ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

Human Journals
Research Article
February 2017 Vol.:8, Issue:3

Comparative Subacute Toxicity Study of an Ayurvedic Formulation Hartal (Orpiment) and Rasa Manikya (Processed Product of Hartal) in Albino Mice

ISSN 2349-7203
IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
HUMAN

Dr. Shanker S. Mishra*[1], Mr.Kumud Kant Awasthi[2], Dr. Inderpal Soni[2], Dr. V. K. Gothecha[3]

[1]*Department of Agad Tantra, Ch. Brahm Prakash Ayurved Charak Sansthan Delhi.*

[2]*Department of Zoology, Rajasthan University, Jaipur (Raj.)*

[3]*Department of Agad Tantra, National Institute of Ayurveda, Jaipur (Raj.)*

Submission: 7 February 2017
Accepted: 12 February 2017
Published: 25 February 2017

Keywords: detoxification, orpiment, hartal, rasamanikya.

ABSTRACT

Hartal or orpiment, an Ayurvedic metallic drug, is taken as an ingredient in many ayurvedic formulations in spite of its direct use and theses formulations are used externally as well as internally. Rasmanikya [RM] is one of the ayurvedic formulations of hartal which is used by ayurvedic physicians forshwasa (Asthma), kasa (cough), kushtha (skin diseases) etc.Aim and objectives of this study are to ascertain the safety vis-à-vis toxicity of Hartal. Also, the effect of shodhan on toxicity profile is to be determined.In this study, 3 experimental compounds namely IH (impure hartal in crude form), PH (pure Hartal detoxified with fruit juice of Benincasahispida) and RM (prepared by putting PH between mica sheet and heated for 5-10 min) were used. On the basis of therapeutic dose, drug dose was decided16.25 mg/kg BW. In experimental part, total 24 albino male mice, each weighing 25-30 gm were taken and randomly divided into 4 groups, 6 in each group (control, IH, PH and RM). After 28 days blood was collected by extirpating eyeball for RFT and LFT there after all animals were sacrificed, dissecting kidney, liver, part of intestine and skin out for histopathological study. Results showed that there is significant abnormality found in LFT and RFT of IH treated group whereas PH treated group shows non-significant changes and RM treated group shows mild significant changes in LFT and RFT parameters.There is fatty degeneration of liver found in IH and RM treated groups. Kidney of IH and RM treated group also shows some abnormal cytoarchitecture. No abnormality of liver & kidney was seen in PH treated group. Thus IH is probably toxic, PH is non-toxic and RM is mildly toxic.

INTRODUCTION

Ayurvedic medicines have been traditionally used for thousands of years in India. Presently Drugs & Cosmetic act of India control ayurvedic medicines.[1] In fact, there is large number of ayurvedic formulations containing poisonous substance as an ingredient and these poisonous substances are placed under schedule E of Drugs and cosmetic act 1940. As per Ayurvedic philosophy, one needs to undertake detoxification called Shodhan during preparing the ayurvedic formulation. It is an Ayurvedic concept that these shodhan procedures mitigate or eliminate the unwanted toxic effect and enhance the therapeutic effect of Ayurvedic formulation containing poisonous substances.[2, 3, 4]

Hartal, chemically arsenic trisulphide, is an ayurvedic compound which is taken as ingredient in many Ayurvedic formulations. Since ancient physicians said Hartal toxic [S. Samhita. kapla2] therefore it is subjected to shodhan process to make it least toxic and suitable for better human consumption. There have been several substances such as juice of *Benincasahispida* fruit, juice of *Salmaliamalabarica* root etc. which are used for detoxification of Hartal.

Rasamanikya (RM) is an Ayurvedic formulation of Hartal. It is prepared by putting detoxified (Shodhit) powdered Hartal between two mica sheets and heated for 5 to 10 minutes until red or ruby color is obtained. RM is indicated in various skin diseases, Shwasa (Bronchial Asthma), Kasa (cough) etc.

In present scientific view, Orpiment (arsenic trisulphide) is sulfur compound of arsenic. It is insoluble in water but soluble in hot water and organic solvents. As 2S3 is non-toxic or least toxic and this fact justifies the use of Hartal as a medicine in Ayurvedic system.

Since much outcry is made regarding the toxicity of metals and minerals in Ayurvedic therapeutics, therefore this study was planned to ascertain the safety vis-à-vis toxicity of Hartal. Also, the effect of shodhan on toxicity profile was determined. Since RM is a preparation of Hartal (Orpiment) therefore it was felt necessary to incorporate RM in this study.

Aims and objectives-

1. To compare the 28 days toxicity profile of IH, PH & RM in animal model

2. To study the effect of shodhan on toxicity profile

MATERIALS AND METHODS

Experimental compound- Hartal (Arsenic trisulphide) was procured from National institute of Ayurveda, Jaipur (Rajasthan) in crude form. It was named as impure Hartal (IH). Now 2/3rd part of this was subjected to detoxification with juice of *Benincasahispida* fruit and this sample was named as pure Hartal (PH) [5]. 1/3rd of this PH was further processed to prepare RM and this sample was named as RM. In this way, total numbers of experimental compound were 3 IH, PH and RM. Here shodhan was done with single material *Benincasahispida* fruit juice due to some limitations.

Experimental animals- 24 male swiss albino mice each of 20-25 g weight were used at animal house of department of Zoology, Rajasthan University, Jaipur. These animals were maintained under normal climatic condition, ambient temperature, humidity and exposed to natural day and night cycle.

The animals were fed with standard balanced diet and tap water was provided *ad libitum*. The experiments were carried out as per the guidelines of institutional animal ethical committee.

The mice were further divided into 4 groups each group containing 6 animals. Group 1 received only distilled water, group 2, 3 and 4 received IH, PH and RM respectively.

Dose- therapeutic dose of Hartal and RM is 125 mg for human being. So the dose for experimental animal was calculated by extrapolating human dose to animal dose based on Paget & Barnes Body surface ratio.[6]

Dose for mice= adult human dose x BSA ratio convertible factor

=125 x 0.0026

=0.325 mg/ mice of 20 g weight

In order to convert this into mg/kg BW, the above dose was to be multiplied with 50.

Now, 0.325 x 50= 16.25 mg/kg BW

Experimental protocol-dose was given orally for 28 days. After 28 days blood was collected by extirpating eyeball and thereafter all animals were sacrificed, dissecting liver, kidney, part of small intestine and skin out for histopathological study.

Parameters studied-

[1] serum SGOT, SGPT, Alkaline phosphatase, total protein, albumin, globulin, AG ratio for liver functioning and serum creatinine and uric acid for kidney functioning

[2] Histopathological slides of liver, kidney, part of intestine and skin were prepared and studied.

The data were analyzed statistically by applying Analysis of Variance (ANOVA) to test the level of significance.

Observation-

Table-1: The % change in weight of liver and kidney of Albino mice (In comparison to Control group)

Group	**Liver weight (g)**	**% change in weight of Liver**	**Kidney weight (mg)**	**% change in weight of Kidney**
Control	1.815±0.019	-	218.50±2.119	
Impure hartal	1.782±0.025	1.818 ↓	212.19±4.541	2.89 ↓
Pure hartal	1.808±0.020	0.386 ↓	211.11±2.462	3.38 ↓
Rasamanikya	1.812±0.023	0.0165↓	208.15±2.687	4.74 ↓
F value		0.4664		1.975
P value		>0.05		>0.05

Table-2: Biochemical parameters of LFT of albino mice in different treated group

Parameter / Group	SGOT	SGPT	Alk.Phos	TP	A	G	AG ratio
Control	155.5±1.478	121.17± 3.146	193.00± 1.528	121.17± 3.146	4.11± 0.03	4.1± 0.04	1.001±0.001
Impure Hartal	174.17± 2.971	134.33± 2.741	188.17 ±5.394	8.1 ±0.03	4.13± 0.03	4.0 ±0.07	1.024±0.002
Pure Hartal	163.5 ±3.063	133.67±2.883	198.67 ±4.318	8.3 ±0.09	4.23±0.05	4.1 ±0.04	1.017±0.004
Rasamanikya	168.33 ±3.904	132.50±1.893	197.33±2.940	8.3 ±0.11	4.23±0.05	4.1 ±0.04	1.016±0.005
F value	6.999	5.270	1.535	1.145	1.913	0.8389	4.953
P value	<0.01	<0.01	>0.05	>0.05	>0.05	>0.05	<0.01

Table-3: Biochemical parameters of RFT of albino mice in different treated group

Parameter / Group	Serum creatinine	Serum uric acid
Control	0.71± 0.004	7.13± 0.049
Impure Hartal	0.79 ±0.017	7.55 ±0.120
Pure Hartal	0.73 ±0.010	6.95 ±0.286
Rasamanikya	0.73 ±0.016	7.12 ±0.135
F value	7.088	2.230
P value	<0.01	>0.05

One way ANOVA followed by Turkey-Kramer multiple comparisons test for different parameters (parameters in which p-value is lesser than 0.05)

P value Comparison	Serum SGOT	Serum SGPT	Serum Alk. Phos.	Serum AG Ratio	Serum Creatinine
Control Vs IH	P<0.01	P<0.05	P>0.05	P<0.01	P<0.01
Control Vs PH	p>0.05	P<0.05	P>0.05	P>0.05	P>0.05
Control Vs RM	P<0.05	P<0.05	P>0.05	P>0.05	P>0.05
IH Vs PH	p>0.05	p>0.05	p>0.05	p>0.05	P<0.05
IH Vs RM	p>0.05	p>0.05	p>0.05	p>0.05	P<0.05
PH Vs RM	p>0.05	p>0.05	p>0.05	p>0.05	p>0.05

RESULTS AND DISCUSSION

Weight of liver- There is non-significant decrease observed in weight of liver. The entire test drug treated groups do not exert any effect on the weight of liver.

Weight of kidney- There is non-significant decrease in weight of kidney. Maximum change in decrease was found in pure Hartal group (4.74%).

Aminotransferases

The *aminotransferases* (formerly *transaminases*) are the most frequently utilized and specific indicators of hepatocellular necrosis. AST is present in a wide variety of tissues like the heart, skeletal muscle, kidney, and brain and liver.[7, 8]

ALT (SGPT) is primarily localized to the liver. *Transaminases* catalyze the interconversion of amino acids and α-ketoacids by transfer of amino group.

In the present study, significant increases in SGOT level were observed in IH and RM treated group in comparison to control group. Mild elevation in SGOT levels (1-3 times) are usually seen in sepsis-induced neonatal hepatitis, fatty liver, drug toxicity, extrahepatic biliary atresia (EBHA), cirrhosis, non-alcoholic steatohepatitis (NASH), Duchenne muscular dystrophy. It means IH and RM drugs exert toxic effects on heart, skeletal muscles, kidney, brain etc.

Mild but significant elevation in SGPT levels were observed in IH, PH and RM treated group in comparison to control group. The observed elevation of mild intensity in SGPT level may be indicative of hepatic toxicological effect**.**

Alkaline phosphatase activity: In the present study non-significant elevation was observed in PH and RM administered group in comparison to control group. IH treated group shows non-significant decrease in serum Alkaline Phosphatase levels. The elevation indicates towards possible degenerative changes in liver, kidney, and intestine. Also, the histopathological findings support this assumption because all the three organs were found to have changed cytoarchitecture.

Total Proteins-

In present study, the non-significant decrease was observed in IH-treated group. While there is non-significant increase in serum Total protein observed in PH and RM treated group in comparison to control group. Hence it might be possible that IH, PH ad RM do not exert any effect on serum total protein.

Serum Albumin and Serum Globulin:

There is non-significant increase observed in serum albumin level in all three IH, PH and RM treated group in comparison to control group. In this connection change in serum globulin level was also found to be statistically non-significant in all three IH, PH and RM treated group

A: G Ratio-

In the present study very significant increase in A: G ratio was observed in IH-treated group in comparison to control group. The exact reason is difficult to determine the increase in AG ratio.

Serum creatinine-

It is a normal alkaline constituent of urine and blood. It is the decomposition product of the metabolism of phosphorus-creatine, a source of energy for muscles contraction. Increased quantities of it are suggestive of renal disease.

In this study, very significant increase was observed in serum creatinine level in IH-treated group in comparison to control group. It means IH exerts some deleterious effect on kidney which further leads to renal failure. This result also justifies the importance of shodhan of poisonous drugs. Histopathological findings also coincide with this observation.

Serum uric acid:

Uric acid is crystalline acid occurring as an end product of purine metabolism. Increased elimination in human being is observed after ingestion of proteins and nitrogenous foods, after exercise, after administration of cytotoxic agents and in leukemia and gout.

Decreased elimination is observed in kidney failure, lead poisoning and people who eat protein free diet.

Non-significant elevation in serum uric acid was observed in IH administered group. While non-significant decrease was observed in rest two groups PH and RM treated group.

It has been reported that long-term administration of inorganic arsenic compound has produced liver lesions, anemia, and pathological skin changes in animal models.[9]

Table-4: Consolidated statement on the effect of test drugs on Histopathological profile

Parameter	IH	PH	RM
Liver	Some sections are normal with central vein congestion and some showed mild degree of necrotic and degenerative changes.	No necrotic and degenerative changes were observed	mild fatty changes with moderate expansion of sinusoidal spaces
Kidney	Mild degree of the lesion of hemorrhages. The renocytes of the PT and DT were showing mild hydropic and fatty degeneration of the cells.	No necrotic and degenerative changes were observed	No necrotic and degenerative changes were observed
Intestine	Villous atrophy with ulceration is seen. Lymphocytic infiltration between mucosal cells	Normal villi and crypts are seen. Some mild villous atrophy are also observed	Villi and crypts are seen normal
Skin	No histopathological changes are seen	No histopathological changes are seen.	No histopathological changes are seen.

Evaluation of histopathological parameters shows that impure [Ausuddha] Hartal produced mixed response with some mice showing normal cytoarchitecture and some showed mild to moderate fatty change. Rasamanikya produced mild fatty changes with expanded sinusoidal spaces. Cirrhosis was absent. The observed changes may be due to diversion of lipids to the liver leading to their accumulation. Liver is one of the organs which are readily affected in arsenic poisoning. It has been mentioned in literature (Klaassen, C.D. 1992) that inorganic arsenicals are particularly toxic to the liver. They produce fatty infiltration, central necrosis and cirrhosis. The liver parenchyma is normally affected.[10]

In a study, when Rasamanikya was administered with water (M.D. Dissertation submitted to Gujarat Ayurved University, Jamnagar, Gujarat State, India.) at therapeutically equivalent and

double doses for 15 days to rats had produced mild to moderate fatty changes in the liver, significant increase in cholesterol and blood sugar level and decrease in the liver weight.[11]

From above discussion, it might be concluded that internal use of IH is certainly hepatotoxic and internal use of RM is also hepatotoxic to some extent in long term use. Therefore it is suggestive that internal medication of RM must be done cautiously and under medical supervision in people with compromised liver.

IH causes mild degree of the lesion of hemorrhages and hydropic& fatty degeneration in kidney, while PH and RM do not produce any pathological changes. The changes observed in IH-treated group might be due to increase glomerular filtration and capillary permeability by arsenic toxicity as a result of which leakage of protein occurs that causes necrosis [12]. This result clearly indicated that the shodhan reduces the toxicity of Hartal. It also reflects that detoxified Hartal (PH) and RM are safe and least toxic to kidney.

IH, PH and RM all do not harm skin. No abnormality was seen in skin. However, some sections of IH and RM showed reduced number of hair follicle because Hartal is a potent depilatory agent and was used externally as a hair removing agent in ancient period.

Hartal causes irritation of epithelial cells of intestine resulting in damage to villi and ulceration formation. PH causes the same but in low intensity. RM does not affect the intestine. Therefore it could be concluded that shodhan reduces the toxicity of Hartal and make it suitable for its better absorption without causing the epithelial cells damage of GIT.

CONCLUSION

Hartal, chemically arsenic trisulphide, is a non-toxic or least toxic compound. So its ingestion as a drug must be totally safe. However, in commercial form, arsenic oxide contamination renders it poisonous. In fact, the concept of shodhan is to decontaminate the Hartal so that it can be made non-toxic. PH is detoxified form and RM is the heat processed product of PH i.e. both are detoxified and that's why both are non-toxic, while IH is toxic.

In present study, it is concluded that IH is toxic to liver, kidney and also causes ulceration in GIT tract. It also depilates the hair. While PH is completely non-toxic to kidney, liver and could be recommended for therapeutic purposes for a month safely. RM is found mild toxic to kidney and

liver in some cases as it increases serum SGOT, SGPT and Creatinine level. Since RM is prepared by heating PH, there might be little chance that Arsenic in PH gets oxidized and converted into Arsenic trioxide partly and which results in its increased toxicity as compared to PH.

Moreover, it may be concluded that shodhan with single material *Benincasahispida* is not sufficient and repetitive shodhan (detoxification) with other shodhan materials must be carried out for detoxification. Study suggests that detoxification or shodhan really works in reduction of toxicity by removing contamination and some others way. After detoxification PH and RM can be used safely for long duration.

The present study reveals that IH has toxic effect due to contamination of As_2O_3 and after detoxification (shodhan) it is almost safe and can be used for long duration but with caution in liver compromised patient.

This study is not a complete toxicity study; it emphasizes the need for carrying out repetitive detoxification of Hartal and then to study toxicity. In further study, biochemical screening of blood, urine, and nail should be carried out by using sophisticated analytical instruments like Atomic absorption spectrophotometry, Inductively coupled plasma analyzer etc.

REFERENCES

1. Malik Vijay, Drugs & Cosmetics Act, 1940, (Eastern Book Company, Lucknow), (2003)
2. Madhav Upadhyaya, Ayurveda Prakash, commented by Gulraj Sharma, 1982, The Chaukhambha Bhawan, Varanasi, verse 6/47-48
3. Sadana D Sharma, Rasa Tarangini, commented by Kashiram Shashtri, Motilal Banarasidas, 2000, verse 02/52 pp22
4. Sarkar PK, Chaudhary AK, role of milk in shodhana (detoxification) w.s.r. to nux-vomica, Aryavaidyan 2007, XX, 2, pp100-104)
5. Vagbhatt, Ras Ratna Samuchchaya, commented by Ambika Datta Shastri, 1988, Chaukhambha Amarbharati Prakashan, Varanasi, verse III/70-72 P. 54
6. Paget GE &Barnes JM, 1964, Toxicity Tests. In evaluation of Drug activity; Pharmacometrics, Academic Press pp: 135-156
7. Friedman SF, Martin P, Munoz JS. Laboratory evaluation of the patient with liver disease.*Hepatology, a textbook of liver disease*. Philadelphia; Saunders Publication, 2003; 1 : 661-709.
8. Rosalki SB, Mcintyre N. Biochemical investigations in the management of liver disease. *Oxford textbook of clinical hepatology*, 2nd ed. New York; Oxford university press, 1999; 503-521.].
9. International Programme on Chemical Safety (IPCS) (1992). Inorganic Arsenic Compounds other than arsine. Health and safety guide No. 70. World Health Organisation. Geneva

10.Klassen, C.D. (1992). Heavy Metals and Heavy –metal antagonists. In 'The Pharmacological Basis of Therapeutics' EDs: Gilman, A.G., Rall, T.W., Nies, A.S. and Taylor, P). McGraw-Hill International Editions; pp-1602.
11. Mishra D. K. (1999) M.D. Dissertation submitted to Gujarat Ayurved University.
Jamnagar, Gujarat State, India.
12. Cullen et al. 1995, pediatric arsenic ingestion, Am. J. Emerg. Med., 13:432