

STUDY THE EFFECT OF SWEDAN SANSKAR ON ASHUDDHA MANASHILA

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ABSTRACT

Manashila (Realgar) is one of the mineral drugs which are used in the treatment of various diseases. As per modern science Arsenic is a toxic substance when it is in compound form. It is documented that absorbed Arsenic is accumulated in liver, kidneys, heart, lungs and deposited in gastrointestinal tract, spleen, lungs, and keratin rich tissues. Hence there is a need to determine toxicological profile of *Manashila*. In *Ayurved*, *Shodhan Sanskar* [detoxification process] is done on *Manashila* before using as a medicine to remove toxic effects. Present study focuses on acute toxicity study of *Shuddha Manashila*. Raw drugs were identified, selected, procured and authenticated by *Ayurvedic* and modern parameters. Detoxification of *Manashila* was done by boiling it in *Bhringaraj*, *Agasti*, *Jayanti* and *Ardraka swaras* sequentially, each for 12 hours in *Dolayantra*. Change in Colour, weight loss and analysis of *Manashila* indicate changes in properties of *Manashila* during *Swedan Sanskar*. Acute toxicity study was completed following OECD guidelines, with prior permission of Animal Ethics Committee. Effect of *Manashila* on body weight of rats, behaviour, food consumption was not significant. Haematology and blood chemistry KFT, LFT, BSL, Lipid profile were done on 29 day. Values of blood urea, serum creatinine do not change. SEM EDAX, XRD reports and results of toxicity study revealed that LD50 dose of *Manashila* is > 5000mg/kg of rat. *Manashila* is non-toxic for therapeutic dose.

Keywords: *Manashila*, *Swedan sanskara*, Toxicity, XRD.

INTRODUCTION

As per modern science Arsenic is a known toxic substance when it is in compound form. Chemically *Manashila* is Arsenic bi-sulphide. The prominent features of acute toxicity of Arsenic are nausea, vomiting, colicky abdominal pain, profuse watery diarrhea, and excessive salivation. Renal failure, respiratory failure, and pulmonary edema are also commonly seen. Absorbed Arsenic is accumulated in liver, kidneys, heart and lungs. It is also deposited in the muscles, nervous system, gastrointestinal tract, lungs, keratin rich tissues viz. nails,

hair and skin.^[1]

In *Ayurvedic* pharmaceutical science different processes are conducted on the mineral raw drugs to make it safe for internal and external use by removing unwanted effects. *Shodhan Sanskar* [detoxification process] is done on *Manashila* before it is used as medicine. Various detoxification methods of *Manashila* such as *Bhavana* [Trituration with liquids], *Swedan* [Boiling in liquids], *Nimajjan* [Immersion in liquids] are recommended in *Ayurvedic* science. One reference advocates conducting *Swedan*

method using four herbal drugs viz. *Bhringraj*, *Agasti*, *Jayanti* and *Ardraka* for *Manashila* to remove its toxic effects.^[2] Till date no work has been reported regarding toxicological study of *Shuddha Manashila*. Hence there is a need to determine toxicological profile of *Manashila*.

Present study focuses on acute toxicity study of *Manashila* obtained by sequential Swedan Sanskar of *Bhringaraj*, *Agasti*, *Jayanti* and *Ardraka swaras* on *Ashuddha Manashila* for 12 hours each.

Materials and methods

Materials: - Raw drugs -1) *Ashuddha Manashila (Realgar)* 2) *Bhringaraj (Whole plant)* *Eclipta prostate* 3) *Agasti (Leaves)* *Sesbania grandiflora* 4) *Jayanti (Leaves)* *Sesbania sesban*

Physicochemical tests	Bhringaraj	Agasti	Jayanti	Ardraka
Foreign matter	1%	0.4%	0.5%	0.35 %
Total Ash	17%	6.4%	8%	5 %
Acid-insoluble ash	6%	2%	0.5%	0.72 %
Alcohol-soluble extractive	14.8 %	18%	10%	6.42 %
Water-soluble extractive	17.8 %	25.4%	27.2%	4 %
Moisture content	NA	NA	NA	83 %

2. *Manashila* was triturated to 120 mesh size for particle size reduction.^[4]

3. Detoxification of *Ashuddha Manashila* (400gm) was done by boiling it in *Bhringaraj*, *Agasti*, *Jayanti* and *Ardraka swaras* sequentially, each for 12 hours in *Dolayantra*.

Detoxified *Manashila* was then washed with *Kanjika* and kept in airtight container after drying.^[5]

Drug Name	pH before processing	pH after processing
<i>Bhringaraja swaras</i>	6.23	6.40
<i>Agastipatra swaras</i>	6.30	5.94
<i>Jyantipatra Swaras</i>	6.20	6.11
<i>Ardraka swaras</i>	6.30	6.30

5) *Ardraka (Rhizome)* *Zinziber officinale*
Pharmaceutical Instruments- Mixer, Mortar pestle, *Dolayantra*, Sieves, Utensils
Analytical Instruments- Microscope, Digital pH Meter, Muffle furnace, IR moisture Analyzer, Thermometer etc.

Animals- Species of animals: Wistar rat

Methodology: -

A) Shodhan of Ashuddha Manashila-

1. *Manashila* was procured from local vendors. Herbal raw drugs samples were hand-picked. These were identified by experts and the best sample was selected by consensus method. Herbal drugs and *Manashila* were authenticated in laboratory by conducting physicochemical tests.^[3] **Table No. 1 Physicochemical Tests of herbal drugs:**

Weight of *Shuddha Manashila* was 396.26 gm. Averagely 10 liters *swaras* was used every time.

4. Physicochemical analysis of *Shuddha Manashila* was done. Elemental analysis was done by XRD and SEM EDAX of *Shudha manashila*.

Table No.2: Change in pH values of liquids drugs used for Detoxification

Kanjika	2.88	5.43
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Fig. No.1. Graph of Raw Manashila obtained by XRD

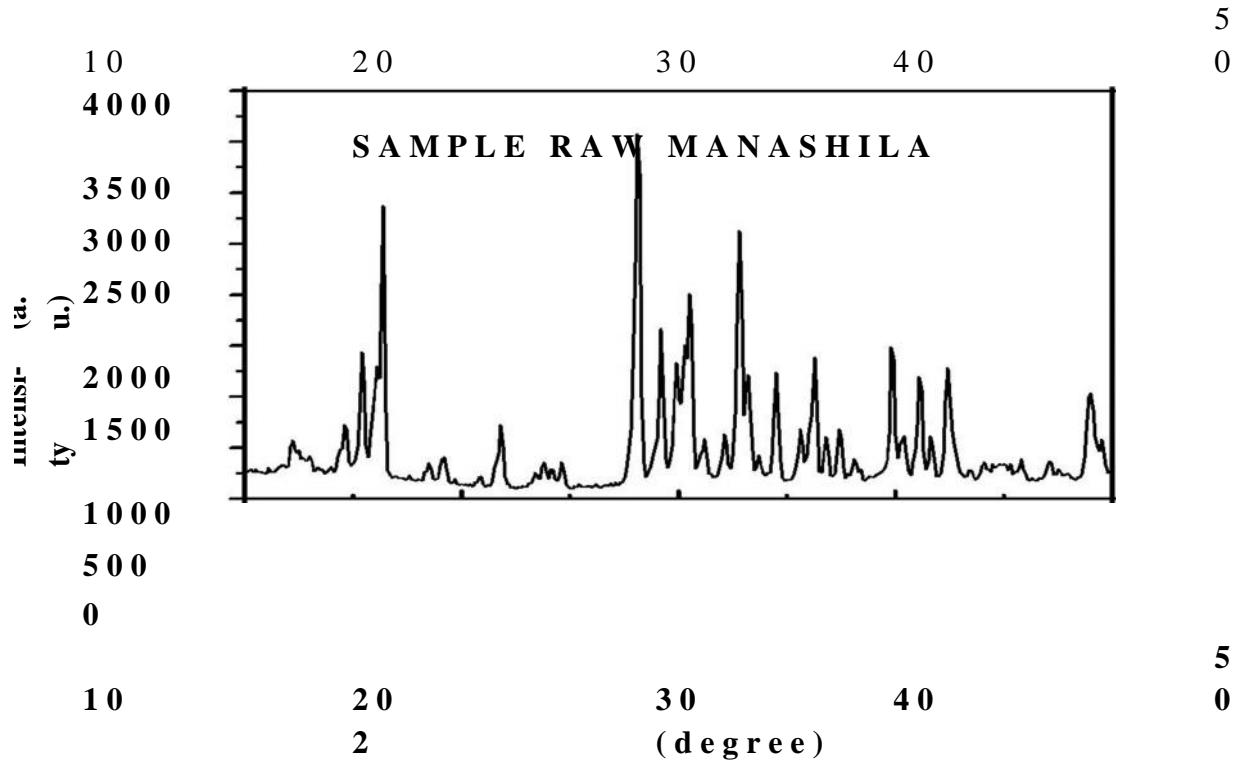
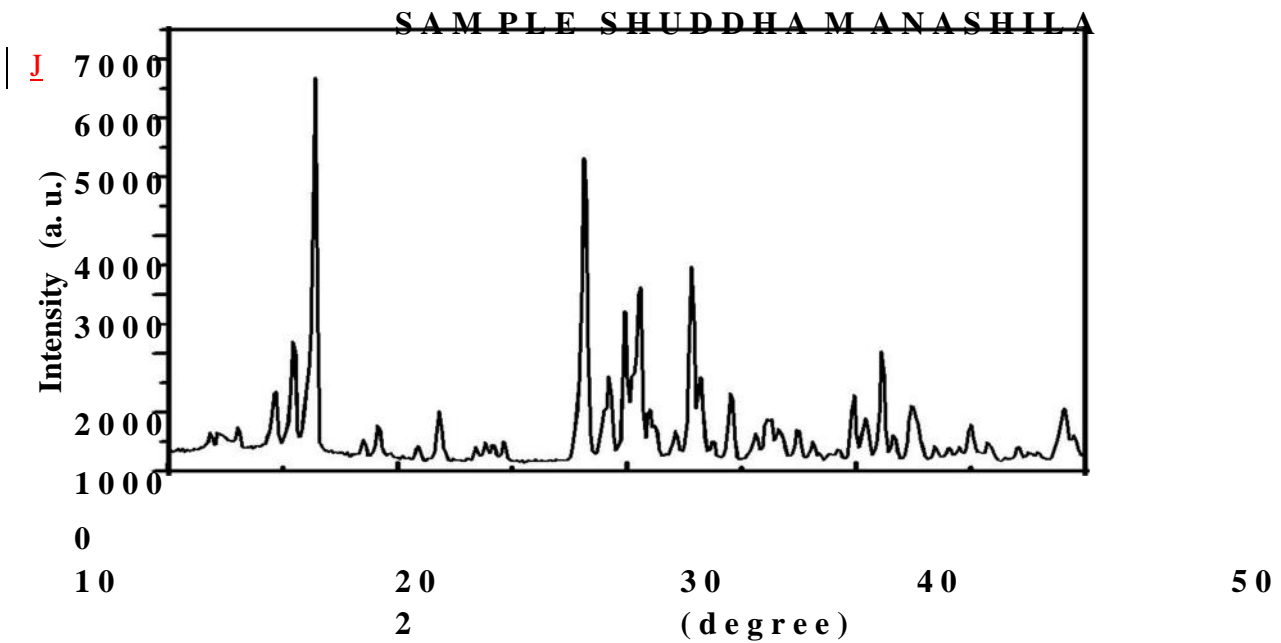


Fig. No.2. Graph of Shuddha Manashila obtained by XRD



Interpretation: 8 of most intense strong peaks from the data were compared for

and relative d values with the ASTM data set. It was found that the *Ashuddha Mana-*

shila is having As and S as major elements and Thorium as another element and some trace elements.

The Shuddha *Manashila* showed similar peaks to that of As S (Arsenic Sulphide)

with small amount of trace elements.

Both types of *Mansahila* structure of mineral was found monoclinic.

Table No.3: Elements detected by EDAX:

Sr.No.	Raw <i>Manashila</i>		Shuddha <i>Manashila</i>	
	Elements	Atomic %	Elements	Atomic %
1	O	13.67	O	31.77
2	S	37.17	S	27.94
3	Cu	2.03	Cu	4.25
4	Ca	1.42	Ca	1.74
5	As	34.19	As	26.29
6	Th	11.22	Th	8.01

Interpretation:

After doing SEM, data detected by EDAX showed that Raw *Manashila* was having Arsenic and Sulphur in major amount with Thorium and Oxygen in minor amount and Copper and Calcium were in trace amount. As and S were almost in equal amount suggesting of the mineral as AsS. Presence of Oxygen was suggestive of possibility of Oxide content (As₂ O₃) of the mineral which may impart the mineral toxic. Presence of Thorium with Copper and Calcium was suggestive of the source of *Manashila* nearby Thorium mines.

Shuddha Manashila was having decreased amount of major elements Arsenic, Sulphur and Thorium with noticeable increased amount of Oxygen and small increase in amount of Copper and Calcium.

B)Experimental Study:

1. Permission was taken from the Animal Ethics Committee before starting the animal experiments IAEC/CPCSEA Project Approval. Proposal Number: 4/2007 .OECD guidelines no.423 was followed for acute toxicity study.

2. Experimental study was carried out at CPCSEA approved Central Animal House, BVU, Pune under the guidance of Dept. of Pharmacology and Dept. of Pathology, Medical College, BVU, Pune.
3. Housing conditions, diet, water for experimental animals were kept as per guidelines. Selection, grouping and coding of animals was done according to guidelines.
4. 4% Gum acacia mucilage and suspension of *Manashila* was prepared by following SOP. Intra gastric drug administration through rubber catheter no.3 by using tuberculin syringe was done at each dose level.

Acute toxicity study- OECD Guideline No.423 (Acute oral toxicity – acute toxic class method) was followed.

Test Animals:

Species /Strain: Rats /Wister Rats
 Source: Central Animal house, B.V.U. Medical college, Pune.

Sex: Female, Age: 8–12 Weeks, Dose: Single, Route: Oral, Vehicle (Anupana) :4%Gum acacia suspension.

The animals were marked using picric acid.
Test substance: *Shuddha Manashila*. (Red-dish orange colored fine powder)

Methodology: *Manashila* is identified as realgar (*Arsenic sulphide*). No references are available about the toxicity of *manashila* on oral ingestion as it is not used therapeutically in modern medicine.

- The Fatal dose of *Arsenic* (in oxide form) ranges between 120 mg to 200 mg considering this a baseline data. LD50 study was carried out in stepwise fashion. Higher LD50 dose of *Arsenic oxide* i.e. 200 mg was extrapolated (extrapolation factor 0.018) as per body weight of rat. First dose (X) i.e. 18mg/kg was administered to rats.
- After administration of *Manashila*, animals were observed for daily food consumption, general appearance, behavioral changes and weight change in the following manner, continuously for 30 min after dosing, and then at intervals of 60 min, 90 min, 2 hrs, 4 hrs and 24 hrs for the first day. For next 14 days animals are observed once daily.

Observation : Parameters for changes in behavior and toxic signs :- Skin, Fur, Eyes, Mucous Membrane, Secretions, Excretions, Lacrimation , Pupil Size, Unusual respiratory pattern, changes in posture, Gait, Response to handling, Presence to clonic / tonic movements, Excessive grooming, Repetitive circling, Self-mutilation, Walking backwards.

No toxicity signs or behavioral changes were observed in first 24 hrs. And till 14 days.

- As per guidelines second dose was 10 times of the first test dose. So the second

test dose (10X) 180mg/kg of rat was administered. No toxicity signs or behavioral changes were observed. So third dose (60X) 1080 mg/kg of rat was administered. This dose did not show any toxicity signs and abnormal changes.

- As per guidelines the fourth test dose (400X) would be 7200mg/kg of rat. This dose was crossing the dose for limit test i.e. 2000mg/kg and the non-toxic dose i.e. 5000mg/kg also. So fourth dose was decided as 5000mg/kg of rat and administered. This dose also did not show any signs of toxicity.
- each dose level 3 female rats / cage were used. Animals were kept fasting overnight prior to dosing.
- At the end of study period i.e. on the 15th day, the animals were sacrificed by cervical dislocation. The body was cut open and inspected for gross necropsy. It was found that there was no congestion or any pathology present in lungs, spleen, kidneys. Stomach, liver, intestine, heart, trachea, kidney, uterus and brain. No death till the end of the experiment. There was steady increase in body weights. General parameters and behavioral pattern showed no change. No congestion or pathology was found in gross necropsy. No other toxicity was seen.

Result:

1) Pharmaceutical Study-

Weight loss of *Manashila* after *Shodhan* was 0.93 %. Brick red colored *Manashila* was obtained. No change in pH values of detoxifying herbal juices.

2) SEM EDAX:

- 1) Decrease in amount of As, S, and Th in *Shuddha Manashila* shows that the toxic el-

elements are reduced in the process of detoxification. As there is no any toxicity sign seen in hematology, blood biochemistry and histopathology, major increase in amount of Oxygen is suggestive of bonding of the *Manashila* with organic material during the processing with herbal juices and heat treatment.

3) Acute Toxicity Study-

1) There were no toxic effects on behavior of rats and no signs of toxicity observed in gross necropsy up to 5000mg/kg body weight for single dose. It indicates LD50 is greater than 5000mg/kg of rat. The signs listed in texts may be for long term dose administration and not for a single dose.

DISCUSSION

Pharmaceutical Study-Weight loss: Weight loss of *Manashila* after *Shodhan* was 0.93 %. It may be due to elimination of impurities.

Change in colour: Colour of *Ashuddha Manashila* changed from reddish orange to

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brick red after *shodhan*. This may be due to heat treatment and elimination of some impurities.

pH: pH of detoxifying herbal juices were not significantly changed.

Acute Toxicity Study-

There were no toxic effects on behaviour of rats and no signs of toxicity observed in gross necropsy up to 5000 mg/kg body weight for single dose. It indicates LD50 is greater than 5000 mg/kg of rat. The signs listed in texts may be for long term dose administration and not for a single dose.

CONCLUSION

Manashila shows weight loss and change of colour after *shodhan* treatment. In both types of *Manashila* structure was found monoclinic. *Manashila* detoxified by sequential *swedana sanskar* did not show any toxicity signs. There are no significant changes in histopathology, hematology and blood biochemistry of rats. LD 50 of *shuddha Manashila* is greater than 5000 mg/kg of rat.

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