Study Protocol

Pharmaceutical and Analytical study of *Tryushanadya Lauha* & modified form as *Tryushanadya Mandura* and their comparative evaluation for antidiabetic activity in Wistar rats.

Abstract

Background: *Loha* is a metal which is used in many preparations after transforming it into non-metallic form by purification and incineration method uses to treat different kind of diseases. Mandura is the rusting of iron.*Tryushanadya lauha(TL)* is one among the *Ayurvedic* herbo-mineral formulations describe in Bhaishajya Ratnavalli and as modified dosage form as Tryushanadya mandura(TM). The herbal contents are *tryushana (i.e pippali(Piper longum* Linn),*maricha (Piper nigrum Linn)and shunti (Zingiber Officinale* Roscoe), *cavya (Piper chaba* Hunter), *bakuchi (Psoralea Corylifolia* Linn*), bhang(cannabis sativum* Linn), and *lavana* like *saindhava, aubhida, vida* and *sauvarchala ,*and *loha bhasma* is the main ingredient.

Aim: Pharmaceutical and Analytical study of *Tryushanadya Lauha* & modified form as *Tryushanadya Mandura* and their comparative to evaluation for antidiabetic activity in Wistar rats.

Material and method: All herbal drugs will be collected, verified and primarily authenticated by Department of *Dravyaguna*. *Loha* and *mandura* will be procured from vender and authenticated by Department of Rasashastra and Bhaishajya kalpana Mahatma Gandhi Ayurved College Hospital Research Centre, Salod(H),Wardha, and they will be prepared as per reference. Organoleptic, *bhasma pariksha*, physico-chemical, XRD and FEG-SEM parameters will be evaluate. To assess Tryushanadya lauha and Tryushanadya mandura antidiabetic action will be conducted in 30 Wistar rats in 5 groups and will be compared them.

Observation and Results: The study will be assessed *Tryushanadya lauha* and *Tryushanadya mandura* antidiabetic action in 30 Wistar rats by using one way ANOVA.

Conclusion: Pharmaceutical and Analytical study of *Tryushanadya Lauha* & modified form as *Tryushanadya Mandura* will provide the standard parameters.

Keywords: *Tryushanadya lauha ,Tryushanadya mandura*, organoleptic, XRD,antidiabetic, wistar rats.

Introduction:

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

As Rasashastra and Bhaishajya kalpana is one of the branch of Ayurveda which deal with the preparation of herbo-mineral, metal etc. where they will be process 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 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 is one among the *Ayurvedic* herbo-mineral formulations describe in Bhaishajya Ratnavalli .The herbal contents are *tryushana (i.e pippali(Piper longum* Linn),*maricha (Piper nigrum Linn)and shunti (Zingiber Officinale* Roscoe), *cavya (Piper chaba* Hunter), *bakuchi (Psoralea Corylifolia* Linn*), bhang (cannabis sativum* Linn), and *lavana* like *saindhava, aubhida, vida* and *sauvarchala* ,and *loha bhasma* is the main ingredient.*Tryushanadya lauha* has the properties of increase *bala,varna* and *agni*.It is indicated in *sthaulya* (obesity) *prameha* (diabetes),*kustha* (skin disorder) and many others diseases. Its act as *rasayana*.[3]

Loha is a metal which is used in many preparations after transforming it into non-metallic form by purification and incineration method .It will be in *bhasma* form and non-toxic which help to cure many diseases. As *Loha bhasma* is one of the ingredient which is having properties like *lekhana* (scrape), *balya* (improves the physical strength),*vrushya* (aphrodisiac), *varnya* (good complexion) and *medhya* (promote intellectual) etc. It cures *kaphapittahara* diseases, *medo* and *prameharoga* (correlated to obesity, metabolic syndrome and diabetes mellitus) and it also useful in many diseases. *Mandura* is the rust of iron which forms by the reaction of iron and oxygen in the presence of water or air moisture. It has synonym like *kitta, lohabhava, lohakitta, lohamala* and *lohacchista*.[4]

Diabetes is chronic and incurable disease. The hyperglycaemia is one of the diabetic symptoms, which in turn damages many of the body system leading to complication which further exacerbate the condition and affects the quality of life. In Adults there is increase 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 attribute to diabetes each year.[6]

In *Ayurvedic* classic diabetes can be correlated with *prameha* later stage is *madhumeha*. It is one of the *yapya vyadhi* which can treated in early stage and by follow the *apathy pathya*. To contribute a safe and effective antidiabetic *Ayurvedic* formulation, *Tryushanadya lauha* is taken as it is herbo-mineral formulation which is indicated in *prameha* and *mandura* is one of the *lohakitta* , with the question can it be compare and have the quality of *loha* comparative study is taken.As the previous study of *loha bhasma* and *mandura bhasma* in *panduhara* effect and haematinic evaluation shows that *mandura bhasma* have more significant effect than *loha bhasma* on haemoglobin level and *mandura bhasma* had better haematinic compared to *loha bhasma*.[7] [8].The process of preparation of *loha bhasma* consume a lot of times when compare to the preparation of *mandura bhasma*.[9] Pharmacuetical preparation of *loha bhasma* is a tedious process involving many steps in conversion of *loha bhasma* from *loha*.It is very costly and time consuming also. *Loha bhasma* if not given sufficient number of *puta* it causes constipation, while in *mandura bhasma* is not there.[10] The properties which *manda lauha bhasma* have the same properties will be there in *suddha manda mandura* in minute form, so to treat the disease *mandura bhasma* can be used.[11] 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 *Trushnadya lauha* will be prepared by adding *Mandura bhasma* instead of *Loha bhasma*. If *Tryushanadya Mandura* is having same or better efficacy as compared to *Tryushanadya Lauha* ,a cost effective, less time consuming but efficacious product can be used in clinical studies.

Aim:

Pharmaceutical-Analytical study of *Tryushanadya Lauha* and modified form as *Tryushanadya Mandura* and their comparative evaluation for antidiabetes activities in wistar rats.

Objectives:

1. To prepare *Tryushanadya lauha.*
2. To prepare *Tryushanadya mandura*.
3. To Analyse and compare of *Tryushanadya lauha* & *Tryushanadya mandura* on different parameters.
4. To evaluation of *Tryushanadya lauha* & *Tryushanadya mandura* for anti-diabetic study.
5. To Compare and assess *Tryushanadya lauha* & *Tryushanadya mandura* for anti-diabetic study.

Hypoyhesis

a. Null hypothesis [H_0]:

Tryushandya mandura and Tryushanadya lauha not having any antidiabetic action.

b. Alternate hypothesis [H_1]:

Both *Tryushanadya mandura* and *Tryushanadya lauha* have antidiabetic action.

MATERIAL

The reference of *Tryushanadya lauha* is taken from Bhashajya Ratnavalli from 38 chapters of *Medovikara* 26-28 shloka.

Drugs Review

Trushana-i.e pippali(Piper longum Linn),*maricha (Piper nigrum Linn)and shunti (Zingiber Officinale* Roscoe).

Table No.1

Dravya	*Latin name/ Family*	Part use	*Rasa*	*Guna*	*Veerya*	*Vipaka*	*Karma*
Pippali	*Piper longum* Linn *Piparaceae*	Fruit	*Katu*	*Laghu Snigdha Tikshna*	*Usna*	*Madhura*	*Dipana,vrisya Rasayana pramehagulmaghna*
Maricha	*Piper nigrum Linn Piparaceae*	Fruit	*Katu*	*Laghu Tiksna*	*Usna*	*Katu*	*Kaphavatahara Dipana Pramathi*
Shunti	*Zingiber Officinale* Roscoe *Zingeberaceae*	Rhizome	*Katu*	*Guru Ruksha Tikshna*	*Usna*	*Madhura*	*Vatakaphahara Dipana Bhedana*
Vijaya	*Cannabis Sativa* Linn *Cannabacea*	Leaves,seed	*Tikta*	*Laghu Tikshna Vyavayi*	*Usna*	*Katu*	*Vatakaphahara*

	e						
Cavya[12]	*Piper chaba* Hunter *Piperaceae*	Roots	*Katu*	*Laghu, Ruksha*	*Usna*	*Katu*	*Kaphavatahara Dipana Pacana*
Vida lavana	Black salt	Whole	*Lavana*	*Ruksha*	*Ushna*	*katu*	*Dipana,vibanda Urdhvaadha Kaphvatanulomana Anahavistambha Shoolajauravanasha*
Aubhidha Lavana	Reha salt	Whole	*Tikta, katu kshara*	*Tikshna Utkedi*	*ushna*	*katu*	*Utkedi*
Bakuchi	*Psoralea Corylifolia* Linn *Fabaceae*	Fruits	*Katu Tikta*	*Laghu Ruksha*	*Sheeta*	*Katu*	*Kaphavatahara Rasayana Twachya*
Saindhava Lavana	*Rock salt*	Whole	*Madura*	*Laghu*	*Anusna*	-	*Vrusya,hrudya tridoshahara dipana,avidaha*
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,vrushya Kaphapittahara Mehahara Varnya,medhya Sarvarogahara*
Mandura[14]	*Rubrum (Fe2O3)*	*Bhasma*	-	*Sheeta*	-	-	*Ruchikaraka Agnidipaka Pittashamaka Raktavruddhikara Pandukamalarogahara*

***Trikatu*:** They are having *katu* (pungent) taste and *tishna* (sharp) property which penetrating the deeper *dhatu* (tissue) and subside the *kaphadosha*.

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

Chavya: By its *katu,laghu,ushna* property its will counteract the *kaphadosha.*

***Bakuchi*:** It's also has the property of *rasayana* which help to rejunivate the body.

***Lavana*:** It's has the ability to enter the minute channels of the body and help in mobilizing the *kapha* from upper part of body and *vata anulomana.* Its cure the constipation and increase taste.

***Loha*:** It's possesses *ruksha* and *lekhana* (scraping) property which subside *medadhatu* and *kaphadosha*.Its improves the physical strength and aphrodisiac and beneficial to *kshaya* of *dhatu*.It is also beneficial in reinstating the physical strength after suffering with any chronic or acute ailments.

Mandura:Its will increase *agni* and taste.It is having aphrodisiac and increase *raktadhatu* in body.Its may have same property as that of *loha*.

- **Drugs collection and authentication:**

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

b.*Loha* and *mandura* will be procure from vender and authenticated by 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 VII.

PREPARATION OF LOHA BHASMA AND MANDURA BHASMA

A. i.***Shodhana*** of ***loha***[15]

- *Loha churna* will be taken in *loha darvi*
- Heat over high flame till red hot
- Dip it into a vessel containing *triphala kwatha*
- Iron powder from *triphla kwatha* will be collect back
- Heat it again till red hot and dip in *triphala kwatha*
- This process will be repeats for 7 times.

ii. *Marana* of *loha*

- Purified *loha churna* will be taken in *khalva yantra*
- Give *bhavana* with *nimbu swarasa*
- *Cakrikas* of even size and shape will be prepare and dry under the sun
- Enclose *sarava samputa* and subject to *gaja puta*.

B.i. *shodhana* of *mandura*

- *Mandura* will be heat red hot over glowing charcoal
- Then it will dip in vessel containing *gomutra*
- This process will be repeats for 7 times.

ii. ***Marana*** of ***mandura***

- Fine powder of *suddha mandura* will be taken in clean *khalva yantra*
- *Bhavana* will be given with *kumara swarasa*
- *Chakrikas* of even size and shape will be prepare and dry under the sun
- Enclose *sarava samputa* and subject to *gaja puta*.

C.Preparation of *Tryushanadya lauha /mandura*[16]

Table No 2

Sl.No	Drugs	Part use	Dose
1	*Tryushana (Piper longum* Linn , *Piper nigrum Linn, Zingiber Officinale* Roscoe*)*	Fruit	1 part
2	*Bhanga (cannabis sativum* Linn*)*	Leaves/seed	1 part
3	*Cavya(Piper chaba* Hunter)	Root	1 part
4	*Vida lavana (*black salt*)*	Whole	1 part
5	*Aubhidha lavana*	Whole	1 part
6	*Bakuchi (Psoralea Corylifolia* Linn)	Fruits	1 part
7	*Saindhava lavana(*rock salt*)*	Whole	1 part
8	*Sauvarchala lavana (*sochal salt*)*	Whole	1 part
9	*Ayas churna*	Bhasma	12 part
10	*Mandura bhasma*	Bhasma	12 part

- All the drugs mentioned above (1-7) will be taken and make them into powder
- Then add *ayas churna/mandura bhasma* equal to the combine weight of the above drugs.
- Mixing all the materials together and store.

Standardization parameters:

Organoleptic parameters:

- Colour
- Odour
- Taste
- Touch
- Appearance

***Bhasmapariksha*[17]**

1. *Rekhapurnatva* (fine enough to enter the crevices of finger).

To ascertain the fineness of prepared *bhasma*, when *bhasma* rubbed in between the thumb and index finger *bhasma* enter and embed in finger prints which consider enough fine and accepted as standard.

2. *Nirdhooma* (smokeless)

Bhasma are 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*.

Physico-chemical analysis:[18][19]

1. Moisture analysis

-To ensure and control the quality of product as the moisture content will affect the processibility ,shelf-life, usability and quality of product.

2. Total ash

-To detect inorganic substance and also gives an estimation about purity and quality of drugs.

3. pH

-To know how much the pH value whether it is acidity or alkalinity.

4. Acid –insoluble ash

-To quantify the amount of siliceous compound in sample.

Modern sophisticated analysis:[20]

- XRD

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

- FEG-SEM

-To observe the surface of sample.

Methods:

I. **Study centres**:

1. Department of Rasashastra and Bhaishajya Kalpana , 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 animal house, DMCP, DMIMS (DU), Wardha.
4. According to the need of study, analysis or experiments will be carried out in laboratory or research institute of national repute as listed in DMIMS (DU) profile.

Study design:

Experimental study will be done in five groups containing 6 Wistar rats (3 males and 3 females), total 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 (*Tryushanadya lauha)TL*
- Group V-Test group 2 (*Tryushanadya mandura)TM*

Table 3

Groups	**Name of groups**	**Drugs**	**No.of Animals**	**Dose**	**Anupana**	**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	*Madhu & grita*	6	18mg 36mg	-	15 days

Group IV	*Test group1*	*Trushanadya lauha*	6	9mg/g	18mg madhu 36mg grita	15 days
Group V	*Test group 2*	*Trushanadya mandura*	6	9mg/g	18mg madhu 36mg grita	15 days

12]Dose calculation[21][22]:

As drugs dosage is different from one species to another. FDA guideline is use for calculation of dose in experimental studies by using rat conversion factor (Paget & Barnes).

Human dose x 0.018/250g of rats

=500x0.018/250g of rats

=9mg/g.

Oral Glucose Tolerance Test [23]

It will be perform in same group of rats. Glucose (4g/kg) will be fed orally for 1 day. One hour after the administration of drug. Blood will be withdrawn after glucose administration and fasting plasma glucose levels will be estimate at 0,30,60 &120 minutes.

The 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 shows how well the body handles sugar from foods and risk for diabetes.[24]

Sample size

- 30 (5x6) 3 males and 3 females of Wistar rats will be used as an animal model.
- Thirty Wistar rats will be used as animal model.
- All animals experiment will be carried out in accordance with the guidelines of CPCSEA after the approval of Institutional Animal Ethical Committee (IAEC).
- The sample in animals study is 6 is the smaller sample for any experimental study in each groups so according IEAC the reduced the number use of animals in study as much as possible.

Inclusion and exclusion criteria

Inclusion criteria

- Rats weighing 200-250 grams of either sex.

Exclusion criteria

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

Withdrawal criteria

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

Randomisation:

The animals will be taken randomly.

Analytical and experimental study:

- Blood glucose level
- OGTT(oral glucose tolerance test)

Outcome measures:

- Blood glucose levels will be estimated at the intervals.

Statistical methods:

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

Experimental animals:

- Healthy adult Wistar rats weigh of 200-250 grams of either sex between 2 and 3 months 0f age will be uses for the study.
- 30 (5x6) 3 males and 3 females of Wistar rats will be used as an animal model.

Housing and husbandry:

- All the rats will be healthy and will be kept in standard environment.
- They will be house in group in polypropylene cages and maintain under standard condition.
- Food will be feed with rat pellet diet.

Animal care and monitoring:

- All animals will be acclimatised before the study.
- While withdraw the blood from the animals care will be taken not to cause pain and blood will be withdraw at 0.5-1ml
- To reduce pain anaesthesia can be use

- Side effect may be seen during the study but all the standardisation will be taken before giving the medicine to animals.

Interpretation/ scientific implications

- If the result come out are as follow

1. *Tryushanadya lauha* is having more antidiabetic action when compare to *Tryushananadya mandura* then what is explain in Bhaishajya Ratna Valli stand right.
2. *TM* and *TL* having both antidiabetic action but TM is more effective then *Tryushananadya mandura* can be use instead of TL for preparation and further study.

- As Rasa Ratna Samucchaya stated that *mandura* is the *kitta* of *loha* expected to have its quality so can be used to treat disease as *loha* instead.

Limitation :

The study is an experimental study in 30 Wistar rats as it is pre-clinical study to obtain preliminary efficacy, toxicity and pharmacokinetic information. sample size is less.

For safety of human being so it has to study in animals and later clinical study in humans.

- If this Antidiabetic study is successful then this data will be used in another clinical study for intervention of Anti diabetic study.

Experimental procedures:

- Group I Non-diabetic healthy control group received normal saline intravenously.
- The hyperglycemia will be induce by alloxan monohydrate at a dose of 65mg/kg.
- Group II vehicle group will be give with *madhu* 1ml (18mg) & *grita* 2ml (36mg) ,
- Test drug treated group i.e Test group 1 will be give with *Tryushanadya lauha* 500mg/kg (9mg/kg) with *madhu* 1ml (18mg) & *Grita* 2ml (36mg) and
- Test group 2 with *Tryushanadya mandura* 500mg/kg (9mg/kg) with *madhu* 1ml (18mg) & *Grita* 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 (11^{th} day) and 360 h (15th day) and fasting plasma glucose levels were estimated at all intervals.

Oral Glucose Tolerance Test

- On the 16th days Oral Glucose Tolerance Test will be perform in same group of rats.
- Glucose (4g/kg) will be fed orally for 1 day. One hour after the administration of drug.
- Blood will be withdrawn after glucose administration and fasting 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]

Discussion:

TM is herbo-mineral formulation which is indicated in *prameha* and *mandura* is one of the *lohakitta* use as dosage form TM , with the question can it be compare and have the quality of *loha* comparative study is taken. As the previous study of *loha bhasma* and *mandura bhasma* in *panduhara* effect and haematinic evaluation shows that *mandura bhasma* have more significant effect than *loha bhasma* on haemoglobin level and *mandura bhasma* had better haematinic compared to *loha bhasma*.[25] [26]

The process of preparation of *loha bhasma* consume a lot of times when compare to the preparation of *mandura bhasma*. Pharmacuetical preparation of *loha bhasma* is a tedious process involving many steps in conversion of *loha bhasma* from *loha*.It is very costly and time consuming also.*Loha bhasma* if not given sufficient number of *puta* it causes constipation, while in *mandura bhasma* is not there.The properties which *manda lauha bhasma* 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 recognised in both the samples that is *TL and TM.*[28]Even in analytical study XRD and FEGSEM may show nano-particles size of *mandura* is less than *loha* as the number of *puta* is given more in *loha*.[29][30]

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.

In this study, TL and TM are the two formulation use to evaluate their antidiabetic action in induced hyperglycemia 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 among *lauha* and *mandura* which one is having better action in antidiabetic.

Conclusion:

If this Antidiabetic study is successful then this data will be used in another clinical study for intervention of Antidiabetic study, as it is Herbo-mineral medicine so it may/may be not show toxic effect in animal models. So after the pre-clinical study it can be study as clinical trial in human being.

Expected outcome -

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

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.

References:

1. AK A, Sabu NJ, Bindu KK. International Journal of Ayurveda and Pharma Research. Int. J. Ayur. Pharma Research. 2019;7(4):39-48..)

2. YOU S, KANG M. A Study on Methods to Prevent Pima Indians Diabetes using SVM. Korea Journal of Artificial Intelligence. 2020;8(2):7-10..

3. Shastri AD, Shastri R. Bhaishajya ratnavali. Edition Chauk hamba Prakashana, Varanasi. 2008;26:551-3.
4. Vilas D, Prakash P. A text Book of Rasashastra. Ist ed., Chaukhamba Surbharati Prakashan, Varanasi. 2004:383.
5. Savarese G, Lund LH. Global public health burden of heart failure. Cardiac failure review. 2017 Apr;3(1):7.
6. Naz H, Ahuja S. Deep learning approach for diabetes prediction using PIMA Indian dataset. Journal of Diabetes & Metabolic Disorders. 2020 Jun;19(1):391-403.

7. Sarkar PK, Prajapati PK, Choudhary AK, De S, Ravishankar B. A comparative pharmaceutico-pharmaco-clinical study of Lauha Bhasma and Mandura Bhasma wsr to its Panduhara Effect. AYU (An international quarterly journal of research in Ayurveda). 2007 Jan 1;28(1):11.

8. Sarkar PK, Prajapati PK, Choudhary AK, Shukla VJ, Ravishankar B. Haematinic evaluation of Lauha bhasma and Mandura bhasma on HgCl 2-induced anemia in rats. Indian Journal of Pharmaceutical Sciences. 2007;69(6):791.

9. Sharma SN, Tarangini R, Shastri KN. Hindi commentary. Delhi: Motilal Banarasi Das. 2004;6:149.

10. Rajendra prasad ML, Shekhar S, Subramanya AR. Pharmaceutical and analytical study on loha bhasma. Int J Ayurvedic Med. 2010;1:47-59.

11. Vagbhatacarya RR. Hindi commentary by Mishra S. Varanasi, Chaukhambha Orientalia. 2011;2:19.

12. Sastry JL, Chunekar KC. Dravyaguna vijnana. Edn. 2008;3:128-31.
13. Murthy S. English translation of Astanga Hridaya. Varanasi, India: Choukhamba Orientalia. 1991.
14. Chandrabhushan DZ. Text book of Rasashastra. Revised Edition. Varanasi: Chaukhambha Surbharti Prakashan. 2007.
15. Belge RS, Belge AR. Ayurvedic shodhana treatments and their applied aspect with special reference to loha. IOSR–J Pharm Biol Sci. 2012;2:45-9.
16. Sen G, Shastri AD, Shastri RD. Bhaishajya ratnavali. Hindi commentary of Ambika datta Sastry, verse-(Jwara). 2008;5:1162-9.
17. Chaudhari N, Sathe N. PHARMACEUTICO-ANALYTICAL STUDY OF KANTA LAUHA BHASMA: BIO-SYNTHESIZED TRADITIONAL NANOPARTICLES USING CLASSICAL AND MODERN METHODS.
18. Ayurvedic Pharmacopoeia Committee. The ayurvedic pharmacopoeia of India. Government of India, Ministry of Health and Family Welfare. New Delhi, India: Department of AYUSH. 2008,17,25,74

19. Wanjari AS, Bhutada S, Desai P, Chouragade NB, Wanjari DS. Standardization of Herbal Products in Relation to Indian Market. Research Journal of Pharmacognosy and Phytochemistry. 2016;8(4):245-51.

20. Mulik SB, Jha CB. Physicochemical characterization of an Iron based Indian traditional medicine: Mandura Bhasma. Ancient science of life. 2011 Oct;31(2):52.

21. Paget GE. Evaluation of Drug Activities. Pharmacometrics.. 1964.

22. Food and Drug Administration. Guidance for industry: estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers. Centre for Drug Evaluation and Research (CDER). 2005 Jul;7.

23. Wanjari MM, Mishra S, Dey YN, Sharma D, Gaidhani SN, Jadhav AD. Antidiabetic activity of Chandraprabhavati–A classical Ayurvedic formulation. Journal of Ayurveda and integrative medicine. 2016 Jul 1;7(3):144-50.

24. Gittelsohn J, Wolever TM, Harris SB, Harris-Giraldo R, Hanley AJ, Zinman B. Specific patterns of food consumption and preparation are associated with diabetes and obesity in a Native Canadian community. The Journal of nutrition. 1998 Mar 1;128(3):541-7.

25. Potbhare M, Khobragade D. In Vitro Evaluation of Antioxidant Potential of Ayurvedic Preparations Lauha Bhasma and Mandura Bhasma. Asian Journal of Pharmaceutical Research. 2017;7(2):63-6.

26. Khobragade DS, Potbhare MS, Lote SB, Pardeshi KS, Wankhede SB, Tenpe CR. Preclinical Evaluation of the Effect of Antioxidant N-acetyl-D Glucosamine on Haematinic Potentials of Lauha Bhasma and Mandura Bhasma. Biomedical and Pharmacology Journal. 2021 Mar 1;14(1):163-74.

27. Singh TR, Gupta LN, Kumar N. Standard manufacturing procedure of Teekshna lauha bhasma. Journal of Ayurveda and integrative medicine. 2016 Apr 1;7(2):100-8.

28. Kamble S, Wanjari A, Rathi B, Rajput D. Pharmaceutico-Analytical Study of Mukta shukti Pishti and Mukta shukti bhasma and Comparative Evaluation of their Relative Oral Bioavailability. Journal of Pharmaceutical Research International. 2021 Jun 11:1-9.

29. Bamoriya H, Singh R, Chandil S. CONCEPT OF NANOTECHNOLOGY IN AYURVEDA WSR TO RASA AUSHADHIES.

30. Virupaksha GK, Kumar N. Characterization of Tarakeshwara rasa: An Ayurvedic herbomineral formulation. Ayu. 2012 Jul;33(3):406.

Tables:

Table 1*:*Drugs review

Table 2*:* Preparation of *tryushanadya loha/mandura*

Table 3*:* Grouping of study animals, dose of drugs and *anupana.*