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# COMPARATIVE TOXICOLOGICAL EVALUATION OF *SHUDDHA MANASHILA* PROCESSED IN *BHRINGRAJ SWARAS* AND *BHRINGRAJ SWARAS* WITH *AJA MUTRA*

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## ABSTRACT

*Manashila* (realgar) ($As_2S_2$) is an Arsenic compound. To formulate '*Shuddha* (detoxified) form of *Manashila*' in the present study '*Swedan*' (boiling in liquid) procedure was conducted using various vehicles like *Bhringraj Swaras* (Eclipta prostrata) and mixture of *Bhringraj Swaras* with *Aja mutra* (Goat's urine). Three samples were analyzed using physicochemical, SEM EDAX and XRD technique. Physicochemical properties of *Ashuddha Manashila* were matched with standards. Atomic % of different elements was observed in *Ashuddha* and both samples of *Shuddha Manashila* in SEM EDAX. XRD technique showed the structural change in *Ashuddha* and both samples of *Shuddha Manashila*. Toxicity profile of *Ashuddha* and two samples of *Shuddha Manashila* were determined using OECD guidelines 423 and 407 for Acute and Sub acute toxicity respectively. In acute toxicity study Ladder system was followed. In the study $LD_{50}$ of *Ashuddha Manashila* in rat was greater than 5000mg/kg rat. In sub acute toxicity study limit test dose was considered and 1000mg/kg drug dose was administered to animals up to 28 days. Out of 8 parameters studied no significant abnormalities were observed in any of the 7 standard parameters viz. food consumption, average body weight, general observations, hematological investigation, percentage organ weight, gross necropsy and Histo-pathological investigations of all animals in all groups. Thus, *Ashuddha* and two samples of *Shuddha Manashila* did not show toxicity in Sub Acute Toxicity Study. Therefore, it is not possible to find out the role of *'Swedan'* process and liquid drugs used in detoxification of *Manashila*. This process might be helpful to change the structure of *Manashila.*

**Keywords:** *Manashila, Bhiringraj Swaras, Aja mutra, Swedan* Process

## INTRODUCTION

In Ayurvedic pharmaceutics varieties of natural products such as herbal, mineral and animal drugs are used to prepare herbal and herbomineral formulations. It is reviewed that before 8th century herbal sources are maximally used than mineral and metal drugs. After this period herbo-mineral

formulations became a vital part in Ayurved practice. As 'Rasashastra' is a separate branch evolved different Mineral, Metallic formulations have been documented for its desired activities and for untoward effects.

*Manashila* ($As_2S_2$) is an Arsenic compound is one of the important mineral drugs which are an ingredient of approximately 396 formulations. Formulations containing *Manashila* have wide range of dosage forms like *Taila, Ghrita, Lepa, Avaleha, Varti* etc. Maximally *Manashila* is used in compound formulations to treat Respiratory disorders and Skin disorders. In Ayurved and in modern science, compounds of Arsenic said to be toxic. Specific toxicity signs of Arsenic compounds are documented viz. nausea, vomiting, colicky abdominal pain, profuse watery diarrhea and excessive salivation.

*Shodhan* is a basic concept of Rasashastra Bhaishajya Kalpana. To conduct *Shodhan 'Sanskara'* different processes such as *Bhawana, Bharjan, Swedan, Nirvap, Dhalan* etc. are carried out to convert raw drug into "*Shuddha* form".

In Ayurvedic pharmaceutics detoxification (*Shodhan*) of *Manashila* is mentioned to eliminate untoward effects and make it safe for use. Three processes like Swedan (boiling in liquid); *Bhawana* (Trituration) and *Nimajjan* (Immersion) are advocated to formulate *'shuddha Manashila'*

Literature study strongly recommends the use of *'Swedan'* process in detoxification of *Manashila.* "*Swedan"* is a process in which powdered drug is kept in cloth and suspended in liquid herbal or animal drugs. What kind of change *Swedan (Shodhan* process) brings in *Ashuddha* is not yet completely explored. Therefore, to generate evidence for the 'process of *Swedan'* experimental studies are conducted.

Eight liquid drugs are advocated to detoxify *Manashila.* Among them the use of *Bhringraj Swaras* and mixture of *Bhringraj Swaras* and *Aja Mutra* for detoxification of *Manashila* is not analyzed till date. Rationality in the use of these drugs might be helpful to remove unwanted effects and facilitate the targeted action of *Manashila.*

Previously research work on pharmaceutical and clinical study of *Manashila Dhoomra*[1&2], efficacy of *Manashila Ghrita*[3&4], *Manashiladi Yoga*[5&6], *Manashiladi Lepa*[7&8], *Manashiladi Anjana*[9] and Physico-chemical analysis of *Shuddha Manashila*[10] have been conducted.

Thus, in the present study toxicity profile of *Ashuddha* and two samples of *Shuddha Manashila* obtained by conduction of '*Swedan Sanskara'* using *Bhringraj swaras*[11] and Mixture of *Bhringraj Swaras* and *Aja mutra*[12] is determined.

**AIM:**

Compare the effect of *Swedan Sanskara* on *Ashuddha Manashila* with *Bhringraj swaras* and mixture of *Bhringraj swaras* with *Aja mutra* by using experimental model.

**OBJECTIVES:**

1) To procure raw materials from the market
2) To authenticate mineral, herbal and animal drugs
3) To prepare *Shuddha Manashila* by standard methods
4) To analyze physical and chemical properties of *Shuddha Manashila*
5) To determine $LD_{50}$ dose of *Ashuddha Manashila*
6) To assess the oral Sub acute toxicological profile of *Ashuddha* and two samples of *Shuddha Manashila.*

**Pharmaceutical study:**

**MATERIALS:**

**Drugs –** Raw *Manashila* (Realgar- $AS_2S_2$), *Bhringraj swaras* (Eclipta prostrata) and *Aja mutra* (Goat urine).

**Pharmaceutical Instruments** - *Dola yantra* assembly, Mixer, Sieves, Steel vessels, silk cloths, measuring cylinders etc.

**Instruments used for analytical testing-** I.R moisture analyzer, Digital balance, hot water bath, crucibles, muffle furnace, test tubes, conical flasks,

Digital pH meter, filter paper, x-ray diffractometer, etc.

## METHODOLOGY:

### A] Pharmacognosy and Pharmaceutical study:

**Authentication of *Manashila* (Realgar):**

Five samples were coded. Identification and selection of *Manashila* was done using consensus method. These samples were assessed using ayurvedic and geological parameters. The parameters were Color, Heaviness, Smoothness, Lustre and Redness Parameters assessed by Geologists were Hardness, Cleavage and Streak etc. Organoleptic Characteristics of selected sample of *Ashuddha* (Raw) *Manashila* was done**.** Physical Parameters of selected sample of Raw *Manashila* were studied. XRD analysis and SEM EDAX analysis of selected sample was done.

**Authentication of *Bhringra*j (Eclipta prostrata Linn.):**

Identification of handpicked sample of *Bhringraj* was done by Ayurvedic experts. Selected sample was tested in analytical laboratory using API parameters. Test was- Foreign matter, Total ash content, Alcohol soluble extractive value and Water soluble extractive value. The sample was authenticated.

**Authentication of *Aja Mutra*:**

Fresh *Aja mutra* was collected in the morning before experiment. PH and Specific gravity was tested for three times.

### PHARMACEUTICAL PROCESS:

- Raw *Manashila* was finely powdered using *Khalva Yantra* (Mortar & Pestle) and passed through 120 mesh size. *Swaras* was prepared from freshly procured *Bhringraj* by standard method.
- Two assembly of *Dola yantra* was prepared using *Ghata*, having capacity 16 times of the drug[13].
- In first assembly powdered *Manashila* was kept in *Kadali patra* and tied with thread. Then it was placed in *pottali* prepared with four layered silk cloths. *Bhringraj Swaras* was poured in *Dola yantra*. *Pottali* was immersed in *Bhringraj Swaras.* It was kept 2 *angulas* [5cm] above from the bottom of *Dola yantra. Dola yantra* was subjected to low heating for 12 hours. (sample D1)
- In second assembly of *Dola yantra* above said procedure was followed and instead of *Bhringraj Swaras,* mixture of *Bhringraj Swaras* and *Aja Mutra* was used. (sample D2)

## RESULTS:

**Table 1:** In house authentication of *Bhringraj*:

| Parameters | Obtained values |
|---|---|
| Foreign matter | 0.4 % |
| Total ash | 6.4 % |
| Acid Insoluble ash | 2 % |
| Alcohol soluble extractive | 18 % |
| Water soluble extractive | 25.4 % |
| pH value | 6.23 |
| Specific gravity | 1.00 |

**Interpretation:**

Authentication of *Bhringraj* was done and obtained values were within normal limit as per API.

**Table 2: In house authentication of Aja Mutra:**

| Parameters | Obtained values |
|---|---|
| pH value | 7.26 |
| Specific gravity | 1.01 |

**Interpretation:**
Authentication of *Aja Mutra* was done and obtained values were within normal limit.

**Table 3:** Physical Properties of *Ashuddha Manashila*[14]:

| | | | |
|---|---|---|---|
| **Colour** | Reddish Orange | **Fracture** | Conchoidal |
| **Luster** | Resinous | **Cleavage** | Good |
| **Tenacity** | Brittle | **Habit:** | Massive Granular |
| **Inclusions** | None | **Crystallography** | Monoclinic |
| **Streak** | Reddish Orange | **Specific Gravity:** | ~3.55 |
| **Diaphaneity** | Transparent to Translucent | **Hardness** | Between 1 & 2 on Moh's Scale |

**Interpretation:**
All the physical properties of selected sample of *Manashila* were matched with geological standards.

**Table 4:** Comparison between Organoleptic Characters of *Ashuddha* and two samples of *Shuddha Manashila*

| **Parameters** | ***Ashuddha Manashila*** | ***Shuddha Manashila*(D1)** | ***Shuddha Manashila*(D2)** |
|---|---|---|---|
| ***Shabda* –** (Parameters analyzed by auditory system) Sound produced on breaking. | On breaking specific "KAT" sound | Not Applicable as drug is in powder form | Not Applicable as drug is in powder form |
| ***Sparsh*-** (Parameters analyzed by tactile system)<br>Smoothness/Roughness<br>Soft/Hard<br>Heavy/ Light. | Rough<br>Brittle<br>Heavy | **Smooth** | **Smooth** |
| ***Rupa*-** (Parameters analyzed by visual system)<br>Color<br>Luster<br>Shape<br>Opacity | Reddish<br>Resinous<br>Opaque<br>Irregular | **Reddish orange** | **break red**<br>Powder form |
| ***Rasa*-** (Parameters analyzed by gustatory system) | Not applicable | Not applicable | Not applicable |
| ***Gandha*-** (Parameters analyzed by olfactory system) | Specific Sulfur smell | **Specific *Bhringraj* smell** | **Specific *Aja Mutra* smell** |

**Interpretation:**
Change in the color of sample D1 i.e. reddish orange and sample D2 i.e. brick red was noted. Specific odor is may be due to the liquid drugs used in processing.

- **Comparison between XRD analysis of *Ashuddha* and two samples of *Shuddha Manashila***

**Fig. No.1.** XRD analysis of *Ashuddha* (Raw) *Manashila*

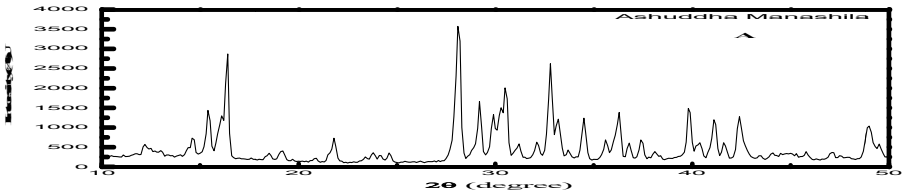



**Fig. No. 2.** XRD analysis of *Shuddha Manashila* (D1)

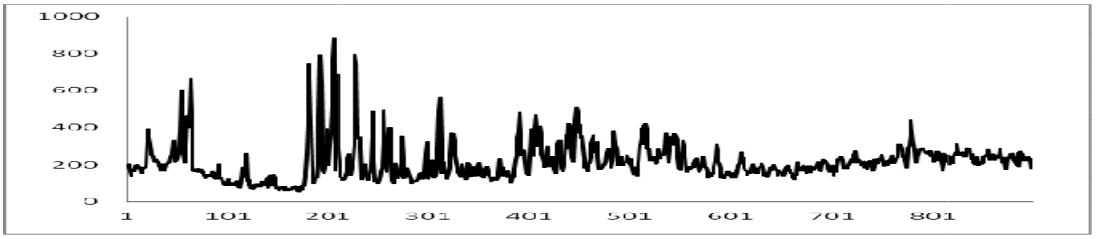
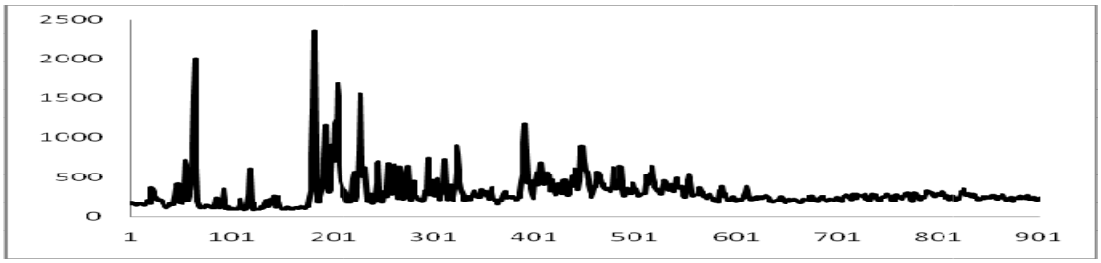



**Fig. No. 3.** XRD analysis of *Shuddha Manashila* (D2)




**Interpretation:**
The peaks of Arsenic and Sulphur were similar in all three samples. In both *Shuddha* samples, peaks of other elements were also noted. This change may be due to structural change in *Manashila* after processing.

**Table 5:** Comparison between values of *Ashuddha* and *Shuddha Manashila*

| **Elements** | | O | S | Cu | Ca | As | Th |
|---|---|---|---|---|---|---|---|
| **Atomic %** | ***Ashuddha Manashila*** | 13.67 | 37.17 | 2.03 | 1.42 | 34.49 | 11.22 |
| | ***Shuddha Manashila* (D1)** | 29.38 | 26.92 | 2.36 | 1.90 | 31.29 | 8.16 |
| | ***Shuddha Manashila* (D2)** | 22.28 | 27.93 | 2.19 | 1.92 | 32.97 | 6.71 |

**Interpretation:**
SEM EDAX technique was utilized to find out the % of different elements present in *Ashuddha* and two samples of *Shuddha Manashila*. The % of Sulphur, Arsenic and Thorium were reduced considerably, and percentage of Oxygen, Copper and Calcium were raised after *shodhan* process.

## DISCUSSION

**PHARMACEUTICAL STUDY:**

- Consensus method proved useful to identify the best sample of *Manashila*.
- Obtained values of *Bhringraj* were matched with standard values.

- pH and specific gravity of Aja mutra were matched with standard values.
- Weight variation was seen in both samples of *Shuddha Manashila* after processing.
- Organoleptic tests of two samples of *Shuddha Manashila* were changed after processing.
- In XRD analysis peaks of Arsenic and Sulphur were similar in all three samples of *Manashila*. In both '*shuddha* samples' of *Manashila* peaks of other elements were also noted. It might be due to change in the structure of *Manashila* after processing.
- In SEM EDAX technique atomic % of Sulphur, Arsenic and Thorium were reduced and % of Oxygen, Copper and Calcium were raised after *shodhan* process in both *shuddha* samples.

**EXPERIMENTAL STUDY:**

Permission was taken from the Animal Ethics Committee.

OECD Guidelines number 423 for Acute Oral Toxicity study and number 407 for Sub-Acute Oral Toxicity Study was followed.[15]

Test Details for Acute and Sub Acute toxicity study: The study was conducted at CPCSEA approved Central Animal House, BVU.

- The species chosen was Wistar Rat (Age 8-12 weeks)
- Animals were coded by using Picric acid.
- The drug was administered in the form of mucilaginous suspension prepared using 4% Gum Acacia in water with No. 3 rubber catheter and 1ml Tuberculin syringe.
- SOP for dose preparation and intra gastric dose administration were followed throughout the study.
- Standard Housing of Animals, Environmental Condition, Diet and Water was maintained for animals as per guidelines.

**ACUTE TOXICITY STUDY:**

**MATERIAL:**

Animal: Wistar rats (all females)

Drug: *Ashuddha Manashila*

Vehicle: Gum acacia

Instruments: No. 3 rubber catheter, specially prepared wooden stand, syringe, beaker etc.

**METHODOLOGY:**

OECD Guidelines number 423 for Acute Oral Toxicity study was followed.

- 3 animals, all females, were used at each dose level. Animals were kept fasting overnight prior to dosing. The animals were observed for 14 days after single dose administration at each level. Dose for each Animal was calculated according to its body weight.
- Ladder system of dosing was followed as per OECD Guideline to find out $LD_{50}$ of *'Ashuddha Manashila'* (Raw Realgar)

**Dose selection of *Ashuddha Manashila*:**

Reported LD50 of Arsenic (Arsenious oxide-$As_2O_3$) is 120mg to 200mg and that of Arsenic sulphide [$As_4S_4$] is 185mg. The data on LD50 of $As_2S_2$ is not available.

Hence in the present study the maximum dose level i.e. 200 mg in humans was extrapolated for rats using 'extrapolation factor'

The dose levels were selected as 18 mg/kg **(X)**, 180mg/kg **(10X),** and 1080mg/kg **(60 X).** In all this dose level no death was observed in animals within 24 hours and no signs of toxicity were observed in next 14 days. Therefore, further dose was **400X** was considered but it has crossed non-toxic dose i.e. 5000mg/kg. Hence in the present study 5000mg/kg dose in rat was chosen.

**General Observations:** The animals were observed for changes in any of the following parameters:

**Table 6:**

| | | | |
|---|---|---|---|
| Skin | Secretions | Mucous Membrane | Unusual respiratory pattern |
| Fur | Excretions | Response to Handling | Presence to clonic / tonic movements |
| Eyes | Lacrimation | Changes in posture | Excessive grooming |
| Pupil Size | Piloerection | Gait | Repetitive circling |

**Sacrifice of Test Animals:**

Rats were sacrificed by cervical dislocation on fifteenth day. The organs lung, liver, stomach, intestine, heart, spleen, kidney, brain, uterus was collected and observed for gross necropsy.

**RESULTS:**

No death of any animal was observed till the end of the experiment. Food consumption and body weight of rats increased gradually in all the four groups during study duration. Behavioral patterns showed no signs of toxic effects. No other toxicity signs were seen in any animal.

**INTERPRETATION:**

Acute toxicity study revealed that signs of toxicity occurring due to consumption of unprocessed *Manashila* documented in 'Rasagranthas' may not be for single dose administration. Rather toxicity signs may be seen after administration of *Manashila* for longer duration. The reported data strengthened the thought that long term administration of Arsenic compounds exhibit signs of toxicity.

Thus, it was felt worth while not to perform Acute Toxicity Study of *Shuddha Manashila* in a single dose level.

Also, it was decided to evaluate Sub acute toxicity of *Ashuddha Manashila.*

**SUB ACUTE TOXICITY STUDY:**

**MATERIAL:**

**Animal:** Wistar rats (3 male and 3 females in each group)

**Drug:** *Ashuddha Manashila,* two samples of *shuddha manashila* (D1 and D2)

**Vehicle:** Gum acacia

**Instruments:** No. 3 rubber catheter, specially prepared wooden stand, syringe, beaker etc.

**METHODOLOGY:**

OECD Guidelines number 407 for Sub Acute Oral Toxicity study was followed.

- To conduct sub acute study three test drug groups and a control group were used. But considering the results of acute toxicity study of *Manashila*, it was decided to perform 'limit test' at a dose of 1000mg/kg; according to the guideline.
- Rats were randomly allocated into four group viz. *Ashuddha Manashila*, two samples of *Shuddha Manashila* and Control. Number of animals per group was 6, comprising of 3 males, 3 females. Dosing period was 28 days.
- SOPs for acute toxicity study were followed in this study. Weights of Animals were recorded just before dosing. Dosing was done according to weight of respective Animal. Daily food consumption was recorded. The general behavior of animals was observed for changes in skin, eyes, Secretions, Excretions, Lacrimation etc.
- Blood samples were collected after 24 hours of last dose administration on 29$^{th}$ day. The animals were sacrificed by cervical dislocation and dissected. Lung, heart, liver, spleen, kidney, adrenal glands, testes, ovary, intestines, stomach, bladder, skin and brain were collected very carefully and weighed. Organs were preserved into the 10% buffered formalin as per standard procedure. These organs were sent to Department of Pathology B. V. Medical College, Pune.

**RESULS:**

- Out of 8 parameters studied significant abnormalities were not observed in 7 parameters

viz. average body weight per group, general observations, food consumption, hematological investigation, percentage organ weight, gross necropsy and histo-pathological investigations.

- In Biochemical parameters 18 values were studied out of 14 values were found to be normal in all animals from all groups.
- Remaining 4 biochemical values viz. Urea, Serum Creatinine, Serum Alkaline Phosphatage and Serum Triglycerides were altered when compared in all groups. But these values were not on a higher level of the normal range. Hence did not signify functional changes.
- Therefore, both the samples of *Shuddha* and *Ashuddha Manashila* did not show any toxicity signs in Sub Acute Toxicity Study.

Therefore, it is not possible to find out the role of '*Swedan*' process and liquid drugs used in detoxification of *Manashila*. This process might be helpful to change the structure of *Manashila.*

## CONCLUSION

**Acute Toxicity Study shows that the** $LD_{50}$ of *Ashuddha Manashila* is greater than 5000 mg/kg of rat.

**Sub Acute Toxicity Study shows that,** the dose of 1000 mg/kg *Ashuddha* and two samples of *Shuddha Manashila* does not show any significant toxicity in sub acute toxicity study

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