

In Vitro Study of Antibacterial Activity of Twak and Shunthi Churna in *Leptospira Interrogans* W.S.R. To Mooshaka Visha

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Introduction

Ayurveda is a science which deals with knowledge of life. Our Acharyas explained Ashtanga Ayurveda and Agadtantra is one of them. Agadtantra describes the features of bites by poisonous animals and diseases due to different kind of poisons and their treatment including study of sthavara, jangma and krutrimavisha.^[1]

Visha

A substance on administration affects all functions of body i.e. Dosha, Dhātu, Mala and makes them abnormal which degrades the health of human being.

There are two types of Visha 1) Sthavara 2) Jangama.

In Ayurvedic texts Rat bite is described as Mooshaka visha. Bruhatrayee describes its signs, symptoms, Eighteen types of Mooshaka, incurable features and treatment in detail.

There are five modes of spread of Mooshaka visha- semen, faeces, urine, scratched by nails and bites with teeth of rat.^[2]

Symptoms of Mooshika Visha

Vitiation of blood, pallor, rashes and reddish patches on skin, giddiness, loss of taste, fever with rigors, violent epileptic fits, severe pain, anaemia, aversion to food, difficulty in breathing, shivering, arthralgia, exudations, fainting of long duration and repeated vomiting of large quantity.^[3]

Incurable Signs

Fainting, oedema all over body, discolouration of skin, exudation, loss of hearing, fever, heaviness of head, excessive salivation, hematemesis, retention of urine, discolouration of lips, body covered with small nodules resembling rat body, emitting smell of rat.^[4]

Leptospirosis

Leptospirosis is an infection caused by corkscrew shaped bacteria called *Leptospira interrogans* often referred as Rat fever occurs in endemic and epidemic forms. Rodent is the most important reservoir of infection. Rats shed *Leptospira* in urine for prolonged period also transmitted by contaminated water, soil coming in contact with eyes, skin, mouth or nose. Farmers, veterinarians are at high risk and increased chances in monsoon. Symptoms of Leptospirosis are fever, severe headache, nausea, vomiting, diarrhoea, abdominal pain, myalgia, subconjunctival haemorrhage, sore throat, maculopapular skin rash.

Severe infection known as Weil's disease shows symptoms of severe jaundice, acute renal failure, haemorrhagic manifestation include sub-conjunctival haemorrhage, epistaxis, haemoptysis, ecchymosis and gastrointestinal haemorrhage, haemorrhagic pneumonitis. For treatment antibiotics like Doxycycline, Penicillin, Ceftriaxone, Cefotaxime, Erythromycin are used. Vaccines do not offer widespread protection.^[5]

The description of Leptospirosis shows similarity with Mooshaka visha in Ayurveda like mode of transmission and symptoms. The involvement of liver and kidney is evidenced by the presence of symptoms like discolouration and oedema all over body, Myalgia, tenderness, haematemeses, petechial haemorrhage on skin, mucous membrane are common in both disease.^[6]

Need For Study

- In Agadtantra there is description of Mooshaka visha therefore it is necessary to study it.
- Very few researches are available on Mooshaka visha till date.
- Description of Mooshaka visha and Leptospirosis shows similarity.
- The disease was considered inconsequential till recent but it is emerging as important health problem during last decade in sudden upsurge in number of reported cases and outbreaks.

The data on Leptospirosis shows more than 2000 cases have been reported from Maharashtra, Karnataka, Kerala, Tamil-Nadu in India mostly in coastal region from 1998-2005 and 394 deaths^[7].

A cross sectional study shows from Feb 2013-Jan 2016 out of 1527 patients 562 (36.8%) diagnosed for Leptospirosis.^[8]

Twaka and Shunthi

Twaka and Shunthi churna in equal quantity with warm water is very useful in all types of Mooshaka visha.^[9]

Twaka: *Cinnamomum zeylanicum*

Shunthi: *Zingiber officinale*

Review of Literature

Recent Research Relevant To Present Study

- Sabu H. A Comparative invitro study on the inhibitory effect of Sindhuvaradi kwatha on the *Leptospira Interrogans* bacteria in the context of Mooshika visha. Vaidyaratnam PS Varior College Kottakal, Kerala 2011.
- Thakre M, Pharmacological screening of some medicinal plants as antimicrobial and food activities including *Cinnamomum zeylanicum*. [Internet] 2004 July 16. Available from: <http://thesis.lib.vt.edu.com>

- Rekha N, Effect of Cinnamomum zeylanicum and Seyzium cumini on Gastrointestinal Diabetic Rats [Internet] 2010 Aug Doctor of Physiology Department of Industrial Biotechnology. Dr. MGR Educational and research Institute University. Available on: <http://www.drmgrdu.eu.in>
- Nimje P.D, Garg H, Gupta A, Srivastva N, Katiyar M, Ramlingam C Comparison of antimicrobial activity of Cinnamomum zeylanicum and Cinnamomum cassia on food spoilage bacteria and water born bacteria [Internet] 2013 Available on: <http://www.scholarsresearchlibrary.com>
- Rajeev P, Thomas L, Ginger- Extension pamphlet. [Internet] 2015 Nov Available on: <http://www.spices.res.in>

Difference from Previous Work

In previous work Sindhuvaradi kwatha was used and antibacterial effect on other species carried out. No other study mentioned specific antibacterial action of Twaka and Shunthi on Leptospira interrogans w.s.r. to Mooshaka visha.

1) अगदतंत्रनामसर्पकीटलूतामूषकादिदष्टः।

विषव्यंजनार्थविविधविषसंयोगशमनार्थचासुसू.१/१४

2) शुक्रपतितियत्रैषांशुक्रस्पृष्टैःस्पृशन्तिवा।

नखदन्तादिभिस्तस्मिन्गात्रेरक्तप्रदुष्यति।।सु.क.७/७

3) जायन्तेग्रन्थयःशोफाःकर्णिकामण्डलानिच।

पीडकोपचपश्चोग्रोविसर्पाःकिटिभानिच।।

पर्वभेदोरुजस्तीव्रामूर्च्छाङ्गसदनंज्वर।

दौर्बल्यमरुचिःश्वासोवमथुर्लोमघर्षणम्।।सु.क.७/८,९।।

4) मूर्च्छाङ्गिशोथवैवर्ण्यक्लेदशब्दाश्रुतिज्वराः।

शिरोगुरुत्वंलालासूकछर्दिश्चासाध्यमूषिकैः।।च.चि.२३/१४८

5) National guidelines. Diagnosis Case Management Prevention and Control of Leptospirosis. National Centre for Disease Control. [Internet] 2015. Available from <http://www.ncdc.gov.in>

6) Chaugule S.B. Ayurvedic aspect of Rat Bite and its management Unique Journal of Ayurvedic and Herbal Medicine. [Internet] Aug 2015 Available on <http://www.ujconline.net>

7) Shivkumar S. Leptospirosis Current scenario in India. API Medicine. [Internet] cited date 2008. Available on <http://www.apiindia.org>

8) Saha R, Sil (Mullik) S, Guha (Thakurta) R. Incidence of Leptospirosis in India. European Journal of Pharmaceutical and Research vol 3 [Internet] 2016 June 16 available on <http://www.ejpmr.com>

9) त्वचंचनागरंचैवसमांशश्लेष्मपेषितम्।

पेयमुष्णाम्बुनासर्वमूषिकानांविषापहम्॥ च.चि.२३/२०५

- 10) Guidelines for Prevention and Control of Leptospirosis. Zoonosis Division. National Institute of Communicable Diseases. World Health Organization Country office India. [Internet] 2006. Available From <http://www.ncdc.gov.in>
- 11) Ningal S, Kothule M, Jadhav N, Kadam S, Kature Y, Hapse S. A Review on Leptospirosis- World of Journal of Pharmacy and Pharmaceutical Science [Internet] Aug 2015 Available on <http://www.wjpps.com>
- 12) Rao R. S., Gupta N, Bhalla P, Agarwal S.K. Leptospirosis in India and the rest of world. Brazilian Journal of infectious disease. Vol 7. [Internet] 2003 June Available on; <http://www.scielo.br>
- 13) Leptospirosis | authorSTREAM [Internet] 15 June 2011 Available on <http://www.authorstream.com>
- 14) Leptospirosis | CDC [Internet] 2014 Nov. 18 Available on: <http://www.cdc.gov/leptospirosis>
- 15) Leptospirosis Information [Internet] Available on <http://www.leptospirosis.org>
- 16) Gogate M. Dravyaguna vidnyan. 3rd ed. Vaidyarnitra Prakashan. 2014 Feb 14. p. 272-274 and 440-441

Objectives

Primary Objective

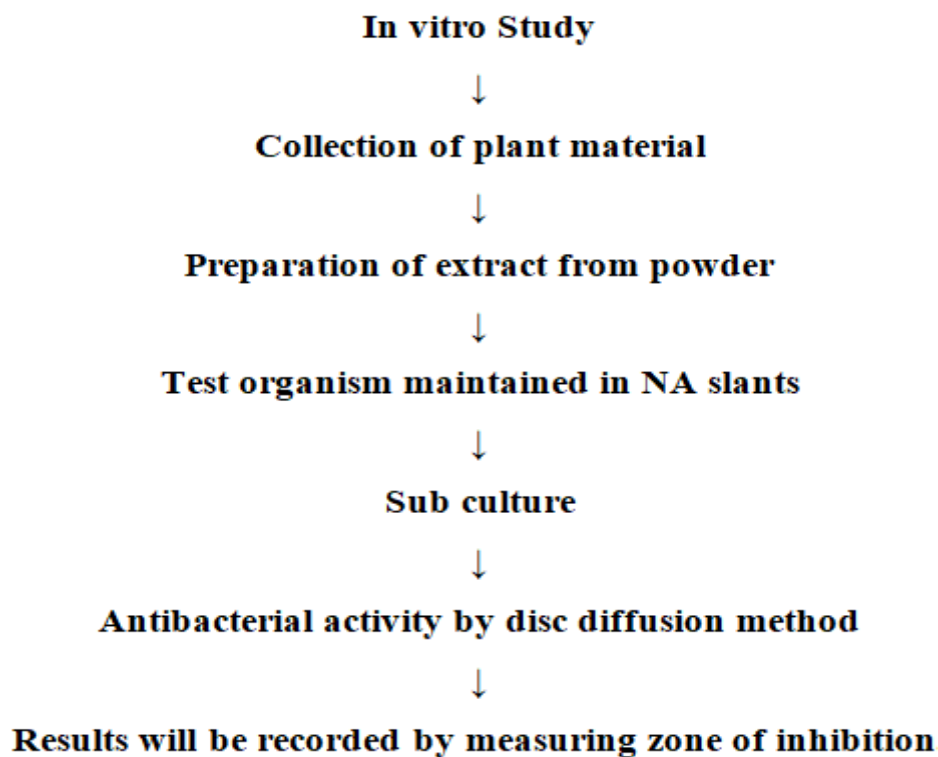
- To review Mooshaka visha through Ayurveda.
- To Evaluate antibacterial activity of Twaka and Shunthi churna in context of Mooshaka visha.

Secondary Objectives

- To co-relate Mooshaka visha with Leptospirosis.

Methodology

Types of Study Design : Study will be carried out by *In vitro* with Twaka and Shunthi churna on *Leptospira* Interrerogans



Location of Study : Lab of Institute.

- Duration of Study : 18 months.
- Eligibility Criteria : a. Inclusion criteria- Leptospira Interrogans
b. Exclusion criteria - Other Bacterias.

Materials

Conceptual Study: will be assessed for literature aspect of Mooshaka visha

1. Ayurveda literature – Charak Samhita Chikitsa sthana 23
Sushrut Samhita Kalpasthana 1,3,7
Ashanga Sangraha Uttarsthana 46
Ashtanga hridaya Uttarsthana 38
2. Medicines formulation will be revised through Charak Samhita Chikitsasthan23/205

Contents

| Sr.No | Dravya | Botanical Name | Family | Part Used |
|-------|---------|-----------------------|---------------|-----------|
| 1 | Twaka | Cinnamomum zeylanicum | Lauraceae | Stem bark |
| 2 | Shunthi | Zingiber officinale | Zinziberaceae | Rhizome |

Method

- 1) Collection and processing of Twaka stem bark and Shunthi rhizome.
- 2) Both Drugs will be identified and authenticated by a Botanist. There after both Drugs will be washed and allowed to dry under shed. The dried Drugs will be grounded in fine powder in equal quantity by electric blender and will be stored in air tight container.
- 3) Preparation of Extract
The dried powder will be macerated with 95% Ethanol for 48 hours in room temp. Drugs extract will be concentrated using the rotary evaporator at 45 °C and under vacuum for solvent removal. The crude extract obtained will be investigated further for Antibacterial Activity.
- 4) Test Organism
The strains of Leptospira interrogans gram-negative bacteria will be used to evaluate antibacterial activity. Its culture will be maintained in NA slants/plates stored at 4°C and periodically sub-cultured.

Antimicrobial Activity Test

1. Antimicrobial activity will be tested using a modified disc diffusion assay (DDA) method originally described by Bauer (1966) and Ncube et al (2008).
2. Drugs extracts will be dissolved in 20% DMSO treated water.
3. The inoculums for Leptospira interrogans will be prepared from broth cultures (10 CFU/ml).

4. A loop of culture from the NA slant stock will be cultured in LB medium overnight and spread with a sterile swab into Petri-dishes.
5. Sterile disc will be impregnated with the plants extracts(5mg/ml)will be placed on the cultured plates and incubated for 24h at 37°C.
6. Positive control for bacterial culture will be carried out under similar condition by using Ceftriaxone.(250mg/ml)
7. The results will be recorded by measuring the zones of growth inhibition. Test will be repeated.

Instruments: Electric Blender, Ethanol, Petri dish, Rotary evaporator, Sterile disc, Incubator, LB medium.

Research Methodology Specified and Explained for Data Collection

Methods for Data Collection Relevant to Objectives: Primary/direct method

Study Instrument: Laboratory test- Descriptive observation.

Data Management and Analysis Procedure: Data collected by observations of lab tests will be entered into Microsoft Excel and statistical analysis will be done. It will be presented in the form of tables, charts, graphs etc.

Plan for Statistical Analysis

The statistical tests will be applied in following manner.

Non parametric Tests like Wilcoxon sign Test, Run Test etc.

References

1. ShastriA.Shushrut Samhita,Reprint. Chaukhamba Sanskrit Sansthan. Varanasi. 2005 Sutrasthan 1/14 p.4
2. ShastriA.Shushrut Samhita.Reprint Chaukhamba Sanskrit Sansthan. Varanasi. 2005. Shushrut Samhita Kalpasthan 7/7 p.56-57
3. ShastriA.Shushrut Samhita.Reprint Chaukhamba Sanskrit Sansthan Varanasi. 2005. Shushrut Samhita Kalpasthan 7/8-9 p.56-57
4. Tripathi B. CharakSamhita part 2.Reprint. Chaukhamba Surbharti Prakashan Varanasi. 2003. Chikitsasthan 23/147 p.148
5. Sharma S. Ashtangsangraha. 2nded. Chaukhamba Sanskrit series Varanasi. 2008. Uttarsthan 46 p. 1to 3.
6. Gupta A.Ashtanga Hridayam. 13thed. Chaukhamba Sanskrit Sansthan. Uttarsthan 38/4-7 p.592
7. Shastri K. CharakSamhita. part 2. Chaukhamba Sanskrit Sansthan Varanasi. Reprint 2000. Chikitsasthan 23/147 p.148
8. Tripathi B. CharakSamhita part 2. Reprint. Chaukhamba Surbharti Prakashan Varanasi. 2003 .Chikitsasthan 23/205 p.793.
9. Tripathi B. CharakSamhita part 2.Reprint. Chaukhamba Surbharti Prakashan Varanasi. 2003. Chikitsasthan 23/147 p.148.
10. Shastri B. Bhavprakash Nighantu.9thed.Chaukhamba Sanskrit Sansthan. 1999 p. 13-14 . 226 – 228.
11. Gogate M. Dravyaguna vidnyan. 3rded. Vaidyamitra Prakashan. 2014 feb 14.p.272-274 and 440-441.
12. Innes J.A. Davidson's Essentials of Medicine. 2nd ed .2016 ch 5 p.81
13. Park K.Park's textbook of Preventive and Social Medicine. 23rd ed. ch5/4 p.291
14. Shaha S. N. Textbook of Medicine Vol. 1 The Association of Physicians of India 8th ed ch36 p. 83,84.

15. National guidelines. Diagnosis Case Management Prevention and Control of Leptospirosis. National Centre for Disease Control. [Internet] 2015 Available from <http://www.ncdc.gov.in>
16. Chaugule S.B. Ayurvedic aspect of Rat Bite and it's management Unique Journal of Ayurvedic and Herbal Medicine. [Internet] Aug 2015 Available on <http://www.ujconline.net>
17. Shivkumar S. Leptospirosis Current sceranio in India. API Medicine.[Internet] cited date 2008. Available on <http://www.apiindia.org>
18. Saha R, Sil(Mullick) S, Guha(Thakurta) R. Incidance of Leptospirosis in India. European Journal of Phamaceutical and Research vol 3 [Internet] 2016 june 16 available on [http:// www.ejpmr.com](http://www.ejpmr.com)