CHAPTER 2

LITRATURE REVIEW
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The increasing morbidity and mortality from coronary heart disease is the biggest challenge to medical scientists all over the world. There is no single etiology to this multifaceted problem. A bit of work has been done in this field. However there is many more work has to be done to explore its mechanism.

2.1 Pathophysiology of thrombus and embolism.

2.1.1 Thrombus

![Fig. 2.1: Thrombus in venous system](image)

A thrombus, or blood clot, is the final product of the blood coagulation step in hemostasis. It results due to the aggregation of platelets that forms a platelet plug, followed by activation of the humoral coagulation cascade mechanism (i.e. clotting factors).
Fig. 2.2: Schematic diagram showing coagulation mechanism.
2.1.2 Myocardial infarction

Fig. 2.3: Myocardial infarction

Myocardial infarction (MI or AMI), commonly known as a heart attack, occurs when the blood supply to part of the heart is interrupted. This is most commonly due to occlusion (blockage) of a coronary artery following the rupture of a vulnerable atherosclerotic plaque, which is an unstable collection of lipids (like cholesterol) and white blood cells (especially macrophages) in the wall of an artery. The resulting ischemia (restriction in blood supply) and oxygen shortage, if left untreated for a sufficient period, can cause damage and/or death (infarction) of heart muscle tissue (myocardium).

2.1.3 Pulmonary embolism

Pulmonary embolism (PE) is a blockage of the pulmonary artery or one of its branches, usually occurring when a deep vein thrombus (blood clot from a vein) becomes dislodged from its site of formation and travels, or embolizes, to the arterial blood supply of one of the lungs. Present mode of treatment is typically with anticoagulant medication, including heparin and warfarin. Severe cases may require thrombolysis
with drugs such as tissue plasminogen activator (tPA) or may require surgical intervention via pulmonary thrombectomy.

2.1.4 Deep vein thrombosis

Deep vein thrombosis (usually abbreviated as DVT) is the formation of a blood clot ("thrombus") in a deep vein. It is a form of thrombophlebitis (inflammation of a vein with clot formation). Deep vein thrombosis commonly affects the leg veins (such as the femoral vein or the popliteal vein) or the deep veins of the pelvis. Occasionally the veins of the arm are affected.

2.1.5 Fibrinolysis

Fibrinolysis is the process wherein a fibrin clot, the product of coagulation, is broken down. [1] It is plasmin which cuts the fibrin mesh at various places, leading to the production of circulating fragments that are cleared by other proteases or by the kidney and liver. Plasmin is produced in an inactive form, plasminogen, in the liver. Although plasminogen cannot cleave fibrin, it still has an affinity for it, and is incorporated into the clot when it is formed. Plasminogen contains secondary structure motifs known as kringle s, which bind specifically to lysine and arginine residues on fibrinogen. When converted from plasminogen into plasmin, it functions as a serine protease, cutting C-terminal to these lysine and arginine residues. Fibrin monomers, when polymerized, form protofibrils. These protofibrils contain two strands, anti-parallel, associated non-covalently. Within a single strand, the fibrin monomers are covalently linked through the actions of coagulation factor XIII. Thus, plasmin action on a clot initially creates nicks in the fibrin and further digestion leads to solubilization.[2]
Tissue plasminogen activator (t-PA) and urokinase are the agents that convert plasminogen to the active plasmin, thus allowing fibrinolysis to occur. t-PA is released into the blood very slowly by the damaged endothelium of the blood vessels, such that, after several days (when the bleeding has stopped), the clot is broken down[3]. This occurs because plasminogen became entrapped within the clot when it formed; as it is slowly activated, it breaks down the fibrin mesh. t-PA and urokinase are themselves inhibited by plasminogen activator inhibitor-1 and plasminogen activator inhibitor-2 (PAI-1 and PAI-2). In contrast, plasmin further stimulates plasmin generation by producing more active forms of both tPA and urokinase. [3]

![Diagram of Fibrinolysis]

Fig. 2.4: Fibrinolysis
Alpha 2-antiplasmin and alpha 2-macroglobulin inactivate plasmin. Plasmin activity is also reduced by thrombin-activatable fibrinolysis inhibitor (TAFI), which modifies fibrin to make a less potent cofactor for the tPA-mediated plasminogen.

Anticoagulant and antiplatelets are only moderately efficient for acceleration of lysis and prevention of reocclusion, but are safe. Selective thrombin inhibitors and antiplatelet agents are more potent, but their safety remains to be confirmed [5]. Continued investigation in this area will provide new insights and promote progress toward the development of the ideal thrombolytic therapy, characterized by maximized stable coronary arterial thrombolysis with minimal bleeding [5].

Several new thrombolytic agents have been developed. Compared with older agents (alteplase), newer thrombolytic agents such as monoteplase, tenecteplase, reteplase, lanoteplase, pamiteplase, and staphylokinase result in a greater angiographic potency rate in patients with acute myocardial infarction, although, thus far, mortality rates have been similar for those few drugs that have been studied in large-scale trials. Bleeding risk, however, may be greater. Recently, preventive measures against thrombosis have been tried. Oral administration of the fibrinolytic enzyme nattokinase has been reported to enhance fibrinolytic activity in plasma and the production of tPA [6].

*Terminalia belerica* is one of the constituents of commonly prescribed preparation "Triphala". Triphala is used in wide array of areas ranging from laxative, headache, leucorrhoea, liver diseases to gastro-intestinal complaints and it is the best herb for controlling Kapha. It has been also used as a rejuvenative herb that nourishes the lungs, throat, voice, eyes
and hair. Researches have shown that it can expel stones or other kapha-type accumulations in the digestive, urinary, and respiratory tracts. It is unique in being both laxative and astringent, so it purges the bowels, while simultaneously toning the tissues of the digestive tract. It provides strength to the tissues of the sense organs. The overall tonic effect of this fruit has been known for thousands of years in India and other Asian countries.[8]

Triphala acts as anthelmintic, antiseptic, astringent, expectorant, laxative, lithotriptic, rejuvenative, tonic. It is useful in asthma, biliousness, bronchitis, inflammations, sore throat, and treating the diseases of eyes, nose, heart and bladder. Triphala and its constituents act as cardio-tonic, control blood pressure, improves blood circulation and reduces cholesterol levels [8][12].

The Ayurvedic Pharmacopoeia of India recommends the drug *Terminalia belerica* in powder form in emesis and worm infestation, in addition to other therapeutic applications. [9]

The fruits of *Terminalia belerica* contain beta-sitosterol, gallic and ellagic acids, ethyl gallate, galloyl glucose, chebulagic acid and a cardiac glycoside, bellaricanin. The fruits produce hepato-protective effect in CCl₄-induced liver injury in mice. Alcoholic extract of the fruit exerts a negative chrono-and inotropic and hypotensive effect of varying magnitude in a dose dependent fashion on isolated rat and frog atria and rabbit heart. [9]

*Nepeta hindostana* also known as Baadranjboya, Billilotan, (belong family *Labiatae*) is generally found in Punjab, Uttar Pradesh, Bihar, West Bengal, Madhya Pradesh and South India.[10]
The Avicenna recommended it for heart problems. Its main action is as a tranquillizer. It is a soothing and calming agent for stressed nerves. The herb is a common constituent of relaxants, nerveine and sleeping aids throughout the world. It is primarily indicated where there is dyspepsia associated with anxiety or depression [14]. It relieves tension and stress reactions, is widely valued for its calming properties and has a tonic effect on the heart and circulatory system causing mild vasodilation of peripheral vessels, thus lowering blood pressure. It is also used in migraine associated with tension, neuralgia, anxiety-induced palpitation and insomnia. It is useful in liver diseases and weakness of eyesight. Also used as a spermatogenic tonic. The plant also shows an effect on the thyroid gland and has been used to treat hyperthyroidism. It is also useful in sinusitis. [12]

The alcoholic extract of the plant yielded a triterpenoid aldehyde, nepeninal.

Nepetidone, nepedinol, and a triterpenic acid have also been reported. The plant contains flavonoids including nepitrin, dinatin, nepetin. Flowers and stem gave napetol, hentriacontane and beta-sitosterol. An aqueous extract of flowers contained Na, K, Ca, Mg, Zn, Cd, Cu, Ni, Mn and Fe. Alcoholic extract of the plant produced marked hypocholesterolaemic effect in experimental animals. It also produced beneficial effects in the histopathology of myocardial infarction. Aqueous extract of the plant, given intravenously, lowered the blood pressure in dogs. It also showed CNS depressant and sedative activities. In Indian medicine, the plant is used in various cardiac conditions including cardiac asthma. [10]
Fig. 2.5: *Nepeta hindostana* plant

Fig. 2.6: *Nepeta hindostana* flowers
*Nigella sativa* (belong family *Ranunculaceae*) also known as Black Cumin, Small Fennel, Kaalaajaaji, Kalikaa, Prthvikaa, Sthulajiraka, Sushavi, Upkunchikaa, Kalonji, Kamaazaruuus, is generally found in Punjab, Bengal, Assam and Bihar.\[11\]

The seeds of *Nigella sativa* used as stimulant, carminative, diuretic, lactiferous, emmenagogue (stimulate uterine contractions). It is also used in puerperal fever. The powder of seeds is applied externally for treatment of boils. \[11\]

Essential oil of *Nigella sativa* is used in common cold, cough and broncho spasm. The essential oil from seeds contains nigellone and 2-methyl-4-isopropyl-\(p\)-quinone. The oil contains carvone (45–60%), \(d\)-limonene and cymene. Seeds contain fatty acids including palmitic, myristic, stearic, oleic, linoleic and linolenic. Beta-sitosterol is also present in the seeds. \[11\]

Low concentration of nigellone has been shown to inhibit the release of histamine from mast cells in animals. \[11\]

The ethanolic extract of the seeds and the volatile oil from seeds showed antispasmodic activity in experimental animals, possibly due to a calcium antagonistic effect. The oil exhibited CNS depressant and potent analgesic effects on experimental animals, possibly due to the presence of an opioid principle in the oil. \[11\]

Karen et al.\[15\] worked on onion induced antiplatelet activity and reported that onion extracts inhibit in vitro, in vivo and ex vivo human platelet aggregation. Much of the antiplatelet activity in onions has been attributed to unique organosulfur compounds, thiosulfinates. Upon disruption of onion bulb tissue either by cutting or crushing, the
Salk(en)yl-L-cysteine sulfoxides react with alliinase to form thiosulfimates. Thiosulfimates are responsible for onion flavor and a significant portion of the antiplatelet activity induced by onions. [15] Yamamoto et al.[16] worked on antithrombotic effect of carrot filtrates in rats and mice and found no significant correlation between antithrombotic activity and the levels of polyphenolics and any other biochemical parameter, including antioxidant activity, alpha-carotene and beta-carotene, alpha-tocopherol and ascorbic acid.[16] Yang et al. [17] worked on suppressive effect of Resveratrol on ADP induced human platelet aggregation and its active mechanism. Resveratrol is a polyphenolic compound present in native plants such as grape, fleeceflower root, and peanut, etc. They investigated the in vitro effects of Resveratrol on adenosine diphosphate (ADP)-induced platelet aggregation, platelet membrane-bound fibrinogen and its mechanism of action. Their methods involve platelet aggregometer, flow cytometry and Western blotting, respectively. All their experiments suggest that Resveratrol inhibited platelet aggregation and platelet membrane-bound fibrinogen induced by ADP partly through decreasing the activity of phospholipase-C beta of platelets, and that Resveratrol had definite effect of antiplatelet and might be developed as a novel antithrombotic agent. [17] Shaila et al.[18] experimentally induced hypercholesterolaemia and atherosclerosis in rabbits by cholesterol feeding and evaluated the effect of Terminalia belerica, which reduced the levels of lipids in hypercholesterolemic animals. A significant decrease in liver lipids and heart lipids (P <0.05) in the drug-treated animals was also reported. [18]
Fibrinolytic therapy is an option in the treatment of patients with PE due to its ability to rapidly dissolve thromboemboli clots and there is a clear benefit/risk ratio for fibrinolytic therapy in patients with PE who present with cardiac arrest and in those who are hemodynamically unstable from a massive PE. [19]

Effects of alginate microencapsulation on the fibrinolytic activity of fermented soybean paste have been studied by Ko et al.[20] Prepared Alginate microparticles was evaluated for the effect on the fibrinolytic activity of Korean fermented soybean paste. Fibrinolytic activities of encapsulated and non-encapsulated soybean extract were measured at various ranges of pH and temperature. When non-encapsulated soybean extract was exposed to simulated gastric juice of pH 2.0, the fibrinolytic activity was rapidly reduced. However, fibrinolytic activity of encapsulated soybean extract was significantly higher than that of non-encapsulated soybean extract. The fibrinolytic activity of non-encapsulated soybean extract decreased rapidly with increased temperature, but stability of fibrinolytic activity of encapsulated soybean paste was improved under high temperature conditions. These results indicate that the microencapsulation technique is an effective tool to protect the fibrinolytic activity of soybean extract from ingestion and heating effects. [20]

Work on antiplatelet and antithrombotic activity of l-3-n-butylphthalide in rats was carried out by Ying Peng et al.[21] and reported that 3-n-butylphthalide (NBP) is a potentially beneficial drug for the treatment of ischemic stroke with multiple actions on different pathophysiological processes. They investigated the effect of l-, d-, and dl-NBP on ADP, collagen, and Arachidonic acid induced platelet aggregation. In their
experiment the l-NBP was the most potent among l-, d-, and dl-NBP. At higher concentration, the effect of dl-NBP on platelet aggregation was greater than that of l- or d-NBP alone. The ex vivo anti-aggregatory activity of l-NBP (100mg/kg) declined gradually after 2 hours, but a considerable antiplatelet activity was still observed 4h after l-NBP administration. NBP was given orally and resulted in a dose-dependent inhibition of thrombus formation. Of the two isomers, l-NBP was the most potent. It significantly protected mice from a mixture of collagen and epinephrine induced thromboembolic death. When 100 mg/kg of l-NBP were administered orally to rats, the bleeding time increased 2.1-fold compared with the control group. At the same dose, ex vivo platelet aggregation induced by ADP, collagen, and AA was inhibited by l-NBP and the antithrombotic effects of the compound were also observed. Thus, NBP exerts oral anti-platelet and anti-thrombotic efficacy without disturbing systemic hemostasis in rats. [21]

Fibrinolysis, the result of activation of normally circulating human plasminogen, occurs spontaneously under natural conditions. The mechanism, by which this clinical condition occurs, however, is not known. In the laboratory, the activation of plasminogen can be demonstrated quite readily by the lysis of the fibrin clot, which may be achieved in the following ways; (1) by tissue activators (tissue kinases); (2) by urinary activators (urokinase) and other body fluids such as tears and milk; (3) by trypsin- and chloroform-treated plasma; and (4) by streptokinase and other bacterial filtrate in the presence of a proactivator. The resultant product of this activation is plasmin, which splits the peptide bond of fibrin and thus causes lysis. [23]
Antiplatelet activity of various extracts obtained from Jordanian *Achillea falcata* has been reported [24]. Chemical composition of the volatile oil hydrodistilled from the plant aerial parts was also evaluated by GC and GC/MS and showed significant inhibitory effect against platelet aggregation induced by collagen and ADP while the aqueous extract showed no inhibition against blood aggregation induced either by collagen or ADP. [24]

The spontaneous fibrinolytic activity of blood is due to a labile activator which appears to be stabilized by adsorption to fibrin. At present, activator activity can be measured only indirectly through its conversion of plasminogen to plasmin which in turn causes fibrinolysis. It is essential to cool the blood sample to 0°C immediately after collection, and to carry out any subsequent experiment at this temperature in order to preserve the activator which disappears rather rapidly from fluid blood kept at room temperature. It is shown that those two factors inhibit spontaneous fibrinolysis, the natural inhibitory capacity of the blood, which is probably complex, and the presence of calcium. The first can be diminished by dilution or by separation of the euglobulin fraction, the inhibitors remaining in the supernatant and the calcium can be removed by precipitation. [25]

Activated protein C stimulates the fibrinolytic activity of cultured endothelial cells and decreases anti-activator activity. Bovine activated protein C (APC) was investigated on the fibrinolytic activity of cultured bovine aortic endothelial cells. In these experiments confluent monolayers were incubated with purified APC under various conditions and changes in total fibrinolytic activity and in the level of plasminogen activator and plasminogen activator inhibitor (antiactivator) were
monitored. The addition of APC to the cells in the absence of other blood or plasma components led to a rapid, dose-dependent increase of fibrinolytic activity both in the media and in cellular extracts. This enhancement in fibrinolytic activity indicates increase in both urokinase-related and tissue-type plasminogen activators produced by these cells. It has been reported that diisopropyl fluorophosphate- inactivated APC did not decrease antiactivator or increase plasminogen activator. Although a small but significant direct (i.e., cell-independent) effect of APC on both fibrinolytic activity and antiactivator activity could be demonstrated, the major portion of these changes appeared to be cell-mediated. These observations indicate that the fibrinolytic potential of cultured endothelial cells is increased by APC and that the enzyme active site is essential for this change. Moreover, the results suggest that one of the primary mechanisms for this stimulation of endothelial cell fibrinolytic activity involves an APC-mediated decrease in antiactivator. [26] Studies on enhanced fibrinolytic activity in man has shown that enhanced fibrinolytic activity occurs in the blood of almost all patients following electroshock, pyrogens and severe exercise and that a less consistent response occurs following epinephrine, acetylcholine and ischemia. Furthermore, it is possible to demonstrate the appearance in the plasma of a plasminogen activator following each of the circumstances mentioned above. Following methods were used in these experiments:

**Whole blood clot lysis:** Whole blood clots were made with 0.9 ml. of oxalated blood and 0.1 ml. of thrombin (Parke-Davis; 10 units per ml. in 0.01 M veronal buffer, pH 7.4). Clots were incubated at 370 in a water bath and the time for complete lysis recorded. Samples of spontaneously
clotted whole blood were compared with oxalated samples clotted with thrombin. [28]

Brassica oleracea, Capsicum frutescens, and combination of Honey & Nigella sativa has been reported to produce significant clot lysis effect with reference to standard drug Streptokinase in in-vitro thrombolytic models [29].

Use of fibrinolytic agents is a widely used tool in the treatment of thrombotic diseases. Fibrinolytic agents such as streptokinase or tissue-type plasminogen activator are used to activate the fibrinolytic system by converting the plasminogen to active plasmin, which is responsible to cleave the fibrin clot into soluble components. But the administration of these agents often produces secondary hemorrhagic events that may be life-threatening. The development of new thrombolytic agents with greater clot specificity is an area of intense study. [30]
REFERENCES:


human platelet aggregation and its active mechanism’, *Yao Xue Xue Bao.*; 43:4, pp 356-60.


24- Talal Aburjai and Mohammad Hudaib, (2006), ‘Antiplatelet, antibacterial and antifungal activities of *Achillea falcata*
extracts and evaluation of volatile oil composition’, *Pharmacognosy Magazine*; 2: 7, pp-191-198


