:1 ratio and centrifuged at 1000 g for 10 minutes (OPN-8 centrifuge, rotor RU180 L, 2000 g) [23]. Platelet aggregation was determined in an aggregometer (С/Ф5027358/1от 03,11,2021 Biola, Russia) based on the Born method, which records changes in light transmission in platelet-rich plasma and analyzes light transmission oscillations that occur as a result of random changes in the number of particles [6-10]. In our subsequent experiments, macroscopic examination of the internal organs of the rats was performed one week after the administration of the thrombotic agent and the compounds under investigation. Histological preparations were prepared from samples taken from the liver, lungs, and heart of the model and control rats.

The obtained samples were stained with hematoxylin-eosin stain [24]. This stain is widely used for staining histological preparations. Sections from paraffin blocks are cleared in paraffin-chloroform and, after washing in water, hematoxylin stain is applied to them for 3 minutes. The hematoxylin is washed off in water for 10 minutes and, depending on the dye uptake, is held in eosin for 2-3 minutes. The sections are dehydrated in alcohols with increasing concentrations from 70% to 96%, cleared with carbol-xylene, and mounted with balsam. Result: the nuclei of the cells are stained bluish-black, and the cytoplasm is stained violet. Subsequently, the condition of the tissues of the heart, lungs, and liver of the control and model rats is studied.

RESULTS AND DISCUSSIONS

A comparison of the effects of K-39 (5 mg/kg) and D-38 (5 mg/kg) derivatives with heparin (1 mg/kg) on partial thromboplastin time (PTT) revealed that heparin prolonged the PTT by 22-25 seconds compared to the control, while K-39 and D-38 derivatives prolonged it by 46 seconds and 76 seconds, respectively. A comparison of the effects on thrombin time (TT) revealed that heparin prolonged the TT by 25-30 seconds compared to the control, while K-39 and D-38 derivatives prolonged it by 40 seconds and 60 seconds, respectively. A comparison of the effects on prothrombin time (PT) revealed that heparin prolonged the PT by 25-45 seconds compared to the control, while K-39 and D-38 derivatives prolonged it by 55 seconds and 85 seconds, respectively (Figure 1).

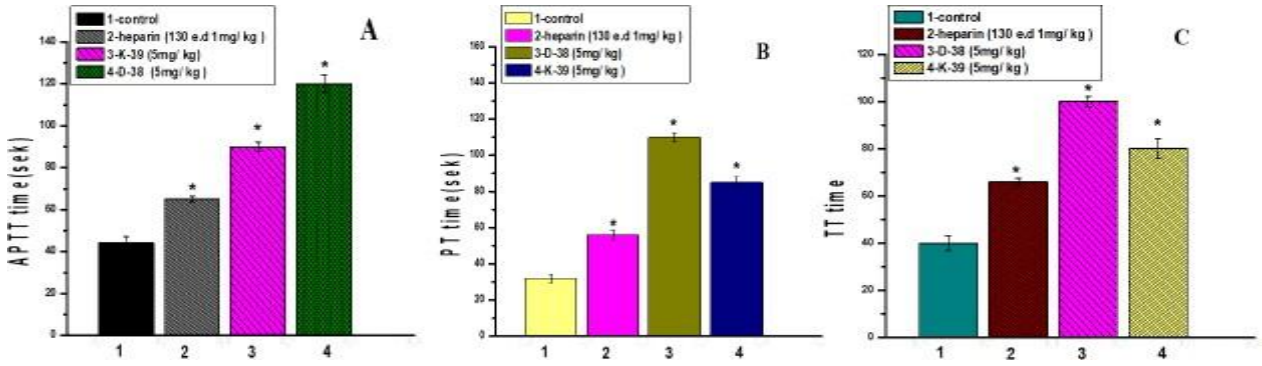

Figure 1. Clotting time in partial thromboplastin time (PTT) (A), prothrombin time (PT) (B), and thrombin time (TT) (C) tests in a thromboplastinemia model: 1 - control, 2 - heparin, 3 - D-38, 4 - K-39 derivatives. * -P> 0.05

It is known that prolonged clotting time in normal PTT, PT, and TT tests indicates a deficiency or inhibition of factors VIII, IX, XI, XII, as well as prekallikrein and high molecular weight kininogen [18]. Similarly, prothrombin time or prothrombin index, a measure of the extrinsic pathway of coagulation, reflects a deficiency or reduced activity of prothrombin complex factors (VII, X, V, II) [14]. When the effect of D-38 and K-39 derivatives on prothrombin time was observed, the prolongation of prothrombin time indicates a partial inhibition of the activation of the extrinsic pathway of coagulation, specifically a partial inhibition of the activity of factors V and II. Prolongation of PTT and PT is only indicative of an effect of indirect anticoagulants on factor VII [12]. In the thromboplastinemia model, the prolonged clotting time observed with D-38 and K-39 derivatives, compared to the control and heparin, suggests an inhibition or deficiency of one of the factors II, V, VII, or X.

In our subsequent research, we investigated the effect of blood plasma from the model rats on spontaneous aggregation and aggregation induced by ADP and adrenaline inducers [16-17]. Spontaneous aggregation occurred in the thrombosis model. When ADP inducer was added to the obtained plasma, the degree of aggregation increased in a concentration-dependent manner (5-10 μmol), showing a primary one-phase and secondary two-phase curve-shaped aggregation, with irreversible platelet aggregation observed at higher concentrations (10 μmol) (Figure 2)..

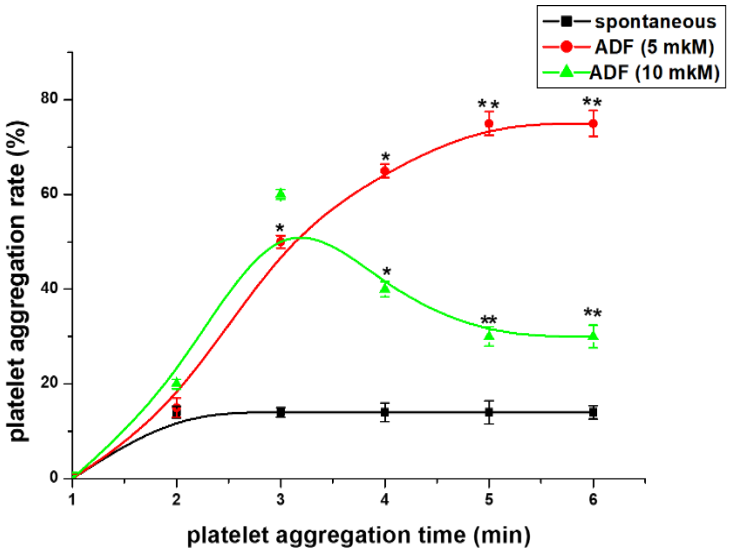

Figure 2. Platelet aggregation induced by ADP. *- P <0.05; **- P <0.01. (n=6)

investigating the effects of D-38 and K-39 derivatives in the presence of ADP inducer, a significant inhibition of platelet aggregation was observed (Figure 3). It is known that ADP, by binding to its P2Y1 and P2Y12 receptors on the surface of platelet membranes, causes a conformational change leading to platelet aggregation. This results in the inhibition of adenylate cyclase and a reduction in cAMP production, leading to an increase in [Ca+2] release from the depot and subsequent release of Ca2+ ions into the cytoplasm, ultimately causing platelet aggregation. [6-11]**.**

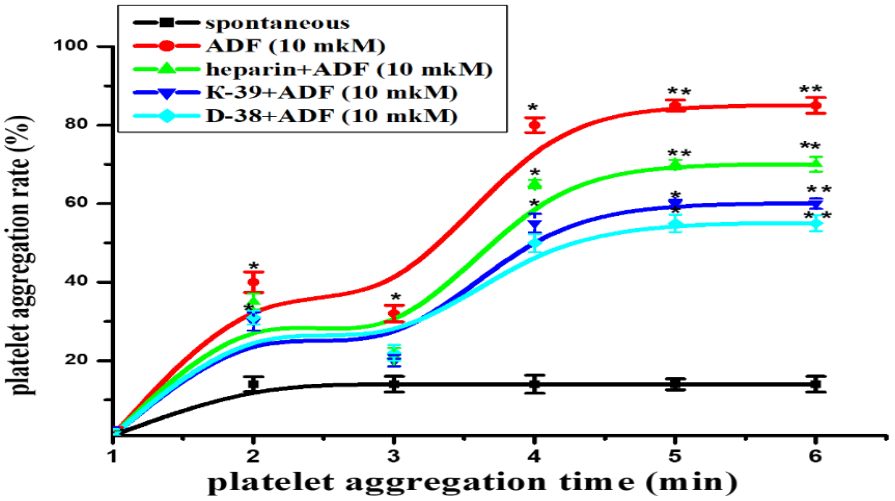

Figure 3. Effect of D-38 and K-39 polysaccharide on ADP-induced platelet aggregation in a dose-dependent manner. *- P <0.05; **- P <0.01. (n=6).

In our subsequent research, when adrenaline inducer was administered to blood plasma from model rats at concentrations of 2-5 μmol, platelet aggregation was observed. At a concentration of 2 μmol, adrenaline induced aggregation in a two-phase curve shape, leading to irreversible aggregation of platelets isolated from blood of rats with a modeled thrombosis at higher concentrations.
Figure 4

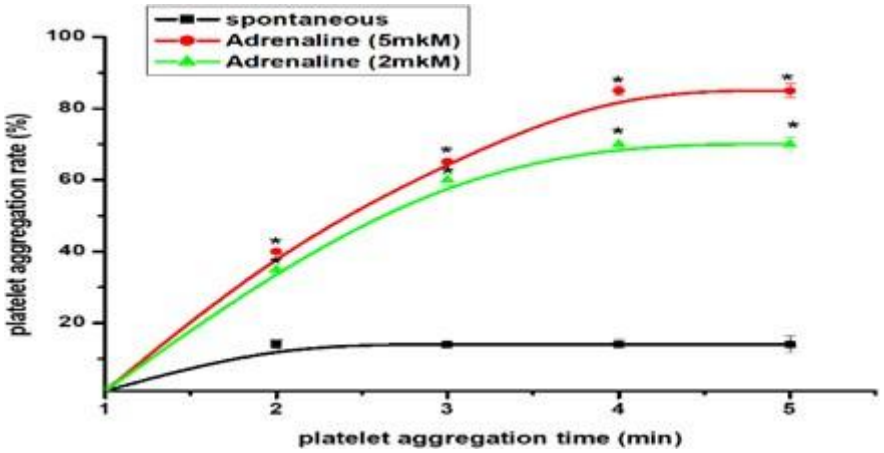

Figure 4. Platelet aggregation induced by adrenaline. *- P <0.05; **- P <0.01. (n=6)

Adrenaline interacts with α2 adrenergic receptors on the platelet membrane, significantly inhibiting adenylate cyclase activity, promoting the production of thromboxane A2, and increasing cell membrane permeability and calcium ion transport [13.14.15.17]. These inducer effects lead to an increase in intracellular calcium concentration, resulting in the phosphorylation of proteins within platelets. The sharp increase in Ca2+ concentration from the endoplasmic reticulum plays a crucial role in platelet activation, including aggregation and the release of coagulation factors. In this scenario, the primary element of platelet activation is the release of calcium ions from intracellular stores into the extracellular space, ultimately resulting in platelet aggregation [11.12.16].

In the thromboplastinemia model, the mechanisms of action of derivatives D-38 (5 mg/kg) and K-39 (5 mg/kg) involve the activation of adenylate cyclase, a decrease in thromboxane A2 production, and a reduction in the efflux of intracellular calcium ions. Ultimately, this leads to a decrease in platelet aggregation (Figure 5).

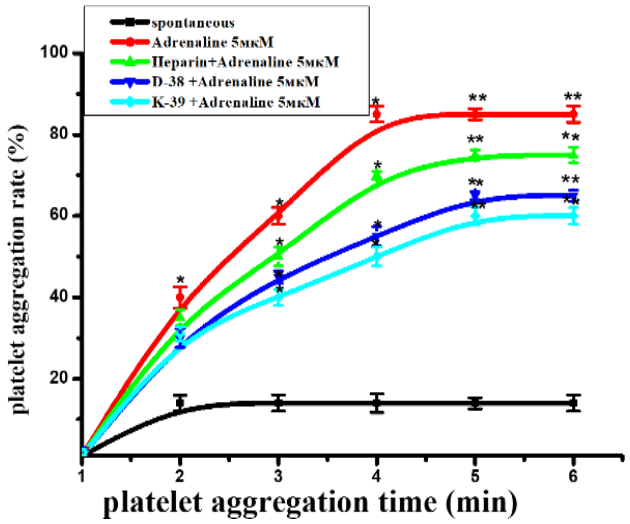

Figure 5. Effect of D-38 and K-39 Derivatives on Adrenaline-Induced Platelet Aggregation.** *- P<0.05; **- P<0.01. (n=6).

In our subsequent experiments, we conducted a histological analysis of liver tissue in control rats and rats treated with heparin, D-38, and K-39 derivatives in a thromboplastinemia model.

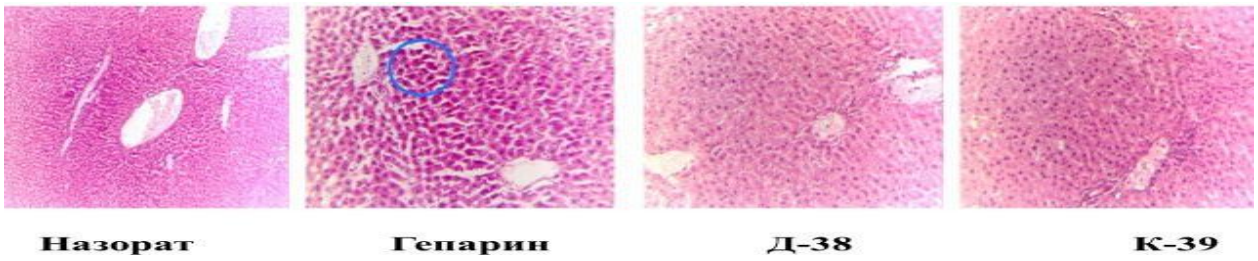

Figure 6. Histological Analysis of Liver Tissue in a Thromboplastinemia Model.

Liver tissue from control rats showed signs of edema (swelling), inflammation, blood residue, and tissue damage (Figure 6). In the model rats treated with heparin, liver tissue showed partial damage. However, liver tissue from rats treated with D-38 and K-39 derivatives appeared normal compared to the control and heparin-treated rats.

CONCLUSION

Research has demonstrated that the investigated D-38 and K-39 derivatives exhibit anticoagulant and antithrombotic effects by inhibiting coagulation factors II, V, and X. They may also influence Ca2+-binding factors in the extrinsic pathway of coagulation. Furthermore, these derivatives potentially bind to glycoprotein receptors (P2Y1 and P2Y12) and adrenergic receptors (α2-adrenergic), activating adenylate cyclase and decreasing thromboxane A2 production. This, in turn, inhibits the efflux of Ca2+ from the cell membrane, reducing platelet aggregation and potentially preventing thrombus formation in blood vessels.

REFERENCES

1. Papayan, lp Normal hemostasis // Hematology. National Guide on Hematology. / Ed. prof. O. A. Rukavitsin. - M: GEOTAR-Media, 2015. S. 90-100.
2. / D. M. Monroe, M. Hoffman // Arterioscler. Thromb. Vasc. Biol. – 2006. - Vol. 26. - P. 41-48.
3. Gracher AHP, Santana AG, Cipriani TR, lacomini M. Aprocoagulant chemical sulfated mannan. Carbohydrate Polymers. 2015.136:177-186.
4. Sedinkin VA, KLIGUNENKO EN. The role and significance of exogenous factors of blood clotting in the obstetric flow. State Institution "Dnepropetrovsk Medical Academy of the Ministry of Health of Ukraine", Dnepr, Ukraine. (93).,
5. Sedinkin VA, KLIGUNENKO EN. The role and significance of exogenous factors of blood clotting in the obstetric flow. State Institution "Dnepropetrovsk Medical Academy of the Ministry of Health of Ukraine", Dnepr, Ukraine. (93).
6. Patel S. Therapeutic Potential of Sulfated Polysaccharides from Seaweeds: An Update on Recent Findings. 3 Biotech, 2012. 2(3), 171–185. https://doi.org/10.1007/s13205-012-0061-9
7. Dorea CMPG, Alvesa MGCF, Iroda LSEP, Kosta TG, Sabryb DA, et al. Carbohydrate Polymers 2012. 91: 467-475.
8. Council of Europe, European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes. 1986, available at: http://conventions.coe.int, accessed December 2023
9. Wang Z.M., Li L., Zheng B.S., Normaxamatov N., Guo S.Y. Preparation and anticoagulant activity of sodium cellulose sulfate // Int. J. Biol. Macromol. 2007. Vol. 41 (2). - P. 376-382.
10. Participation of glycoprotein IIb-IIIa in spontaneous platelet aggregation / O.V. Sirotkina [et al.] // Bulletin of Experimental Biology and Medicine. – 2007. – Vol. 143, No. 4. – P. 398–401.
11. RaimovaG.M.,MusaevaM.K.,KhoshimovN.N.,VypovaN.L.,NasirovK.E.Selection and study of influence of preparation “CMC” on the process of cooking of blood.RussianJournalofBiologicalResearch.2019.P.16-22.DOI:10.13187/ejbr.2019.1.16
12. Guli M. Raimova, Nozim N. Khoshimov, Kabil E. Nasirov, Abbaskhan S. Turaev, Malokhat E. Savutova. Anti-thrombotic action of sulfated polysaccharides on thrombosis caused by thromboplastin. Res.J. Pharmacy Tech. 2021; 14(11):6085-8. doi: 10.52711/0974-360X.2021.01057
13. Раимова Г.М., Насиров К.Э., Шодмонова Д.Ш., Хотамов Т.Н., Махмудов Р.Р.Исследование антидиабетический активности полифенолов ANK-1,ANK-2 иITL-2 примоделированиисахарногодиабета 2 типа. Инфекция иммунитет и фармакология. Ташкент. №6.2023.С.103-109
14. Э.К. Насиров, М.Г. Раимова, Л.Н. Выпова, К.М. Мусаева, М.М. Ортиков, С.С. Ходжиев, Р.Р. Махмудов Действие некоторых полифенолов на ферментные и плазменные системы крови крыс, моделированных сахарном диабетеом 2 типа.Узбекский биологический журнал.2023,№ 6.С.32-41

15. Raimova G.M., Nasirov K.E., Khodjiyev S.S., Ortikov M.M., Maxmudov R.R., Toshtemirova M.A. ANK-1, ANK-2, ITL-2 Polyphenols in a Dexamethasone-Induced Rat Model of Type 2 Diabetes Mellitus Journal of Angiotherapy (2024). Treatment, Journal of Angiotherapy, 8(7), 1-11, 9778. Scopus, импакт-фактор 0,4)
16. Nozim N. Khoshimov, Guli M. Raimova, Kabul E. Nasirov, Zulayho A. Mamatova, Nodira I. Mamadaliyeva, Abbaskhan S. Turaev. The effect of Sulphated cellulose on System of Haemostasis. Research Journal of Pharmacy and Technology. 2021; 14(6):3283-9. doi: 10.52711/0974-360X.2021.00571
17. Guli M. Raimova, Nozim N. Khoshimov, Kabil E. Nasirov, Abbaskhan S. Turaev, Malokhat E. Savutova. Anti-thrombotic action of sulfated polysaccharides on thrombosis caused by thromboplastin. Research Journal of Pharmacy and Technology. 2021; 14(11):6085-8. doi: 10.52711/0974-360X.2021.01057
18. Nozim N. Khoshimov, Alisher A. Mukhtorov, Kabil E. Nasirov, Rakhmatilla N. Rakhimov, Rahmatjon R. Mamadaminov. Effects of Polyphenols on changes in the transport of Ca^{2+} NMDA-receptors under the influence of L-glutamate. ResearchJournalofPharmacyandTechnology 2023; 16(3):1205-3. doi: 10.52711/0974-360X.2023.00200.