REVIEW OF LITERATURE
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The salivary gland of Hirudo medicinalis contain hirudin, a powerful thrombin inhibitor (Bagdy et al., 1976; Dodt et al., 1984, 1985). Their secretion also found to contain an inhibitor of the intrinsic mechanism of blood clotting (Baskova et al., 1984 a) as well as inhibitors of thrombin and trypsin (Baskova et al., 1984 b).

The saliva of the leech Hirudo medicinalis also contain eglin, hyluronidase, apyrase and collagenase (Rigbi et al., 1987). Building on observation that the saliva of blood sucking animals is also constituted as to maintain the ingested blood in the fluid state and modulate the inflammatory response (Riberio et al., 1985). Baskova et al., (1984,1984C) state that the saliva of blood sucking leech, Hirudo medicinalis strongly inhibits human platelet
aggregation induced by ADP. The saliva also inhibits platelet aggregation induced by collagen and epinephrine. Evidently, the inhibition by leech saliva covers a range of aggregating agents. Therefore, apart from inhibition by enzymes such as apyrase which has been shown to inhibit ADP induced platelet aggregation (Haslam, 1967); Orevi et.al., (1992) and leech collagenase (Rigbi et al., 1987) which presumably does so (by interacting with collagen). The saliva contain an additional factor which inhibits epinephrine induced platelet aggregation. Epinephrine activate platelets by binding to the $\alpha_2$- adrenergic receptor on the platelet membrane.

Rigbi et al., (1987) reported that the ingested blood by maintaining in the liquid state, leech saliva inhibits some leukocyte activities. Leech saliva contains eglin a powerful inhibitor of leukocyte elastase and cathepsin G. It inhibits the production of super oxide radical and consequently of resultant oxygen metabolites, an activity to be due at least in part to eglin.

Leech saliva strongly inhibits human platelet aggregation in a dose related manner when induced by collagen, ADP or epinephrine. The initial lag in collagen induced aggregation represents the latent phase which leads to the release of ADP by platelets (Zucker, 1983). When ADP and epinephrine are added, platelets begin to aggregate immediately. A secondary phase follows due to release of endogenous ADP and Thromboxane A$_2$ (Eldor et al., 1983).
Eglin is also known to inhibit leukocyte elastase and cathepsin G (Seemuller et al., 1977).

The anaesthetic effects of leech bites, and dilute leech saliva for their effects on the sensitivity thresholds to heat, touch and pain and/or the evoked CAP following stimulation of peripheral nerve. The results are negative. Leech saliva may however, possess kinase activity which would reduce the pain producing effect of bradykinins (Ribeiro et al., 1985).

The anticoagulant of Hirudo medicinalis is a specific thrombin inhibitor (Markwardt, 1970). The serine protease inhibitors of the leech have been the subject of extensive studies (Fritz and Krejci, 1976; Seemuller et al., 1977; Jochum et al., 1983). Four different types of protease inhibitors have been characterized,

a) hirudin (Markwardt, 1970) with specificity against thrombin and trypsin

b) bdellin, a low molecular weight protein with inhibitory activity against trypsin and plasmin. (Fritz and Krejci, 1976),

c) a high molecular weight protein with inhibitory activity against trypsin (Seemuller et al., 1977) and

d) eglins, two low molecular weight polypeptides with inhibitory activity against chymotrypsin, granulocyte elastase, subtilisin and cathepsin G (Seemuller et al., 1977).
Hyaluronidase, another pharmacologically active agent secreted by leeches, acts as a spreading factor in the host tissues. (Linker et al., 1960). This substance is also known as orgelase. It increases the blood flow in the region of the wound produced by the feeding leech.

Hirudin and orgelase is useful in reestablishing blood flow in occluded arteries. Several proteinase inhibitors, have been isolated from H. medicinalis.

Quattan et al., (1993); Rapaport et al., (1993) reported that the saliva of the leech has a lipid profile that is compatible with and necessary for its functions. These functions permit the easy flow of blood and of other nutritional product from the attacked individuals and may help to prevent the closing of wound or coagulation that would help the flow of fluids to the leech.

Lee, Peckit (1996) reported that the saliva of leech Hirudo medicinalis contains vasodilator which aids in the flow of blood in the capillaries and a “prostaglandin that reduces swelling”. Once the leech has completed feeding, it promptly falls off the host.

Chmiel et al., (1989) stated that the blood viscoelasticity is decreased after leech application, although no significant changes of hematocrit plasma viscosity and fibrinogen concentration observed. The aggregation tendency and flexibility of RBC showed a significant decrease after leech treatment. Therefore, leeching is not a
hemodilution effect alone apart from its antithrombin effect. It’s remaining effect is on hemorheological properties. (Markwardt et al., 1984). Leech prostaglandin as main antihemostatic agent have a protective antithrombic ability, which diminishes after 28 hours. (Nikonov, 1987).

Bottenberg, (1935) and Wanke, (1951) reported that salivary gland secretion of leeches have a complex structure consisting of various substances (not a single substance but a combination of several). Due to its blood cleansing and metabolism stimulating effect the leech treatment can induce a new formation in the bone marrow. Therefore, under the influence of the active substance of the leech salivary gland secretion, the erythropoiesis is stimulated and that the new formed red blood cells are possibly endowed with normalized hemorheological properties.

_Hirudo medicinalis_ suck polling blood. The calcite teeth of the leech jaw rip up the skin tissue of the host. The contraction of the injured blood vessels is blocked by histamine (Lindemann 1929; Damas 1974). Damas (1974) reported gelatinolytic activity to be present in the salivary glands.

The fast and specific reaction of hirudin with thrombin was utilized for quantitative determination of the inhibitor. One mole hirudin complexes with one mole enzyme corresponds to the antithrombin activity of pure hirudin of which 1mg inhibits about 15 units of human thrombin. Therefore, the activity of hirudin is
measured in antithrombin units (ATU). Where 1 ATU is the amount of hirudin which neutralises 1 U of thrombin. (Markwardt, 1970; Walmann, 1988).

The anticoagulant effect of hirudin is highly specific for a thrombin without affecting other closely related serine proteinase or further enzymatic activities due to its high affinity for thrombin. Relatively low inhibitor concentrations are necessary to prevent coagulation. In the hirudin-thrombin complex, all proteolytic functions of the enzyme are blocked. Thus hirudin presents not only fibrinogen clotting but also further thrombin catalysed hemostatic reaction such as activation of clotting factor V, VII, XIII and the thrombin induced platelet reaction. Therefore, by instantaneous inhibition of the small amount of thrombin generated after activation of the coagulation system, the positive feed back on prothrombin activation is prevented that otherwise would lead to accelerated generation of further thrombin. (Markwardt, 1956).

Hoffmann (1984) stated that depending on the hirudin concentration in blood, coagulation is retarded or completely inhibited. Accordingly, the coagulation variables, thrombin time, partial thromboplastin time and prothrombin time are prolonged. He further confirmed that after complexing with hirudin, thrombin loses its effect on platelet i.e. the thrombin induced platelet aggregation and release reaction are prevented. Glusa (1990) observed that no
influence is exerted on the platelet reactions induced by other activators like ADP, collagen and adrenaline or platelet adhesion.

Earlier, Glusa (1988) has shown that thrombin is an agonist for various cellular activities, such as proliferation of fibroblasts, stimulation of endothelial cells and contraction of smooth muscle cells. After complexing with hirudin, thrombin loses cellular non hemostatic effects as well. For thrombogenesis, it is of importance that hirudin is able to inhibit the vasoconstriction activity of thrombin in de-endothelialized vessels.

Toxicology studies in experimental animals and in human volunteers showed that, apart from its anticoagulant effect, hirudin is pharmaco-dynamically inert and is well tolerated (Markwardt et al., 1984; Bichler et al., 1988). Hemorrhagic complications and signs of sensitization are not observed during leeching. The intravenous injection is tolerated without any changes in heart rate and respiratory rate. The blood pressure remained constant. Hirudin did not cause any changes in platelet count, fibrinogen level and plasma hemoglobin. (Kaiser, 1986).

For pharmaco kinetic studies, hirudin content in blood is based on its complexing with thrombin and on determination of the residual thrombin activity by measuring the clotting time or the chromogenic activity (Griessbach et al., 1985) Immunological methods are used by other workers (Spinner et al., 1986).
Pharmaco kinetic data resulting from the evolution of its blood level and urinary excretion have shown that hirudin given intravenously to experimental animals is eliminated in active form through the kidney by glomerular filtration (Henschen et al., 1988). The results of pharmaco-kinetic studies with hirudin in man agreed with those obtained in animal experiment (Markwardt et al., 1987).

After genetically engineered recombinant desulfato - hirudins proved to be as potent anticoagulant as native hirudin, preclinical studies were started. Identical thrombosis models were used to examine r - hirudin and naive hirudin for their efficacy. It is shown that depending on its blood level, r hirudin is anti thrombotically effective in different models of experimental thrombosis. (Markwardt et al., 1988; Talbot et al., 1989).

Markwardt et al., (1977) reported that the hirudin is superior to heparin in that it anticoagulant effect does not require the presence of antithrombin III further more, it is not bound by the heparin neutralising platelet factor IV. The beneficial effect of hirudin become particularly apparent in DIC. (Disseminated Intravascular Coagulation).

Munro et al., (1991) described a potent inhibitor of collegen mediated platelet adhesion / aggregation in leech saliva which they named calin.
The use of leeches in the management of acute problems related to the venous congestion in patients with traumatic injuries and surgical problems, They have demonstrated that the salivary secretion from the leeches contain anaesthetic substance and the bite of all blood sucking leeches is painless. This substance is not identified yet, but it is known that this is different from Hirudin (Varshney et al., 1999).

The leech saliva also contains vasodilator. This Vasodilator is histamine like substance. In Hirudo medicinalis, this substance is secreted from the salivary cells and it is called as orgelase hyaluronidase. The hyaluronidase located in the salivary cells of Hirudo medicinalis plays more important role in the efficacy of leeching than even the anticoagulant. Nobel laureate Claude (1937) showed that this substance is not hirudin and that it is located in the anterior part of the leech, and others showed that this substance is mucolytic and specific for hyaluronic acid (Damas 1974). Leech hyaluronidase has powerful antibiotic properties as well as good effect in the treatment of glaucoma. Proteolytic inhibitors are also isolated from saliva of Hirudo medicinalis. These are hirudin, bdellin, and eglin. These proteolytic inhibitors are immunologically distinguishable and mature. Hirudo medicinalis contains 285 ATU of hirudin, varying with feeding state.

The study was also carried out in 23 children’s during 10 years suffering from appendicular infiltration, treated with leech
application and had shown considerably better results. (Solov’ev et al., 1989).

Medicinal leeches (\textit{Hirudo medicinalis}) are being used with increasing frequency for the treatment of venous congestion in flaps. (Dereganc and Zdravic, 1960; Dickson et al., 1984), and microvascular tissue transfer (Mercer et al., 1987; Henderson et al., 1983; Mutimer et al., 1987). Coincident with increased use of leeches, soft tissue infections following leech application are being reported, with one group noting a 20% incidence of infection in patents receiving leeches. (Dickson et al., 1984; Mercer et al., 1987). The bacterium isolated from these infections is \textit{Aeromonas hydrophila}; a gram negative that resides in the leech gut and symbiotically assists in the digestion of ingested blood. (Jenning and Vanderlande, 1967; Whitlock et al., 1983). The \textit{Aeromonas hydrophila} infections following leech use have resulted in abscess formation and tissue necrosis (Dickson et al., 1984; Mercer et al., 1987). Abrutyn (1988) state that \textit{Aeromonas hydrophila} is a normal flora symbiotic in the gut of medicinal leeches.

A wound infection in an immunologically normal host caused by \textit{Aeromonas hydrophila} derived from the medicinal leech, which is the vector because leeches are being increasing used after plastic surgery to relieve venous congestion, the possibility of infection from them is a constant threat (Admas, 1988).
Whitlock et al., (1983) found *A. hydrophila* in the mucous secretion, suckers and gut of the medicinal leech. They predicted that *A. hydrophila* infection could result from the use of these animals.

Dickson et al., (1984) reported the first case of *A. hydrophila* wound infection associated with the medicinal leeches. It occurred in a skin flap placed during breast reconstruction. Mercer et al., (1987) reported the first case of *A. hydrophila* wound infection associated with the medicinal leech. It occurred in a skin flap placed during breast reconstruction. Mercer et al., (1987) reported six cases of *A. hydrophila* wound infection in 30 patients with plastic surgical repairs who treated with leeches.

*Aeromonas hydrophila* has been implicated in three types of infection. It has been reported to be the causative organism in 2% of patients with diarrhoeal and infection may occur after injuries sustained while swimming in contaminated water and in immunocompromised patients. The organism is occasionally carried in faeces. (Millership, 1983; Bulger 1966). Whitlock et al., (1983) suggested that as there is a risk of *Aeromonas hydrophila* infection the clinical use of leech should be prohibited.

According to David et al., (1980). The Aeromonas group is associated in man with septicemia, pneumonia and moderate to server gastroenteritis and that their recognised indigence in severe human disease has been steadily increasing. Serious infections in
man due to Aeromonas hydrophila have been reported by Lion et al., 1979; Beaune et al., (1978).

However, because of its apparent clinical efficacy, use of leeches will continue to increase (Foucher et al., 1981; Henderson et al., 1983). If secondary infection proves to be a significant problem, the use of antibiotic for prophylaxis against Aeromonas hydrophila infection suggested by Mutimer et al., (1987). One study reconfirm that first generation cephalosporins, commonly used as prophylactic antibiotic in microsurgical procedure (Lineaweaver et al., 1988b), do not provide reliable coverage of Aeromonas hydrophila.

It has been suggested that leeches should not be applied to tissue which has a severely compromised arterial blood supply, to prevent the seeding of neurotic tissue with Aeromonas spp. Furthermore, the prophylactic administration of antibiotic has been proposed when leeches are applied. A hydrophila isolated from 25 leeches were all susceptible to ciprofloxacin, tetracycline and co-trimoxazole (Braga et al., 1990). Interestingly, antibiotic administered orally to patients before the application of leeches has subsequently been detected in leech enteric contents, and this is associated with a reduction in the number of gut A. hydrophila (Lineaweaver et al., 1992 a).

Clinical isolates of Aeromonas spp. are susceptible to a wide range of antimicrobial agents; they are universally resistant to
penicillin, ampicillin, carbenicillin, erythromycin, streptomycin and clindamycin. Among other antimicrobial agents examined, chloramphenicol, ciprofloxacin and co-trimoxazole have been consistently active against *A. hydrophila*, *A. sobria* and *A. caviae* (Overman, 1980; Fass and Barnisham, 1981; Motyl et al. 1985, Kuijper et al., 1989).

Medicinal leeches (*Hirudo medicinalis*) are often used by microvascular surgeons to decongest flaps when venous drainage is inadequate. Infection with *A. hydrophila* may be acquired from numerous sources, mainly from fresh water and exceptionally from leeches (Mc Cracken and Barkley, 1972; Whitlock et al., 1983).

A central platelet aggregating agent is ADP. Apyrase (ATP diphosphohydrolase, EC 3.6.1.5) which hydrolyses ATP and ADP to AMP has been reported to be present in the salivary secretions of *R. prolixus*. (Ribeiro and Garcia, 1980), idammini (Ribeiro et al., 1985). The salivary secretion of *R. prolixus* (Ribeiro and Garcia, 1981) and idmini (Ribeiro et al., 1985). possess platelet antiaggregating activity which is attributed to the action of apyrase. Potato apyrase has in fact been found to have antiinflammatory and immune suppressive effects (Ribeiro et al., 1985). A general concept has emerged that the saliva of blood sucking animals is a composite system which has evolved in such a way as to maintain the blood in the liquid state during ingestion and storage to prevent inflammation and suppress the host’s immune response with this concept in mind and against
the background here presented to investigate the biologically active compound of *Hirudo saliva*.

Of the leech salivary enzymes apyrase and colagenase, apyrase is shown to exist in two forms with molecular weights of 45KD and probably over 400 KD respectively. The large enzyme may be an oligomer of the smaller one, or it may be a different enzyme altogether. It is worth mentioning that two apyrases, with molecular weights of about 120 and 12000 KD were found in the saliva of the bug, *R. prolixus* (Smith and Parke, 1958). Two apyrases were also found in white potatoes, and two others in red potatoes (Molnar and Lorand, 1961). Optimum activity of leech salivary apyrase is around pH 7.5, which is different from that of *R. prolixus* (pH 7.5-8.5; Ribeiro and Garica, 1980) and that of *A. geyptii* (pH 9.0; Ribeiro et al., 1984). The presence of apyrase in leech saliva, as in the saliva of blood sucking arthropods is strongly suggestive of its function as a platelet anti-aggregant (Ribeiro et al., 1984).

The interest in the protease inhibitors of *H. medicinalis* has not caused analysis of protease inhibitors from other types of leeches. One exception is the south American bloodsucker *Haementeria ghilianii*, which was shown by Budzynski et al., (1981, 1981a) to contain an enzyme in its salivary glands that degraded fibrin and fibrinogen; thus preventing coagulation of the host's blood. This leech did not contain a hirudin type protease inhibitor. Murer et al., (1984) showed that salivary gland extracts
from *H. ghilianii* and the related species *H. officinalis* contained inhibitors of trypsin, aymotrypsin, plasmin and granulocyte elastase.

Bunker (1981) described medicinal leech in the management of periorbital haematoma in an accident and emergency department. Foucher et al., (1987) reported 14 cases of digital replantation in which venous repair had been impossible. In all these cases medicinal leeches were applied to the wound, twice a day for five days, and satisfactory survival was achieved in all.

Thus at present leeches are used widely in parasurgery and looking towards leech as a host of *Aeromonas* and many more bacteria, it was thought to be essential to study the hematological changes after leeching.