



ALTERATIONS IN IMMUNOLOGICAL PROTEIN VALUE AFTER LEECH THERAPY

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Abstract:

The leech has been used extensively among people for bloodletting since ancient times in the treatment of various disorders. *Hirudino medicinalis*, has also proven effects in medicine and pharmacology. Medicinal leeches secrete saliva containing about 60 different proteins. These achieve a wide variety of goals useful to the leech as it feeds, helping to keep the blood in liquid form and increasing blood flow in the affected area. Several of these secreted proteins serve as anticoagulant (such as hirudin), platelet aggregation inhibitors (most notably apyrase, collagenase, and calin), vasodilators, and proteinase inhibitors. The saliva contains an anesthetic substance, as leech bites are generally not painful. The physiological effect of this secretion was determined not only by hirudin which inhibits coagulation by thrombin binding but also by components blocking the contact stage of blood coagulation.

Key word : Blood-letting, hirudin, *Hirudo medicinalis*, protein, hirudotherapy

Introduction

The blood-sucking annelids, leeches have been used for therapeutic purposes since the beginning of civilization. Ancient Egyptian, Indian, Greek and Arab physicians used leeches for a wide range of diseases starting from the conventional use for bleeding to systemic ailments, such as skin diseases, nervous system abnormalities, urinary and reproductive system problems, inflammation, and dental problems. Kaiser, Markward (1986) Recently, extensive researches on leech saliva unveiled the presence of a variety of bioactive peptides and proteins involving antithrombin (hirudin, bufrudin), antiplatelet (calin, saratin), factor Xa inhibitors (lefaxin), antibacterial (theromacin, theromyzin) and others. Haycraft (1984) Consequently, leech has made a comeback as a new remedy for many chronic and life-threatening abnormalities, such as cardiovascular problems, cancer, metastasis, and infectious diseases. Baskova *etal* (1988) Medicinal leech therapy made an international comeback in the 1970s in microsurgery, used to stimulate circulation to salvage skin grafts and other tissue threatened by postoperative venous congestion, particularly in finger reattachment and reconstructive surgery of the ear, nose, lip, and eyelid. Other clinical applications of medicinal leech therapy include varicose vein, muscle cramps, thrombophlebitis, and osteoarthritis, among many varied conditions. Griessbach *etal* (1985) The therapeutic effect is not from the blood taken in the meal, but from the continued and steady bleeding from the wound left after the leech has detached, as well as the anesthetizing, anti-inflammatory, and

vasodilating properties of the secreted leech saliva. Adams (1988) The most common complication from leech treatment is prolonged bleeding, which can easily be treated, although allergic reactions and bacterial infections may also occur. when leeches bite a victim, their unique saliva causes blood flow to increase and prevents clotting. As a result, once bitten, victims can bleed for hours, allowing oxygenated blood to enter the wound area until veins regrow and regain circulation. Leech saliva contains biologically active compounds that are mainly proteins & peptides. Marshall (1984).

The use of leeches is not without risks, such as serious wound infection, but many medical practitioners and researchers believe the benefits often outweigh the risks and that risks can be minimized (e.g., with the use of prophylactic antibiotics) 2004 was really the greatest year for the surgeons and other physicians who have worked with leeches in micro and constructive surgery. That was the year the FDA approved the request of the French company, Ricarimpex, for the commercialization of medicinal leeches in the United States. This approval of medicinal leeches as a medical device in the field of plastic and reconstructive surgery has helped leech USA to do the commercialization of the medicinal leeches in United States for the French company. Leech USA has been selling Many clinics for plastic surgery all over the world started to use leeches for cosmetic purposes. Lion *etal* (1979) Despite the efficacious properties of leech therapy, the safety, and complications of leeching are still

controversial. Despite being a comparatively simple creature, the leech, so it seemed to medical practitioners, was capable of considerable behavioral unpredictability that had to be controlled in order to make it a safe and useful medical tool.

Method and Material

The present study was carried out on starved *Poecilobdella viridis* weighing about 2.5 to 3 gms. Leeches were collected locally. Leeches were kept in the laboratory without food at 20 °C in the dark avoiding direct sunlight. They were kept in suitable containers with sufficient dechlorinated tap water. The water was changed every other day. temperature was upto 45° F. Recommended method for Leech Application.

Seven healthy male and female volunteers from age ranged 20 to 50 years with weight (range 54 to 80 kg.) were selected for the present study. Disinfected and starved adult *Poecilobdella viridis* of similar size were applied to the fore arm volar surface of drug free male and female volunteers (n=7) . The head (biting end) of the animal can be recognised by its searching movement, while the tail end is used mostly as a sucker for fixation. Before the application of leeches . Once the leech is attached, it remained safely in place until fully distended. During their period a care was taken and disturbance was avoided . Leeches were simply dropped off the skin when satisfied. Blood samples from volunteers before and immediately after leech application were collected in small eppendorf tubes. Serum was prepared from venous blood within 30 min of collection by centrifugation at – 20° C for 10 minutes. When anticoagulated blood was centrifuged in a hematocrit tube at high speed, the erythrocytes. Sediment at the bottom. The red cell column is called as packed cell volume (PCV) or hematocrit (Cell volume percent). The reading was multiplied by 100 for volume percent. The serum proteins are collidal in nature. At pH 8.6 when subjected to an electrical current, all the serum proteins behave like anions and move towards the anode. The rate of migration depends upon their different molecular weights and sizes. Thus, albumin is the fastest moving component, followed by alpha-1, beta-2, and gamma globulin.

Reagents – Buffer solution, sodium diammonium sulphate. Staining solution – Brilliant blue stain Destaining solution – Methanol, acetic acid, Distilled water.

Procedure: -

- 1.About 100 to 150 ml of buffer was added in anode and cathode compartments respectively.
- 2.The paper strip was arranged horizontally on the holder.
- 3.The lid of the buffer tank was placed in the position and left the paper strips for about 15 min for equilibrium to be reached.
- 4.Few drops of serum were added uniformly along the pencil line drawn on the strip.
- 5.The current was switched on and allowed to run for one hour
- 6.The strip was removed from the bath and dried at about 80 °c for 30 minutes.
- 7.The dried strip was carefully placed in a 250-ml beaker with sufficient quantity of the staining solution. (The strip was dipped completely) strip was kept at room temperature for one hour.
- 8.The strip was destained by occasional rinsing in the destaining solution till the background of the paper appeared white. Then strip was dipped in liquid paraffin for few minutes.
- 9.After that the strip was dried at about 80° C for 10-15 minutes.
10. After drying, the strip was scanned in the densitometer to get the densitogram of immunological proteins. The scanning was carried out as per the total amount of protein in the sample.

Observation and result

Blood samples from normal healthy male and female were taken before and after application of leech to know the alterations in total protein (g/dL), Albumin (%), α 1 globulin (%), α 2 globulin (%), β globulin, γ globulin and A/G ratio. The analysis was done by paper electrophoresis and then by using densitometer. Decrease in protein values and albumin (%) was observed after leeching in all volunteers. This decline in proteins was moderately significant. (p < 0.05) .Significant decrease in A/ G ratio was obtained in all the volunteers after leeching.

In present study the gamma globulin was found to be increased in all the volunteers after leeching and the densitogram exhibited 4 peaks indicating formation of polyclonal antibodies. Thus this indicates that the leech saliva contains various protein ingredients and they are antigenic. Hirudin forms a complex with thrombin, inhabiting its activity, resulting into bleeding for long time affecting the coagulation process. We have observed increased bleeding time and clotting time in all the volunteers who were applied with one and

more than two leeches. However, prothrombin time remained unaffected. The present results and the characterization of hirudin from various tools of bioinformatics strongly indicate the antigenic nature of hirudin and hence it is

suggested that utmost precaution should be taken during application leech with respect to size and number of leeches so that adequate amount of leech saliva is administered in venous blood.

Table 1- Alterations in total protein, Albumin and Immunological Proteins in male and female volunteers before and after application of one leech.

No. of volunteers	Total protein		Albumin (%)		α_1 Globulin %	
	Before	After	Before	After	Before	After
1	6.43	5.92	56.01	46.62	3.53	3.46
2	6.21	6.00	54.33	45.00	6.22	6.01
3	6.55	6.49	58.09	50.99	4.50	4.44
4	6.96	5.02	48.65	43.25	3.18	3.05
5	7.02	5.44	50.15	40.11	4.16	4.10

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