

PLANT AS ANTICOAGULANT/ANTITHROMBOTIC AGENT

***Shikha Jain¹, CBS Dangi¹, Manpreet Kaur¹, Hardev Singh², Jaya Peter¹ & Sushmit Kosta¹**

¹Department of Biotechnology, RKDF University, Bhopal

²Casualty Department, Chirayu Medical College & Hospital, Bhaishakhedi, Bhopal.

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Correspondence for*Author****Shikha Jain**

Department of Biotechnology,
RKDF University, Bhopal,
India.

ABSTRACT

Thrombotic diseases such as myocardial or cerebral infarction are serious consequences of the thrombus formed in blood vessels. These diseases are important cause of mortality in developed and third world countries. Antithrombotic agents are used to prevent thrombosis and thrombolytic agents are used to dissolve the already formed clots in the blood vessels however, these drugs have certain limitations which cause serious and sometimes fatal consequences. Herbal preparations have been used since ancient times for the treatment of several diseases. Several plants used for the treatment of thromboembolic diseases in different systems of traditional medicine have shown

anticoagulant or antithrombotic activity and such plants claimed in the traditional system still remain to be scientifically investigated. The review explains few traditional antithrombotic drugs and their side effects and mentioned few plants showing antithrombotic and anticoagulant *in-vitro* and *in-vivo*.

Key words: Anti-coagulant activity, Antithrombolytic Activity, Prothrombin, Plants, Coagulation factors.

INTRODUCTION

Blood is a connective tissues which flows through vertebrate's arteries and veins smoothly and efficiently, but if a clot, or thrombus, blocks the smooth flow of blood, resulting thrombosis which can be serious and even cause death. Diseases arising from clots in blood vessels include pulmonary emboli, deep vein thrombosis, CVA and myocardial infarction which are the major causes of mortality and morbidity [1]. These disorders collectively are the most common cause of death and disability in the developed world. [2] Haemostasis is the

process that retains the blood within the vascular system during periods of injury. The coagulation mechanism may be thought of as a complex series of cascading reactions involving development of enzymes from their precursor (zymogens, procoagulants proenzymes). Most of the substances which are necessary for coagulation are present in an inert form and must be converted to an activated state. As one enzyme is formed it then becomes available to convert the next zymogen to its activated enzyme (serine protease). This process continues until a fibrin meshwork clot is formed. In addition to the zymogens, protein cofactors and surface membrane phospholipids, and calcium ions play an active role in the final development of the fibrin clot.[3]

Antithrombotic Therapy

Atherothrombotic coronary artery disease and deep vein thrombosis are most common causes of death worldwide .[4] Atherothrombotic diseases such as myocardial or cerebral infarction are serious consequences of the thrombus formed in blood vessels. Thrombolytic agents are used to dissolve the already formed clots in the blood vessels. However, these drugs have certain limitations which cause serious and sometimes fatal consequences. [5]

Types of Thrombosis

There are two types of thrombosis: Arterial thrombosis (that form in arteries) and venous thrombosis (that form in the veins). In venous thrombosis of the lower limbs, stasis, local inflammation on activated vascular endothelial cells induced by adhering leukocytes and platelets and in some cases direct vascular damage, promotes local thrombus formation .[6] while in arterial thrombosis, local flow changes and particularly vascular wall damage are the main patho-physiological elements. Alterations in composition of the arterial blood are also involved but the specific role and importance of blood coagulation is an ongoing matter of debate. [7,8]

Process of Blood Clotting

Blood coagulation occurs when the enzyme thrombin is generated that proteolyzes soluble plasma fibrinogen, forming the insoluble fibrin polymer or clot. Mechanisms that restrict the formation of platelet aggregates and fibrin clots to sites of injury are necessary to maintain the fluidity of the blood. As depicted in Figure No. 1 [9]

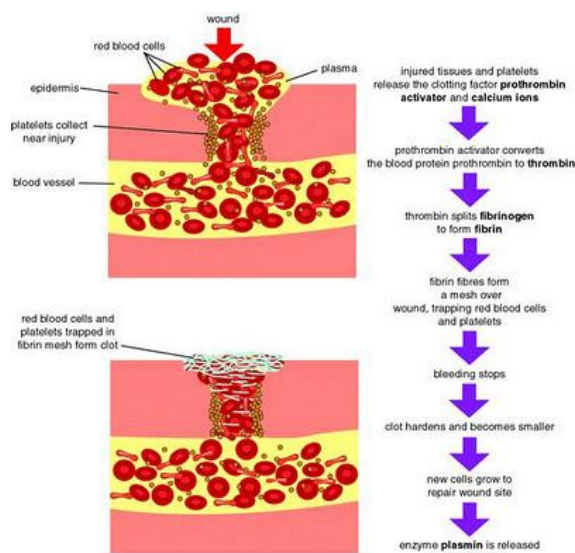


Figure No.1 Process of blood clotting (<http://biologymozac.blogspot.in/2012/01/blood-clotting-mechanism-flow-chart.html>)

Reactions of the blood coagulation cascade are propagated by complex enzymes containing a vitamin K dependent serine protease and an accessory cofactor protein that are assembled on membrane surface in a calcium dependent manner. These complexes are 10^5 - 10^9 fold more efficient in proteolyses of their natural substrates than enzymes alone. Based upon data acquired using several in vitro models of blood coagulation, tissue factor initiated thrombin generation can be divided into two phases: an initiation phase and a propagation phase.

The initiation phase is characterized by the generation of nanomolar amounts of thrombin, femto to picomolar amounts of factors VIIa, IXa, Xa, and XIa, partial activation of platelets, and almost quantitative activation of pro-cofactors, factors V and VIII as described in Figure No. 2 . The duration of this phase is primarily influenced by concentrations of tissue factor and TFPI. The characteristic features of the propagation phase are: almost quantitative prothrombin activation at a high rate, completion of platelet activation, and solid clot formation. This phase is primarily regulated by antithrombin III and the protein C system. Thrombin generation during the propagation phase is remarkably suppressed in the absence of factor VIII and IX (haemophilia A and B, respectively) and at platelet counts $<5\%$ of mean plasma concentration. [10]

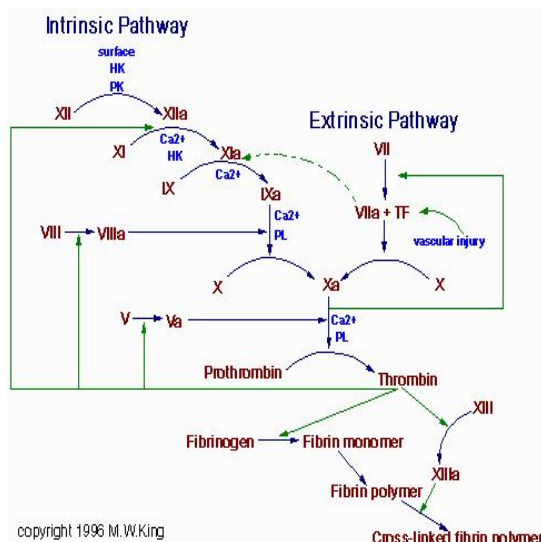


Figure 2. Representing the Intrinsic and Extrinsic Factors

Categorization of Antithrombotic Drugs

Antithrombotic agents fall in three categories:

1. Drugs which prevent fibrin formation (the anticoagulants and defibrinating enzymes)
2. Drugs which prevent platelet adhesion or aggregation (the antiplatelet drugs) and
3. Thrombolytic drugs which induce fibrin degradation.

Antithrombotic drugs work by reducing thrombus formation. They can be used therapeutically for primary prevention, secondary prevention, or treatment of an acute thrombus. Arterial and venous thrombi are composed of platelet aggregates, fibrin, and trapped red cells. Because arterial thrombus forms under high-shear conditions, platelets are abundant and fibrin is relatively sparse. In contrast, venous thrombi, which form under low-shear conditions, are rich in fibrin and trapped red cells and contain fewer platelets.[11] These features have important implications for antithrombotic therapy. Targeting the components of both arterial and venous thrombi, antithrombotic drugs encompass antiplatelets, anticoagulants, and fibrinolytic drugs. [12]

Anticoagulant- An anticoagulant is a substance that prevents coagulation (clotting) of blood. Such substances occur naturally in leeches and blood-sucking insects. A group of pharmaceuticals called anticoagulants can be used in-vivo as a medication for thrombotic disorders. Anticoagulants reduce blood clotting. This prevents deep vein thrombosis, pulmonary embolism, myocardial infarction and stroke. Thromboembolic disorders such as pulmonary emboli, deep vein thrombosis, strokes and heart attacks are the main causes of

morbidity and mortality in developed countries. [1] Hence, anticoagulants play a vital role as agents for the prevention and treatment of thromboembolic disorders. [13]. For more than five decades, anticoagulant drugs consisting of heparins, Coumarins, Ardeparin etc. their derivatives have been the major players in the clinical setting. Although their efficacy remains undisputed, the deleterious life-threatening side effects of these drugs have also been well documented. [14]


Drugs available in market as anticoagulants and their side effects are depicted in tabular form in Table No.1:





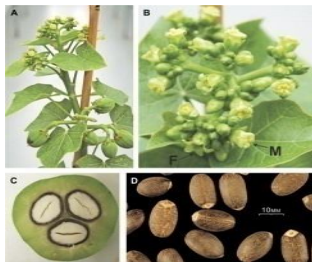

Table No. 1. Drugs available in market and their sideeffects		
S.no.	Available drugs	Side effects
1.	Aspirin [15]	Major bleeding, Gastrointestinal- Nausea, Stomach pain, heart burn, Nausea, Consipation, hematemesis, and melena [15]
2.	Dalteparin [16]	Skin- Hair loss, skin necrosis. Genitourinary- Blood in urine.(Haematuria) Blood- Any bleeding event, blood clot in the spine. Hypersensitivity- Allergic reactions, including itching, rash, fever, injection-site reaction, hypersensitivity reactions. Local- Injection-site blood clot, wound hematoma, injection-site pain. [16]
3.	Heparin [17]	Major bleeding. Heparin-induced thrombocytopenia (HIT), Paradoxical Thrombosis. [17]
4.	Coumarins [18-20]	Coumarins is a vascular purpura that causes skin necrosis. This is associated with protein C deficiency and malignancy. [18-20] Coumarins cross the placenta and cause spontaneous abortion and specific embryo abnormalities if administered in the first trimester of pregnancy. [21,22]
5.	Lepirudin [23]	Heart- Heart failure. Skin- Bleeding in injection site, wounds and allergic skin reactions. Eye and ENT- Nosebleed. (Epistaxis) Gastrointestinal- Gastrointestinal and rectal bleeding. Genitourinary- Abnormal kidney function, blood in urine and vaginal bleeding. Blood- Anemia and sepsis, Liver- Abnormal liver function. Respiratory- Pneumonia. Miscellaneous- Fever and infection. [23]
6.	Urokinase [24-25]	Side Effects : Most Common- Severe bleeding. (Haemorrhage) Heart - Heart attack, pulmonary embolism. Blood- Decreased red blood cells and platelets. Miscellaneous- Excess sweating.





		Potentially Fatal- Severe bleeding, severe allergic reactions, cholesterol embolization. Other side effects such as cardiac arrest and irregular heart rate(arrhythmia) have also been seen in patients with urokinase injection. [24-25]
7.	Fondaparinux [26]	Side Effects : Most Common- Mild bleeding, reduced platelet levels (Thrombocytopenia), irritation, rash or itching at the injection site. Blood- Bleeding, anemia, blood clot formation, post-operative bleeding and bruising. Central Nervous system- Sleeplessness, dizziness and confusion. Miscellaneous- Low blood pressure (hypotension), low potassium in blood, increase in liver enzymes (elevations of hepatic enzymes) and no excess of cardiovascular events. [26]
8.	Warfarin [27]	Most Common – Tingling sensation, headache, chest, abdomen, joint, muscle pain, dizziness, shortness of breath, difficulty in breathing and swallowing, weakness, low blood pressure and shock. Severe active bleeding during pregnancy; documented hypersensitivity - fever, rash and hair loss. Gastrointestinal - Nausea, vomiting, diarrhea and abdominal pain. Central Nervous System - Fatigue, tiredness, uneasiness, weakness, headache, dizziness, loss of consciousness, fainting, coma and taste perversion. [27]





Plants Acting as Anticoagulant agent

Plants may serve as the best alternative sources for the development of new anticoagulant agents due to their biological activities. [28] Phytochemicals present in plants with having anticoagulant properties can ultimately reduce or eliminate the risk of thrombo embolic diseases. List of few plants having anticoagulant properties and their mode of action is depicted in Table No. 2.

Table No.2 Showing the plant and its mode of action as anticoagulant		
1.	<p><i>Careya arborea</i> [29] White Teak., Lecythidaceae, Plant part used: Bark extract Mode of Action: The methanolic bark extract of <i>Careya arborea</i> exhibited anticoagulant activities when compared with the standard warfarin Prolongation in PT and prolongation of aPTT may be due to decrease in coagulation factors like V, VII and X involved in extrinsic pathway, and there is also decrease in coagulation factors such as VIII, IX, XI, XII. [30-31]</p>	

2.	<p><i>Melastoma malabathricum</i> [32] , Senduduk, Melastomataceae</p> <p>Plant part used : Leaves extract</p> <p>Mode of Action: The aqueous extract of leaves prolonged aPTT and thrombin time (TT) Further investigations evaluated the bioactive compound(s) responsible for the anticoagulant activity as well as the determination of the coagulation factor (s) affected. [32]</p>	
3.	<p><i>Gloriosa superb</i> [33]. Flame lily, Lilaceae</p> <p>Plant part used : Leaves extract</p> <p>Mode of Action: The leaves extracts displayed anticoagulant properties by inhibiting thrombin induced clotting, with IC50 value of 2.97 mg/ml</p> <p>It decreases the fibrin clot formation. [33]</p>	
4.	<p><i>Eichhornia crassipe</i> [34] water-hyacinth, Pontederiaceae</p> <p>Plant part used : Leaves extract</p> <p>Mode of Action: Anticoagulant activity by acting on the intrinsic pathway of the coagulation cascade. [34]</p>	
5.	<p><i>Bauhinia forficata</i> [35] Pata de vaca, Leguminosae</p> <p>Plant part used: Leaves extract</p> <p>Mode of Action: Act as inhibitors of serine-protease involved in blood clotting disturbances induced by snake venoms. [35]</p>	
6.	<p><i>Jatropha curcas</i> [36], Barbados nut, Euphorbiaceae</p> <p>Plant part used: Leaves and Fruits extract</p> <p>Mode of Action: Coagulant activity of the latex of <i>Jatropha curcas</i> showed that whole latex significantly reduced the clotting time of human blood Prolonged the clotting time: at high dilutions, This indicates that <i>Jatropha curcas</i> latex possesses both procoagulant and anticoagulant activities. [36]</p>	
7.	<p><i>Porana volubilis</i> (Horse-Tail Creeper) Convolvulaceae .</p> <p>Plant part used : Flowers and leaves extract</p> <p>Mode of Action: Its anticoagulant activity is mediated by the enhancement of thrombin inhibition that in turn is mediated by heparin cofactor II but not by antithrombin Anticoagulant activity is mediated by the enhancement of thrombin inhibition that in turn is mediated by heparin cofactor II. [37]</p>	

8.	<p><i>Synclisia scabrada</i> [38-39] , Menispermaceae</p> <p>Plant part used: leaves, stem, bark and root extract</p> <p>Mode of Action: The aqueous and ethanol extract of <i>Synclisia scabrada</i> significantly ($P<0.05$) prolonged Prothrombin Time (PT) of normal plasma, which suggests that both extracts of <i>Synclisia scabrada</i> have anticoagulant properties. The aqueous and ethanol extract of <i>S. scabrada</i> significantly ($p<0.05$) prolonged the PT of normal Plasma Compared to the ethanol extract, This suggests that the aqueous and ethanol leaf extracts of <i>S. scabrada</i> have anticoagulant properties which compares well with heparin. [38-39]</p>	
9.	<p><i>Codium fragil</i> & <i>Sargassum horeri</i> [40] Marine Algae, Gracilariaceae</p> <p>Plant part used: Algae</p> <p>Mode of Action: Algal anticoagulant polysaccharides exert their anticoagulant activity through potentiating antithrombin III (AT III) and/or heparin cofactor II (HC II) that are important endogenous inhibitors, called SERPIN. The anticoagulant mechanism is the one by which heparin, heparin sulfate and dermatan sulfate exert their activity. On the other hand, some algal anticoagulant polysaccharides exert anticoagulant activity through directly inhibiting fibrin polymerization and/or thrombin activity without potentiating AT III and HC II. Recent studies conducted on marine algal biologically active compounds have shown anti-platelet and anticoagulant proteins and fibrinolytic enzymes.[40]</p>	
10.	<p><i>Allium sativum</i>, [41] Garlic, Lilliacae</p> <p>Plant part used: Whole fresh bulb, dried bulb.</p> <p>Mode of Action : Bulbs that have been dried and re-moistened ferment into various types of oils. Oils that are act as clot-preventing agents. At the high dose of garlic and (500 mg/kg), a further decrease of TXB 2 levels in the serum of the rats was observed. Boiled garlic and at high concentration (500 mg/kg) had very little effect on TXB2 synthesis. A high dose of garlic and onion produces toxicity in the rats . These results show that garlic can be taken frequently in low doses without any side effects, and can still produce a significant antithrombotic effect. Raw garlic is preferred since bioactive compounds are destroyed while cooking. [41]</p>	
11.	<p><i>Allium cepa</i>, [41] L., Red onion, Amaryllidaceae</p> <p>Plant part used: Bulb extract</p> <p>Mode of Action: The aqueous extract of red onion was found to inhibit coagulation process in vitro and significantly prolonged prothrombin time in a dose-dependent manner. The prothrombin time test (also known as the pro test or PT test) is a useful screening procedure for the extrinsic coagulation mechanism including the common pathway. It detects deficiencies in factor II, V, VII, and X. Prolongation indicates a deficiency in one or more of II, V, VII, and X factors. [41]</p>	

12.	<p><i>Curcuma longa</i>, [42] Turmeric, Zingiberaceae</p> <p>Plant part used: Root and rhizome extract</p> <p>Mode of Action: Turmeric suppresses the ability of platelets to stick together to form clots, which may help to boost circulation. Curcumin prolonged aPTT and PT significantly and inhibited thrombin and FXa activities. These anticoagulant effects of curcumin were better than the other derivative of <i>Curcuma longa</i>. this is indicating that methoxy group in curcumin positively regulated anticoagulant function of curcumin. [42]</p>	
13.	<p><i>Tulbaghia violaceae</i>, [43] Wild Garlic, Violaceae</p> <p>Plant part used for Extract: Rhizomes, leaves & Bulbs extract</p> <p>Mode of Action: <i>T. violacea</i> plant displayed the best anticoagulant activity, secondly if an aqueous or organic extraction method would be most suited and thirdly, to determine how the coagulation pathway and platelet aggregation were affected using both an in vitro and ex vivo rat model. In clinical tests of blood coagulation, PT is used to evaluate the overall efficiency of the extrinsic clotting pathway; a prolonged PT indicates a deficiency in coagulation factors V, VII and X. On the other hand, APTT is a test of the intrinsic clotting activity; a prolonged APTT usually represents a deficiency in factors VIII, IX, XI, XII and V. [43]</p>	
14.	<p><i>Terminalia belerica</i>, [44] Bahera, Combretaceae</p> <p>Plant part used: Fruit extract</p> <p>Mode of Action: <i>Terminalia belerica</i> fruits possess thrombolytic and antithrombotic activity in vitro; however in vivo clot dissolving properties and active component of <i>Terminalia belerica</i> for clot lysis are yet to be discovered. (Vaseem A Ansari, H H Siddiqui, Satya Prakash Singh., 2012). In case of antithrombotic experiment, the clot was formed in normal time or slight delay when NS was added to the control Whereas tube, to which SK was added, the clot was not formed and in case of extract solutions, significant delay in clot formation time is noted as according to concentration. [44]</p>	
15.	<p><i>Molinieria recurpata</i> [45] Palm Grass, Hypoxidaceae</p> <p>Plant part used: Leaf extract</p> <p>Mode of Action: The anticoagulant activity of <i>Molinieria recurpata</i> (Family: Hypoxidaceae) leaf extract (Methanol) on fresh human blood. Both the extracts were found to have sufficient anti-coagulant activity. But the concentration of 2g/ml methanolic leaf extract showed the maximum effect with respect to others. It detects deficiencies in factor and X. Thus prolongation indicates a deficiency in one or more of these factors II, V, VII, & X. [46]</p>	

CONCLUSION

The present review mainly focuses on many natural and traditional anticoagulant agents used in allopathic system of medicine which is based on the fast therapeutic actions of various antithrombotic and anticoagulant agents such as heparin and aspirin. These drugs have various side effects. Taking cost factor and hospitalization it becomes very difficult for poor population to afford this treatment. Traditional plant medicines have been used to alleviate the suffering of human beings since long. These plant products are safe and cost effective. Despite their wide spread usage traditional medicines have not been evaluated scientifically with regard to their safety and efficacy and has many limitations. The review explored *invitro* and *invivo* activity of various plant extracts. The plant medicine requires exploitation up to desired level to reach some conclusion regarding their use in pharmacopeia.

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