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Methanolic Extract of Terminalia Arjuna Seeeds against Anticoagulant Activity

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Abstract: ***In an environment that predominately favours an anticoagulant state, coagulation inculdes the controlled proteolytic activation of a number of zymogens in order to produce suitable and prompt hemostasis in a damaged vessel. The inciting event in the non - pathological state involves the exposure of circulating factor VII/VIIa to extravascularly expressed tissue factor, sets in motion a series of events that amplifies the initial stimulus and leads to the conversion of fibrinogen to fibrin and clot formation. A set of anticogulant processes balances the well timed chain of events and ensures that the haemostatic effect is controlled and does not go too far. In contrast, due to inherited or acquired abnormalities, these processes may escape normal regulatory systems in diseased situations. That result in thrombosis. Despite being based on drugs that have been around for more than 80 years, current anticoagulant therapy is shifting towards targeted therapy for particular coagulation factors and events in the coagulation cascade due to the current understanding of the primary triggers and crucial events within the series of reactions that results in haemostasis. In an environment that predominately favours an anticoagulant state, coagulation inculdes the controlled proteolytic activation of a number of zymogens in order to produce suitable and prompt hemostasis in a damaged vessel. The inciting event in the non - pathological state involves the exposure of circulating factor VII/VIIa to extravascularly expressed tissue factor, sets in motion a series of events that amplifies the initial stimulus and leads to the conversion of fibrinogen to fibrin and clot formation. A set of anticogulant processes balances the well timed chain of events and ensures that the haemostatic effect is controlled and does not go too far. In contrast, due to inherited or acquired abnormalities, these processes may escape normal regulatory systems in diseased situations. That result in thrombosis. Despite being based on drugs that have been around for more than 80 years, current anticoagulant therapy is shifting towards targeted therapy for particular coagulation factors and events in the coagulation cascade due to the current understanding of the primary triggers and crucial events within the series of reactions that results in haemostasis***.

Keywords: Terminalia arjuna, EDTA, Coagulation factors, Rivaroxaban, Megakaryocytes, Ticlopidine, Clopidogrel, Ticagrelor and Prasugrel.

1. Introduction

Coagulation

The process through which blood turns from a liquid to a gel and forms a blood clot is knownas coagulation, often known as clotting. It may lead to hemostasis, the halting of blood loss from a damaged vessel, followed by repair. Platelet activation, adhesion, and aggregation, as well as fibrin deposition and maturation, are all components of the coagulation mechanism.

After a damage to the endothelium lining a blood artery. Coagulation starts nearly immediately Two processes are triggered when blood is exposed to the subendothelial space; alterations in platelets and the exposure of subendothelial tissue factor to plasma factor VII, which ultimately results in the creation of cross - linked fibrin. Primary hemostasis refers to the initial formation of a plug by platelets at the site of damage. In addition to factor VII, other coagulation (clotting) factors also function in a cascade to generate fibrin strands, which reinforce the platelet plug. This secondary hemostasis takes place concurrently.

The production of clots is referred to as hemostasis. There are four distinct stages. The first stage involves the formation of a platelet plug as a result of a damage to the vascular endothelium caused by injuries from smoking, diabetes, hypertension, and tearing of the vascular wall. Following vascular wall damage, endothelial cells and megakaryocytes produce the von willibrand factor, which facilitates platelet adherence to the injured vascular surface and platelet aggregation.

The second stage involves the spread of clots through the conversion of different proenzymesto their active state.

The third stage of the clotting process is the termination of clot formation and the antithrombin control mechanism, which are intended to stop and moderate the extent of clot formation and so stop actions that can result in thrombosis, vascular inflammation, and tissue damage. The fluidity of the blood is ensured throughout this stage of the clotting process. The final stage in clot formation is the removal of the clot by fibrinolysis. Plasmin clearance of organised clots, wound healing, and tissue remodelling are all ensured during this period.

The most often prescribed drugs include anticoagulants and antiplatelet agents. Aspirin, dipyridamole, ticlopidine, clopidogrel, ticagrelor, and prasugrel are anti - platelet medications, and parenteral glycoprotein IIb/IIIa inhibitors are used during angioplasty. Warfarin (and associated coumarins) and heparin are the two anticoagulants that are most frequently used. Proteins C and S as well as the vitamin K - dependent coagulation factors II, VII, IX, X are all impacted by warfarin, whilst thrombin and factor Xa are made more reactive by antithrombin due to the use of heparin and similar substances.

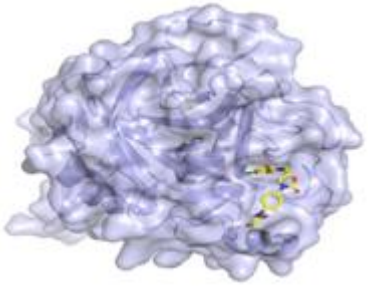

Figure 1: Rivaroxaban drug bound to the coagulation factor Xa.

2. Materials and Method

2.1 Materials

Centrifuge, EDTA (Ethylene Diamine Tetra Acetic Acid), Sodium Chloride, Calcium Chloride, Test tubes, Capillary tubes, Glass Slides, Syringes, Needles, Spirit, Cotton, Filter paper, Micropipettes, Methanol, Soxhlet apparatus, Saline Water, Sew 40, Weighing machine, Terminalia Arjuna seed powder, Beakers.

2.2 Method

1) Selection of Plant Material

In the present work Terminalia Arjuna which is the plant of family Combretaceae was selected. Healthy seeds of the above medicinal plant were collected in and around Ghatkesar, Telangana. Then seeds were shade dried for about 2 days. The shade dried seeds was further coarsely powdered and the powder was sieved through the mesh 40 and stored in airtight container for further analysis.

Figure 2: Terminalia Arjuna seeds

Figure 3: Terminalia Arjuna seed powder

2) Extaction of Terminalia Arjuna Seeds

30g of the seed powder was weighed accurately and placed in Soxhlet extraction chamber which was suspended above the flask containing 200ml of 80% Methanol for 12h under (600c - 800c) using Soxhlet apparatus. The extraction was carried out until the extract becomes colourless. The extract is then concentrated and dried under reduced pressure.

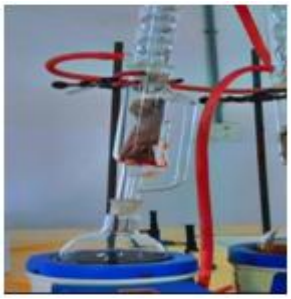

Figure 4: Soxhlet Apparatus

3) Aqueous Extraction of Terminalia Arjuna Seeds

30g of the seed powder was weighed accurately and mixed in 200ml of distilled water at constant temperature of 950c under continuous stirring. The supernant was subsequently filtered through whatman No.1 filter paper.

4) Blood Sample Collection and Preparation of Plasma

Blood samples were drawn from a healthy donor (age 18 - 35 years old) via vein puncture. The blood placed separately in containers containing EDTA to prevent the clotting process. Centrifugation (15 minutes at rate 3000 rpm) was carried out to separate the blood cells from plasma in order to obtain pure platelet plasma (ppp) for Prothrombin time test. The plasma was separated and stored in the refrigerator at - 40c until use.

5) Anticoagulant Assay

Collection of Blood and Plasma Re - Calcification

0.2ml plasma, 0.1 ml of alkali extract of different concentration and different volume of Cacl2 (25 Mm) were added together in a clean fusion tube and incubated at 370c in water bath. For control experiment extract solution was replaced by same volume of 0.9% saline water. The clotting time was recorded with stopwatch by tilting the test tubes every 5 seconds. This time is called Prothrombin time.

Determination of Coagulation Time

Table 1: Aqueous/Organic/Standard extract of Terminalia Arjuna

Group	*Amount of Plasma*	*Amount of extract*	*Cacl2 solution*
Group I	0.2	0.1	0.3
Group II	0.2	0.2	0.3
Group III	0.2	0.5	0.3
Group IV	0.2	1	0.3
Group V	0.2	2	0.3
Control	0.2	0.1	0.3

3. Results

The below table consists of coagulation time of aqueous extract, organic extract, control and standard drug. Control is used as Cacl2 and standard drug as warfarin drug (anticoagulant drug).

Table 2: The table indicates the time intervals of aqueous extract, organic extract, standard and control

Group	*Amount, of extracts (ml)*	*Coagulation time of organic extract (sec)*	*Coagulation time of aqeous extract (sec)*	*Coagulation time of standard (sec)*
Group I	0.1	27	22	15
Group II	0.2	35	38	38
Group III	0.5	38	51	52
Group IV	1	42	1.00	1.50
Group V	2	1.31	1.59	1.35
Control	0.1	50	56	60

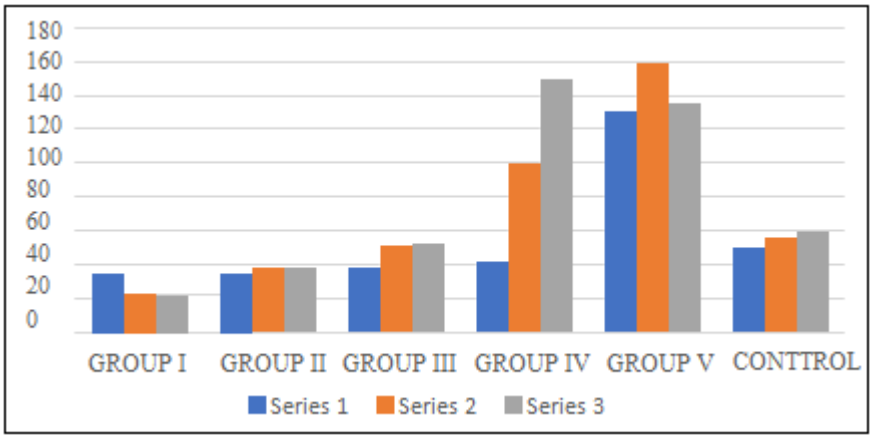

Figure 5: The graph indicates the time intervals of aqueous extract, organic extract and control

4. Conclusion

The Anticoagulant activities of all extracts are shown significant anticoagulant properties was reported. The group IV and group V shown good promising activity when compared to control. The daily intake of Terminalia Arjuna plant seeds extract may help full to prevent the cardiovascular diseases. It requires further investigation to find out active molecules and their pharmacological properties and other effects.

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