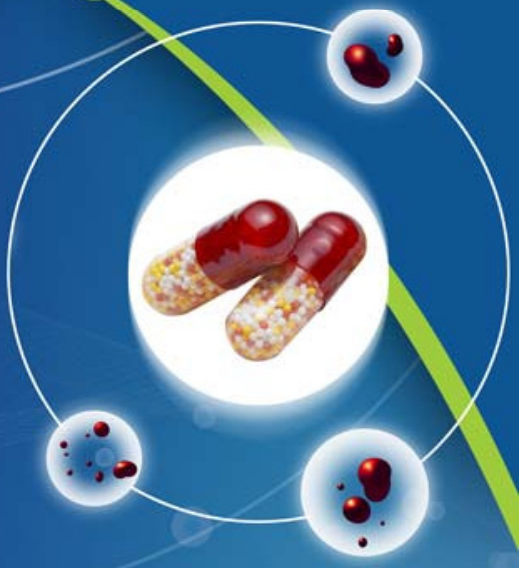
ISSN : 2320 4850

BI MONTHLY

Asian Journal of Pharmaceutical Research And Development

(An International Peer Reviewed Journal of Pharmaceutical Research and Development)

AJPRD



Volume - 01 Issue - 05 SEP-OCT 2013

website: www.ajprd.com
editor@ajprd.com

Asian Journal of Pharmaceutical Research and Development
(An International Peer-Reviewed Journal of Pharmaceutical Research and Development)
www.ajprd.com
ISSN 2320-4850

Research Article

TOXICOLOGICAL STUDY OF MERCURIALS WITH SPECIAL REFERENCE TO *SAMAGANDHA KAJJALI*

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***Received:* 12 Sept. 2013,** ***Revised and Accepted:* 28 Oct. 2013**

ABSTRACT

Rasashastra, a mercury centered branch of Ayurveda mainly deals with the preparation of safer metallic medicinal preparations. Among all metals described in Ayurveda, Rasa i.e. mercury has unique importance due to its wide usage and comparatively potent therapeutic efficacy. In India, mercurials have been in use since time immemorial and curing many dreadful diseases till. But in present era, therapeutic usage of mercury is strongly condemned by modern scientific community as it is heavy metal and heavy metal causes toxicity in patients. So to ensure the modern scientist and patients using Ayurveda formulations, that the mercurials are safer at therapeutic dose level, here, study of acute toxicity of Samagandha Kajjali which is said to be the weakest bond of mercury was carried out.

Keywords: *Acute toxicity, Mercurials, Samagandha Kajjali*

INTRODUCTION

Rasashastra deals with the science of *Rasa* (Mercury). Thus it is priority to study effect of *Rasa* preparations i.e. mercurials which are nothing but Rasa *murchchhana*. *Murchchhana* are of four types i.e. *Kharaliya*, *Kupipakwa*, *Parpati* and *Pottali*. Among them *Kajjali* is the prime and basic *Kharaliya Murchchnana* of *Parada*. *Kajjali* is amalgam of purified sulphur and purified mercury, having weaker bond between them than that of other three *Murchchhanas*. It is said that, *murchchhita Parada* is uniquely capable of eliminating all sorts of diseases. Hence; a need to determine the safety of these *mercurials* was there.

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Oral toxicity study of Kajjali would provide the much needed data of maximum toxicity which might have been caused by any *Rasa Murchchhana* or *mercurials*. Commonly *Kajjali* is prepared from equal quantity of purified mercury and purified sulphur. So '*Samagandha Kajjali'* was used for the acute toxicity study. This study will definitely help to prove the therapeutic safety of *Mercurials*.

AIM & OBJECTIVES

- To determine the overall acute toxicity of prepared *Samagandha Kajjali*.
- To study behavioral changes induced if any, in Swiss albino mice due to feeding of *Kajjali* after a single dose.
- To determine LD50 value of prepared *Samagandha Kajjali* according to OECD guideline No. 423, adopted on 17th Dec. 2001.

MATERIAL & METHODS

The study was performed in three phases as follows

Preparation of *Samagandha Kajjali*

Mercury was purified by triturating it with garlic juice for about 7 days, 8 hours daily. It was regained from triturated mixture by washing into hot water. Sulphur was purified by liquefying it along with cow ghee on low flame and followed by its *Dhalana* into cow milk through muslin cloth. For purification, this process was repeated for further two times. Then sulphur was powdered and washed with hot water to remove oily part from it. After *Shodhana* mercury was observed lustrous, while sulphur looked pale than earlier. Such a medically purified sulphur and mercury were taken in equal quantity in *Khalvayantra*. This mixture was ground in *Khalvayantra* till it become soft, amorphous, solid and perfectly black and structure like soot. Observations during the procedure were noted. [1, 2]

Physico-chemical Testing:

Prepared *Samgandha Kajjali* was tested for total ash, acid insoluble ash, water soluble ash, Atomic absorption spectroscopy (AAS) and solubility.

Toxicological Study

Acute oral toxicity study

The study was done at National Toxicological center (NTC), Pune by following the OECD guidelines No. 423, Adopted on 17th Dec. 2001 [4] under the supervision of the study director - Dr. Apte K.G.

Experimental Procedure

Test substance: *Samagandha Kajjali*

Test animal: -

S.N.	Species	Albino mice
1.	Strain	Swiss Albino
2.	Source	NTC, Pune
3.	Weight range	22-26 gm
4.	Age	6 to 8 weeks
5.	Sex	Female
6.	Number	15
7.	Housing	3 of similar sex per cage
8.	Diet	Pelleted feed.
9.	Water	Community tap water ad libitum.
10.	Room temp	20-24^0 C
11.	Relative humidity	40-60%
12.	Light cycle	12 hour light & 12 hour's dark
13.	Vehicle used	Corn oil 10ml / kg body wt
14.	Dose Volume	1 ml / 100gm
15.	Treatment	300 mg / kg, 300 mg / kg, 2000 mg / kg, 5000 mg / kg of body wt.

The study was performed in a stepwise manner

Step: 1

Vehicle control (corn oil): 10.0 ml / kg

A group of three female mice were administered the vehicle to show that the vehicle as per se did not cause any toxicity.

Step: 2

Three female mice were administered *Samagandha Kajjali* diluted in corn oil at the dose of 300 mg / kg body weight. The mice were deprived of feed overnight before and 3-4 hours after dosing. Water was allowed ad libitum.

Step: 3

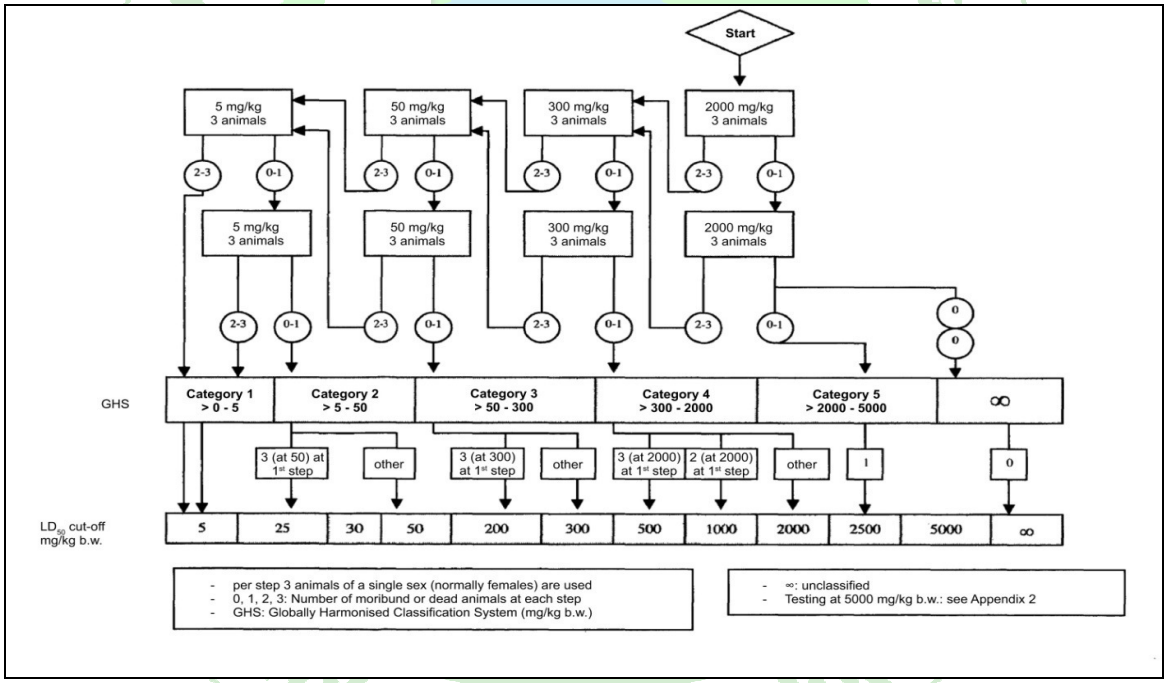
Another group of three female mice were administered the test material diluted in corn oil at the dose of 300 mg / kg body weight.

Step: 4

Another group of three female mice were administered the test material diluted in corn oil at the dose of 2000 mg / kg body weight.

Step: 5

Another group of three female mice were administered the test material diluted in corn oil at the dose of 5000 mg / kg body weight.



The mice were observed for toxic symptoms and mortality, if any, for 14 days after dosing. The animals in each group were observed for behavioral changes, changes in skin, fur, eyes and mucous membranes, signs of tremors, convulsions, salivation, lacrimation, diarrhoea, lethargy, sleep or coma.

OBSERVATIONS & RESULTS

Organoleptic Characters of *Samagandha Kajjali*

Color	Odor	Taste	Touch	Luster
Perfect Black	Not specific	Irritant	Soft, amorphous	N o

Table No.1- Prepared *Samagandha Kajjali* passed following *Parikshas*

Sr. No.	*Parikshas*	*Results*
1	*Shlakshnatva*	Passed
2	*Nishchandratva*	100%

Table No.2- Physicochemical Analysis of *Samagandha Kajjali*

Sr.No.	Test	Results
1	Total Ash	0.10%
2	Acid Insoluble Ash	0.06%
3	Water Soluble Ash	0.32%

Table No.3- Solubility of *Samagandha Kajjali*

Sr.No.	Solvent	Insoluble/Soluble
1	Water	Insoluble
2	Alcohol	Insoluble
3	Petroleum ether	Insoluble
4	Hydrochloric Acid	Insoluble
5	Sulphuric Acid	Insoluble
6	Nitric Acid	Insoluble
7	Aquaregia	Soluble

Table No.4- Atomic Absorption Spectroscopy (AAS):-

Sample	Mercury	Sulphur
Samagandha Kajjali	49.02%	49.07%

Table No.5 - Body weight changes (Mean)

Group	Sex	Group Mean Weight (Day)			% Weight Change
		0^{th}	7^{th}	14^{th}	
I	Female	24.16	27.4	30.6	26.65
II	Female	23.5	26.63	29.6	25.95
III	Female	24.66	27.66	31.16	26.38
IV	Female	24.06	27.06	30.46	26.66
V	Female	24.33	27.15	30.40	24.94

Table No.6- Clinical Signs - Sex: Female Mice

Group & Dose (Mg/kg)	Clinical Signs Observed	Signs Observed after dosing																		
		Hours					Days													
		½	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14
I (0.00)	NO CST	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
II (300)	NO CST	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
III (300)	NO CST	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
IV(2000)	NO CST	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
V (5000)	NO CST	3	3	3	3	3	0	2	2	2	2	2	2	2	2	2	2	2	2	2
	DS, U, D	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0

No CST – No Clinical signs of toxicity

DS – Dark Stool, **U** – Urination, **D**- Diarrhea

Table No.7- Mortality Data - Sex: Female Mice

Group & Dose (mg/kg)	Mortality Observed after dosing																			No. AD / No. AT	% Mortality
	Hours					Days															
	½	1	2	3	4	1	2	3	4	5	6	7	8	9	10	11	12	13	14		
I (0.00)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3	0.00
II (300)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3	0.00
III (300)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3	0.00
IV(2000)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0/3	0.00
V (5000)	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	1/3	33.33

No. AD/ No. AT – Number of Animals died / Number of Animals treated

Table No.8- Necropsy Finding - Fate: Terminal Sacrifice

Group	Dose mg/kg	Sex	Findings	No. of Animals
I	0.00 Vehicle Control	Female	NAD	3
II	300 mg / kg	Female	NAD	3
III	300 mg / kg	Female	NAD	3
IV	2000 mg / kg	Female	NAD	3
V	5000 mg / kg	Female	NAD	2

NAD - No Abnormality Detected

Table No.9- LD 50 Values

Sex	mg / kg
Female	> 2000 mg / kg

DISCUSSION

Rasagandha Kajjali being of prime among other mercurial preparation, it was selected as the test substance. Acute oral toxicity involves evaluation of the effect of administration of test drugs at higher dose for short term effect on different parameters. Lethal dose of *Samagandha Kajjali* was determined as one animal died out of 3 animals when the dose of 5000 mg / kg of body weight was administered. But no death was observed at dose of 2000 mg / kg of body weight. Hence based on the results and according to GHS (Globally Harmonized Classification System) the GHS value of *Samagandha Kajjali* in mice by the oral route was found to be in GHS category 5 >2000-5000 mg / kg of body weights, with LD50 cut off at 2000 mg / kg of body weight.

Selection of start dose of 300 mg / kg of body weight for acute toxicity study was done on the basis of its wide usage in human being since longer duration and its dose range of 120 mg to 720 mg in adult human being. Animals used for this oral toxicity study were Swiss albino female mice as they are the smallest laboratory animals, that can be bred uniformly and female mice are more prone to any toxic evidences than male mice. [3] Acute oral toxicity of Samagandha Kajjali has created a platform for sub-acute toxicity study.

CONCLUSION

Based on AAS analysis, it can be said that prepared *Samagandha Kajjali* was homogeneous in nature. Based on Organoleptic tests and observations, it can be said that prepared *Samagandha Kajjali* passes satisfactorily through *Ayurvedic* parameters. Solubility of *Samagandha Kajjali*, has confirmed the consideration on that, *Ayurvedic* metallic preparations are insoluble in acids, ether, alcohol and water. The LD50 value of *Samagandha Kajjali* according to the OECD guidelines no. 423. Adopted 17th Dec. 2001 in albino mice by the oral route, following the method of Litchfield and Wilcoxan (1949) was found to be in GHS category 5, >2000-5000 mg / kg body weight with a LD50 cut off at 2000 mg / kg body weight.

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