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Standardization of Hinguliya Manikyarasa

Research Article

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Abstract

Standard is a numerical value which quantifies the parameter and thus denotes the purity of the material. Standardization of *Ayurvedic* drugs is a need of the hour in this changing Global scenario. In *Ayurvedic* texts the parameters given are mostly subjective. For this reason some objective parameters should be established through exhaustive pharmaceutical study and Analytical study. A drug selected for present study is *Hinguliya Manikyarasa* and some objective parameters like measurement of temperature, the yield of finished product were studied. Physical ,Chemical analysis along with the toxicological study were carried out. The findings will be helpful to establish objective parameters for Standardization of *Hinguliya Manikyarasa* and toxicological study will be helpful to establish its safety profile. This study will provide guidelines to establish such type of standards to other Herbomineral formulations, so that the manufacturer can give best quality *Ayurvedic* products to our society and to the people all over the world.

Key Words: *Rasashastra, Kupipakwa rasayana, Hinguliya Manikyarasa,* toxicity study.

Introduction

Our ancient *Acharyas* understood the importance of physical well-being for achieving the means of life. To maintain the health or to keep physical well-being man learned the art of designing and manufacturing the medicines. *Acharya Charaka* preaches *Trisutra Ayurveda* which consists of science of Causes (*Hetu*), Symptoms (*Linga*) and Medication (*Aushadha*).[1] The Drug and pharmaceutical preparations comes under third category. Standard is a numerical value which quantify the parameter and thus denotes the purity of the material. Standardization of *Ayurvedic* drugs is a need of the hour in this changing Global scenario. This standardization can be carried out in three phases i.e. at raw material level, at manufacturing process level and at finished product level. In *Ayurvedic* texts for *Ayurvediya oushadhikarana*, the parameters given are mostly subjective. For this reason some objective parameters should be established through exhaustive pharmaceutical study and Analytical study. *Rasashastra* has its origin in *Vedic* period.[2] The main substance used was Mercury and other minerals and inorganic substances were involved in various processes and these medicines were used extensively in the therapeutics. It is given that there are number of remedies for curable disorders but for incurable disorders the only

remedies are the *Rasaushadhis* 3. These *Rasaushadhis* are of four types viz. *Kharaliya*, *Parpati*, *Kupipakwa* and *Pottali*.4 Out of that *kupipakwa rasayana* is important formulation. So for the present study the drug selected for pharmaceutical, analytical and toxicological study is *Hinguliya Manikyarasa* which is a *Kupipakwa rasayana* depicted in *Rasatarangini*.5

Aim & Objectives:-

To study pharmaceutical and analytical aspects of *Hinguliya Manikyarasa* and its establishment of safety profile by toxicological study.

Objective of the Study –

- Pharmaceutical study of *Hinguliya Manikyarasa* and
- Analytical study of *Hinguliya Manikyarasa*.
- To conduct toxicological study of *Hinguliya Manikyarasa*

Materials and Methodology:-

The ingredients of *Hinguliya Manikyarasa* i.e. the raw materials *Hingula*, *Haritala* and *Gandhaka* were selected after careful observation of *grahya lakshanas* and authentication was done and then used for present study. As all we know it is important that crude drugs both of vegetable and mineral origin should be subjected to purification process i.e. to remove the *doshas* by procedures like trituration etc.

i) Purification of *Hingula*- Purification of *Hingula* was carried out as per the reference given in *Rasatarangini*. Unpurified *Hingula* was triturated well with juice of *Zingiber officinalis*.8 *bhavanas* were given. The observations of *Hingula* before and after purification were noted.

ii) Purification of *Gandhaka*- Purification of *Gandhaka* was carried out as per the reference given in *Ayurved Prakash*. Powdered *Gandhaka* ,*Goghruta* and Milk was used. The solid mass of *Gandhaka* was washed thoroughly in hot water and kept for drying. The same procedure was followed for 3 times.6 The observations of *Gandhaka* before and after purification were noted.

iii) Purification of *Harital* -*Haritala* was purified by the process given in *Rasatangini* by using *Kushmand Swarasa* in *Dolayantra*. The procedure was repeated in *dolayantra* by using *Churnodaka* (Lime water). The observations of *Harital* before and after purification were noted.

Preparation of *Hinguliya Manikyarasa* by *Kupipakwa Rasayana* method -

i) **Preparation of *Hinguliya Manikyarasa kajjali*-** Purified *Hingula*, purified *Gandhak* and purified *Haritala* were taken in equal quantity in mortal and pestle and juice of flowers of Butea monosperma i.e. *Palasha puspa* was added and *bhavanas* were given for 7 days .

ii) Preparation of *Hinguliya Manikyarasa*-

240 Gms.*Hinguliya Manikyarasa kajjali* was taken and filled in *kachakupi*. *Valukayantra* was placed exactly at the Centre of the furnace and *kupi* placed at the Centre of the *valukayantra*. Heat was gradually increased by adding hard and soft coals at regular interval. When the Sulphur fumes ceased at the mouth of the *kupi*, red hot rod was introduced frequently to ignite the Sulphur and clear the pathway. Cold rod test was performed as per the requirement to observe the status of *kajjali* and subsequent stages. When the blue flame appeared on the mouth of the bottle the temperature was maintained. When the flame, fumes were completely stopped, Cold rod test was positive, Copper coin test was negative and base of the bottle was red hot like rising Sun, the mouth of the bottle was corked. The sand layer of about 2-3

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inches surrounding the bottle neck was move aside. Gradually increasing Heating Pattern was strictly maintained throughout the practical as 24 Hrs Mild, 24 Hrs. Moderate and 24 Hrs. Severe heating. After self-cooling the bottle was removed and *Hinguliya Manikyarasa* was collected from the neck region and powdered.

Observations and Results:-

Table no. 1 Table showing the average temperature in ^{0}C during the preparation of *Hinguliya Manikyarasa*

Type of *Agni*	**Temp. in ^{0} C**
Mrudu	132.23
Madhyama	303.66
Tivra	499.83

Table no. 2 Table showing the time in hrs. And temperature in ^{0}C recorded during 10 cardinal stages of *Hinguliya Manikyarasa*

Sr.no.	**Cardinal stages**	**Time in hrs**	**Temp. in ^{0} C**
1	Initial stage	00	42
2	Fumes started	02	65
3	Yellow fumes	08	110
4	Profuse fumes	42	350
5	Blue flame	56	462
6	Flame stopped	64	530
7	Red hot base	66	560
8	Corking	66	560
9	Completion	66	560
10	Self-cooled	94	44

Table no. 3 Table showing the total fuel consumed, wt. of *H M Kajjali* taken, wt. of *H M* obtained, wt. of residue and their percentage.

1	Total fuel consumed	91.34 kgs
2	Wt. of *H M* Kajjali taken	240 gms
3	Wt. of *H M* obtained	92 gms
4	Wt. of residue remained	40 gms
5	Percentage of *H M* obtained	38.33
6	Percentage of residue remained	16.66

Table no. 4 Table showing the analytical data of *Hinguliya Manikyarasa kajjali*

Parameters	***Hinguliya Manikyarasa kajjali* (% w/w)**
Mercury as Hg	25.77
Arsenic as As	9.88
Total Sulphur as S	43.23
Free Sulphur	29.32
Combined Sulphur	13.91
Ash value	4.63
Water soluble extractive	9.20

Table no. 5 Table showing the analytical values of *Hinguliya Manikyarasa*

Parameters	**Upper part (% w/w)**	**Lower part (% w/w)**
Mercury as Hg	48.05	0.17
Arsenic as As	17.84	25.11
Total Sulphur as S	27.50	27.73
Free Sulphur	0.86	2.30
Combined Sulphur	26.64	25.43
Ash value	0.33	39.65

Table no. 6 Table showing the organoleptic parameters for all samples of *Hinguliya Manikyarasa*

Organoleptic parameter	***Hinguliya Manikyarasa***
Appearance	Sublimed crystal having one face adhered to *kupi* shining, smooth and other is rough, rhombohedral or conconical crystal type shape

Color	The ruby red or crimson red
Tactility	Heavy, hard, cool and smooth
Sound	No specific sound, brittle and forms crystals in longitudinal shape
Taste	Tasteless
Smell	Odorless but slight Sulphuric smell may be observed

Crystallography Study By X-Ray Diffraction Method-

This method is used to determine the atomic and molecular structure of organic, inorganic material or metal, mineral or salts.[7] The result showed that Sulphur was present most probably in orthorhombic form. Arsenic Sulphide was most probably present in monoclinic form and Mercuric Sulphide was in Hexagonal form. *Hinguliya Manikyarasa* may be a mixture of Sulphur, Arsenic Sulphide and Mercuric Sulphide. Another observation was As_2S_3 was found converted to AsS irreversibly.

Nps Test Study-

The Nambury Phased Spot Test was carried out to identify and establish spot standards for the samples of *Hinguliya Manikyarasa*. The reagent for drug solution was Aquaregia. . Another 3 samples namely *Shilasindur*, *Mallasindur* and *Samirapannaga rasa* which also contains Arsenic, Mercury and Sulphur were taken for comparison.[8]

A) Whatman paper impregnated with 10 % Potassium Iodide- The pattern of the spot was central reddish orange colored spot surrounded by whitish cream colored margin surrounded by brown colored circle.

B) Whatman paper impregnated with 5% Potassium Ferocyanide- Central blue coloured spot surrounded by whitish or light blue coloured space limited by thick blue coloured ring and periphery of bluish green coloured.

C) Whatman paper impregnated with 5% alcoholic extract of Curcuma longa- The pattern was brown coloured central spot for all the four samples and almost remained same in all the three phases.

Toxicological Study-

Short Term Chronic Toxicity Study Was Carried Out On Experimental Animals

- Drug dose was fixed, dose was converted from human dose to animal dose.
- Drug suspension was prepared by adding tween 80 solution in it.
- For control group only tween 80 solution and water was given.

Route of Administration- Daily at specific time drug was administered orally by gavage method. The drugs were administered for 15 consecutive days.

Dose- The normal adult dose of *Hinguliya Manikyarasa* = 60-180 mg/day
So the suitable rat dose was calculated by referring to the table of Paget and Barnes i.e.
Human Dose X Body surface area ratio convertibility factor.
=180 X 0.018
=3.24 mg/rat (200 gm body weight)

By converting to mg/kg the dose multiplied with suitable factor i.e. 5
=3.25 mg X 5
=16.20 mg/kg

Table no. 7 Effect of *Hinguliya Manikyarasa* on Body weight Parameter in albino rats

Treatment	Body weight
3% Tween 80 control	46.00 ± 4.83
Hinguliya Manikyarasa	40.8 ± 5.994

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Table no.8 Effect of *Hinguliya Manikyarasa* on weight of various organs:

Treatment	Liver	Spleen	Heart	Kidneys	Testis
Hinguliya Manikyarasa	Decreased	Increased	Increased	No changes	Increased

Table no.9 Effect of *Hinguliya Manikyarasa* on Hemoglobin level, total WBC count, Differential count and Blood Urea in albino rats

Treatment	3% Tween 80 control	*Hinguliya Manikyarasa*
Hemoglobin	12.5 ± 0.15	13.68 ± 0.407
WBC	4800 ± 825	3870 ± 211.896
Neutrophils	43.5 ± 3.1	43.6 ± 4.501
Lymphocytes	55.0 ± 3.25	55.6 ± 4.632
Eosinophils	----	1.5 ± 0.5
Blood Urea	44.2 ± 1.63	35.68 ± 4.571

Table no.10 Effect of *Hinguliya Manikyarasa* on histopathological changes of various organs:

Treatment	Liver	Spleen	Heart	Kidneys	Testis
Hinguliya Manikyarasa	Mild Toxicity	No Toxicity	Mild Toxicity	No Toxicity	No Toxicity

Discussion

Table no. 1 shows that for the preparation of *Hinguliya Manikyarasa* the average temperature for Mrudu Agni was 132.23^{0C}, for Madhyamagni was 303.66^{0C} and that for Tivra Agni was 499.83^{0C}

Table no. 2 shows that the temperature recorded and the time required for every cardinal stage. Profuse fumes were came out from bottle at 350^{0C} and the blue flame was appeared at $462^{0C.}$. The corking was done after 66 hours and the temperature was 560^{0C} the bottle was removed after self-cooling and for that total 94 hours required.

The table no.3 suggests that total 92 Gms. *Hinguliya Manikyarasa* was obtained, the percentage was 38.33. The Total fuel consumed was 91.34 kgs.

Table no. 4 reveals that *Hinguliya Manikyarasa kajjali* contains about 9.20 % water soluble extractive. Arsenic content was less than the expected value considering the Arsenic content in Harital.

Table no. 5 suggests the analytical values of *Hinguliya Manikyarasa*. The percentage of Mercury was 48.05 i.e. much lesser than it was in *Hingula*. The percentage of Arsenic was 17.84 it was also less as compared to the Arsenic content of *Haritala*. The percentage of free Sulphur was too less as compared to that in purified *Gandhak*.

Table no. 6 gives the organoleptic characteristics of *Hinguliya Manikyarasa*. It was having ruby red colored, heavy, hard and having some Sulphur smell.

Table no. 7 shows that the test drug *Hinguliya Manikyarasa* when administered to the animals the weight gain was almost similar to the control group which suggests that this drug may not produce any tissue destruction and degenerative changes in the organ.

Table no. 8 gives the data on ponderal changes in certain organs which shows that there was increase in weight in almost all organs like Speen,Heart,Testes excluding Liver .

Table no. 9 shows the effect of the test drug on various hematological parameters which depicts that the increase in Hemoglobin percentage is statistically significant, the total leucocyte count is

decreased the differential count remained unchanged. The blood urea level was found to be decreased in test drug which indicates a positive nitrogen balance and more importance is it indicates that there is no tissue destruction.

Table no. 10 shows that *Hinguliya Manikyarasa* showed mild toxic changes in Liver tissue and Heart tissue whereas did not show any change in other tissue.

Conclusion

Hinguliya Manikyarasa can be prepared by Textual method as described in *Rasatarangini* and it requires 94 hours. The average temperature for *Mrudu Agni* was 132.23^{0C}, for *Madhyamagni* was 303.66^{0C} and that for *Tivra Agni* was 499.83^{0C}All the parameters like Time, Temperature, and the amount of Fuel can be used as objective parameters for standardization of *Hinguliya Manikyarasa*. The Nambury Phased Spot Test can be used to identify and establish spot standards for the samples of *Hinguliya Manikyarasa*. The crystallographic study suggests that the compound *Hinguliya Manikyarasa* may be a mixture of Sulphur, Arsenic Sulphide and Mercuric Sulphide. Another observation was As_2S_3 was found converted to AsS irreversibly. *Hinguliya Manikyarasa* produces least toxic changes, as the dose used for toxicity study was higher dose, the lower therapeutic dose level may not produce toxic changes.

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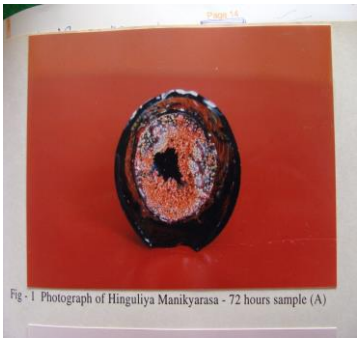

Fig - 1 Photograph of Hinguliya Manikyarasa - 72 hours sample (A)