GLYCOSAMINOGLYCANS FROM OREOCHROMIS NILOTICUS SKIN: ISOLATION, PHYSICAL-CHEMICAL CHARACTERIZATION AND IN VITRO INHIBITION OF THROMBIN GENERATION USING A CONTINUOUS ASSAY


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INTRODUCTION. Glycosaminoglycans-(GAGs) comprise the extracellular-matrix of animal-tissues, and residues derived from processing fish commercially-important-harvested may offer potential-therapeutic-alternatives to heparin-(HEP), a drug widely used in diverse clinical-practices with high-rates of bleeding-episodes. Thrombin-generation-(TG)-inhibition-protocols provide relevant-data to analysis alternative-sources of anticoagulants. OBJECTIVE. This study isolated, physically-chemical characterized and evaluated the in vitro-inhibitory-effects of TG by GAGs-fractions from the skin of Nile Tilapia, Oreochromis niloticus-(On-GAGs), using a continuous-detection-assay. MATERIALS AND METHODS. Specimens of O. niloticus-(Weigh:495.6±58.13g;Total-length:29.90±0.87cm,n=5) were obtained from experimental-farming at Federal University of Ceará. On-GAGs were extracted with papain-digestion-(24h,60°C) in 100mM-sodium-acetate-buffer-(pH5) containing cysteine+EDTA(both-5mM), followed by anion-exchange-chromatography-(DEAE-cellulose) applying a NaCl-stepwise(0→0.75M, with 0.25M of intervals). Fractions were lyophilized and submitted to both 0.5%-agarose-gel-electrophoresis and 6%-polyacrylamide-gel-electrophoresis-(PAGE) and compared to standards HEP, chondroitin-4-sulfate-(40kDa), chondroitin-6-sulfate-(60kDa) and dextran-sulfate-(8kDa). The in vitro-anticoagulant-effects were assessed by both activated-partial-thromboplastin-time-(APTT) and prothrombin-time-(PT)-tests using citrated-normal-human-plasma and unfractionated-HEP. TG-protocol was carried-out in a microplate containing cephalin-or-thromboplastin.830-μg/well-plate(10μL)+Tris-HCl/PEG-buffer-(30μL,pH7.4)+polysaccharides-(On-GAGs:0.4,1,8,3,41.6 and 83.3μg/well-plate or HEP:2μg/well-plate)(10μL)+CaCl₂(20mM)/substract.S2288(0.33mM)-(60μL). The in vitro-reaction was triggered (37°C) by addition of 60-fold-diluted-plasma-(10μL), and the absorbance (405nm) was recorded for 60min. The inhibition of TG by polysaccharides was determined by peak-thrombin, endogenous-thrombin-potential and time-to-peak. RESULTS AND DISCUSSION. The yield was 0.095%-On-GAGs. The chromatographic-profile showed two-fractions-(On-I(52.97%)-0.5M and On-II(6.48%)-0.75M of NaCl, respectively), revealing between them different-pattern on charge-density and mobility similar to chondroitin-4-sulfate by agarose-gel, and a dispersion in their molecular-masses as chondroitin-6-sulfate by PAGE, respectively. These techniques, associated with the use of Stains-All, also revealed the presence of hexuronic-acid in the fractions. The APTT of the fractions (On-I-(1mg/mL:53.5±0.3s) and On-II-(1mg/mL:42.0±0.4s), respectively) was discretely-modified when compared with HEP-(2.5μg/mL:38.20±0.6s), but they had no action on PT. In-contrast, fractions had potential-inhibitory-effects in
concentration-dependent-manner on TG stimulated by cephalin or thromboplastin after diluted-plasma-addition, whereas TG in plasma in the presence of HEP was abolished. Findings demonstrated both intrinsic/extrinsic-inhibitory-actions of On-GAGs. **CONCLUSION.** On-GAGs alter the TG-response in both intrinsic/extrinsic-coagulation-pathways as potential-tools to assess anticoagulatory-mechanisms paralelly-HEP-testing in subsequent-investigations.

**Keywords:** coagulation-pathways, polysulfated, thrombin.

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