

Açaí-derived low-molecular-weight sulfated xylan: structural optimization and anticoagulant/antithrombotic activity

Genilza da Silva Mello

genilza.mello@ufpr.br

Universidade Federal do Paraná

Guilherme Lanzi Sassaki

Universidade Federal do Paraná

Guilherme Fadel Picheth

Universidade Federal do Paraná

Leociley Rocha Alencar Menezes

Universidade Federal do Paraná

Alexandra Acco

Universidade Federal do Paraná

Giuliana Cozzella Campo Grande

Universidade Federal do Paraná

Philippe Rodrigues Benedetti

Universidade Federal do Paraná

Julia Teixeira Lucena

Universidade Federal do Paraná

Thaís Moro Cantu-Jungles

Universidade Federal do Paraná

Lucimara Mach Cortes Cordeiro

Universidade Federal do Paraná

Kauê Marcel de Oliveira

Universidade Federal do Paraná

Ricardo Stefani

Universidade Federal de Ouro Preto

Thales Ricardo Cipriani

Universidade Federal do Paraná

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Abstract

Hemostasis depends on a tightly regulated balance between procoagulant and anticoagulant mechanisms, and disruption of this equilibrium may result in thromboembolic events. Sulfated polysaccharides from non-animal sources have attracted interest as potential anticoagulant scaffolds; however, low-molecular-weight linear sulfated xylans remain insufficiently characterized regarding their anticoagulant mechanism, antithrombotic efficacy, and parenteral tolerability. In this study, a low-molecular-weight sulfated xylan derivative obtained from *Euterpe oleracea* pulp, designated NXSo, was prepared by partial acid hydrolysis of native xylan using 1.0 mol L^{-1} HCl at 100°C for 90 min, followed by $\text{SO}_3\text{-Py}$ -mediated chemical sulfation in DMF at a 6:1 $\text{SO}_3\text{-Py}$:OH molar ratio for 4 h at 50°C . Its anticoagulant activity was evaluated by clotting assays and chromogenic residual activity assays against factor IIa and factor Xa in the presence or absence of antithrombin and heparin cofactor II. NXSo inhibited FIIa activity in an AT-dependent manner, reaching 63% residual activity at $1 \text{ }\mu\text{g mL}^{-1}$, and produced approximately 35% FIIa residual activity via HCII in a dose-independent manner. *In vitro*, NXSo prolonged aPTT, PT, and TT in a concentration-dependent manner, increasing aPTT up to approximately 2.9-fold at $5 \text{ }\mu\text{g mL}^{-1}$, PT up to approximately 6.3-fold at $80 \text{ }\mu\text{g mL}^{-1}$, and TT up to approximately 3.3-fold at $12 \text{ }\mu\text{g mL}^{-1}$, compared with basal clotting times of $41.9 \pm 1.6 \text{ s}$, $13.7 \pm 0.4 \text{ s}$, and $14.3 \pm 1.4 \text{ s}$ for aPTT, PT, and TT, respectively. After subcutaneous administration, NXSo prolonged ex vivo aPTT for up to 12 h, whereas intravenous administration reduced thrombus formation by more than 90% in a stasis- and thromboplastin-induced venous thrombosis model. Docking simulations using a simplified persulfated xylan pentasaccharide model suggested plausible interactions with glycosaminoglycan-recognition regions in antithrombin-containing complexes. However, repeated subcutaneous administration induced localized inflammatory nodules with histological evidence of panniculitis, necrosis, and hemorrhage. Overall, NXSo represents a promising non-animal sulfated polysaccharide scaffold with anticoagulant and antithrombotic activity, but further purification, formulation, and local tolerability optimization are required before therapeutic translation.

1. Introduction

The development of thrombosis is driven by the interplay of three fundamental factors: endothelial injury, hypercoagulability, and abnormal blood flow [1, 2]. Acquired risk factors including cancer, surgery, immobility, fractures, and pregnancy as well as genetic variants in coagulation factors, contribute to a hypercoagulable state [3].

Unfractionated heparin (UFH), one of the most widely used anticoagulants, potentiates the activity of antithrombin to inhibit both thrombin (IIa) and factor Xa [4]. Its long polysaccharide chains enable the formation of a ternary complex involving antithrombin and thrombin, thereby accelerating thrombin inhibition [5]. UFH also exhibits the suppression of factor IIa via heparin cofactor II (HCII) serpin at elevated concentrations. Low molecular weight heparins (LMWHs), in contrast, are shorter fragments incapable of bridging antithrombin and thrombin efficiently [6]. While UFH exhibits broader anticoagulant activity, its use is limited by variable bioavailability and an increased risk of heparin-induced

thrombocytopenia (HIT), whereas LMWHs offer more predictable pharmacokinetics, reduced nonspecific binding, and an improved safety profile [7]. Nevertheless, since both agents are obtained from animal tissues, the risk of pathogen contamination remains a concern, and their purification is complicated by the frequent presence of other glycosaminoglycans [8]. In addition, their relative heterogeneity results in complex pharmacokinetic, biophysical, and biological properties that impact therapeutics [9]. Particularly, bleeding remains a major complication during UFH and LMWH therapy [10] while other severe adverse effects, such as HIT that occur in approximately 1–6% of patients receiving unfractionated heparin [11]. These limitations highlight the need for alternative anticoagulants derived from renewable sources, with homogeneous structures and efficacy comparable to conventional heparins.

Plant-derived polysaccharides emerge as candidates, not only because they eliminate the risk of pathogen transmission, but also due to their wide availability, lower production costs, structural diversity, and alignment with sustainable biotechnological approaches. Among these, xylans, abundant hemicelluloses with diverse structural variations, have emerged as promising candidates [12]. Chemical sulfation of naturally non-sulfated polysaccharides has been widely reported to impart significant biological properties, especially antithrombotic and anticoagulant functions [13]. Synthetic sulfated xylans have demonstrated broad therapeutic potential, with antiviral activity in marine-derived xylans [14], *in vitro* anticoagulant effects in corn straw xylans [15], and antithrombotic properties in glucuronoxylans and arabinoxylans isolated from beech and oats [16]. More recently, a wheat straw-derived sulfated xylan with a defined sulfation pattern exhibited potent anticoagulant activity, further reinforcing the therapeutic promise of xylan-based derivatives [17].

The capacity of sulfated xylans to promote the formation of AT-FIIa and HCII-FIIa complexes has been confirmed, even at low concentrations [18–20;23–25]. While xylans are typically described as highly branched polysaccharides [21], those isolated from pulp of *Euterpe oleracea* fruits (açai) exhibit a predominantly linear structure [22]. Such linear backbones enable a more uniform distribution of sulfate groups, thereby enhancing anticoagulant activity [23], particularly in the presence of 3-*O*-sulfation, which significantly strengthens this effect [24]. Moreover, low molecular weight xylan sulfates designed to mimic LMWHs may overcome the limitations of high molecular weight polysaccharides, however, their structure–activity relationships, pharmacodynamic behavior, antithrombotic efficacy, and tolerability after parenteral administration remain insufficiently explored.[7].

Among synthetic low molecular weight xylan sulfates analogs, pentosan polysulfate (PPS) (M_w 4.000 to 6.000 g mol⁻¹) obtained from beech wood, has been extensively investigated [25, 26]. Although PPS demonstrates anticoagulant activity *in vitro*, its *in vivo* efficacy is limited, and long-term administration is discouraged due to potential adverse effects such as elevated transaminases and thrombocytopenia [27–30]. Structurally, PPS is a highly branched 4-*O*-methyl glucuronoxylan polysulfate, containing at least 20% acetylated 4-*O*-methyl-glucuronic acid residues and an overall proportion of 4-*O*-methyl-glucuronic acid ranging from 35% to 70%. This composition characterizes PPS as a xylan with a high degree of branching [31].

Although numerous studies have described sulfation techniques and their resulting biological activities, only a few have systematically examined how variations in reaction conditions (such as reagent ratios, temperature, and reaction time) influence both anticoagulant activity and structural integrity [32]. Excessive reaction severity, for instance, can result in polysaccharide degradation [33]. Among available methods, the sulfur trioxide-pyridine (SO₃-Py) complex has emerged as particularly advantageous, offering stability, reproducibility, and high yields when employed in aprotic organic solvents such as dimethylformamide (DMF) [34].

In this context, the present study investigated a chemically sulfated low-molecular-weight xylan derivative obtained from *Euterpe oleracea* pulp. The work aimed to define sulfation conditions associated with anticoagulant activity, characterize the optimized derivative, and evaluate its effects on classical coagulation assays, factor IIa and factor Xa chromogenic activity, *ex vivo* aPTT prolongation, and experimental venous thrombosis in rats. Because parenteral tolerability is a critical requirement for anticoagulant development, local and systemic effects after repeated subcutaneous administration were also assessed. This integrated approach was designed to determine whether açai-derived sulfated xylan can be considered a viable non-animal anticoagulant scaffold and to identify the main limitations for further development.

2. Experimental

2.1 Materials

Native xylan (NX) from açai berries was previously obtained by [35]. *N,N* Dimethylformamide ($\geq 99\%$) was purchased from VETEC (Duque de Caxias, Brazil), MWCO 3.5 kDa dialysis membrane (Spectra/Por®) was purchased from Repligen (Massachusetts, USA), HCl 1 mol L⁻¹ (1 N) and glacial acetic acid (HAc, $> 99\%$) were purchased from Neon (Suzano, Brazil). *M*-hydroxydiphenyl (phenyl phenol, $\geq 90\%$), NaBH₄ ($\geq 98.0\%$), NaOH ($\geq 97\%$), Na₂SO₄ (99%), trifluoroacetic acid (TFA, $\geq 99\%$), DMSO-*d*₆ (99,8%), deuterium oxide (D₂O, 99.9 atom % D) and sulfur trioxide pyridine (SO₃-Py, 98%) were purchased from Sigma-Aldrich (St. Louis, USA).

2.2 Methods

2.2.1 Partial acid hydrolysis

NX was partially hydrolysed with 0.5 mol L⁻¹ HCl for 30, 90 and 180 min, at 100°C resulting in the partially hydrolysed xylans. NX was also partially hydrolysed with 1.0 mol L⁻¹ HCl for 90 min, at 100°C. After hydrolysis, the pH was neutralized with 0.5 mol L⁻¹ NaOH and, when necessary, adjusted with PA glacial acetic acid. The samples were dialyzed against tap water for 72 h and then against distilled water for 48 h (six changes of water), using 3.5 kDa cut-off membrane. After dialysis the samples were freeze-dried.

2.2.2 Chemical sulfation

Chemical sulfation of the partially hydrolysed xylans was performed with adaptations from [29]. Briefly, 10 mg of the partially hydrolysed xylans were suspended in 200 μL of *N,N*-dimethylformamide (DMF) (ratio of 20 $\mu\text{L}/\text{mg}$; DMF/polysaccharide), and stirred for 30 min at 50°C. Then, $\text{SO}_3\text{-Py}$ complex was added to give a molar ratio of sulfation agent to hydroxyl group on the xylan ($\eta\text{SO}_3\text{-Py:OH}$ ratio) of 9:1. The solution was then stirred at 50°C for 2 h. Afterwards, 10% NaHCO_3 was added to quench the reaction and the samples were dialyzed against tap water for 48 h and then distilled water for 48 h (six changes of water), using 3.5 kDa cut-off membrane, then freeze-dried.

2.2.3 Chemical sulfation in different molar ratios of sulfation agent

Chemical sulfation of the partially hydrolysed xylans was carried out with adaptations from [29]. Briefly, 10 mg of the hydrolysed xylan was suspended in 200 μL of *N,N*-dimethylformamide (DMF), and stirred for 30 min at 50°C. Then, $\text{SO}_3\text{-Py}$ complex was added, and the solution was then stirred at 50°C for 2 h. The 1:1, 3:1, 6:1 and 9:1 $\eta\text{SO}_3\text{-Py:OH}$ ratios were evaluated. Next, 10% NaHCO_3 was added to interrupt the reaction, and the samples were dialyzed against tap water for 48 h and then distilled water for 48 h (six changes of water), using 3.5 kDa cut-off membrane, then freeze-dried.

2.2.4 Chemical sulfation in different reaction times

NSX was chemically sulfated using 6:1 $\eta\text{SO}_3\text{-Py:OH}$ ratio, at reaction times of 2, 4, 8 and 16 h, following the other conditions as described in section 2.2.2.

2.2.5 Homogeneity analysis and determination of molecular weight

Homogeneity analysis of the chemically sulfated xylans was performed by high performance size exclusion chromatography (HPSEC) in a WATERS equipment, with a differential refractive index (IR) detector. To determine the molecular weight (M_w) of the samples, the elution times of the peaks were compared with those of standard dextrans of 5.220, 9.400, 17.200, 40.200, 72.200, 124.000 and 266.000 g mol^{-1} obtained from Sigma. Analyses were performed using four Ultrahydrogel™ columns in series, with cut-off sizes of 7×10^6 , 4×10^5 , 8×10^4 , and 5×10^3 g mol^{-1} . A 0.1 mol L^{-1} sodium nitrite solution containing 0.2 g L^{-1} sodium azide was used as mobile phase, with a flow rate of 0.6 mL min^{-1} . The samples were solubilized using mobile phase as solvent, generating a concentration of 1 mg mL^{-1} , and filtered through cellulose acetate membranes with pores of 0.45 μm . Finally, 100 μL of the sample were injected for analysis.

2.2.6 Nuclear Magnetic Resonance (NMR)

1D and 2D NMR experiments were acquired in a Bruker AVANCE III HD 400 MHz spectrometer operating at 9.4 T and observing ^1H and ^{13}C at 400.13 and 100.62 MHz, respectively. The spectrometer was equipped with a 5 mm inverse detection multinuclear BBI probe with z-gradient. Each NMR tube was

filled with 10 mg of polysaccharide in 0.5 mL deuterated solvent. Partially hydrolysed xylans were dissolved in D₂O or DMSO-*d*₆. ¹H ¹³C correlation from heteronuclear single quantum coherence edited (HSQCed) NMR experiments were acquired using a gradient selected and sensitivity enhanced pulse program (*hsqcetgpsisp2.2*). The chemical shifts (δ) were expressed relative to acetone at 30.2/2.22 (¹³C/¹H) or DMSO-*d*₆ at 39.70/2.50 (¹³C/¹H) and the temperature of the experiments was set at 70 °C for unsulfated samples and 30 °C for sulfated samples.

To obtain the 2D Diffusion Ordered NMR Spectroscopy (DOSY) spectra, a Bruker LED pulse sequence (*ledbpgp2s*) was used. 2D DOSY spectra were baseline corrected and were calibrated to TSP signal at -9.3 m² s⁻¹ and 0 ppm. 10 mg of sulphated xylan was dissolved in 500 µL of D₂O, while 10 mg of unsulfated xylans were solubilized in 500 µL of DMSO-*d*₆. The temperature of DOSY experiments was set at 30 °C for all samples.

The molecular weight (M_w) was determined by diffusion coefficient D (m² s⁻¹), with a calibration curve [Log Mw x Log D] of dextrans from Sigma, with Mw of 5,220; 9,400; 40,200; 72,200; 266,000 and 487,000 g mol⁻¹ for unsulfated samples, while the M_w of sulfated samples was determined using a calibration curve of enoxaparin (4,500 g mol⁻¹), dalteparin (6,000 g mol⁻¹), tinzaparin (6,500 g mol⁻¹), bovine heparin (13,000 g mol⁻¹), and USP heparin (16,200 g mol⁻¹).

2.2.7 Degree of Substitution by Sulfate Groups (DS)

The sulfate content of the chemically sulfated xylans was determined using the turbidimetric barium chloride-gelatin method [36]. Samples were hydrolyzed overnight with 1 mol L⁻¹ HCl at 110 °C to release inorganic sulfate. The sulfate percentage of samples was quantified using a Na₂SO₄ calibration curve, and the degree of substitution (DS) was calculated according to a previously described procedure [37], using Eq. 1:

$$DS = (132 \times S\%)/(3200 - 102 \times S\%) \quad (1)$$

2.2.8 Anticoagulant assays

aPTT, PT and TT times were evaluated in a COAG-A-MATE XM coagulometer, using Hemosil® kits. For aPTT, 50 µL of citrated sheep plasma and 50 µL of chemically sulfated xylan in saline solution (to a final concentration of 1, 2, 3, 4, 5, 100, 200, 400 and 500 µg mL⁻¹) were incubated at 37 °C for 150 s. Then, 50 µL of aPTT reagent was added and incubated for 150 s. Finally, 50 µL of CaCl₂ (25 mmol L⁻¹) were added to start the clotting process. For PT test, 30 µL of citrated sheep plasma and 20 µL of chemically sulfated xylan in saline solution (to a final concentration of 20, 40, 50, 60 and 80 µg mL⁻¹) were incubated at 37 °C for 150 s. Then, 100 µL of PT reagent was added to start the clotting process. To carry out TT test, 80 µL of citrated sheep plasma and 20 µL of chemically sulfated xylan in saline solution (to a final concentration of 4, 6, 8, 10 and 12 µg mL⁻¹) were incubated at 37 °C for 60 s. Then, 100 µL of TT reagent at a final thrombin concentration of 3 UNIH mL⁻¹ was added to start the clotting process. Saline

solution was used to verify the normal clotting time on all tests (negative control). Enoxaparin (Versa®; 100 mg mL⁻¹, Eurofarma) was used as positive control. The results are expressed as mean ± SEM (n = 4) of T₁/T₀, with T₀= value (s) in the absence of the polysaccharide (blank), and T₁= value (s) in the presence of the polysaccharide.

2.3. *In vitro* FIIa and FXa assay

The assays were conducted in 96-well plates with a final reaction volume of 100 µL, composed of the following components: 25 µL of enzyme solution (FIIa or FXa), 25 µL of inhibitor or control solution (AT, HCII, UFH, LMWH, NXSo, or buffer), 25 µL of chromogenic substrate solution (S-2238 or S-2222), and 25 µL of Tris/HCl buffer (0.15 M, pH 7.4) containing 1 mg mL⁻¹ PEG 8000 and 0.15 M NaCl.

In thrombin (FIIa) inhibition assays, 6 nM human α-thrombin (FIIa, 5.7 mg mL⁻¹, Invitrogen) was incubated with either 100 nM antithrombin (AT, 8.3 mg mL⁻¹, Invitrogen) or 15 nM heparin cofactor II (HCII, 6.9 mg mL⁻¹, Invitrogen), in the presence of varying concentrations of NXSo (0.1–10.0 µg mL⁻¹), for 1 minute at 37°C. The reaction was initiated by the addition of the chromogenic substrate S-2238 (100 µM, Chromogenix), and absorbance was monitored at 405 nm for 15 minutes using a Spectra Max Plus 384 spectrophotometer.

For FXa inhibition assays, 8 nM human factor Xa (FXa, 7.2 mg mL⁻¹, Invitrogen) was incubated with 100 nM AT and varying concentrations of NXSo (0.1–10.0 µg mL⁻¹) under the same conditions described above, followed by addition of the chromogenic substrate S-2222 (100 µM, Chromogenix) and absorbance monitoring as previously described.

Negative control reactions were performed by replacing NXSo, AT, or HCII with buffer solution. As positive controls, NXSo was replaced by 1 µg mL⁻¹ of unfractionated heparin (UFH, Hepamax-S 5000 IU, Blau) or Enoxaparin (Versa, Eurofarma).

Residual enzymatic activity was calculated based on the slope of the linear portion of the absorbance curve as described by [38]. For each condition (n = 3), the residual activity of FIIa or FXa was expressed as mean ± standard error of the mean (SEM).

2.4. *In vivo* Experiments

Animal experiments were conducted using male Wistar rats (150–200 g) from the *vivarium* of Federal University of Paraná (UFPR), Curitiba, Brazil. They were maintained under standard laboratory conditions, 12 h light/dark cycle and a temperature of 22 ± 2 °C, with access to standard pellet food and water *ad libitum*. All experimental procedures were approved by the Institutional Ethics Committee of UFPR, with authorization number 1432, ensuring adherence to ethical standards throughout the study.

2.4.1. *Ex vivo* aPTT

Blood was collected (1 mL of blood in 100 µL of 3.8% sodium citrate) from the right carotid artery at 60, 120, and 180 min after subcutaneous administration (dorsal region) of saline (vehicle) or NXSo (5, 8, 10,

and 20 mg kg⁻¹), according to [39]. These early time points (1–3 h) were used both to establish the dose-response profile and to define the optimal NXSo dose. For blood collection, animals (n = 3 per group) were intramuscularly anesthetized, the right carotid artery was cannulated, and anesthesia was maintained throughout the experimental period. Following each withdrawal, blood was centrifuged at 5000 rpm for 15 min to obtain citrated plasma. aPTT was determined as described above. Results are expressed as mean ± SEM (n = 3 animals per group; quadruplicate determinations per animal). In addition, to evaluate the *ex vivo* aPTT profile over an extended period, blood was also collected at 6, 12, and 24 h after subcutaneous administration of 8 mg kg⁻¹ NXSo or 8 mg kg⁻¹ enoxaparin (Versa, Eurofarma) (n = 3 animals per time).

2.4.2 Antithrombotic assay

Venous thrombosis was induced in rats using a modified version of the method described by [40]. Animals were anesthetized via intramuscular injection, and the right carotid artery was cannulated for intravenous administration of treatments. The rats received saline (vehicle), NXSo (2, 4, or 8 mg kg⁻¹), or enoxaparin (Versa, Eurofarma; 2, 4, or 8 mg kg⁻¹), followed by thromboplastin (5 mg kg⁻¹) to induce coagulation. Next, the abdominal vena cava was exposed, and loose ligatures were placed around the vessel between the right renal and femoral veins, as well as around the left renal vein. This procedure isolated a segment of approximately 0.7 cm of the abdominal vena cava. After drug administration through the carotid artery, animals underwent a 5-minute circulation period. Thrombus formation was induced by thromboplastin injection, and 20 seconds later, vascular stasis was induced by tightening the ligatures, occluding the isolated segment. After 20 minutes, the formed thrombus was removed from the occluded segment, rinsed with saline, freeze-dried, and weighed. For each experimental group (n = 6), thrombus weight was determined (mean ± SEM) and expressed as a percentage relative to the control (vehicle-treated) group. The control group was defined as 100%.

2.4.3 Histopathological analysis

The skin nodules formed in the NXSo s.c. injection were surgically removed from three animals, and approximately 2 cm of each sample was stored in 4% formalin solution in carbonate buffer, pH neutral, for 48 h. Subsequently, the collected tissues were dehydrated and exposed to molten paraffin at 65 °C, using an automated apparatus. The total processing time was around 14 h, using the equipment OMA Tissue Processor, Model D40. The samples were cut in 4 µm slices (American Optical Manual Rotary Microtome, Model 820). The sections were stretched in a histological water bath maintained at 50 °C, fixed to glass slides and dried at 65 °C for 30 min. To eliminate the paraffin, the slides underwent immersion in a xylene solution at 65 °C for 5 min, and then staining with H&E (HE - Harris Hematoxylin and Eosin-Floxine at 2%).

2.4.4 Measurement of acute phase proteins and organ weight evaluation

Wistar male rats (~150 g) were divided into two groups (n = 9 per group). 1 mL of saline or NXSo (8 mg kg⁻¹) were subcutaneously administered in the dorsal region once a day, for 30 days. On the 29th day

the animals were fasted for 12 h, with water *ad libitum*. On the 30th day, they were intraperitoneally anesthetized, and the inferior vena cava was surgically exposed for blood collection (2 mL) for measurement of Haptoglobin (E-EL-R0473) and Rat C-reactive protein (E-EL-R0506), obtained from Elabscience (USA). After the euthanasia, with deepening anesthesia, the skin, lungs, kidneys, spleen, and liver were collected for organ weighing.

2.5. Molecular docking

2.5.1. Preparation of Macromolecular Systems and Ligands

A persulfated xylan pentasaccharide was employed as a low-molecular-weight structural model of NXSo for molecular docking studies. The three-dimensional coordinates of the protein receptors were obtained from the Protein Data Bank, corresponding to the codes 1TB6 (Antithrombin-Thrombin-Heparin Complex) [41] and 2GD4 (Antithrombin-Factor Xa-Pentasaccharide Complex) [53]. Polar hydrogens were added, and atomic partial Gasteiger charges were calculated, with the files converted to the .pdbqt format. The highly sulfated oligosaccharide ligands (heparin and pentasaccharide analogs) were treated as flexible ligands, with their roots detected and rotatable torsions parameterized for the same computational format.

2.5.2. Molecular Docking Protocol

Molecular docking simulations were performed using the search algorithm and scoring function of the AutoDock VinaCarb software. The definition of the search space (grid box) and operational parameters were customized for each system, aiming at adequate conformational sampling for the specific site topologies. For the 1TB6 system, the center of the search grid was positioned at Cartesian coordinates (X: 40.19, Y: 19.87, Z: 33.45), delimited within a volume of 20 x 35 x 35 Å. The exhaustiveness of the algorithm was set to 10, generating a maximum of 100 binding modes (*num_modes*), focusing on a rigorous exploration within a delimited pocket. For the 2GD4 complex, a significantly broader grid was adopted to explore a larger space at the protein interface, centered at (X: -63.67, Y: -14.35, Z: 10.80) with expanded dimensions of 45 x 65 x 65 Å. Exhaustiveness was set to 8, and the binding modes were limited to 10. In both protocols, the maximum energy range tolerance (*energy_range*) between the optimal pose and the lowest affinity conformation reported was fixed at 3 kcal/mol.

2.5.3 Analysis of Intermolecular Interactions

The poses with the most favorable predicted binding scores were selected for three-dimensional visual inspection and 2D topological extraction using Discovery Studio Visualizer 2025. Profiles of the hydrogen bond network (classic and carbon-hydrogen contacts), charge attraction interactions (salt bridges), and electrostatic map profiles were thoroughly mapped.

2.6. Statistical analysis

The data were expressed as the mean $\pm$ standard error of the mean (SEM). Statistical significance with $p < 0.05$ was determined using t Student or one-way analysis of variance (ANOVA) followed by Tukey's test, performed with GraphPad Prism 8.0.1 software.

3. Results and Discussion

3.1 Partial Hydrolysis and Chemical Sulfation

The NX used in this study was previously extracted from açai (*Euterpe oleracea* Mart.) fruit pulp. The NX was structurally characterized by ^{13}C NMR, revealing a β -D-(1 $\rightarrow$ 4)-linked xylan backbone, mainly composed of xylose ($\sim 97\%$) and glucuronic acid ($\sim 2.8\%$), evidencing a predominantly linear structure with minor substitutions [33].

To fully explore the anticoagulant activity of this polysaccharide, the NX was initially submitted to partial acid hydrolysis to reduce the molar mass. The NX was hydrolysed under 4 different experimental conditions according to HCl concentration and hydrolysis time as displayed in Table S1.

Afterwards, all the partly hydrolysed fractions underwent the sulfation process using 9:1 $\eta\text{SO}_3\text{-Py:OH}$ ratio. Following the reactive process, all samples exhibited water solubility and were analysed by SEC. The results are presented in Fig. S1 and Table S3. The elution profile displays similar distribution between all samples and enoxaparin (Fig. S1A). The sulfated samples showed a pronounced M_w reduction compared to their non-sulfated precursors.

The sample hydrolysed at 1.0 mol L^{-1} HCl for 90 min and subsequently sulphated with 9:1 $\eta\text{SO}_3\text{-Py:OH}$ ratio was selected for the next steps - henceforth designed as NXS. Prior to the sulfation process, the HSQCed NMR spectra of this sample showed correlations at δ 101.4/4.30 (C1/H1), 75.5/3.54 (C4/H4), 74.1/3.31 (C3/H3), 72.6/3.10 (C2/H2), 63.1/3.91 (C5/H5eq) and 63.1/3.22 (C5/H5ax), a typical profile of β -D-(1 $\rightarrow$ 4) xylans [18] (Fig. S1B blue and green). After the sulfation reaction, the sample exhibited five $^{13}\text{C}/^1\text{H}$ correlations at δ 99.5/5.13 (C1/H1), 73.0/4.43 (C2/H2), 72.5/4.75 (C3/H3), 72.3/3.93 (C4/H4), 58.9/4.39 (C5/H5eq) and 58.9/3.81 (C5/H5ax), displaying significant shifts before the procedure [34, 41], indicating effective sulfation (Fig. S1B red and pink). The absence of other $^{13}\text{C}/^1\text{H}$ correlations is suggestive of homogeneous sulfation pattern in NXS, with a DS of 0.45 ± 0.05 (Table 1–9:1 $\eta\text{SO}_3\text{-Py:OH}$ ratio with reaction time of 2h).

Table 1
Degree of substitution (DS) and anticoagulant activity (aPTT slope) of the samples obtained at different SO₃·Py/OH molar ratios and reaction times.

SO ₃ ·Py/OH ratio	Reaction time (h)	DS	aPTT slope
1:1	2	0.24 ± 0.04	2.8 ± 0.1 × 10 ⁻³
3:1	2	0.34 ± 0.07	2.9 ± 0.03 × 10 ⁻¹
6:1	2	0.45 ± 0.06	3.1 ± 0.05 × 10 ⁻¹
9:1	2	0.45 ± 0.05	2.7 ± 0.2 × 10 ⁻¹
6:1	4	0.51 ± 0.06	4.3 ± 0.1 × 10 ⁻¹
6:1	8	0.62 ± 0.07	3.0 ± 0.2 × 10 ⁻¹
6:1	16	0.73 ± 0.04	2.4 ± 0.2 × 10 ⁻¹

3.2 *In vitro* anticoagulant activity

The anticoagulant activity of NXS was assessed by the aPTT analysis [42].

To verify the impact of DS on the aPTT activity, the sulfation reaction conditions were varied using different molar ratios of SO₃·Py:OH (1:1, 3:1, 6:1, and 9:1) with a fixed reaction time of 2 h. Among these samples, the 6:1 ratio exhibited the highest slope in the aPTT assay ($3.1 \pm 0.05 \times 10^{-1}$), indicating superior anticoagulant performance compared to other conditions as well as enoxaparin (Fig. S2). It is possible that the sulfating agent excess in the 9:1 ratio destabilized labile sulfate esters via hydrolysis, which were subsequently removed during dialysis, thereby maintaining the DS with similar values compared to 6:1 ratio, despite the greater reagent availability [43].

Following, the sample prepared using 6:1 ratio was further investigated under different reaction times (4, 8, and 16 h) to determine optimal slope. The DS value for each condition gradually increased from 0.45 to 0.73 at reaction times of 2h and 16 h, respectively (Table 1). However, despite the increase in DS, no further improvement in the aPTT slope was observed, suggesting that functional sulfation motifs relevant for anticoagulant activity are established early in the reaction, whereas additional substitution at prolonged reaction times provides no significant contribution to the anticoagulant activity [42].

The sample obtained at a 6:1 ratio with a 4 h reaction time showed the best aPTT performance (Fig. 1A), with T_1/T_0 of $4.3 \pm 0.1 \times 10^{-1}$ - henceforth designed as NXSo. Following, the NXSo was also assessed by the PT and TT (Fig. 1B and C). The results show that the sample increases the PT compared to enoxaparin, though in a lower concentration regime, whereas TT revealed a similar profile between these two samples. These findings indicate that NXSo interferes with coagulation parameters involving the intrinsic and common pathways and suggest a relevant effect on thrombin-dependent clot formation.

Structurally, the NXSo sample presents a dense and relatively homogeneous substitution of sulfate groups along the β -(1→4)-linked xylose backbone at the C-2 and C-3 hydroxyl positions, displaying

significant shifts before the sulfation procedure [34]. This configuration may generate a high negative charge density and an extended molecular conformation in solution, which maximizes electrostatic interactions with coagulation cofactors [44]. To confirm the M_w of NXSo, a DOSY study was performed, and the diffusion coefficient ($m^2 s^{-1}$) of standard glycosaminoglycans was used to prepare a calibration curve. The M_w of NXSo was determined as $9.730 g mol^{-1}$ ($D = 9.078 \times 10^{-11} m^2 s^{-1}$) (Fig. S3).

3.3 Anti-FIIa and Anti-FXa effects

In general, the anticoagulant mechanism of heparins and others sulfated polysaccharides involves binding to AT and HCII, enhancing their inhibitory effects over FIIa and/or FXa [45]. Therefore, the effect of XS on FIIa and FXa activities was evaluated in the presence or absence of AT and HCII.

As displayed in Fig. 2, NXSo significantly enhanced the inhibitory effect of AT on FIIa in a dose-dependent way: the FIIa residual activity decreased to $\sim 36\%$ and $\sim 6\%$ in the presence of NXSo at 1 and $10 \mu g mL^{-1}$, respectively (Fig. 2A). Comparatively, enoxaparin increases the AT effect up to $\sim 42\%$, whereas UFH causes similar effect enhancing it to $\sim 8.3\%$, both at $1 \mu g mL^{-1}$ (Fig. 2B). The residual activity of FIIa was not affected by the presence of HCII, even when enoxaparin and UFH were added (Fig. 2C and D). However, in the presence of both NXSo and HCII, the residual activity of FIIa decreased to about 35% at all concentrations.

In addition, the NXSo sample prepared at $10 \mu g mL^{-1}$ significantly inhibited the FXa activity, even in the absence of AT, reducing it to $\sim 20.5\%$, suggesting that NXSo may also interfere with FXa activity through a serpin-independent component (Fig. 2E and F). Furthermore, in the presence of AT, NXSo also reduces the enzyme activity, from 0.1 to $10 \mu g mL^{-1}$.

NXSo enhanced the anti-FIIa activity of AT, possibly via a ternary complex as previously observed by [41]. NXSo also showed direct anti-FXa activity in absence of AT. This effect is probably related to the high negative charge density, which enables direct electrostatic interaction with cationic domains of FXa [46]. Such AT-independent inhibition indicates that NXSo exerts anticoagulant activity not only via the classical AT-mediated mechanism but also through direct modulation of coagulation proteases. Moreover, different from the heparins, NXSo enhanced the anti-FIIa activity of HCII in a dose-independent way. This dose-independent profile suggests that NXSo may contain sulfated motifs capable of enhancing HCII-mediated thrombin inhibition, although direct NXSo-HCII binding was not experimentally demonstrated. In addition, its relatively low molar mass and predominantly linear structure may favor continuous and accessible anionic domains, facilitating direct and homogeneous interactions with HCII [47].

3.4 Anticoagulant Effect After NXSo Subcutaneous Injection

The potential anticoagulant effect via subcutaneous administration of NXSo was evaluated in an *ex vivo* experiment in rats. As shown in Fig. 3A, the administration of NXSo significantly increases the aPTT

compared to the saline group: at 5 mg kg^{-1} at all time intervals, NXSo increased the aPTT by 2-fold ($41 \pm 7\text{s}$ vs. $19 \pm 1\text{s}$, respectively). At 8 mg kg^{-1} , the anticoagulant effect augments by 2.8-fold, with $55 \pm 5\text{s}$ at 60 min, although no statistical difference is observed for 120 and 180 min, with $76 \pm 13\text{s}$ and $79 \pm 17\text{s}$, respectively. A further concentration increment, to 10 mg kg^{-1} , causes a 4.2-fold change compared to saline by revealing higher aPTT values: $84 \pm 13\text{s}$ at 60 min, $152 \pm 52\text{s}$ at 120 min and $195 \pm 50\text{s}$ at 180 min. The highest NXSo concentration leads to a 10-fold increase in aPTT for the 60 min time interval, with $212 \pm 70\text{s}$.

The anticoagulant effect of NXSo reached a plateau at 3 h for the doses of 5 and 8 mg kg^{-1} , whereas at 10 and 20 mg kg^{-1} the aPTT values continued to increase beyond this time point. For the lower doses, the rapid absorption enabled early attainment of peak plasma levels and saturation of serpin targets, explaining the stabilization of the effect at 3 h [48]. The delayed increase in aPTT observed at 10 and 20 mg kg^{-1} is probably related to differences in absorption from the injection site, local retention, or dose-dependent systemic exposure. These mechanisms may have sustained systemic availability of NXSo beyond 3 h, shifting the pharmacodynamic peak.

To evaluate the anticoagulant effect, the animals were treated with NXSo or enoxaparin (both at 8 mg kg^{-1}), since in clinical practice an anticoagulant is expected to prolong the activated partial thromboplastin time (aPTT) by approximately 1.5–2.5 fold [49], and the effect was subsequently monitored over a 24 h period (Fig. 3B).

The anticoagulant activity was sustained by both samples by 12h with ≥ 1.5 -fold higher compared to the control group. Possibly, the predominant linear conformation of NXSo combined with a more uniform sulfation pattern provides a higher density of accessible negative charges, favoring immediate interaction with coagulation proteins. In contrast, enoxaparin is composed of a heterogeneous mixture of oligosaccharide chains with variable sulfation motifs, resulting in distributed affinities for different binding sites. These data suggest that the samples provide a locally sustained release of the active components. After 24 hours, however, there's no significant difference between the samples and the control group, indicating that the measurable anticoagulant effect had returned toward baseline by 24 h.

The antithrombotic effect of NXSo was evaluated in a rat model of venous thrombosis induced by stasis and thromboplastin, and the results are presented in Fig. 3C. Accordingly, 100% of thrombosis corresponds to the thrombus weight of the saline group, with $8.0 \pm 3.7 \text{ mg}$ in accordance with previous reports [50]. NXSo reduced thrombus formation by 93.2% at 8 mg kg^{-1} . In comparison, the animals treated with enoxaparin showed complete inhibition of thrombus formation at the same doses.

As exhibited in Table S4, there was no modification in the weight of any of the evaluated organs. The absence of changes in the weight of organs suggests that the polysaccharide did not induce organomegaly, atrophy, or gross pathological alterations in the main target organs of systemic toxicity [51]. Additionally, the concentrations of acute-phase proteins such as haptoglobin and CRP remained unchanged. These findings indicate that NXSo has relevant anticoagulant and antithrombotic activity,

but its parenteral tolerability requires formulation optimization before further therapeutic development. [52].

3.5 Subchronic subcutaneous NXSo administration induces localized inflammatory nodules with post-treatment regression

To evaluate possible side effects of a continuous therapy with NXSo, the polysaccharide was daily administered in rats at a dose of 8 mg kg⁻¹ subcutaneously, alternating the injection site daily in the dorsal area. After 30 days of treatment, hematological and biochemical parameters, and acute phase proteins were analyzed, the animal organs weighed, and skin histological analyzes were performed.

However, after the 10th day of treatment, 88% (n = 8) of the animals treated with NXSo exhibited a firm and well-circumscribed nodules in the injection locals. The palpable nodules were painless, without ulceration, fluid drainage, hyperthermia, or alopecia. Histologically this tissue presented pyogranulomatous panniculitis, along with diffuse and pronounced necrosis and hemorrhage on the dorsal skin region (Fig. 4). These alterations are compatible with the hematological findings, mainly leukocytosis (Table S4). It is worth mentioning that throughout the treatment, the rats maintained normal behavior and regular ingestion of water and food.

To monitor the reversible or permanent character of dermal lesions, two animals treated with XS were followed for more 20 days to evaluate the nodules post-treatment dynamics. From the 5th day the lesions started regression, and on the 20th day the nodules were no longer visible macroscopically. Subsequent histological analyzes revealed infiltrates that were multifocal, coalescing, and discrete in the dermis, along with rare perivascular mast cells in degranulation, and residual histological changes were less pronounced after the recovery period (Fig. 4D). These findings indicated that the lesion was self-limiting and healed spontaneously, suggesting a benign course.

The mechanism underlying the local reaction remains uncertain. A sterile inflammatory response to a highly anionic polysaccharide, local accumulation of the compound, residual processing-related impurities, solvent or reagent traces, or higher-molecular-weight fractions may have contributed to tissue reactivity. Histologically, these lesions are marked by foamy macrophage infiltration and granulomatous architecture, consistent with an immune reaction to localized, non-biological stimuli [58]. Although not detected in the current analytical workflow, the presence of such impurities cannot be entirely excluded and may help explain the sterile inflammatory response observed. These preliminary findings highlight the need for further refinement of the purification process and deeper characterization of the compound, aiming to improve its biocompatibility and reduce potential local tissue reactivity.

3.6 Hematological and biochemical effects of subchronic NXSo administration

As indicated in Table S4, NXSo treatment did not induce thrombocytopenia. In contrast, hematological adverse effects associated with the long-term use of enoxaparin or PPS are well-established, with a notable mention of heparin-induced thrombocytopenia (HIT), commonly arising from the fifth day of treatment [59]. Similarly, adverse effects linked to the prolonged use of PPS have also been reported, including thrombocytopenia and alterations in liver tests in humans [28].

Despite the increase in granulocyte and monocyte counts induced by NXSo treatment, along with mild anemia, the concentrations of acute-phase proteins such as haptoglobin and CRP remained unchanged (Fig. S4). This suggests the absence of a significant inflammatory process or cardiovascular impairment [60].

Regarding the biochemical parameters, 30 days treatment with NXSo induced an increase in plasmatic GGT levels (Table S5), while the levels of the other hepatic biomarkers (e.g. ALT, AST and bilirubin) remained unaltered. Significant alterations in liver markers have been observed in patients using LMWH, associated with an increase in the HMGB1 (high mobility group box 1 protein), acting as a signal of the innate immune response [61]. These characteristics are associated with extensive liver damage that has not been observed in animals treated with NXSo.

Interestingly, subchronic treatment with 8 mg kg^{-1} XS induced a significant reduction in glycemia (-48%), triglycerides (-53%) and VLDL (-53%) levels (Table S5). These results indicate a prospective investigation of NXSo as a hypoglycemic and hypolipidemic agent. These findings align with existing evidence that modified and natural sulfated polysaccharides have previously demonstrated hypoglycemic and hypolipidemic properties. For instance, low molecular weight fucoidans have been shown to improve glucose metabolism and lipid profiles in diabetic rat models [62].

3.5 Docking Simulations

Docking simulations results (Table 2) demonstrate thermodynamic affinity driven by the high density of negative charges inherent to poly-sulfated oligosaccharides.

Table 2
Molecular docking binding affinities ($\text{kcal}\cdot\text{mol}^{-1}$) of heparin and a persulfated xylan pentasaccharide model (NXSo) against antithrombin-containing crystallographic complexes.

Code	Target complex	Heparin	NXSo
1TB6	Antithrombin-Thrombin	-6.6	-7.2
2GD4	Antithrombin-FXa	-7.2	-6.7

The docking results revealed predicted binding scores in a range similar to those obtained for heparin-derived ligands under the same computational protocol, indicating that the persulfated xylan pentasaccharide model is capable of occupying canonical glycosaminoglycan-recognition sites. The analysis of the two-dimensional interactions of heparin in the 1TB6 active site (Fig. 5A-B) confirms the

accentuation of residues with basic character anchoring the molecule. The formation of conventional hydrogen bonds with Thr386, Asn233, Gly219, Ser230, and Trp60D was identified. Furthermore, stabilizing network of electrostatic attractions is observed pairing the sulfate groups directly to the basic residues of the receptor, through arginines (Arg97, Arg393, Arg221A) and Lys275. Van der Waals contacts (Val389, Ser227, Lys226) complement the overall stabilization. This interaction profile is consistent with the well-established mechanism of heparin recognition by antithrombin-containing complexes, in which sulfate groups are preferentially coordinated by clusters of basic residues, particularly arginines [41, 44].

(A) Three-dimensional binding pose of heparin. (B) Two-dimensional interaction map of heparin. (C) Three-dimensional binding pose of the persulfated xylan pentasaccharide model (NXSo). (D) Two-dimensional interaction map of NXSo.

Inspection of the docking of the 1TB6-NXSo reveals a divergent anchoring mode within the macromolecular interface in comparison with its mode on heparin (Fig. 5C-D). Unlike heparin ligand, the primary electrostatic recognition abandons the strict dependence on arginines and becomes coordinated almost exclusively by lysine residues, specifically Lys136, Lys139, and Lys193, through strong hydrogen bonds. The thermodynamic and mechanistic aspect that emerges from this system is the explicit participation of water molecules (HOH872 and HOH885) in mediating polar interactions. These structural waters act as hydration bridges, connecting the polar groups of the analog to the receptor network (e.g., Asn127 and Asn144). Additionally, the Tyr131 residue contributes significantly to the stabilization of the pose through conventional hydrogen bonds.

Docking analysis of NXSo pentasaccharide performed in the 2GD4 system demonstrated that the pentasaccharide pose remains in the central binding groove (Fig. 6A-B). This orientation is primarily supported by structural hydrogen bonds involving Ile22, Asn96, Gly328, Asp327, Asn75, and Ser330. The major saline electrostatic component of this system is far more focused, centering on the interaction with a single prominent basic residue, Lys370. These findings indicate a greater contribution of neutral polar interactions in this system, with hydrogen-bond networks playing a dominant role in ligand accommodation.

The inclusion of NXSo derivative in the scope of the analysis reveals the plasticity of heparin-binding sites. That is, the direct comparison elucidates distinct pharmacophoric strategies operating in the stabilization of these polymers. While heparin in 1TB6 predominantly recruits arginines, favoring rigid bidentate interactions with the sulfates, NXSo optimizes its pose through lysines. This shift suggests that the ligand's topology dictates the need for an amino acid side chain with greater conformational flexibility, such as lysine, to properly accommodate the specific sulfation pattern of the NXSo derivative. Such differences suggest that anticoagulant activity is not determined solely by sulfate density but also by the three-dimensional distribution of anionic groups and their compatibility with the local architecture of the binding pocket [23, 34, 46].

Furthermore, water-mediated interactions in the 1TB6-NXSo complex highlights a clear enthalpic-entropic compensation. The immobilization of water molecules (HOH872 and HOH885) in the binding site incurs an entropic penalty, which must be overcome by the enthalpic gain of the newly formed hydrogen bonds, conferring high specificity to the complex. Moreover, both 1TB6 and 2GD4 systems appear to rely more on solvent exclusion (the hydrophobic effect) and direct ligand-protein contacts. Finally, in contrast to the massive ionic network observed in the 1TB6 complexes, the anchoring in the 2GD4 system relies more prominently on neutral polar interactions provided by residues such as asparagine and serine, being electrostatically anchored solely by Lys370. Therefore, the structural rationalization for the design of new biomaterials or inhibitors inspired by these polysaccharide scaffolds must go beyond the simple maximization of anionic charge density. Success in modulating affinity will depend on strict control of the steric arrangement to selectively exploit specific basic residues (Arg vs. Lys) and consider the strategic recruitment of hydration waters at the binding interface.

4 Conclusions

This study demonstrates that a low-molecular-weight sulfated xylan derived from *Euterpe oleracea* exhibits relevant anticoagulant and antithrombotic activity, associated with a sulfated β -(1 $\rightarrow$ 4)-xylan backbone and functional modulation of FIIa/FXa-related pathways. NXSo prolonged ex vivo aPTT after subcutaneous administration and reduced thrombus formation in a rat venous thrombosis model. However, repeated subcutaneous administration induced localized inflammatory nodules with histological evidence of panniculitis, necrosis, and hemorrhage, indicating that parenteral tolerability remains a key limitation. Overall, NXSo represents a promising non-animal sulfated polysaccharide scaffold for further anticoagulant development, particularly after formulation and safety optimization.

Declarations

Author Contribution

GSM: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing—original draft, Writing—review and editing. GLS: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing. GFP: Methodology, Investigation, Formal analysis, Writing—review and editing. LRAM: Conceptualization, Formal analysis, Writing—review and editing. AA: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Validation, Visualization, Writing—review and editing. GCCG: Writing—review and editing. PRB: Methodology, Investigation, Writing—review and editing. JTL: Methodology, Investigation, Writing—review and editing. TMCJ: Writing—review and editing. LMCC: Writing—review and editing. KMO: Methodology, Investigation, Formal analysis, Writing—review and editing. RS: Methodology, Formal analysis, Visualization, Writing—review and editing. TRC: Conceptualization, Formal analysis, Funding acquisition, Investigation, Methodology, Project

administration, Resources, Supervision, Validation, Visualization, Writing—original draft, Writing—review and editing.

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Data Availability

The data generated and analyzed during the current study are available within the article and its Supplementary Information files. Additional data supporting the findings of this study are available from the corresponding author upon reasonable request.

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Figures

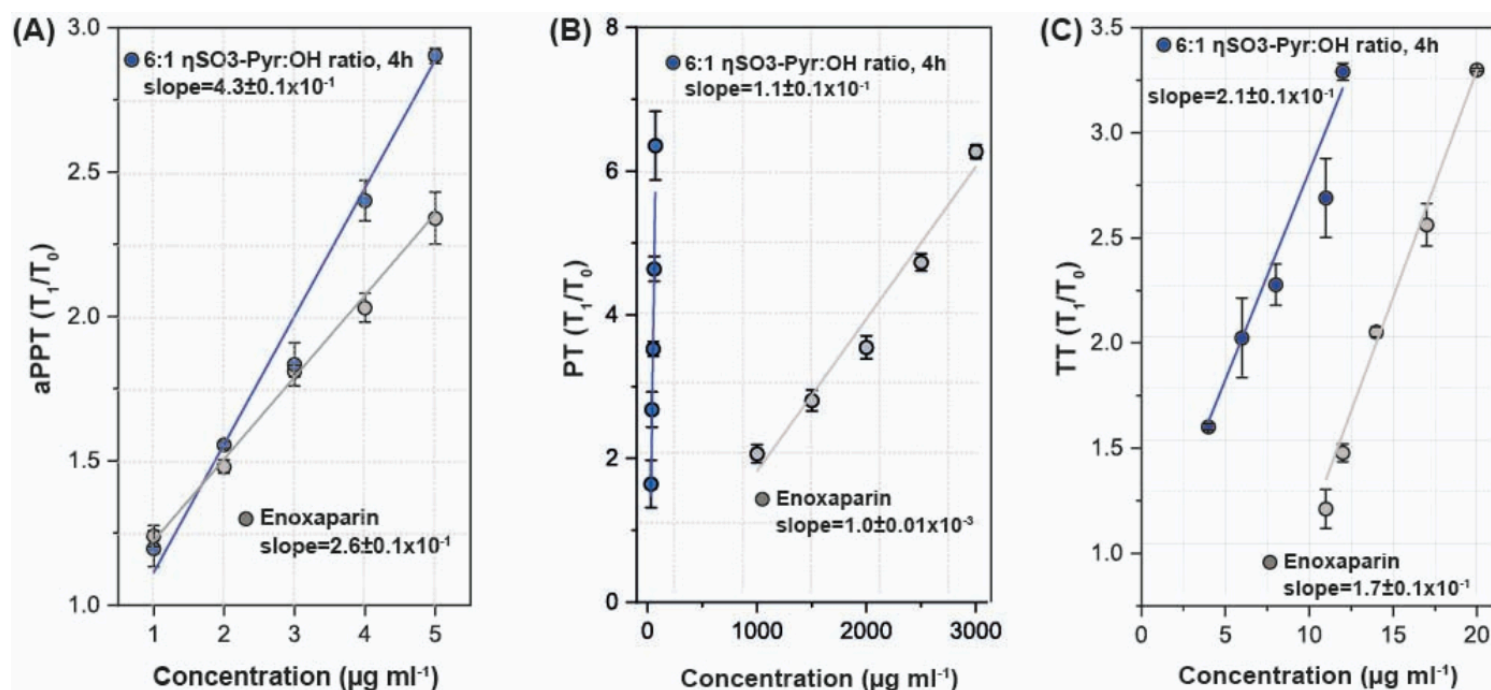


Figure 1

Dose-response curves of aPTT (A), PT (B) and TT (C) with NXSo and enoxaparin. aPTT in the absence of NXSo was 41.9 ± 1.6 s; PT in the absence of NXSo was 13.7 ± 0.4 s; TT in the absence of NXSo was 14.3 ± 1.4 s. All data are expressed as mean $\pm$ SEM (n=4) of T_1/T_0 .

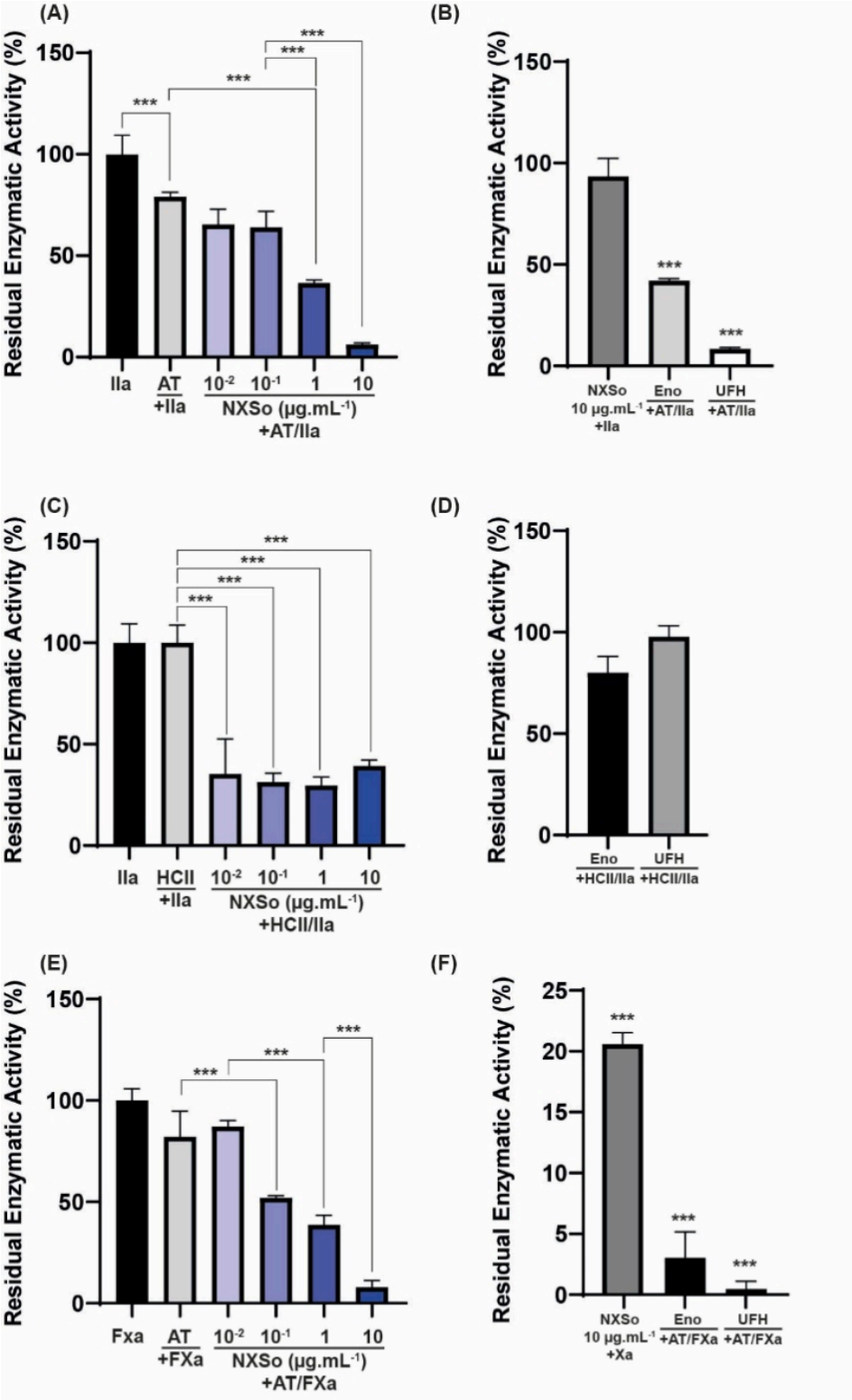


Figure 2

Effect of NXSo on FIIa and FXa activities. Residual enzymatic activity of FIIa in the presence of AT with NXSo, Enoxaparin at 1 $\mu\text{g mL}^{-1}$ (Eno) or UFH 1 $\mu\text{g mL}^{-1}$ (A and B). Residual enzymatic activity of FIIa in the presence of HCII with NXSo, Enoxaparin at 1 $\mu\text{g mL}^{-1}$ (Eno) or UFH 1 $\mu\text{g mL}^{-1}$ (C and D). Residual enzymatic activity of FXa in the presence of AT with NXSo, Enoxaparin at 1 $\mu\text{g mL}^{-1}$ (Eno) and UFH 1 $\mu\text{g mL}^{-1}$ (E and F). All data is expressed as mean SEM (n=3). *** p<0.001

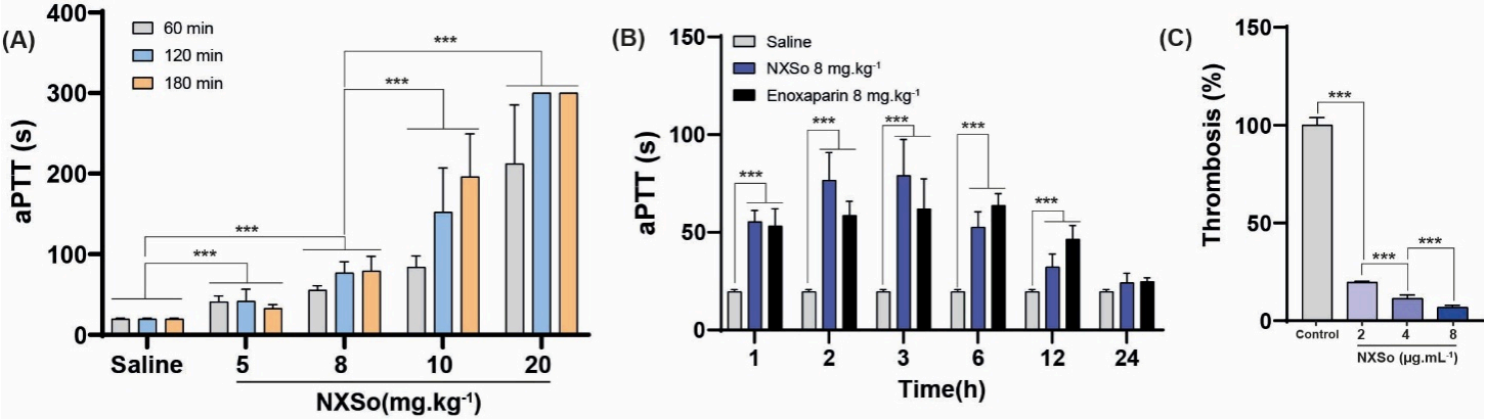


Figure 3

Ex vivo aPTT and antithrombotic effects of NXSo in rats. Different concentrations of NXSo tested up to 180 min after subcutaneous administration (A). aPTT time-dependent analysis of NXSo and enoxaparin at 8 mg kg⁻¹ after subcutaneous administration (B). Antithrombotic effect of NXSo in rats (C). All results are expressed as mean±SEM (n=3 animals per group; for each animal, aPTT was evaluated in quadruplicate). *** p<0.001.

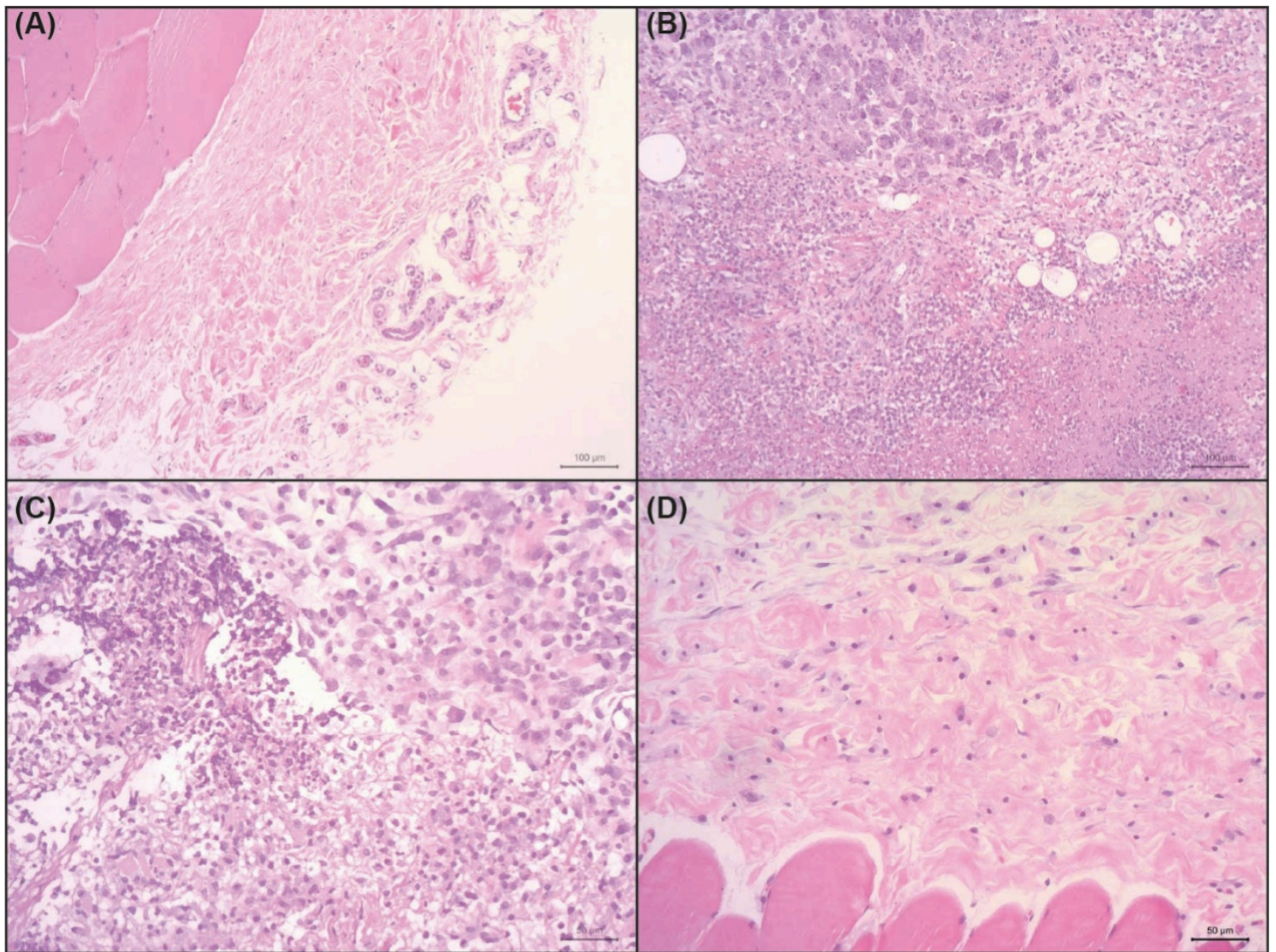


Figure 4

Skin histopathology of a saline-treated, XS-treated and a recovered animal. Saline-treated animal, showing dermis and skeletal muscle without changes (hematoxylin and eosin; original magnification 100×) (A). XS-treated animal, showing macrophages and neutrophils obliterating the subcutaneous tissue with areas of liquefactive necrosis (hematoxylin and eosin; original magnification 100×) (B). Epidermis and dermis, unchanged. XS-treated animal, showing macrophages and neutrophils obliterating the subcutaneous tissue with proliferation of fibroblasts and areas of liquefactive necrosis (hematoxylin and eosin; original magnification 200×) (C). Epidermis and dermis, unchanged. Tissue of an animal in recovery, showing foamy macrophages infiltrating the dermis with multifocal to coalescent distribution (hematoxylin and eosin; original magnification 200×) (D).

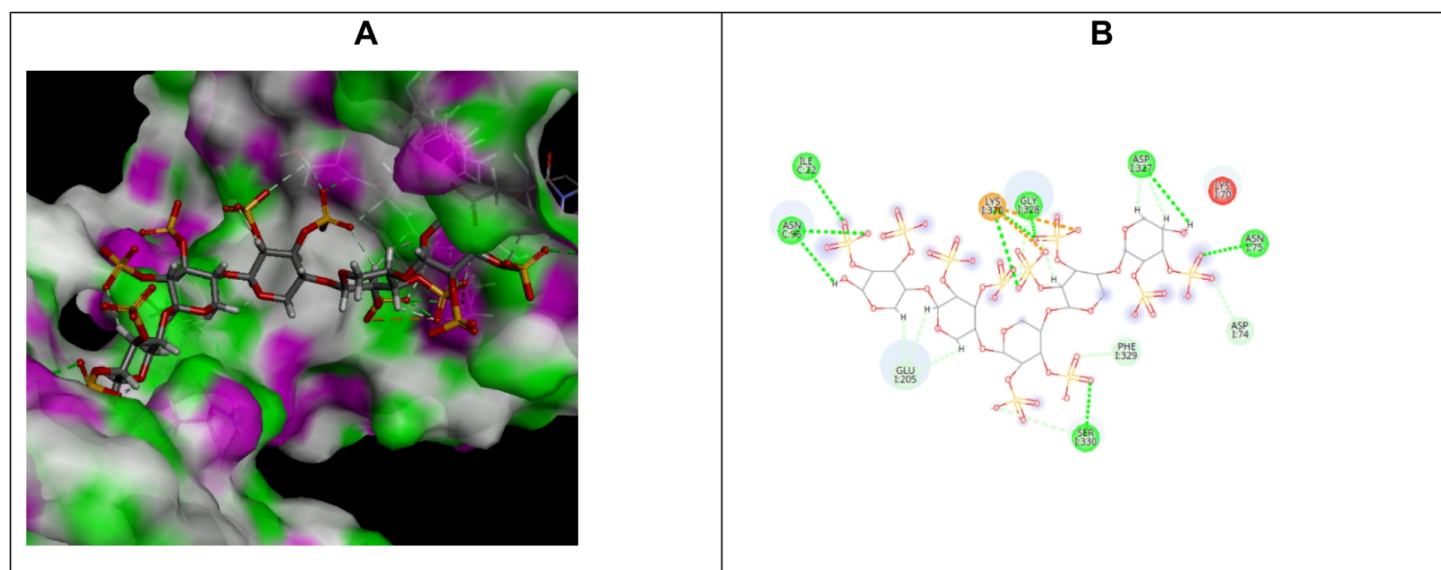


Figure 6

Molecular docking analysis of the persulfated xylan pentasaccharide model in the antithrombin–factor Xa complex (PDB: 2GD4). (A) Three-dimensional binding pose within the glycosaminoglycan-binding groove. (B) Two-dimensional interaction map highlighting hydrogen-bond and electrostatic interactions.