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Experimental study on dissection of gut of the leech for isolation of microorganism present in western ghaat leech

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ABSTRACT

Leech application in various diseases shows remarkable results. Now in this scientific era the area of use of leeches is wide, sometimes the application of leech produces local as well as systemic infection. To rule out such complication and myths this study was conducted. The purpose of study is isolation of microorganism which cause such complication.

Key words: Leech, Jalauka, Jalaukavacharana.

INTRODUCTION

Ayurveda is ancient science which gives healthy life and longevity. The term Veda denotes knowledge and Ayus is the continuence of consciousness, sustaining of body thus the Ayurveda encompasses the knowledge of different facet of life. Ayurveda advocate in various means to protect health and to Disorder. Ayurveda which indicates *Hita, Ahita, Sukha, Dukha* of living being. The core object of Ayurveda is happy long life which devoid of sorrow. Acharya Sushruta believes that the knowledge of both surgery and medicine are essential to constitute good doctor who otherwise "is like a bird with only one wing".^[1]

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The Ayurvedic classical text 'Sushruta Samhita' mention 8 Angas among them the Shalya Tantra is one of specialty. In Shalya Tantra various types of Yantras and Shastras have been mentioned, and also explained the Anushastra, Jaloka is one of them. Raktamokshana is procedure in which blood detoxify i.e. removal of vitiated Dosha. Jaloka are mainly used for Raktamokshana, hence the procedure is known as Jalokawacharana, also it known as Hirudotheraphy or Leech theraphy.

In western countries it is mainly used for cosmetic surgery and organ transplant to increases microcirculation. This is one among the *Panchakarma*. Now a day leech application is widely used. The indication of Jaloka application specially Raktaj and Dushit Pittaj disorder, the patient who were fear for the Shalya Chikitsa, Balak, Hinabala etc. the Jalokawacharan is prime, but now a days while application of Jaloka some local as well as systemic complications like swelling, fever, itching, chill etc. has been observed. To treat such complication antimicrobial drugs are used, which again increase the cost of treatment. To find the solution to treat the complications as well as which reduce the expenses of treatment. Hence to find out such microorganisms is necessary.

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OBJECTIVES

- Isolation of gut organisms from leech dissection.
- Culture and identification

MATERIALS AND METHODS

Study Design

A Experiemental Study consisting of 30 leeches, conducted dissection of gut under sterile environment, then grown flora cultured.

Source of data

Leech collected from various parts of Western ghat.

Intervention

Collected leech dissected and isolated gut organism.

Inclusion criteria

Nirvisha Jaluka

Exclusion criteria

Savish Jaloka

Instrument

Laminar flow chamber, gloves two pairs, sterile facemask, scalpel blade, distilled water, forceps toothed and non-toothed, needles, sterile cotton, test tubes, conical flask, beakers, stirrer, dissecting needle, dissection tray with wax.

Chemicals

- 1. Ether
- 2. Distilled water
- 3. Spirit

Procedure

Preparation

Select the leech for dissection, then clean with normal saline. Check the physical parameter weight, length etc. Keep inside the conical flask. Put the swabs which contain the ether 2ml solution, close the mouth of flask with cotton. Wait for 10 minutes. Keep all required instrument in laminar flow chamber for sterilized then switch on the laminar flow machine, wait for 15 min till proper function. Open the laminar

flow chamber. Clean the dissection tray with spirit solution. Once the leech get anaesthetized with help of stirrer put leech on tray, clean again with distilled water. Then put sterile needle apart 5mm from mouth, then put second needle by stretching the leech till its max stretching capacity to the anus part apart from end portion 5mm and press it deeply. Confirm that leech is not displacing during dissection then with help of scalpel take incision from first pin to second pin superficially to avoiding cut inside organ. Open the flaps slowly by holding by forceps and placed needles with maximum stretch. Use the gentle force to for stretching the flaps that they won't tear. One after one place the needle by stretching flaps. Once all internal organ visualized then with help of probe start dissecting the gut. With help of scoop collect the gut flora in test tube which contain the normal saline. Once the dissection finished then remove the needles one by one and discard the leech.

Preparation of Nutrient agar media

Beef extract (1 g), yeast extract (2 g), peptone (5 g) and Sodium Chloride (5 g) were dissolved in 990 ml of distilled water. The pH was adjusted to 7.2 and the volume was made up to 1000 ml. Finally 15g agar was added to the media and autoclaved at 121°C for 20 minutes.

Preparation of the inoculum

Loopful of 24h old culture from the slants was transferred to sterile saline and mixed well to prepare a homogenous inoculum.

RESULTS

SN	Bacteria	Total
1	Staphylococcus sps	13/30
2	Pseudomonas sps	9/30
3	Citrobacter	8/30

All 30 samples dissected and cultured with nutrient agar among that the isolated gut organism are Staphylococcus, Pseudomonas and Citrobacter respectively 13, 9 and 8 in number.

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Fig. 2: Leech after dissection



Fig. 3: Growned Cuture in Media



Fig. 4: Laminar Flow Chamber



DISCUSSION

Leech's collected from various part of western ghaat but the common organisms were found on gut flora. The Indian cattle leech especially Hirudinaria granulosa is prone for such organism. Till a date no hazardous complication had not seen. In Ayurvedic ORIGINAL ARTICLE July-Aug 2018

context also such fatal complications has not been mentioned. This occurrence of microorganism may be pollution, infected food or environmental changes. The micro-organism may vary from place to place. It depends on environmental condition. It is not necessary that organisms isolated from western ghaat leech may differ from other place of organism. During the study dissecting the gut is difficult task. The proper anesthetic drug and dosage is recommended. In this strictly leeches which are not used or feeded any blood were used to avoid the misevaluation of microorganism.

Possible infection

In the study results, we found the major three types of microorganism. If the microorganism transfer from the leech to human body at site of application then may be the below mentioned diseses may manifest and produce futher complication, summarized as below table according to species.

Organisms	Species	Disease
Staphylococcus	gram positive	Skin infection, Cellulitis, Pneumonia, Bacteremia, Toxic shock syndrome, Impetigo abscess, Meningitis.
Pseudomonas	gram negative	Cellulitis, Folliculitis, Otitis, Pneumonia, Cystic fibrosis, Urinary tract infection, Endocarditis, Bacteremia.
Citrobacter	gram negative	Meningitis, Brain abscess, Urinary tract infection, Pneumonia, Bacteremia Peritonitis.

CONCLUSION

In the present study mainly three types of microorganisms were isolated i.e. Staphylococcus, Pseudomonas, Citrobacter. May be the result varies according to place to place as microorganisms varies from palce to place.

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