INTRODUCTION. The increased-incidence of cardiovascular/thromboembolic-diseases in recent-times and the restricted-administration of heparin-HEP due to its risks (e.g., bleeding-complications/thrombocytopenia) indicate the need to look for alternative-sources of anticoagulants. Acanthophora muscoides (Rhodophyta) contains sulfated-galactans (Am-SGs) that exhibit both anticoagulant/procoagulant-effects. However, their in vitro-effects on thrombin-generation-(TG)-assays, important-tools to measure the ability of a plasma-sample to generate-thrombin, have not been demonstrated. OBJECTIVE. This study evaluated the in vitro-anticoagulant/procoagulant-effects of an Am-4,6-pyruvylated-SGs-fraction on TG in 60-fold-diluted-human-plasma using chromogenic-method by a continuous-detection-system. MATERIALS AND METHODS. Am-SGs were obtained by papain-digestion, and then fractionated by DEAE-cellulose using a NaCl-gradient (0→1M, with 0.25M of intervals). Chemical-analyses of sulfate and hexose and procedures of agarose-gel-electrophoresis and polyacrylamide-gel-electrophoresis of the fractions were performed and compared to standards HEP, chondroitin-6-sulfate.60kDa, chondroitin-4-sulfate.40kDa and dextran-sulfate.8kDa. D2O-solution-Nuclear-Magnetic-Resonance-(NMR:1H, HSQC, COSY, TOCSY and HMQC)-experiments of a rich-fraction (named Am-II,5mg) were performed using Bruker-DRX-800-MHz-apparatus with a triple-resonance-probe. Activated-partial-thromboplastin-time-(APTT) and prothrombin-time-(PT) tests were also conducted using plasma and HEP(193IU/mg). TG-assay was performed in a microplate-format containing cephalin-or-thromboplastin.830µg/well-plate(10μL)/without-activation+Tris HCl/PEG-buffer(30µL,pH7.4)+polysaccharides(Am-II:0.4,1,8.3,41.6 and 83.3µg/well-plate) or HEP:2µg/well-plate)(10µL)+CaCl2(20mM)/substrate.S2288(0.33mM)(60µL). The in vitro-reaction was triggered (37°C) by addition of plasma(10μL), and the absorbance (405nm) was recorded for 80min. The inhibition-or-stimulation of TG by polysaccharides was determined by peak-thrombin, endogenous-thrombin-potential and time-to-peak. DISCUSSION AND RESULTS. Fractionation of Am-SGs by DEAE-cellulose yielded-Am-I(0.5M),-II(0.75M) and-III(1M), respectively, containing differences among the relative-proportions of sulfate (3.6-22.5%) and total-sugars(18-47.4%), and themselves revealing marked differences in charge-density and heterogeneous-molecular-weight based on electrophoretic-analyses, respectively, when compared with standards. NMR-analyses of Am-II revealed GSs with highly-heterogeneous-chemical-structure, and presenting 4,6-pyruvated-sugar-residues. Modest-anticoagulation on APTT (1.5-3IU mg⁻¹ for Am-SGs-fractions, respectively) and lacking on PT, compared with HEP, were recorded. Regarding the TG-
parameters, the dual-potency of Am-II was concentration-dependent, whereas TG in plasma in the presence of HEP was abolished. Findings indicate balance between in vitro-procoagulant and anticoagulant-effects of Am-II. CONCLUSION. Method could be used to examine the Am-SGs-anticoagulation/procoagulation in both intrinsic/extrinsic-pathways parallelly-HEP-testing as a function of its inhibitory-response.

Keywords: coagulation-pathways, polysulfated, thrombin.

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