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TOXICITY STUDY OF *KAJJALI* WITH SPECIAL REFERENCE TO DIFFERENT SAMPLES OF *PARADA*

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ABSTRACT

Ayurveda has used mineral and herbo mineral substances in therapeutics since time immemorial. But with the recent questions raised regarding the toxicity of *Ayurvedic* formulations, there is a need to scientifically evaluate and establish the safety of such preparations. *Kajjali* is a predominantly mineral preparation containing Mercury and Sulphur. It forms the base for numerous preparations and extensively used. As per authoritative texts of *Ayurveda*, mercury extracted from Cinnabar is to be used in all formulations. This extraction may not be economically viable and simple process prompting the use of samples of Mercury available in the market. The present study aims to evaluate the toxicity study of three samples of *Kajjali* prepared using three different samples of Mercury (one extracted from cinnabar and two other samples available in the market). No toxicity signs were observed in Wistar rats either in acute or sub acute toxicity study for any of the samples. No mortality was observed. Histopathology study indicated maximum organ damage in the group administered *Kajjali* prepared from market sample *Parada* and the least in group administered branded sample *Parada* prepared *Kajjali*. *Hingulottha Parada* prepared *Kajjali* showed moderate organ damage.

Key words: *Kajjali, Parada, Hingulottha Parada, Acute toxicity, Sub acute toxicity, Histopathology*

INTRODUCTION

Ayurveda has used minerals and metals in therapeutics since time immemorial. But with the recent questions raised regarding the toxicity of Ayurvedic formulations, there is a need to scientifically evaluate and establish the safety of such preparations. *Kajjali* is a preparation containing Mercury and Sulphur that forms the base for numerous formulations and is extensively used. As per authoritative texts of *Ayurveda*, mercury extracted from Hingula is to be used in all formulations. This extraction may not be simple and economically viable prompting the use of samples of Mercury available in the market. The present study aims to evaluate the acute and sub acute toxicity of three samples of *Kajjali* prepared using three different samples of Mercury (one extracted from cinnabar and two other samples available in the market).

AIMS AND OBJECTIVES

1) To prepare three samples of *Kajjali* using different samples of *Parada*. (One *Hingulottha Parada* and two market samples)

2) To compare the acute and subacute toxicity of the prepared samples on Wistar albino rats.

MATERIALS & METHODOLOGY

The first step was the extraction of *Parada* from *Hingula*. Reference from *Rasaratna Samucchaya*[1] was followed. As per classical references, *Hingulottha Parada* was not subjected to *samanya shodhana*. Two other samples of *Parada* were procured from the market. One was a branded sample which assured 99% purity and the other was randomly selected. These samples were subjected to *samanya shodhana* following the reference from *Rasatarangini*[2]. Sample of *Gandhaka* was also sourced from the market and subjected to *Shodhana* as per reference from *Rasatarangini*[3]. Once the necessary raw materials were ready, *Kajjali* was prepared by *dridha mardana* as per reference from *Rasatarangini*[4].

Prepared samples of *Kajjali* were subjected to physico chemical analysis which included classical tests and modern tests like NPST, XRD and SEM. Following which acute and sub acute toxicity of each of these samples were carried out on Wistar strain albino rats.

The Experimental study was conducted in the Animal House of K.L.E. University's Shri BMKAM Belgaum. The required numbers of animals were procured from Animal House K.L.E. University's Shri BMKAM Belgaum. Rats weighing between 150-250g were procured. The animals were transferred into three separate polypropylene cages with 5-6 animals in each cage. Food *ad libitum* was regularly supplied through food trays forming part of the lid of the cage. Water *ad libitum* was provided through glass bottles with a stainless steel drinking nipple. The temperature of the room was maintained at 20-22°C as well as maintenance of photoperiod of 12 hrs light and 12 hrs dark.

The animals were divided in three groups; *Kajjali* prepared using *Hingulottha Parada*, *Kajjali* using branded *Parada* and *Kajjali* using market *Parada*. Each animal in the group was individually marked for identification.

The finely powdered test drugs each 1000 mg in 10 ml of 1% gum acacia, were prepared. The binding agent used here is devoid of any toxicity. Suspensions of the test drugs are prepared. It was dosed at different level as per the OECD guideline. The test dose was prepared freshly on the day of experimentation. Dose prepared per animal: as per the weight of animal. Oral route using intubation canula attached to a 2 ml plastic syringe. The parameters employed were Behavioral changes and Histopathology.

Determination of LD_{50} (test compound) – Fixed does method (OECD guideline No. 420 of CPCSEA) were followed to carryout LD_{50} for the samples.

Requirement:

Animals – Wister Albino Rats (150-250 g)

Test Drugs - *Kajjali*.

3 Healthy Wister Albino Rats weighing between 150-250g were selected and the test substance was orally administered in a single dose by using a stomach tube. Animals were fasted for 24 hrs prior to dosing. Observation was made for 24 hours. The procedure is repeated again the next day at higher dose if signs of toxicity are not observed. Toxicity signs if any prompt the reduction in dose.

Determination of acute toxicity study:

Requirement:

Animals – Wister Albino Rats (150-250g)

Test Drugs- *Kajjali*

Healthy Wister Albino Rats weighing between 150-250g are selected and

grouped into three groups with each group containing 6 animals.

The test substance is orally administered in a single dose by using a stomach tube. Animals were kept fasting for 24 hrs prior to dosing. And observations are made for 24 hours.

Determination of sub acute toxicity study:

Animals – Wister Albino Rats (150-250g)

Test Drugs- *Kajjali*

Healthy Wister Albino Rats weighing between 150-250g are selected and grouped with each group containing 6 animals.

The test substance is orally administered in a single dose daily for 14 days by using a stomach tube. And observation is made every 24 hours.

At the end of 14 days animals were killed by cervical decapitation Brain, Heart, Liver, kidney, Spleen were excised out and placed in physiological salt solution (0.9% normal saline) and then immersed in 10% formalin & sent for histopathology study to Jeevan Pathological lab, Belgaum. Reports noted.

Statistical data:

In the due course of the experimental study there were no comparable observations with respect to time intervals. The histopathological reports also show incomparable cell damage between the groups. The study also comprises of less subjects, hence , after having taken consent of experts, it was concluded that statistics does not apply to the intended study.

OBSERVATIONS & RESULTS

Table 01: Results of LD_{50} of *Hingulottha Parada Kajjali* at dose level 2000mg/kg

No	Animal weight(g)	Dose(mg)	Observation of toxicity reactions		
			Onset of time	Recovery period	Toxicity signs
1	180	360	--	--	AB
2	180	360	--	--	AB

AB: Absent

Table 02: Results of LD_{50} of Branded *Parada Kajjali* at dose level 2000mg/kg

No	Animal weight(g)	Dose(mg)	Observation of toxicity reactions		
			Onset of time	Recovery period	Toxicity signs
1	200	400	--	--	AB
2	190	380	--	--	AB

AB: Absent

Table 03: Results of LD_{50} of Market *Parada Kajjali* at dose level 2000mg/kg

No	Animal weight(g)	Dose(mg)	Observation of toxicity reactions		
			Onset of time	Recovery period	Toxicity signs
1	200	400	--	--	AB
2	190	380	--	--	AB

AB: Absent

Table 04: Acute Toxicity study observations for *Kajjali* at dose 200mg/kg

Group	Catatonia	Diarrhoea	Narcosis	Respiratory depression	Salivation
A	AB	AB	AB	AB	AB
B	AB	AB	AB	AB	AB
C	AB	AB	AB	AB	AB

Table 05: Sub Acute Toxicity study observations for *Kajjali* at dose 200 mg/ kg

Group	Catatonia	Diarrhoea	Narcosis	Respiratory depression	Salivation
A	AB	AB	AB	AB	AB
B	AB	AB	AB	AB	AB
C	AB	AB	AB	AB	AB

Group A: *Hingulottha Parada Kajjali*
Group B: Branded *Parada Kajjali*
Group C: Market *Parada Kajjali*
AB: Absent

Table 06: Histopathology findings for *Hingulottha Parada Kajjali* group

Group	Brain	Heart	Liver	Kidney	Spleen
A	Mild Congestion	Normal	Mild Congestion	Congestion +++ Hyaline Casts + Glomelular cellularity +	Congestion +++

Table 07: Histopathology findings for Branded *Parada Kajjali* group

Group	Brain	Heart	Liver	Kidney	Spleen
B	Congestion +	Congestion +	Normal	Mild Congestion +	Congestion ++

Table 08: Histopathological findings for Market *Parada Kajjali* group

Group	Brain	Heart	Liver	Kidney	Spleen
C	Normal	Mild Congestion	Mild Congestion	Congestion ++ Casts ++ Glomerular Nephritis + Interstitial Nephritis	Congestion +++

CONCLUSION

No toxicity signs were observed in Wistar rats either in acute or sub acute toxicity study for any of the samples. No mortality was observed. Histopathology study indicated maximum organ damage in the group administered *Kajjali* prepared from market sample *Parada* and the least in group administered branded sample *Parada* prepared *Kajjali*. *Hingulottha Parada* prepared *Kajjali* showed moderate organ damage.

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