

**TOXICOLOGICAL STUDY OF *MUGDHA RASA*, AN AYURVEDIC FORMULATION**Soumya T G¹, Surekha S Medikeri²

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Article Received:12/09/2020 - **Peer Reviewed:**28/09/2020 - **Accepted for Publication:**30/09/2020**ABSTRACT**

Mugdha Rasa is one type of *Kharaliya Rasayana* and comes under *Nirgandha, Niragni Murchana* of *Parada*. *Parada* and *Khatika* are the main ingredients of *Mugdha Rasa*. This investigation is an attempt to perform toxicological study of *Mugdha Rasa*. Acute toxicological study and sub-acute toxicological study were carried out as per OECD guideline 425 and 407 respectively. Oral acute toxicity study was carried out at the limit dose of 2000 mg/ kg orally in Swiss albino mice. Sub- acute toxicity study of *Mugdha Rasa* was carried out in Albino rats and it was administered at therapeutic equivalent dose (TED), TED ×2 and TED×5. No signs of toxicity and mortality were observed *Mugdha Rasa* in acute toxicity study. So, LD₅₀ of *Mugdha Rasa* is greater than 2000 mg/kg body weight and *Mugdha Rasa* can be considered assafe on acute exposure. The data generated during sub-acute toxicity study are indicated that it is not a hazardous substance for sub-acute administration at TED dose level. Higher dose levels show mild changes in parameters.

Keywords: *Mugdha Rasa, Acute Toxicity Study, Sub-Acute Toxicity Study, Kharaliyarasayana*

INTRODUCTION

Rasashastra deals with the preparation of medicines of Mineral and herbo mineral origin. These drugs are having high potency and quick in action. Questions always rise on safety status of *Rasaushadi*. In the present era of globalization to make *Rasa* medicine worldwide acceptable, it is necessary to perform various toxicological studies to assess the safety profile of *Rasa* drugs. *Kupipakva*, *Pottali*, *Parpati* and *Kharaliya Rasayanas* are different Mercurial formulations. Among them *Kharaliya Rasayanas* stand foremost with other three in terms of its popularity, wide range of therapeutic utility and ease of preparation. *Kharaliya Rasayanas* are innumerable and most suitably indicated in majority of diseases. Since this formulation is completely prepared and obtained as end product in *Khalva Yantra* by the act of trituration, it is called as *Kharaliya* or *Khalviya Rasayana*¹. Here *Mugdha Rasa* is one type of *Kharaliya Rasayana* and comes under *Nirgandha*, *Anagni Murchana* of *Parada*². *Parada* [Mercury-Hg] and *Khatika* [Chalk-CaCO₃] are the main ingredients of *Mugdha Rasa* and it is prepared by trituration of one part of *Shuddha Parada* and two parts of *Shodhita Khatika*³. *Hingulotha Parada*⁴ was used for this preparation. *Mugdha Rasa* is indicated in *Udaramaya*, *Vami*, *Sahajotthaphirangaroga*, *Kurangaroga* and *Shishuroga*⁵.

Even though *Mugdha Rasa* is mentioned as highly potent medicine, nowadays this *Yoga* is not in practice because of fear of toxicity. The main issue to be considered is whether *Nirganda*, *Niragni Murchita* preparation mentioned in *Rasashastra* is safe or not. A proper toxicological evaluation of *Mugdha Rasa* would serve as an important tool for better understanding.

Materials and Methods

Hingulotha Parada was used for this study. *Hingula* and *Khatika* were procured from authentic sources. Instrumental analysis of ingredients and final product were conducted at IISc, Malleshwaram, Bengaluru. Extraction of *Parada* from *Hingula*, *Shodhana* of ingredients and preparation of

Mugdharasa were carried out in Dept. of *Rasashastra* and *Bhaishajya Kalpana*, GAMC, Bengaluru.

Experimental Animals

Swiss Albino mice (n=6, weighing 18-25 g) and male and female rats (n=40, weighing 150-200 g) were used in these studies. All the animals were procured from Biogen Laboratory Animal Facility, Bengaluru, Karnataka, CPCSEA reg. no. 971/PO/RcBiBt/S/2006/CPCSEA and were maintained under controlled condition of temperature (23± 2°C), humidity (50±5%) and 12 hour light and dark cycles. The animals were randomized (5 males and 5 females in each groups) into 4 groups. The animals were housed in sanitized polypropylene cage containing sterile paddy husk as bedding. The animals are free access to standard food pellets and water. Animals were allowed a one-week acclimatization period prior to the study. An ethical clearance was obtained from the Institutional Animal Ethical Committee (PESCP/IAEC/89/2019) and the study was conducted according to the guidelines of CPCSEA, New Delhi.

Dose Selection

Acute toxicity study – The dose was calculated according to body weight. All the animals were dosed constant dose volume i.e., 175 mg/kg, 550mg/kg bw po and 2000 mg/kg.

Sub – Acute toxicity study - The group I animals were served as vehicle control group and received 1%w/v carboxy methyl cellulose (CMC) vehicle orally at a dose of 10ml/kg body weight. Therapeutic dose of *Mugdha Rasa* is 125 mg/day, the therapeutic equivalent dose for rats was calculated by using equation for conversion of human dose to rat dose. The rats in groups II, III and IV were treated with *Mugdha Rasa* at the doses of 0.35, 0.70 and 1.75 mg/kg bw po daily up to 28days by suspending in 1% w/v CMC.

Experimental Study

Acute Toxicity Study^{6,7}-Overnight fasted three healthy Albino swiss mice were used in this study. Mice No1 treated as normal control. The sample

(dose 175mg/kg b.w P.O) was administered orally to Mice No 2 and 3 by using rat oral gavaging needle. The mortality was observed for a period of 30min. Again, the sample (dose 550mg/kg b.w P.O.) was administered and the mortality does not take place within 30 min after the treatment. Again, the sample (dose 2000mg/kg b.w P.O.) was administered and the mortality does not take place within 30 min. After the administration of sample feed was withheld for further period of 3-4h. Wellness parameters were observed continuously during the first 30 min after dosing and after that observed periodically (4h, 24 h, 48 h, 1st week and 2nd week). Changes in wellness parameters were compared with that of control group animal. Individual rat weights were measured on 1st, 7th and 14th day of experiment.

Sub-acute toxicity study^{8,9} –

Sub-acute toxicity study was carried out according to OECD407 guidelines. Both sexes of rats were divided into four groups with 10 rats in each group (5 males plus 5 females in each group). The group I animals received 1%w/v carboxy methyl cellulose (CMC) vehicle orally at a dose of 10ml/kg body weight and served as vehicle control group whereas the rats in groups II, III and IV were treated with *Mugdha Rasa* at the doses of 0.35, 0.70 and 1.75 mg/kg b.w po daily up to 28days by suspending in 1% w/v CMC. Animals of all groups were observed twice daily for clinical signs of rats and the time of onset, duration of these symptoms. The mortality and morbidity till 28th day were observed. Body weights of the rats in all groups were recorded once before the start of dosing, once weekly during the treatment period and finally after 24h of the 28th day treatment. The food and water intake were recorded daily, and the data were expressed as 7 days cumulative value. At the end of the experiment (on 29th day), 24h urine was collected after hydration to each animals using metabolic cages and used for urine analysis^{10,11}. Blood samples were collected from the rats after overnight fasted (*but water ad libitum*). The blood and serum was used for hematological and serum biochemical parameters respectively^{12,13,14}. Then animals were sacrificed

using overdose of ketamine (150mg/kg ip) and the liver, heart, spleen, brain and kidneys were isolated and these organs were processed for the tissue parameters and histopathological observations.

Statistical Analysis

All data were expressed as the standard error of the mean (S.E. \pm mean). Comparisons among the control and treatment groups were made using analysis of variance (ANOVA) followed by a Bonferroni method of Statistics using the Graph pad prism statistical program. The results were considered statistically significant if 'p' value was ≤ 0.05 or less.

RESULTS AND DISCUSSION

Acute toxicity test assesses the adverse effects that occur within a short time after administration of a single dose of a test substance. It is usually done to provide information on its potential toxicity. No sign of toxicity and mortality was observed at *Mugdha Rasa* 2000mg/kg bw po. Wellness parameters and body weight analysis- no acute oral toxicity was found. Therefore, Ld50 is greater than 2000 mg/kg. So, it can be considered relatively safe on acute exposure.

Sub-acute toxicity study examines toxicity caused by repeated dosing over an extended period of 28 days of oral administration in rodents. This test provides information on target organs and on the potential of the test chemical to accumulate in the organism and then is used as the basis for the determination of the no observed effect level (NOEL). In the present sub-acute study, the rats that were treated with *Mugdha Rasa* at doses 350 μ g/kg bw po, 700 μ g/kg bw po and 1750 μ g /kg bw po showed no signs of morbidity and mortality. During the experimental period no death or no apparent behavioral changes were observed compared with the control group. Changes in body weight of the animals are an important index for assessment of toxicity. Weight difference between control group and treated groups was statistically insignificant. Food and water intake difference between control groups was statistically insignificant

It was observed that, the urine parameters (Table no. 1) like colour, turbidity, sediment, ketone body and bilirubin were found to be unaltered with TED, TED \times 2 and TED \times 5 doses. Other parameters like glucose and proteins were present in urine sample of treated group (TED, TED \times 2 and TED \times 5). "Renal glycosuria is a rare condition in which the simple sugar glucose is excreted in the urine. With normal renal function, glucose is excreted in the urine only when there are abnormally elevated levels of glucose in the blood"¹⁵. However, in those with renal glucosuria, glucose is abnormally elevated in the urine due to improper functioning of the renal tubules, which are primarily components of nephrons. "Proteinuria is the presence of excess proteins in the urine"¹⁶. In healthy rats, urine contains very little protein; an excess is suggestive of illness. Proteinuria can also be a sign of renal damage. Diabetes also may have damaged nephrons and develop proteinuria. Thus, the analysis of the urine parameters clearly shows that there is some untoward effect is there in the functioning of the kidney.

It was observed that, 3 out of 4 hematological parameters (Table no.2) were found to be unaltered with TED, TED \times 2 and TED \times 5 doses. Only one parameter showed significant change in comparison to the control group, significant decrease was observed in the WBC. Other parameters like Hb, RBC and Platelet count shows mild increase or decrease in its value which is statistically not significant indicates that there were no harmful effects observed in these parameters of all treated groups. A decreased WBC count was observed in this study when compared with normal group. But the WBC counts come under normal range in all group. So, clinically it is insignificant.

The IV (MR-11750 μ g/kg) group rats have shown significant increase in ALT levels when compared to normal control group. All three group (MR) rats have shown significant decrease in the AST levels when compared to normal group. These suggest the protective efficacy of MR. The rats of Group II, III, and IV have not shown any significant deference in

serum total cholesterol and serum creatinine levels when compared to normal rats. This confirms the safety of the SR with respect to total cholesterol and renal creatinine. The rats of Group II, III and IV treated with MR have not shown significant deference in triglyceride, Bilirubin and Total protein when compared to normal vehicle group. The rats of Group II, III and IV treated with MR have shown significant increase in blood urea nitrogen when compared to normal vehicle group. Generally, a high blood urea nitrogen level indicates kidney malfunction and can also be due to urinary tract obstruction, congestive heart failure etc. Here in this study BUN is statistically increased when compared to control group, but the values are in normal range. So, clinically it is insignificant.

The rats of Group II, III, and IV have not shown any significant deference in Catalase level (Table no.3) when compared to normal rats. Catalase is a common enzyme found in nearly all living organism exposed to oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species. The Rats of Group II shown significant increase in SOD levels, when compared to normal group. The rats of Group III and Group IV shown significant decrease in SOD level (Table no.3) when compared with control group. Superoxide is produced as by product of oxygen metabolism. SOD is an important antioxidant defense in nearly all living cells exposed to oxygen. The rats of Group I and Group IV have not shown any significant deference in Lipid peroxide level, but Group III shown significant increase in Lipid peroxide level when compared to control group. Histopathology study (fig. no.1, 2, 3 and 4) of TED group showed no significant changes in cytoarchitecture of liver, kidney and heart. But there were significant changes in cytoarchitecture of liver, kidney and heart of TED \times 2 and TED \times 5 dose levels when compared with control group.

CONCLUSION

LD 50 of *Mugdha Rasa* is greater than 2000mg/kg. The data generated during sub-acute toxicity study

are indicated that it is not a hazardous substance for sub-acute administration at TED dose level. Higher dose levels show mild changes in parameters. It is mentioned in *Rasatarangini* that *Nirgandha Murchita Parada Yogas* should not use after the cure of the disease¹⁷. So, its careful administration is not likely to cause any serious toxic outcomes at the therapeutic dose level in short duration of administration.

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Tables and pictures

Table 1: Showing Effects on urine parameters

Urine Parameters	Control	TED	TED × 2	TED × 5
Colour	<i>Pale yellow</i>	<i>Pale yellow</i>	<i>Pale yellow</i>	<i>Pale yellow</i>
Turbidity	-ve	-ve	-ve	-ve
Sediment	-ve	-ve	-ve	-ve
Ph	7.133±0.06	7.112±0.05	7.089±0.04	7.036±0.03
Protein	-ve	+ve	+ve	+ve
Glucose	-ve	+ve	+ve	+ve
Ketone body	-ve	-ve	-ve	-ve
Bilirubin	-ve	-ve	-ve	-ve

*TED – Therapeutic equivalent dose

Table 2: Showing serum parameters of control and treated group

Parameters	Control	TED	TED × 2	TED × 5
Hemoglobin(g/dl)	14.40±1.56	15.20±1.32 ^{ns}	14.97±1.28 ^{ns}	14.45±1.09 ^{ns}
RBC count(million cells/mm ³)	5.34±0.51	5.75±0.49 ^{ns}	5.40±0.34 ^{ns}	5.76±0.59 ^{ns}
WBC count(cells per cmm)	9000±588.78	5300±586.89***	5050±797.56***	4700±632.45***
Platelet count(lakhs cells/mm ³)	2.55±0.72	2.90±0.61 ^{ns}	2.90±0.81 ^{ns}	3.40±0.96 ^{ns}
TC(mg/dl)	26.975±1.47	30.223±1.98 ^{ns}	30.513±5.14 ^{ns}	29.46±4.10 ^{ns}
Crea(mg/dl)	0.204±0.06	0.182±0.08 ^{ns}	0.207±0.04 ^{ns}	0.206±0.04 ^{ns}
ALT(IU/L)	1.817±0.48	1.797±0.58 ^{ns}	2.834±1.20 ^{ns}	3.917±1.56***
AST(IU/L)	137.797±17.33	111.88±5.54***	84.846±7.03***	77.323±10.09***
BUN(mg/dl)	0.54±0.22	2.48±0.63***	2.927±0.19***	2.836±0.34***
TG(mg/dl)	94.932±20.21	97.538±9.58 ^{ns}	98.681±11.86 ^{ns}	87.4±7.55 ^{ns}
Bil(mg/dl)	0.282±0.12	0.243±0.10 ^{ns}	0.202±0.08 ^{ns}	0.292±0.16 ^{ns}
TP(g/dl)	2.546±0.28	2.425±0.21 ^{ns}	2.494±0.21 ^{ns}	2.446±0.24 ^{ns}

*TED – Therapeutic equivalent dose

Table 3: Showing Effects on Tissue Parameters

Parameters	Control	TED	TED × 2	TED × 5
Catalase (U/mg protein)	1.337±0.33	1.186±0.35	1.149±0.56	1.535±0.55
SOD (U/mg protein)	0.719±0.12***	0.875±0.07***	0.465±0.06***	0.322±0.07***
Lipid peroxidation (nM/mg)	0.085±0.04	0.137±0.02	0.280±0.07	0.037±0.02***

*TED – Therapeutic equivalent dose

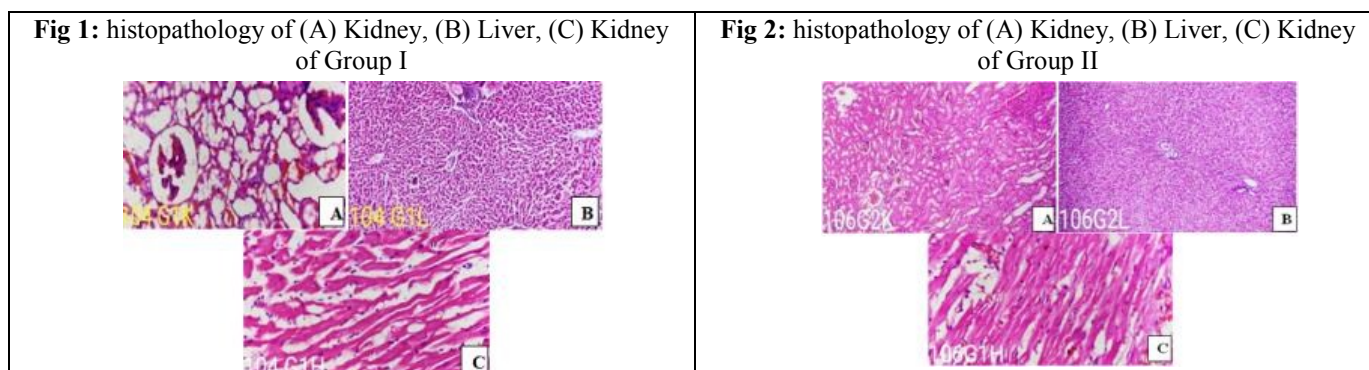


Fig 3: histopathology of (A) Kidney, (B) Liver, (C) Kidney of Group III

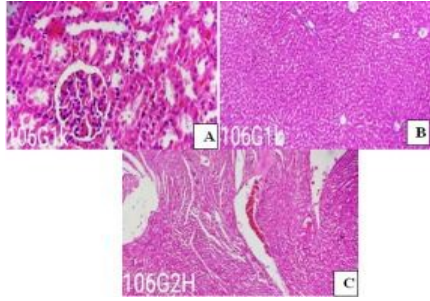
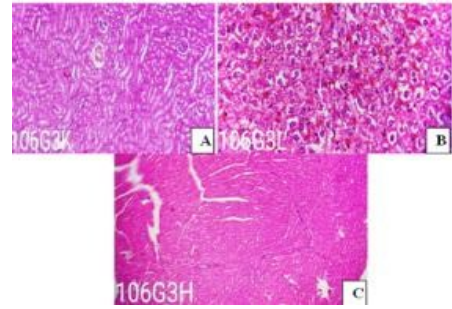


Fig 4: histopathology of (A) Kidney, (B) Liver, (C) Kidney of Group IV



Source of Support: Nil

Conflict of Interest: None Declared

How to cite this URL: Soumya T G & Surekha S Medikeri: Toxicological Study of Mugdha Rasa, An Ayurvedic Formulation. International Ayurvedic Medical Journal {online}; 2020 {cited October, 2020}; Available from: http://www.iamj.in/posts/images/upload/4626_4632.pdf