Extended Abstract

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Pharmacology 2018: The determination of the anti-coagulant property of sulfated glycosaminoglycan from the cephalothorax of white leg shrimp (*Penaeus vannamei)* Family: Penaeidei- Arnold Vincent S- Centro Escolar University

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ABSTRACT

In this study, the anticoagulant property of Sulfated Glycosaminoglycan was evaluated using Plasma Recalcification Test. Extraction of the Sulfated Glycosaminoglycan from the White Leg Shrimp was performed by defatting the sample with acetone. The deffated sample was treated with 0.4M Sodium Sulfate and Aluminum disulfate crystal to collect the supernatant. The supernatant was treated with 90% ethanol. The mixture was centrifuged using a refrigerated centrifuge at 8000 rpm for 3 minutes and the collected precipitate was washed using absolute ethanol. The Sulfated Glycosaminoglycan was tested using Plasma Recalcification Test. The results in the said test showed that at 30ug/mL was significant and at 60ug/mL and 90ug/mL were very significant. The Sulfated Glycosaminoglycan from the White Leg Shrimp exhibited an anticoagulant property. Dermatan sulfates and heparin, almost like the mammalian glycosaminoglycans, but with differences within the degree and position of sulfation were previously isolated from the body of the ascidian Styela plicata and Ascidia nigra. These differences produce profound effects on their anticoagulant properties. S. plicata dermatan sulfate composed by 2-O-sulfated alpha-L-iduronic acid and 4-O-sulfated N-acetyl-beta-D-galactosamine residues may be a potent anticoagulant thanks to a high heparin cofactor II activity. Surprisingly, it's a lower potency to stop thrombus formation on an experimental model and a lower bleeding effect in rats than the mammalian dermatan sulfate. In contrast, A. nigra dermatan sulfate, also enriched in 2-O-sulfated alpha-L-iduronic acid, but during this case sulfated at O-6 of the N-acetyl-beta-D-galactosamine units, has no in vitro or in vivo anticoagulant activity, does not prevent thrombus formation but shows a bleeding effect almost like the mammalian glycosaminoglycan. Ascidian heparin, composed by 2-O-sulfated alpha-L-iduronic acid, N- and 6-O-sulfated glucosamine (75%) and alpha-L-iduronic acid, N- and 6-O-sulfated glucosamine (25%) disaccharide units has an anticoagulant activity 10 times lower than the mammalian heparin, is about 20 times less potent in the inhibition of thrombin by antithrombin, but has the same heparin cofactor II activity as mammalian heparin.

Keywords: Sulfated Glycosaminoglycan, White Leg Shrimp, Anticoagulant, Recalcification Test

Introduction

Cardiovascular diseases are the number one cause of death in the world according to the World Health Organization. In 2012, 7.4 million deaths were reported due to coronary heart diseases and 6.7 million were due to stroke (WHO 2016). In the Philippines, the leading cause of mortality is heart disease. In 2009, 100,908 thousand deaths reported with the rate of 109% (DOH 2013).

The most important behavioral risk factors of heart condition and stroke are unhealthy diet, physical inactivity, tobacco use and harmful use of alcohol. The effects of behavioral risk factors may show up in individuals as raised vital sign, raised blood sugar, raised blood lipids, and overweight and obesity. These “intermediate risks factors” are often measured in medical care facilities and indicate an increased risk of developing an attack, stroke, coronary failure and other complications.

Cessation of tobacco use, reduction of salt within the diet, consuming fruits and vegetables, regular physical activity and avoiding harmful use of alcohol are shown to scale back the danger of cardiovascular disease. In addition, drug treatment of diabetes, hypertension and high blood lipids could also be necessary to scale back cardiovascular risk and stop heart attacks and strokes. Health policies that make conducive environments for creating healthy choices affordable and available are essential for motivating people to adopt and sustain healthy behavior.

There also is variety of underlying determinants of CVDs or "the causes of the causes". These are a mirrored image of the main forces driving social, economic and cultural change – globalization, urbanization and population ageing. Other determinants of CVDs include poverty, stress and hereditary factors.

Anticoagulants are class of drugs that are commonly used in preventing the formation of dangerous clots that could result

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in cardiovascular disease like stroke. Often called "blood thinners", anticoagulants are often the first line medication that the doctors prescribed when the patient is suffering from stroke. Anticoagulants interfere with the proteins in your blood that are involved with the coagulation process. These proteins are called factors. Different anticoagulants interfere with various factors to stop clotting. Anticoagulants have the ability to reduce the blood clot and thereby reducing the likelihood of coronary or vascular emboli. (The Internet Stroke Center, 2016) There are Few side effects for anticoagulants, The most common side effect of treatment with anticoagulant medicine is bleeding. Treatment with these products may cause various degrees of bleeding, including fatal bleeds.

Objectives

1. To isolate the Sulfated Glycosaminoglycan present in *P. vannamei*
2. To identify physical and chemical characteristics of the Sulfated Glycosaminoglycan extract from White Leg Shrimp (*Penaeus vannamei*).
3. To determine the concentration of the Sulfated Glycosaminoglycan that exhibits the anticoagulant property.
4. To determine the anticoagulant property of the Sulfated Glycosaminoglycan from the cephalothorax of *P. Vannamei* by using in vitro tests in human blood plasma sample.

Methods and Procedure

1. Extraction

Extraction of the Sulfated Glycosaminoglycan from the White Leg Shrimp was performed by defatting the sample with acetone. The deffated sample was treated with 0.4M Sodium Sulfate and Aluminum disulfate crystal to collect the supernatant. The supernatant was treated with 90% ethanol. The mixture was centrifuged using a refrigerated centrifuge at 8000 rpm for 3 minutes and the collected precipitate was washed using absolute ethanol.

2. Plasma Recalcification Test

The collected plasma was incubated at 37˚C for 2 to 3 minutes. Mixed with 0.1 ml of 0.85 percent sodium chloride. 0.025 M calcium chloride was added and simultaneously started to record time. The mixture allows remaining in 37˚C water bath, after 90 seconds removed and gently tilted.

Results and Discussion

The Sulfated Glycosaminoglycan was extracted, purified and identified having the percentage yield of 4.54%. The extract obtained from the cephalothorax of White Leg Shrimp was white, odourless, and amorphous powder. Based on the chondroitin 4-sulfate calibration curve, the total Sulfated Glycosaminoglycan was 0.30%. The result showed that 30ug/mL was significant; 60ug/mL and 90ug/mL was very signifant. The Sulfated Glycosaminoglycan of White Leg Shrimp exhibited an anticoagulant property.

Conclusion

The data obtained show that the Sulfated Glycosaminoglycan extract from White Leg Shrimp (*Penaeus vannamei*) possesses anticoagulant (in-vitro) property in human.

Recommendation

1. To perform other method of isolation and purification methods of Sulfated Glycosaminoglycan from White Leg Shrimp.
2. To perform PT and aPTT test as another parameter in determining the anticoagulant property.
3. To make use of HPLC and Electrophoresis for the identification of the Sulfated Glycosaminoglycan.
4. To make use of Thrombin Time (TT) as another parameter in determining the anticoagulant property.
5. To make use of other marine organisms as another source of Sulfated Glycosaminoglycan.

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