Pharmaceutical and Analytical Study of Tryushanadya Lauha & Modified Form as Tryushanadya Mandura and their Comparative Evaluation for Antidiabetic Activity in Wistar Rats

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Authors’ contributions
This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

ABSTRACT

Background: Loha is a metal that is used in many preparations after transforming it into non-metallic form by purification and incineration method uses to treat different kinds of diseases. Mandura is the rusting of iron. Tryushanadyalauha(TL) is one among the Ayurvedicherbo-mineral formulations described in BhaishajyaRatnavalliand as modified dosage form as Tryushanadyamandura(TM). The herbal contents are tryushana (i.epippali( Piper longum Linn ),maricha (Piper nigrum Linn)and shunti (Zingiber Officinale Roscoe), cavya (Piper chabaHunter), bakuchi (Psoralea CorylifoliaLinn),bhang (cannabis sativum Linn),andjavana like saindhava,aubhida,vida and sauvarchala ,andlohabhasma is the main ingredient.

Aim: Pharmaceutical and Analytical study of TryushanadyaLauha& modified form as TryushanadyaMandura and comparative evaluation for anti-diabetic activity in Wistar rats.

Materials and Methods: All herbal drugs will be collected, verified, and primarily authenticated by the Department of Dravyaguna. Lohaand Mandura will be procured from the vendor and...
authenticated by the Department of Rasashastra and BhaishajyaKalpana, Mahatma Gandhi Ayurved College Hospital Research Centre, Salod(H), Wardha, and they will be prepared as per reference. Organoleptic, bhasmapariksha, Physico-chemical,XRD and FEG-SEM parameters will be evaluated. To assess Tryushanadya Lauha (TL) and Tryushanadya Mandura (TM)antidiabetic action will be conducted in 30 Wistar rats in 5 groups and will be compared.

Observation and Results: The study will be assessed Tryushanadya lauha (TL) and Tryushanadya Mandura (TM)antidiabetic action in 30 Wistar rats by using one-way ANOVA.

Conclusion: Pharmaceutical and Analytical study of TryushanadyaLauha (TL) & modified form as TryushanadyaMandura (TM) will provide the standard parameters.

Keywords: TryushanadyaLauha; Tryushanadya Mandura; organoleptic; XRD; antidiabetic; wistar rats.

1. INTRODUCTION

Ayurveda is one of the oldest systems of medicine that contain different classical text where different variety of formulations explains different diseases. Whatever was mentioned in the Ayurvedic classical texts are authentic and had undergone a lot of tests by Acharya and came to conclusion [1].

As Rasashastra and BhaishajyaKalpana is one of the branches of Ayurveda that deal with the preparation of herbo-mineral, metal, etc. where they will be processed in such a way where it will be fit for consumption and give action at proper dose without any harm to the body which is readily absorbed and assimilated.

According to the world health organization, Diabetes is a chronic, metabolic disease characterized by elevated levels of blood glucose, which lead over time to serious damage to the eyes, kidneys, heart, nerves, and blood vessels [2].

Tryushanadya Lauha (TL) is one of the Ayurvedic herbo-mineral formulations described in BhaishajyaRatnavali. The herbal contents are Tryushanai.epippali (Piper longum Linn), Maricha (Piper nigrum Linn) and Sunti (Zingiber Officinale Roscoe), cavya (Piper chaba Hunter), Bakuchi (Psoralea Coryllifolia Linn), Bhang ( Cannabis indica ) etc. It cures kaphapitta diseases, medo, and prameharoga (correlated to obesity, metabolic syndrome, and diabetes mellitus) and is also useful in many diseases. Mandurais the rust of iron that forms by the reaction of iron and oxygen in the presence of water or air moisture. It has synonyms like Kitta, Lohabhava, Lohakitta, Lohama and Lohacchista [4].

Diabetes is a chronic and incurable disease. Hyperglycaemia is one of the diabetic symptoms, which in turn damages many of the body system leading to complications that further exacerbate the condition and affects the quality of life. In Adults, there is an increasing worldwide incidence of diabetes mellitus which constitutes a global public health burden [5].

According to WHO, worldwide 422 million people have diabetes, and 1.6 million deaths are directly attributed to diabetes each year [6].

In Ayurvedic classic diabetes can be correlated with Prameha later stage is Madhumeha. It is one of the critical diseases that can be treated in the early stage and by following the guided regimen of food and code of conduct. To contribute a safe and effective antidiabetic Ayurvedic formulation, TL is taken as it is a herbo-mineral formulation which is indicated in Prameha.

Mandura is derived from Loha. The previous study of Lohabhasma and Mandurabhasma on haematinic evaluation indicates the significant effect of Mandurabhasma than Lohabhasma on hemoglobin level. Mandurabhasma had better haematinic property as compared to Lohabhasma [7,8]. The process of preparation of Lohabhasma consumes a lot of time when
compared to the preparation of Mandurabhasma [9]. The pharmaceutical preparation of Lohabhasma is a tedious process involving many steps in the conversion of Lohabhasma from Loha. It is very costly and time-consuming also. Lohabhasma causes constipation, while in Mandurabhasma it is not observed [10]. The properties which Manda Lohabhasma have the same properties will be there in Manda Mandura in minute form, so to treat the disease Mandura bhasma can be used [11]. From all the above studies, Lohabhasma and Mandurabhasma were compared to assess different therapeutic potentials and indicate better therapeutic efficacy of Mandurabhasma. Considering this TL will be prepared by adding Mandurabhasma instead of Loha bhasma. If TryushanadayaMandura(TM) is having the same or better efficacy as compared to TL, a cost-effective, less time-consuming but efficacious product can be used in clinical studies.

1.1 Aim

Pharmaceutical-Analytical study of TryushanadayaLauha and modified form as TryushanadayaMandura and their comparative evaluation for antidiabetes activities in Wistar rats.

1.2 Objectives

1. To prepare TL.
2. To prepare TM.
3. To Analyse and compare TL&TM on different parameters.
4. To evaluation of TL&TM for anti-diabetic study.
5. To Compare and assess TL&TM for antidiabetic study.

1.3 Hypothesis

a. Null hypothesis [Hₐ]: TM and TL do not have any antidiabetic action.

b. Alternate hypothesis [H₁]: Both TM and TL have antidiabetic action.

2. MATERIALS

The reference of TL is taken from BhashajyaRatnavali from 38 chapters of Medovikara 26-28 shloka.

2.1 Drugs Review

Trushana-i.ePippali (Piper longum Linn), Maricha (Piper nigrum Linn), and Sunti (Zingiber Officinale Roscoe).

Trikatu: They are having katu(pungent) taste and tishna(sharp) property which penetrate the deeper dhatu (tissue) and sub-side the kaphadosha.

Vijaya: Having the vikas property without undergoing the process of digestion will reach faster to the deeper tissue and stimulate the muscle which helps to increase its strength.

Chavya: By its hot potency it will counteract the Kapha dosha.

Bakuchi: It also has the property of Rasayana which helps to rejuvenate the body.

Lavana: It can enter the minute channels of the body and help in mobilizing the Kapha from the upper part of the body. It cures constipation and increases taste.

Loha: It possesses scraping property that sub-sides Medadhatu and Kaphadosha. It improves physical strength and is an aphrodisiac. It is also beneficial in reinstating physical strength after suffering from any chronic or acute ailments.

Mandura: It will increase the digestive capacity and taste. It is aphrodisiac and will improve the haematological parameters in the body. It may have the same property as that of Loha.

2.2 Drugs Collection and Authentication

a. All herbal drugs will be collected, verified, and primarily authenticated by the Department of Dravyaguna.

b. Loha and Mandura will be procured from the vendor and authenticated by the Department of Rasashastra.

c. Raw drugs will be standardized as per API.

d. Animals will be selected as per inclusion and exclusion criteria given in section
| Dravya  | Latin name/ Family | Part use | Rasa | Guna | Veerya | Vipaka | Karma                      |
|---------|--------------------|----------|------|------|--------|--------|----------------------------|
| Pippali | Piper longum Linn Piparaceae | Fruit    | Katu | Laghu Snigdha Tiksha | Usna     | Madhura Dipana, vrisya Rasayana Kaphvatahay Dippana Pramathi Kaphavatahay Dippana Bhedana |
| Maricha | Piper nigrum Linn Piparaceae | Fruit    | Katu | Laghu Tikshna | Usna | Katu | Kaphavatahay Dippana Pramathi Vatakaphahara Dippana Bhedana |
| Shunti  | Zingiber Officinale Roscoe Zingeberaceae | Rhizome  | Katu | Guru Rukshe Tikshna | Usna | Madhura | Vatakaphahara Dippana Bhedana |
| Vijaya  | Cannabis Sativa Linn Cannabaceae | Leaves, seed | Tikta | Laghu Tikshna Vyavayi | Usna | Katu | Vatakaphahara |
| Cavya[12] | Piper chaba Hunter Piparaceae | Roots   | Katu | Laghu Rukshe | Usna | Katu | Kaphavatahay Dippana Pacana Dipana, vibanda Urdhvaadh Kaphvatahay Manoma Anaha, vistambha Utkedi |
| Vida lavana | Black salt | Whole | Lavana | Rukshe | Ushna | katu |                        |
| Aubhidha Lavana | Reha salt | Whole | Tikta, katu kshara Tikshna Utkedi | Ushna | katu |                        |
| Bakuchi  | Psoralea Corylifolia Linn Fabaceae | Fruits   | Katu | Laghu Tikta Rukshe Sheeta | Katu | Kaphavatahay Rasayana Twachya |
| Saindhava Lavana | Rock salt | Whole | Madura | Laghu Anusna | - |                        | Vrusya, hrudyad tridosahay Dippa avidaha |
| Dravya | Latin name/ Family | Part use | Rasa       | Guna   | Veerya | Vipaka | Karma                      |
|--------|-------------------|----------|------------|--------|--------|--------|----------------------------|
| Sauvarchala Lavana[13] | Sochal salt (unaqua Sodium chloride) | Whole | Lavana | Laghu | ushna | Katu | Hrudya,ruchikara Sugandhya,dipana Udgarashodhana Vibandaghna |
| Ayaschoorna (Iron) | Ferrum (Fe) | Bhasma | Kashaya | Ruksha | Guru | Lekhana | Sheeta | Balya,vrshya Kaphapittahara Meharara Varnya,medhya Sarvarogahara |
| Mandura[14] | Rubrum (Fe2O3) | Bhasma | -       | Sheeta | -     | -     | Ruchikara Agnidipaka Pittashamaka Raktavuddhikara Pandukamalarogahara |
3. PREPARATION OF LOHA bhasma AND MANDURA bhasma

3.1 Shodhana of Loha [15]
• Lohachurna will be taken in a pan & heated over high flame till red hot
• Then it will be dipped into a vessel containing Triphalakwatha(decoction)
• Iron powder from triphalakwatha will be collected back
• Again will be heated till red hot and will be dipped in triphalakwatha
• This process will be repeated 7 times.

3.2 Marana of loha
• Purified lohachurna will be taken in a mortar and pestle & triturated with lemon juice.
• After obtaining the semisolid consistency of the slurry, small pellets will be prepared
• Pellets will be dried properly.
• Dried pellets will be enclosed in the saucer of earthen pots.
• These enclosed saucers will be subjected to Gajaputa (unit of heat).

3.3 Shodhana of mandura
• Mandura will be heated red hot over glowing charcoal
• Then it will be dipped in the vessel containing gomutra(cow urine)
• This process will be repeated 7 times.

3.4 Marana of Mandura
• Purified Mandura will be triturated by alovera juice in mortar and pestle.
• Pellets will be prepared from the slurry and dried properly.
• Dried pellets will be enclosed in the saucer of earthen pots.
• These enclosed saucers will be subjected to Gajaputa.

3.5 Preparation of TL and TM [16]
• All the drugs mentioned above (1-7) will be taken and made into powder
• Loha Bhasma will be added to the above powders.
• Similarly, in another preparation, Mandura Bhasma will be added.
• All the materials will be mixed together and will be store.

3.6 Standardization Parameters

3.6.1 Organoleptic parameters
• Colour
• Odor
• Taste
• Touch
• Appearance

Table 2. Preparation of tryushanadyaloha/mandura

| Sl.No | Drugs                              | Part use           | Dose  |
|-------|------------------------------------|--------------------|-------|
| 1     | Tryushana (Piper longum Linn,     | Fruit              | 1 part|
|       | Piper nigrum Linn, Zingiber        |                    |       |
|       | OfficinaleRoscoe)                 |                    |       |
| 2     | Bhang (cannabis sativum Linn)     | Leaves/seed        | 1 part|
| 3     | Cavya(Piper chaba                  | Root               | 1 part|
|       | Hunter)                            |                    |       |
| 4     | Vida lavana(black salt)            | Whole              | 1 part|
| 5     | Aubhidhalavana                     | Whole              | 1 part|
| 6     | Bakuchi(Psoralea Corylifolia Linn)| Fruits             | 1 part|
| 7     | Saindhavalavana(rock salt)        | Whole              | 1 part|
| 8     | Sauvarchalalavna (sochal salt)    | Whole              | 1 part|
| 9     | Loha                               | Bhasma             | 12 part|
| 10    | Mandura bhasma                     | Bhasma             | 12 part|
3.7 Bhasmapariksha [17]

1. **Rekhapurnatva**(fine enough to enter the crevices of finger).
   - To ascertain the fineness of prepared bhasma, when bhasma is rubbed between the thumb and index finger bhasma enter and embed in fingerprints which consider enough fine and accepted as standard.

2. **Nirdhooma** (smokeless)
   - Bhasma is properly prepared.

3. **Niswadu** (tasteless)
   - Bhasma shouldn’t have any taste.

4. **Dantagrekachkachabhava** (it should not produce sound while chewing).
   - To check any other particles in bhasma.

3.8 Physico-chemical analysis [18,19]

1. **Moisture analysis**
   - To ensure and control the quality of the product as the moisture content will affect the process ability, shelf-life, usability, and quality of the product.

3.9 **Total Ash**
   - To detect inorganic substances and also gives an estimation about purity and quality of drugs.

3.10 **pH**
   - To know the pH value, whether it is acidity or alkalinity.

3.11 **Acid – insoluble Ash**
   - To determine the concentration of siliceous compound in the sample.

4. **Modern Sophisticated Analysis** [20]

4.1 **XRD**
   - For identification of crystalline material and analysis of unit cell dimensions and also identify the chemical composition information of metals.

4.2 **FEG-SEM**
   - To observe the surface of the sample.

4.3 **Methods**

4.3.1 **Study centres**

1. Department of Rasashastra and BhaishajyaKalpana, MGACH&RC, Salod (H) Wardha.
2. Analytical study will be carried out at Dattatraya Ayurved Rasashala, MGACH&RC, Salod(H) Wardha.
3. Experimental study will be carried out at the animal house, DMCP, DMIMS (DU), Wardha.
4. According to the need for study, analysis or experiments will be carried out in laboratory or research institute of national repute as listed in DMIMS (DU) profile.

4.4 **Study Design**

An experimental study will be done in five groups containing 6 Wistar rats (3 males and 3 females), a total of 30 Wistar rats.

Animals will be divided into five groups:

- Group I-Normal Control (NC)
- Group II-Standard Control (SC)
- Group III-Vehicle Control (VC)
- Group IV-Test group 1 TL
- Group V-Test group 2 TM

4.5 **Dose calculation** [21,22]

The calculation of dose will be by using rat conversion factor (Paget & Barnes).

Human dose x 0.018/250g of rats

=500x0.018/250g of rats

=9mg/g.

4.6 **Oral Glucose Tolerance Test** [23]

It will be performed in the same group of rats. Glucose (4g/kg) will be fed orally for 1 day. One hour after the administration of the drug. Blood will be withdrawn after glucose administration and fasting plasma glucose levels will be estimated at 0,30,60 & 120 minutes.
Table 3. Grouping of study animals, the dose of drugs, and Vehicle

| Groups       | Name of groups | Drugs       | No. of Animals | Dose     | Vehicle          | Study duration |
|--------------|----------------|-------------|----------------|----------|------------------|----------------|
| Group 1      | Normal control (NC) | -          | 6              | -        | -                | 15 days        |
| Group II     | Standard group  | Metformin   | 6              | 9mg/g    | Water            | 15 days        |
| Group III    | Vehicle group   | Honey & ghrta | 6            | 18mg     | -                | 15 days        |
|              |                |             |                | 36mg     |                  |                |
| Group IV     | Test group 1    | TL          | 6              | 9mg/g    | 18 mg            | 15 days        |
|              |                |             |                |          | Honey            |                |
|              |                |             |                |          | 36 mg            |                |
|              |                |             |                |          | ghrta            |                |
| Group V      | Test group 2    | TM          | 6              | 9mg/g    | 18 mg            | 15 days        |
|              |                |             |                |          | Honey            |                |
|              |                |             |                |          | 36 mg            |                |
|              |                |             |                |          | ghrta            |                |

Glucose tolerance measures the body’s response to sugar (glucose). The glucose tolerance test can be used to screen for diabetes. The glucose tolerance test identifies abnormalities in the way of body handles glucose after a meal—often before fasting blood glucose level becomes abnormal. The glucose tolerance test is performed to show how well the body handles sugar from foods and the risk for diabetes [24].

4.7 Sample Size
- 30 (5x6) 3 males and 3 females of Wistar rats will be used as an animal models.
- Thirty Wistar rats will be used as animal models.
- The sample in animals study is 6 is the smaller sample for any experimental study in each group so according to IEAC the reduced the number use of animals in the study as much as possible.

4.8 Inclusion and Exclusion Criteria

4.8.1 Inclusion criteria
- Rats weighing 200-250 grams of either sex.

4.8.2 Exclusion criteria
- Diseases and pregnant rats.
- Less than 200 grams of weight.
- Weight above 250 grams

4.8.3 Withdrawal criteria
The rats will be withdrawn from the study if any platform of the disease arises in wistar rats.

4.8.4 Randomization
The animals will be taken randomly.

4.9 Analytical and Experimental Study
- Blood glucose level
- OGTT (oral glucose tolerance test)

4.10 Outcome Measures
- Blood glucose levels will be estimated at the intervals.

4.11 Statistical Methods
- Statistical analysis will be done by applying suitable tests (one way ANOVA).

4.12 Experimental Animals
- Healthy adult Wistar rats weigh of 200-250 grams of either sex between 2 and 3 months of age will be used for the study.
- 30 (5x6) 3 males and 3 females of Wistar rats will be used as an animal models.

4.13 Housing and Husbandry
- All the rats will be healthy and will be kept in a standard environment.
- They will be housed in the group in polypropylene cages and maintained under standard conditions.
• Food will be fed with rat pellet diet.

4.14 Animal Care and Monitoring

• All animals will be acclimatized before the study.
• While withdrawing the blood from the animal's care will be taken not to cause pain and blood will be withdrawn at 0.5-1ml
• To reduce pain anaesthesia can be used
• Side effects may be seen during the study but all the standardization will be taken before giving the medicine to animals.

4.15 Interpretation/ Scientific Implications

If the result comes out are as follow

1. TL is having more antidiabetic action when compare to TM than what is explained in BhaishajyaRatnavalli stand right.
2. TM and TL have both antidiabetic actions but TM is more effective than TM can be used instead of TL for preparation and further study.
3. As Rasa Ratna Samuccaya stated that Mandura is the extract of loha expected to have its quality so can be used to treat disease as loha instead.

4.16 Limitation

• The study is an experimental study in 30 Wistar rats as it is a pre-clinical study to obtain preliminary efficacy, toxicity, and pharmacokinetic information the sample size is less.
• For the safety of human beings, it has to be studied in animals and later clinically in humans.
• If this Antidiabetic study is successful then this data will be used in another clinical study for the intervention of the Antidiabetic study.

4.17 Experimental Procedures

• Group I Non-diabetic healthy control group received normal saline intravenously
• The hyperglycaemia will be induced by alloxaan monohydrate at a dose of 65 mg/kg.
• Group II vehicle group will be given with Honey 1ml (18mg) & Ghrita 2ml (36mg).
• Test drug treated group i.e Test group 1 will be give with TL 500mg/kg (9mg/kg) with Honey 1ml (18mg) & Ghrita 2ml (36mg) and
• Test group 2 with TM 500mg/kg (9mg/kg) with Honey 1ml (18mg) & Ghrita 2ml (36mg)
• While standard treated group received Metformin 500mg/kg (9mg/kg).
• The vehicle or drug treatments were given daily orally for 15 days.
• Blood will be withdrawn from fasted rats (10 h) on 0, 1, 3, 12 h, 72 h (3rd day), 168 h (7th day), 264 h (11th day), and 360 h (15th day), and fasting plasma glucose levels were estimated at all intervals.

4.18 Oral Glucose Tolerance Test

• On the 16th day, Oral Glucose Tolerance Test will be performed in the same group of rats.
• Glucose (4g/kg) will be fed orally for 1 day. One hour after the administration of the drug.
• Blood will be withdrawn after glucose administration and fast plasma glucose levels will be estimate at 0,30,60 &120 minutes. The glucose tolerance test is performed to shows how well the body handles sugar from foods and risk for diabetes [24].

4.19 Expected Outcome

If TL or TM shows expected & significant result as Antidiabetic will be helpful to conduct clinical trials in Human being. If TM is having same or better efficacy as compared to TL a cost effective, less time consuming but efficacious product can be used in clinical studies.

5. DISCUSSION

TM is herbo-mineral formulation which is indicated in prameha and mandura is one of the lohakitta used as dosage form TM , with the question can it be compared and have the quality of loha comparative study is taken. Mandurabhasma have a more significant effect than lohabhasma on haemoglobin level and mandurabhasma had better haematinic compared to lohabhasma [25,26].

The process of preparation of loha bhasma consumes a lot of time when compared to the preparation of mandura bhasma. The pharmaceutical preparation of loha bhasma is a
tedious process involving many steps in the conversion of loha bhasma from loha. It is very costly and time-consuming also. Loha bhasma if not given the sufficient number of puta it causes constipation, while in mandura bhasma it is not there. The properties which mandalauhabhasma have the same properties will be there in suddha manda mandura in minute form, so to treat the disease mandura bhasma can be used [27]. From all the above studies, Loha bhasma and Mandura bhasma were compared to assess different therapeutic potentials and indicating better therapeutic efficacy of Mandura bhasma. Considering this TL will be prepared by adding Mandura bhasma instead of Loha bhasma, as in mandura it will be less time consuming, less costly for preparation and it will not cause constipation. With the help of X-Ray Diffraction (XRD) the crystalline fraction of the molecule will be recognized in both the samples that is TL and TM [28]. Even in analytical study XRD and FEG-SEM may show the nanoparticles size of mandura is less than loha as the number of puta is given more in loha [29,30]. If TM is having the same or better efficacy as compared to TL a cost effective, less time consuming but efficacious product can be used in clinical studies. In this study, TL and TM are the two formulation use to evaluate their antidiabetic action in induced hyperglycaemia by alloxan monohydrates. Later the interval blood glucose will be estimate and at last day of study oral glucose tolerance test will be done to check how much the rats can tolerate glucose after the medication. At last to conclude which of the two, Lauha or Mandura, has the better anti-diabetic action.

6. CONCLUSION

If this antidiabetic study is successful, then this data will be used in another clinical study for the intervention of antidiabetic study, as it is herbal-mineral medicine, so it may or may not show toxic effects in animal models. So after the preclinical study, it can be studied as a clinical trial in human beings.

NOTE

The study highlights the efficacy of “Ayurvedic” which is an ancient tradition, used in some parts of India. This ancient concept should be carefully evaluated in the light of modern medical science and can be utilized partially if found suitable.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All animals experiment will be carried out by the guidelines of CPCSEA after the approval of the Institutional Animal Ethical Committee (IAEC). Protocol no: - DMIMS/IAEC/20-2021/16

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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