JETIR.ORG ISSN: 2349-5162 | ESTD Year : 2014 | Monthly Issue
JOURNAL OF EMERGING TECHNOLOGIES AND INNOVATIVE RESEARCH (JETIR)
An International Scholarly Open Access, Peer-reviewed, Refereed Journal

Fibrinolysis & Fibrinolytic agents

J Bindu[1], Raghu Nataraj[*]
[1]PhD Scholar, [*]Assistant Professor
Division of Molecular Biology, School of Life Sciences
JSS Academy of Higher Education & Research, Mysuru-570015, Karnataka, India.

Abstract: Fibrinolysis is a process of degradation of the fibrin clots by Plasmin. Plasmin, a proteolytic enzyme produced from plasminogen (a zymogen) conversion with the influence of fibrinolytic agents like Tissue Plasminogen Activator (tPA). The current review is focused on shedding light on different such fibrinolytic agents, mechanism of action and their therapeutic applications. Different fibrinolytic agents such as Tissue Plasminogen Activator, Streptokinase and Urokinase etc. have been used in Fibrinolytic therapy aiding to minimize the size of clots formed in the arteries of brain, heart, lungs and veins of leg and preventing serious cardiovascular complications. The current review tries to shed light on the mechanism of fibrinolysis, its complications and its related discussion w.r.t several fibrinolytic agents in current use.

Index Terms: Fibrinogen, Fibrin, Plasminogen, Plasmin, Fibrinolytic agent & Fibrinolysis.

INTRODUCTION

Blood coagulation is a natural process wherein fibrinogen is converted into fibrin under the influence of several coagulation factors, enzymes present in our body resulting in a series of interactions leading to the formation of thrombus. Thrombi, composed of platelets, leukocytes and red blood cells where platelets act as phospholipid surface for blood coagulation aiding in the formation of fibrin network with the help of the proteases (clotting factors) generated during coagulation cascade. Leukocytes enter thrombus to secret enzymes such as serine proteases, matrix metalloproteases responsible for platelet adhesion wherein RBCs provide phospholipid surface to secrete pro-coagulant factors for the blood coagulation [1]. Fibrin clot is initiated by thrombin present in our body through the direct activation of coagulation Factor XIII and cleavage of Fibrinopeptide A & Fibrinopeptide B from N-terminal portions of Aα and Bβ chains of fibrinogen [2][3]. Excess formation of fibrin increases the risk of thrombosis which in turn provides a way for the blockage or obstruction of blood flow, manifesting into life-threatening complications such as myocardial infarction, stroke, deep vein thrombosis, pulmonary embolism and hence, fibrinolysis is one such course of action to treat thrombosis via fibrinolytic agents. The review explores our understanding of fibrinolysis, it's mechanism and fibrinolytic agents with their therapeutic applications.

FIBRINOLYSIS

Fibrinolysis, a process of breaking down the fibrin into its degradation products D-dimer and Fibrinopeptide A [2] by the action of plasmin. Degradation of cross-linked fibrin results in release of D-dimer composed of two cross linked D fragment of fibrin protein, a specific Fibrin Degradation Product (FDP) signifying an increase in the level of D-dimer signifies increase in fibrinolysis [4]. But this not the case always, under the lower concentrations of plasmin enzymes in the blood, a fibrinolytic agent is supplied for the activation of plasminogen [5]. Currently, different fibrinolytic agents are in use like, Tissue plasminogen activator (tPA), Streptokinase and Urokinase Plasminogen activator (uPA) [6].

MECHANISM OF FIBRINOLYSIS

Fibrinolytic system comprises of plasminogen, a zymogen which gets converted into plasmin, a serine protease enzyme through its plasminogen binding receptors with the help of plasminogen activators like tPA, uPA etc [7]. Plasminogen, a single-chain glycoprotein, is produced by the liver, occurring in plasma and other tissues. Fibrinolysis is the degradation of fibrinogen into fibrin fragments catalysed by plasmin (Fig.1) [8]. It is initiated by two pathways as coagulation cascades namely, intrinsic pathway and extrinsic pathway. Intrinsic pathway is initiated by clotting factors like Factor XII, Factor XIa and kallikrein, whereas, Extrinsic pathway is initiated by Plasminogen Activators such as trypsin like serine proteases which are mainly tissue Plasminogen Activator (tPA) and urokinase Plasminogen Activator (uPA). Plasminogen Activators bind selectively to fibrin and plasmin produced on the surface of fibrin which immediately interrupts fibrin causing fibrinolysis [9]. The process is regulated by a balance between the activators and specific protease inhibitors occurring in plasma. α_2-antiplasmin & α_2-macroglobulin are the inhibitors inhibiting plasmin activation, wherein, Plasminogen Activator Inhibitor 1 & 2 (PAI-1 & PAI-2) inhibit plasminogen activation (Fig.2) and Thrombin activatable fibrinolysis Inhibitor (TAFI), a inhibitor of fibrinolysis which activates thrombin (serine protease) for blood coagulation (Fig.2) [10]. Fibrinolysis is elevated when plasmin concentration is higher than inhibitors concentration, generating an imbalance which necessitates the need of fibrinolytic agents to induce plasmin activation and in turn

reducing thrombosis complications (Fig.3) (Fig.4). Absence of such lytic agents paves way to enhanced thrombosis complications, responsible for fibrinolytic dysfunctions in the body.

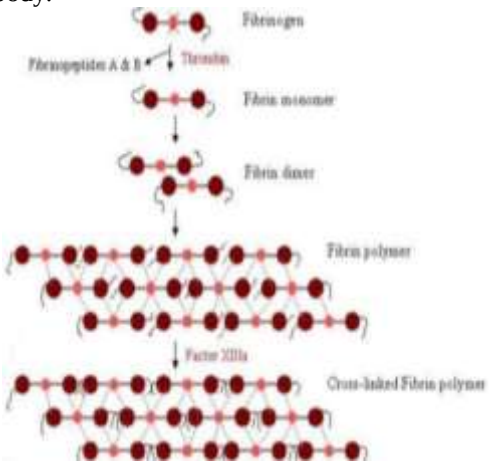

Fig 1: Fibrinogen degradation into fibrin clot [11]

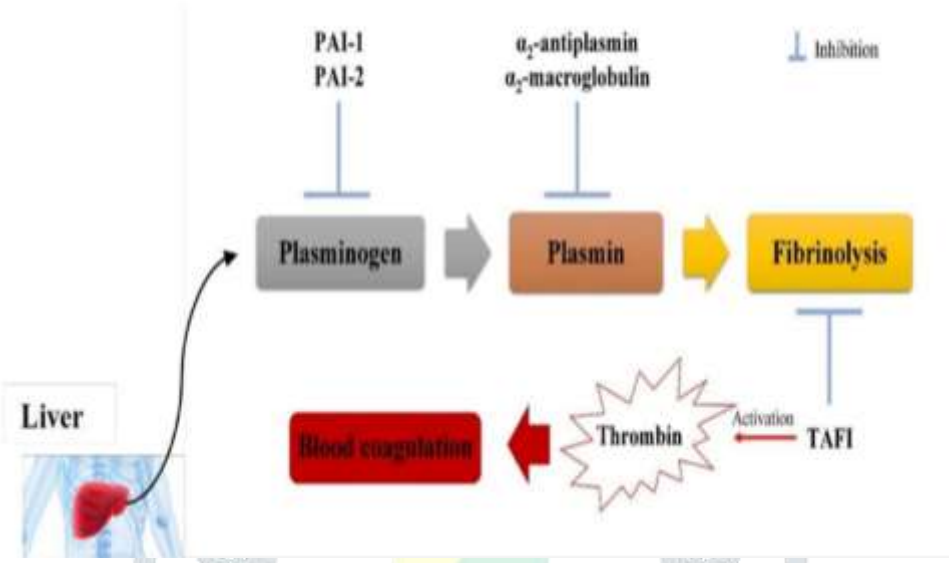

Fig 2: Molecular Mechanism of Fibrinolysis

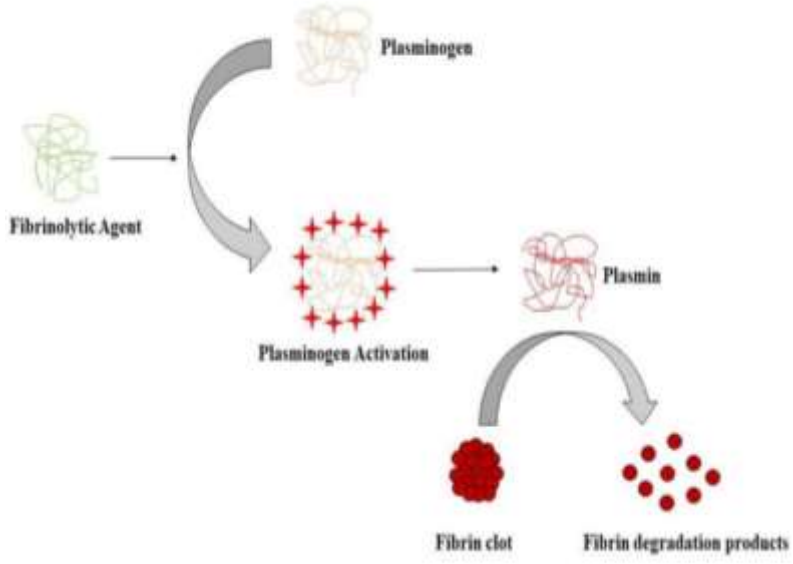

Fig 3: General Mechanism in the presence of Fibrinolysis agent

Fig 4: General Mechanism of Fibrinolysis in the absence of Fibrinolytic agent

Plasminogen deficiency is a condition exhibiting reduced serum plasminogen activity. Hypoplasminogenemia, a Type 1 plasminogen deficiency shows lower plasminogen antigens level (less than 1-9 mg/mL) with decreased plasminogen activity from 1%-51% [12] resulting in impaired extracellular fibrinolysis & chronic mucosal pseudomembranous lesions among ligneous conjunctivitis patients. Dysplasminogemenia, a Type 2 plasminogen deficiency with decreased plasminogen activity and normal plasminogen antigens levels with no clinical manifestation reported yet [13][14]. Plasminogen Activator Inhibitor-1 (PAI-1) deficiency, is a rare autosomal recessive disorder manifested by SERPINE1, a serine protease which down regulates fibrinolysis and controls degradation of blood clots with affected individuals exhibiting bleeding symptoms after trauma or procedure of surgery. Currently no standard assays are available to check PAI-1 activity at lower concentrations [15][16] and can be expected at or more than normal range 5-20ng/mL [17]. tPA, a serine protease encoded by PLAT gene is involved in the activation of inactivation of plasminogen into plasmin during fibrinolysis. Increased tPA is shown to increase fibrinolysis resulting in excessive bleeding whereas decreased tPA is shown to downregulate fibrinolysis which causes thrombosis [18]. Thrombophilia is a condition of hypercoagulation with the increased risk of thrombosis due to HRG deficiency. Histidine-rich glycoprotein (HRG), a plasma glycoprotein binds with plasminogen and heparin with the influence of zinc as cofactor regulates the process of angiogenesis, coagulation & fibrinolysis [19][20][21][22][23]. Hypercoagulation, is also reported to occur due to the deficiency of antithrombin-III, an inhibitor of thrombin [24]. Quebec Platelet Disorder (QPD), is an autosomal dominant bleeding disorder with the absence of systemic fibrinolysis due to increased PLAU (Urokinase-type Plasminogen Activator) levels within platelets responsible for intraplatelet plasmin generation [25][26]. This intraplatelet plasmin generation degrades α-granule proteins in platelets which in turn causes deficiency in platelet factor V (FV) responsible for blood coagulation that directly offers to cause increase in fibrinolysis [27]. Alpha-2-plasmin Inhibitor Deficiency occurs when serine protease inhibitor-2 (SERPINF2) inhibits the activity of plasmin resulting in excess fibrinolysis. It is a rare autosomal recessive disorder with an increased risk of bleeding [28][29].

FIBRINOLYTIC AGENTS

Fibrinolytic agents are used to treat thrombosis wherein different thrombolytic agents are in usage for different types of thrombosis. All fibrinolytic agents are serine proteases functioning as plasminogen activators, converting inactive plasminogen into active plasmin for fibrinolysis process upon their administration through peripheral IV or local release of catheters after spotting the site of clot formed [30].

Streptokinase, is a most widely used fibrinolytic agent to treat acute ST-segment Myocardial Infraction, Deep vein thrombosis, pulmonary embolism, arterial thrombosis & arteriovenous cannula occlusion [31]. It binds to plasminogen to form a complex which converts free circulating plasminogen into plasmin during fibrinolysis. Streptokinase is identified to exhibit allergic reactions as it is an extract of Streptococcus [30]. Urokinase, extracted and purified from human urine directly cleaves plasminogen into plasmin during fibrinolysis. With its low antigenicity property, it is used for peripheral vascular thrombosis and for occluded catheters. Recombinant form of Urokinase is also available as Urokinase-type plasminogen activator [30][32]. Prourokinase is a fibrin specific inactive precursor used to activate urokinase [30]. Advantage of Prourokinase over other plasminogen activator is that it stays in an inactive form within plasma without intervening with other circulating inhibitors [33]. Alteplase is a fibrin specific first-generation recombinant tissue plasminogen activator used to treat acute cardiovascular events, pulmonary embolism and acute ischemic strokes. It is a non-antigenic serine protease present on endothelial cells of the body with moderate risk of bleeding [30][34]. Tenecteplase, a triple combination of mutant alteplase with more fibrin specific used as fibrinolytic agent. It is revealed with lack of antigenicity and lower risk of non-cerebral bleeding [30][35]. Reteplase is a second-generation recombinant plasminogen activator with lower bleeding tendency than first generation alteplase showing non-fibrin specific activity by converting all available plasminogen to plasmin with no antigenicity [30][36]. Anistreplase is an anisoylated plasminogen-streptokinase activator complex (APSAC), a mixture of streptokinase and plasminogen which does not depend on circulating plasminogen to be effective. Similar to streptokinase it has high antigenicity [30][37].

Apart from the above-mentioned fibrinolytic agents, research on plants exhibiting protease activity so far w.r.t fibrinolysis have been reported and mentioned (Table-1). Haemostasis is a process of stages that saves an organism from life threatening events by maintaining the fluidity of the circulating blood containing serine proteases, where one of the main stages includes fibrinolysis. Plant containing proteases has shown the property of dissolving clot which signifies plants have role in fibrinolysis [38][39][40][41][42][43][44]. All these studies have given the knowledge about fibrinolysis, fibrinolytic disorders, current fibrinolytic agents and in continuation role of plant proteases in fibrinolysis was studied where new fibrinolytic agents can be produced from natural products which are easily available than currently being used fibrinolytic agents. Hence the current review tries to shed light on a path to plan and do research on plant protease where no work has been done using that plant.

Table 1: Plant proteases showing Fibrinolytic activity

Plant name	Part of the plant used	Type of protease
Artocarpus heterophyllus [45]	Latex	Serine
Calotropis procera [46]	Latex	Cysteine
Calotropis gigantea [47]	Latex	Cysteine
Asclepias curassavica [48]	Latex	Cysteine
Pergularia extensa [49]	Latex	Cysteine
Cynchum puciflorum [49]	Latex	Cysteine
Ervatamia coronaria [50]	Latex	Cysteine
Euphorbia hirta [51]	Latex	Serine
Ficus carica [52]	Latex	Serine
Carica papaya [53]	Latex	Cysteine

CONCLUSION

Lower concentrations of plasmin enzymes in the blood makes it prone to clot formation and its related complications disturbing the bodily homeostasis. Under such untimely circumstances, several of the explored lytic agents in the current review have always been administered as a potent candidate to counter the probable damages. Understanding fibrinolysis and its related concepts is a foremost relevant area to be worked in developing new fibrinolytic agents against clot related disorders particularly from natural sources.

REFERENCES

1. Wohner N. 2008. Role of cellular elements in thrombus formation and dissolution. Cardiovascular & Hematological Agents in Medicinal Chemistry, 6:224-8.
2. Horan JT, Francis CW. 2001. Fibrin degradation products, fibrin monomer and soluble fibrin in disseminated intravascular coagulation. Seminars in thrombosis and hemostasis, 27:657-666.
3. Weisel JW, Litvinov RI. 2017. Fibrin formation, structure and properties. Subcell Biochem, 82:405–456.
4. Renschler JS. 2019. Fibrinogen (fibrin) degradation products/D-dimer. Clinical Veterinary Advisor. The Horse. Amsterdam: Elsevier, 933.
5. Rogers AA, Burnett S, Moore JC, Shakespeare PG, John Chen WY. 1995. Involvement of proteolytic enzymes—plasminogen activators and matrix metalloproteinases—in the pathophysiology of pressure ulcers. Wound Repair and Regeneration, 3:273-83.
6. Chaudhary S, Godatwar PK, Sharma R. 2015. In vitro thrombolytic activity of Dhamasa (Fagonia arabica Linn.), Kushta (Saussurea lappa Decne.), and Guduchi (Tinospora cordifolia Thunb.). Ayu, 36:421.
7. Fearnley GR. 1971. Fibrinolysis: The Fibrinolytic System. Proceedings of Royal Society of Medicine, 64:923-925.
8. Todd AS. 1980. Plasminogen activators: a morphologist's view. Journal of Clinical Pathology, 14. Supplement (Royal College of Pathologists):18.
9. Elhasade AS, Perkowska-Francka A, Szmidt J, Gaciong Z. 1998. Physiology of fibrinolysis. Medical Science Monitor, 4:555-63.
10. Cesarman-Maus G, Hajjar KA. 2005. Molecular mechanisms of fibrinolysis. British journal of haematology, 129:307-21.
11. McDowall J. 2006. Protein of the month: fibrinogen. Interpro, 205-7.
12. Schuster V, Hügle B, Tefs K. 2007. Plasminogen deficiency. Journal of Thrombosis and Haemostasis, 5:2315-22.
13. Schuster V, Mingers AM, Seidenspinner S, Nüssgens Z, Pukrop T, Kreth HW. 1997. Homozygous mutations in the plasminogen gene of two unrelated girls with ligneous conjunctivitis. Blood, The Journal of the American Society of Hematology, 90:958-66.
14. Schuster V, Seidenspinner S, Zeitler P, Escher C, Pleyer U, Bernauer W *et al.* 1999. Compound-heterozygous mutations in the plasminogen gene predispose to the development of ligneous conjunctivitis: Blood, The Journal of the American Society of Hematology. 93:3457-66.
15. Tjärnlund-Wolf A, Brogren H, Lo EH, Wang X. 2012. Plasminogen activator inhibitor-1 and thrombotic cerebrovascular diseases. Stroke, 43:2833-9.
16. Lee MH, Vosburgh E, Anderson K, McDonagh J. 1993. Deficiency of plasma plasminogen activator inhibitor 1 results in hyperfibrinolytic bleeding, 81:2357-62.
17. Ichinose A, Espling ES, Takamatsu J, Saito H, Shinmyozu K, Maruyama I *et al.* 1991. Two types of abnormal genes for plasminogen in families with a predisposition for thrombosis. Proceedings of the National Academy of Sciences, 88:115-9.
18. Fay WP, Parker AC, Condrey LR, Shapiro AD. 1997. Human plasminogen activator inhibitor-1 (PAI-1) deficiency: characterization of a large kindred with a null mutation in the PAI-1 gene. Blood, The Journal of the American Society of Hematology, 90:204-8.
19. Byeon IJ, Llinás M. 1991. Solution structure of the tissue-type plasminogen activator kringle 2 domain complexed to 6-aminohexanoic acid an antifibrinolytic drug. Journal of molecular biology. 222:1035-51.
20. Poon IK, Olsson AK, Hulett MD, Parish CR. 2009. Regulation of histidine-rich glycoprotein (HRG) function via plasmin-mediated proteolytic cleavage. Biochemical Journal, 424:27-37.
21. Vanwildemeersch M, Olsson AK, Gottfridsson E, Claesson-Welsh L, Lindahl U, Spillmann D. 2006. The anti-angiogenic His/Pro-rich fragment of histidine-rich glycoprotein binds to endothelial cell heparan sulfate in a Zn2+-dependent manner. Journal of Biological Chemistry, 281:10298-304.
22. Dixelius J, Olsson AK, Thulin Å, Lee C, Johansson I, Claesson-Welsh L. 2006. Minimal active domain and mechanism of action of the angiogenesis inhibitor histidine-rich glycoprotein. Cancer research, 66:2089-97.
23. Jones AL, Hulett MD, Altin JG, Hogg P, Parish CR. 2004. Plasminogen is tethered with high affinity to the cell surface by the plasma protein, histidine-rich glycoprotein. Journal of Biological Chemistry, 279:38267-76.
24. Thulin Å, Ringvall M, Dimberg A, Kårehed K, Väisänen T, Väisänen MR *et al.* 2009. Activated platelets provide a functional microenvironment for the antiangiogenic fragment of histidine-rich glycoprotein. Molecular Cancer Research, 7:1792-802.
25. Lane DA, Olds RJ, Thein SL. 1994. Antithrombin III: summary of first database update. Nucleic acids research, 22:3556-9.

26. Paterson AD, Rommens JM, Bharaj B, Blavignac J, Wong I, Diamandis M *et al*. 2010. Persons with Quebec platelet disorder have a tandem duplication of PLAU, the urokinase plasminogen activator gene. Blood, The Journal of the American Society of Hematology, 115:1264-6.
27. Brunet JG, Sharma T, Tasneem S, Liang M, Wilson MD, Rivard GE, Hayward CP. 2020. Thrombin generation abnormalities in Quebec platelet disorder. International Journal of Laboratory Hematology, 42:801-9.
28. Szabo R, Netzel-Arnett S, Hobson JP, Antalis TM, Bugge TH. 2005. Matriptase-3 is a novel phylogenetically preserved membrane-anchored serine protease with broad serpin reactivity. Biochemical Journal, 390:231-42.
29. Lind B, Thorsen S. 1999. A novel missense mutation in the human plasmin inhibitor (alpha2-antiplasmin) gene associated with a bleeding tendency. British journal of haematology, 107:317-22.
30. Miura O, Sugahara Y, Aoki N. 1989. Hereditary α2-plasmin inhibitor deficiency caused by a transport-deficient mutation (α2-PI-Okinawa): Deletion of Glu137 by a trinucleotide deletion blocks intracellular transport. Journal of Biological Chemistry, 264:18213-9.
31. Baig MU, Bodle J. 2020. Thrombolytic therapy. StatPearls.
32. Edwards Z, Nagalli S. 2020. Streptokinase. StatPearls.
33. Schmeltz L, Metzger B. 2007. Therapeutic Areas I: Central Nervous System, Pain, Metabolic Syndrome, Urology, Gastrointestinal and Cardiovascular. Comprehensive Medicinal Chemistry II. Amsterdam: Elsevier Science.
34. Silberstein LE, Anastasi J. 2017. Hematology: Basic Principles and Practice E-Book. Elsevier Health Sciences.
35. Tanswell P, Modi N, Combs D, Danays T. 2002. Pharmacokinetics and pharmacodynamics of tenecteplase in fibrinolytic therapy of acute myocardial infarction. Clinical pharmacokinetics, 41:1229-45.
36. Mohammadi E, Mahnam K, Jahanian-Najafabadi A, Sadeghi HM. 2021. Design and production of new chimeric reteplase with enhanced fibrin affinity: a theoretical and experimental study. Journal of Biomolecular Structure and Dynamics, 39:1321-33.
37. Mohr JP, Wolf PA, Moskowitz MA, Mayberg MR, Von Kummer R. 2011. Stroke E-Book: Pathophysiology, Diagnosis, and Management. Elsevier Health Sciences.
38. Uday P, Achar RR, Bhat PR, Rinimol V, Bindu J, Nafeesa ZO, *et al*. 2015. Laticiferous plant proteases in wound care. International Journal of Pharmacy and Pharmaceutical Sciences, 7:44-9.
39. Al-Snafi AE. 2015. Therapeutic properties of medicinal plants: a review of plants with hypolipidemic, hemostatic, fibrinolytic and anticoagulant effects (part 1). Asian Journal of Pharmaceutical Science & Technology, 5:271-84.
40. Venkatesha SH, Rajaiah R, Vishwanath BS. 2016. Hemostatic interference of plant latex proteases. SM Journal of Clinical Pathology, 1:1002.
41. Pepe A, Guevara MG, Tito FR. 2018. Potential use of plant proteolytic enzymes in hemostasis. In Biotechnological Applications of Plant Proteolytic Enzymes, 129-141.
42. Balakireva AV, Kuznetsova NV, Petushkova AI, Savvateeva LV, Zamyatnin Jr AA. 2019. Trends and prospects of plant proteases in therapeutics. Current medicinal chemistry, 26:465-86.
43. Singh MK, Bindhu OS. 2020. Plant latex: a rich source of haemostatic proteases. In Herbal Medicine in India, 143-153.
44. Ebrahimi F, Torbati M, Mahmoudi J, Valizadeh H. 2020. Medicinal plants as potential hemostatic agents. Journal of Pharmacy & Pharmaceutical Sciences, 23:10-23.
45. Siritapetawee J, Thumanu K, Sojikul P, Thammasirirak S. 2012. A novel serine protease with human fibrino (geno) lytic activities from Artocarpus heterophyllus latex. Biochimica et Biophysica Acta (BBA)-Proteins and Proteomics, 1824(7):907-12.
46. Ramos MV, Viana CA, Silva AF, Freitas CD, Figueiredo IS, Oliveira RS, Alencar NM, Lima-Filho JV, Kumar VL. 2012. Proteins derived from latex of C. procera maintain coagulation homeostasis in septic mice and exhibit thrombin-and plasmin-like activities. Naunyn-Schmiedeberg's archives of pharmacology, 385(5):455-63.
47. Rajesh R, Gowda CR, Nataraju A, Dhananjaya BL, Kemparaju K, Vishwanath BS. 2005. Procoagulant activity of Calotropis gigantea latex associated with fibrin (ogen) olytic activity. Toxicon, 46(1):84-92.
48. Karpagam N, Viswanathan S, Prabhu S, Sulochana S, Sivanandham M. 2013. Biochemical and in silico clotting activity of latex from Asclepias curassavica L. International Journal of Pharma and Bio Sciences, 4(4).
49. Shivaprasad HV, Riyaz M, Kumar RV, Dharmappa KK, Tarannum S, Siddesha JM, Rajesh R, Vishwanath BS. 2009. Cysteine proteases from the Asclepiadaceae plants latex exhibited thrombin and plasmin like activities. Journal of thrombosis and thrombolysis, 28(3):304-8.
50. Sundd M, Kundu S, Pal GP, Medicherla JV. 1998. Purification and characterization of a highly stable cysteine protease from the latex of Ervatamia coronaria. Bioscience, biotechnology, and biochemistry, 62(10):1947-55.
51. Patel GK, Kawale AA, Sharma AK. 2012. Purification and physicochemical characterization of a serine protease with fibrinolytic activity from latex of a medicinal herb Euphorbia hirta. Plant Physiology and Biochemistry, 52:104-11.
52. Hamed MB, El-Badry MO, Kandil EI, Borai IH, Fahmy AS. 2020. A contradictory action of procoagulant ficin by a fibrinolytic serine protease from Egyptian Ficus carica latex. Biotechnology Reports, 27.
53. Asare F, Koffuor GA, Nyansah WB, Gyanfosu L, Abruquah AA. 2016. Anticoagulant and antiplatelet properties of the latex of unripe fruits of Carica papaya L.(Caricaceae). Int J Basic Clin Pharmacol, 4(6):1183-88.