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TOXICOLOGICAL STUDY OF *SURYASHEKHARA RASA*

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ABSTRACT

Suryashekhara Rasa is unique mercurial preparation, which contains *Parada, Gandhaka, Hingula* and *Vatsanabha*. The quantity of *Vatsanabha* is equal to the sum of other ingredients, and also its antidote (*Tankana*) is not mentioned in this formulation. To ensure that the drug is devoid of toxicity and harmful effects, assessing the level of toxicity is important. So, this research work is an attempt to perform acute and sub-acute toxicity evaluation of *Suryashekhara Rasa.* Acute toxicity study of test drug was carried at a limit dose of 2000mg/kg orally in albino mice. For sub-acute toxicity *Suryashekhara rasa* was administered at therapeutic equivalent dose (TED) (0.35mg/kg bw po), TED x 2 (0.70mg/kg bw po) TED x 5 (1.75 mg/kg bw po) for 28 days. Acute toxicity result showed that drug did not produce any signs and symptoms of toxicity or mortality up to an oral dose of 2000 mg/kg in albino mice. The data generated during sub-acute toxicity study are indicated that it is mild toxic substance for sub-acute administration at TED dose level, may be because of alkanes which are found in functional group of aconitum ferox.

Keywords: *Suryashekhara rasa*, acute toxicity, sub-acute toxicity.

INTRODUCTION

Suryashekhara rasa[1] is one among the herbo-mineral formulation which contains *Parada, Gandhaka, Hingula* and *Vatsanabha,* prepared by *kupipakwa* method. *Kajjali* was prepared by using *Hingulottha Parada*[2] and *shudha Gandhaka*[3], then *shoditha hingula*[4] was added and triturated. *Vatsanabha shodhana* was done by immersing *Vatsanabha* in *Gomutra* for 3 days and kept in sunlight, everyday *Gomutra* was changed[5]. Then *Bhavana* is given to *Kajjali* with *vatsanabha kashaya* and subjected for *Kupi. Kupipakwa Rasayana* is a preparation where *Parada* is processed with other *Dravyas* in *Kupi* with *Kramagni.* In this, *Pruthvi* and *Ap Pradhana dravya* will get *Agni samskara* for specific long period of time and it is transformed and attains *Laghu, Sukshma Guna* along with *Tejo Pradhana Guna* by which the drug can easily enter into *Sukshma Srotas* and helps in very fast and effective action of the drug in the body. In other words, it causes *Sroto Shodhana* and gives the *Rasayana* effect resulting in *Dhatuposhana.*

Materials and Methods

Hingula, Gandhaka and *Vatsanbaha*was procured from authenticated sources. Instrumental analysis of ingredients and final product were conducted at IISc, Malleshwaram, Bengaluru.

Instrumental analysis:

X-ray diffraction studies of *Suryashekhara rasa* showed totally 19 peaks at different angles from 23.932 to 75.568 peaks were identified and compared standard as cinnabar (HgS) with hexagonal crystal structure.

Mean particle size of *Suryashekhara rasa* was 123.17nm, and EDX results has showed % of weight of Hg was 79.58, S was 12.87, C was 6.88 and O was 0.67. In FTIR analysis *Suryashekhara rasa* showed C-H bonding present in formulation suggesting the presence of alkanes and C=C bonding suggesting the presence of alkynes.

Experimental Animals

Female Albino swiss mice having weight range 18-25 gm were selected for acute toxicity study and healthy mices of either sex, weighing 150-200g were selected for sub-acute toxicity study. Animals were allowed a one-week acclimatization period prior to the study. Animals were housed under temperature 22 ± 3°C, relative humidity 50-70%, and 12 hours light and 12 hours dark cycle. The animals were housed in sanitized polypropylene cage containing sterile paddy husk as bedding and changed every day. The animals were free access to standard food pellets and water. Animals were provided normal chow diet to all the group of animals throughout experimental period.

Dose selection:[6]

Acute Toxicity Study: The prepared test sample was made into suspension in water with suitable concentration. 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 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 *Suryashekhara Rasa* at the doses of 250, 500 and 1000mg/kg bw po daily up to 28days by suspending in 1% w/v CMC.

Acute Toxicity Study[7]

Healthy female mice (overnight fasted) were used in the study. The dose was calculated according to the body weight. Mice No1 treated as normal control. The sample (dose 175mg/kg b.w P.O) was administered orally (oral gavage) to other mice using oral gavaging needle (not more than 2ml/100g). The mortality was observed for a period of 30min. Mortality does not take place within 30 min after the treatment. Again the sample (dose 550mg/kg b.w P.O) was administered and the mortality not takes place within 30 min after the treatment. Again the sample (dose 2000mg/kg b.w P.O) was administered and the mortality not takes place within 30 min after the treatment. After the administration of sample feed was withheld for further period of 3-4h.

Sub-acute Toxicity Study

It was carried out according to OECD407 guidelines.[8] Both sexes of rats (150-200g) 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 *Suryashekhara Rasa* at the doses of 250, 500 and 1000mg/kg bw po daily up to 28days by suspending in 1% w/v CMC. Animals of all groups were observed twice daily for clinical signs 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 date were expressed as 7 days cumulative value. At the end of the experiment (on 29th day), 24h urine was collected after hydration to each animal using metabolic cages, blood samples were collected from the rats after overnight fasted (*but water ad libitum*). The blood and serum were used for haematological and serum biochemical parameters respectively. 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. ±mean). Comparisons among the control and treatment groups were made using analysis of variance followed by a Dunnett's Multiple Comparison Test of Statistics using the Graph pad prism statistical program. The results were considered statistically significant if 'p' value was =05 or less.

RESULTS AND DISCUSSION

Acute toxicity study of test drug was carried out to record immediate adverse signs and symptoms of drug in female swiss mice at dose levels that are several folds higher than the therapeutic equivalent dose. Administration of *Suryashekhara rasa* did not effect on any behavioural changes and other parameters observed during the acute toxicity test in female mice. No signs and symptoms of toxicity and mortality were observed up to oral dose of 2000mg/kg of test drugs in rats, which suggest that LD50 value may be higher than 2000mg/kg by oral route.

Sub-acute toxicity study examines toxicity caused by repeated dosing over an extended period of 28 days of oral administration in rats. 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 *Suryashekahara Rasa* at doses 350µg/kg bw po, 700 µg/kg bw po and 1750 µg /kg bw po showed no signs of morbidity and mortality.

Table 1: Effects of *Suryashekhara rasa* on Haematological parameters recorded in sub-acute toxicity study

Biological parameters	Control group	TED	TEDx2	TEDx5
Hb%	14.4±1.55	14.6±1.44	15.15±1.35	15.6±1.59
RBC Count	5.34±0.51	5.63±0.52	5.45±0.43	5.81±0.55
WBC Count	9000±588.78	4650±529.67	5400±843.27	5100±906.76
Platelet Count	2.55±0.72	3.55±0.72	3.35±0.81	3.1±0.73

Analysis of effects on haematological parameters was observed that, Hb% and RBC showed non-significant increase in TED, TED x 2, and TED x 5 dose indicating that were no harmful effects observed in these parameters of all groups. There is a highly significant decrease in WBC count in all groups, but values are in normal range, when compare to control group.

Table 2 Effects of *Suryashekhara rasa* on Biological parameters recorded in sub-acute toxicity study

Biological parameters	Control group	TED	TEDx2	TEDx5
Total cholesterol	26.97±1.47	25.84±2.53	31.50±6.27	32.26±4.17
Sr. creatinine mg/dl	0.20±0.06	0.36±0.12	0.38±0.17	0.69±0.06
ALT mg/dl	2.06±0.92	5.74±2.15	5.29±2.01	45.57±14.94
AST mg/dl	145.57±2.56	135.57±6.60	144.64±3.52	119.10±10.39
Blood urea nitrogen	0.54±0.22	2.73±1.55	3.09±2.98	0.48±0.48
Triglycerides	94.93±20.21	92.05±7.91	91.93±3.80	150.67±46.51
Serum bilirubin	0.28±0.12	0.49±0.19	0.52±0.18	0.62±0.20
Serum total protein	2.54±0.28	2.47±0.06	2.94±0.18	3.00±0.60

The effects of *Suryashekhara Rasa* on liver function tests shows that there was non-significant increase in TED and TEDx2 but clinically not merely considered high. But these enzymes rise even before there is onset of structural damage. However, in TEDx5 group has showed highly significant increase, it would be because of temporary and reversible damage to liver parenchyma, and histopathology has shown congestion of blood vessels and destruction of hepatocytes. Bilirubin was found to be statistically significant increase in TED and TEDx2 groups and Highly significant increase in TEDx5 group compared to control groups. Increased bilirubin may be due to hepatic disorders, bile duct problem and destruction of erythrocytes. However, histopathology supports drug toxicity in at higher dose. Total protein was observed was non-significant decrease in TED group, may be due to decreased nutritional conditions since decreased food consumption was observed. In TEDx2 and TEDx5 groups has showed that statistically high but clinically, not merely considered high. Blood urea was found to be statistically significant and moderately significant increase in TED and TEDx2 respectively, may be due to reduced fluid intake and necrosis. In TEDx5 group has shown non-significant decrease in blood urea level but in normal range. An increase in blood urea level was observed in TED, TEDx2 groups. Serum/ plasma urea concentration reflects the balance between urea production in the liver and urea elimination by kidneys, in urine, so increased plasma/serum urea can be caused by increased urea production, decreased urea elimination, or a combination of two. Serum creatinine**:** In present study group 2 and 3 shows statistically and moderately significant increase. Group TEDx5 shows highly significant increase. Elevated increase in not always representative of a true reduction in GFR. A high reading may be due to increased production of creatinine not due to decreased kidney function, to interference with the assay, or to decreased tubular secretion of creatinine. However, in histopathology study of kidney there is haemorrhage in intercellular glomerular space. Total cholesterol: In the present study group TED has shown non-significant decrease in total cholesterol value, where as in TEDx2 and TEDx5 group has shown statistically significant increase compared to control group, but the values are normal in range. But in histopathology of heart section has shown reduced size of cardiac muscles and mild oedema. Triglycerides: In the present study groups TED and TEDx2 has shown non-significant decrease compared to control group. In group TEDx5 has shown highly significant increase in triglyceride value, may be due to changes in histopathology of heart.

Table 3: Effects of *Suryashekhara rasa* on Tissue parameters recorded in sub-acute toxicity study.

Tissue parameters	Control group	TED	TEDx2	TEDx5
Catalase	1.33±0.33	1.20±0.43	1.20±0.56	1.57±0.56
SOD	0.72±0.11	0.90±0.07	0.48±0.07	0.33±0.05
Lipid peroxidation	0.1±0.02	0.14±0.03	0.48±0.07	0.28±0.06

Catalase: Group TED and TEDx2 has shown non-significant decrease, and in group TEDx5 has shown non-significant increase compared to control group. SOD (superoxide dismutase): In present study group TED has shown highly significant increase where as in TEDx2 and TEDx5 groups has shown highly significant decrease. Lipid peroxidation: In present study group TED has shown non-significant increase, in TEDx2 group has shown significant decrease whereas in group TEDx5 has shown highly significant increase. The functional group of aconite, alkanes has found in FTIR analysis the ingestion of alkanes causes the tissue damage may be this is the reason in all above three tests there is significant changes.

Table 4: Effects of *Suryashekhara rasa* on Urine parameters in sub-acute toxicity study.

Urine parameters	Colour	Turbidity	Sedimentation	pH	Protein	Glu	KB	Bilirubin
Control group	Pale yellow	-ve	-ve	7.24±0.07	-ve	-ve	-ve	-ve
TED	Pale yellow	-ve	-ve	7.201±0.3	+ve	+ve	-ve	-ve
TEDx2	Pale yellow	-ve	-ve	6.62±0.21	+ve	+ve	-ve	-ve
TEDx5	Pale yellow	-ve	-ve	6.56±0.10	+ve	+ve	-ve	-ve

Colour of urine was yellow colour in all groups. Turbidity, Sedimentation, Ketone bodies and bilirubin has showed negative, whereas protein and glucose are positive in all groups. Elevated levels of glucose in urine may also be a result of renal glycosuria**.** This is a rare condition in which the kidneys release glucose into the urine. Renal glycosuria can cause urine glucose levels to be high even if blood glucose levels are normal. protein may be excreted in the urine when the kidneys aren't working properly or when high levels of certain proteins are present in the bloodstream. Since there was changes in histopathology study of kidney.

Table 5 Effects of S*uryashekhara rasa* on Physical parameters in sub-acute study

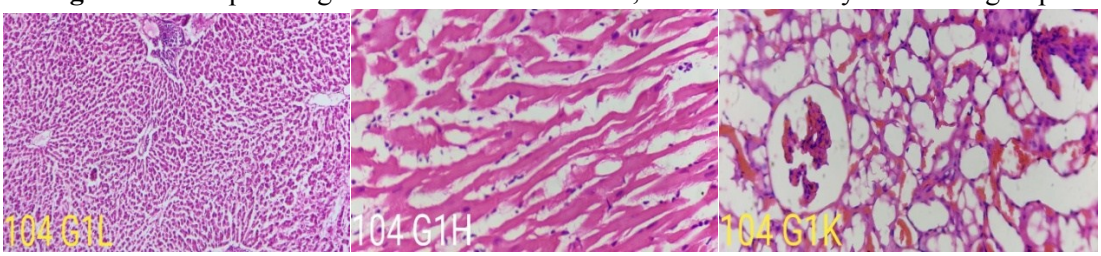
Physical parameters	Control group	TED	TEDx2	TEDx5
Body weight	1.257±1.06	1.117±0.44	1.335±0.44	1.34±1.03
Feed intake	540.6±13.72	533.4±5.35	523±4.57	503.3±6.56
Water intake	1366.8±42.09	1343±53.15	1333.3±47.16	1315.6±83.78

There was gradually decrease in feed intake in all groups in all weeks, may be due to impaired liver function. Since there was impaired function in kidney, so there was a significant decrease in water intake.

Histopathology evaluation: In kidney section from group TED and TEDx2 has shown mild edema and necrosis, also mild hemorrhage is found in intercellular glomerular space. In TEDx5 group has shown more edema, more necrosis, more hemorrhage is found in intercellular glomerular space and compressed blood vessels. Liver section from the group TED and TEDx2 has shown hepatic sinusoids with mild dilation and in group TEDx5 has shown more hemorrhage with increased edema, and also showed congestion of blood vessels and many hepatocytes are washed out. Heart section from group TED has shown reduced cardiac muscle fibers, and more reduced size of cardiac muscle fibers with mild edema and necrosis of few muscles are shown in TEDx5 group.

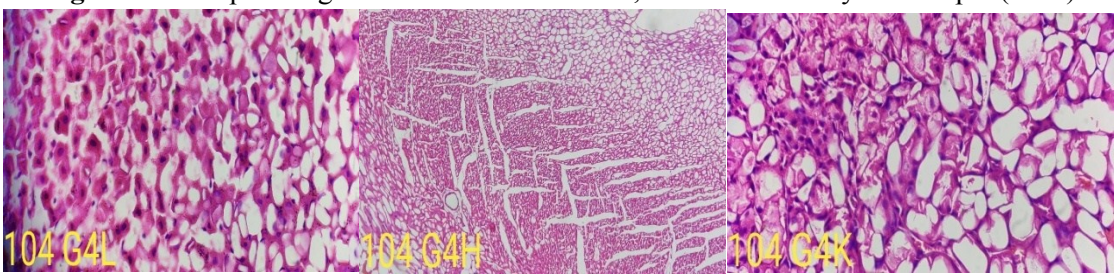
For further study Different purification can be adopted and different pharmaceutical method can be used to standardize this formulation and later toxicological study can be carried out.

Figure 1: Histopathological observations of Liver, Heart and Kidney of control group.



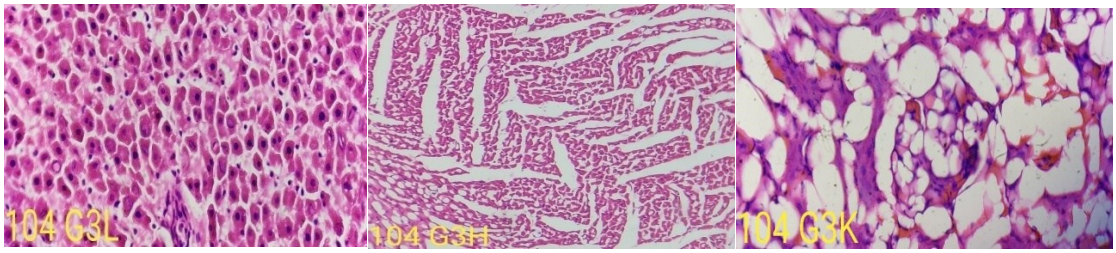
Liver Heart Kidney

Figure 2: Histopathological observations of Liver, Heart and Kidney of Group 2 (TED)



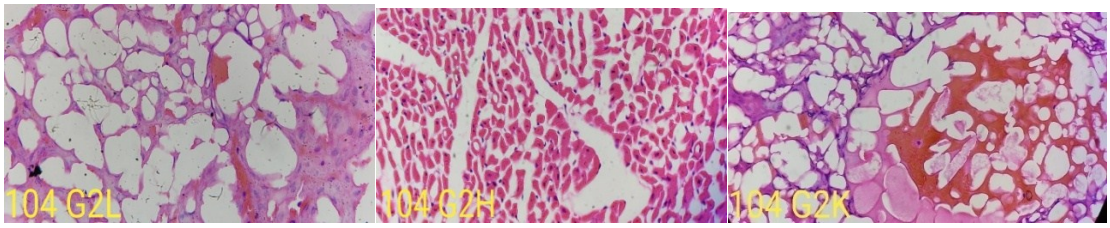
Liver Heart Kidney

Figure 3: Histopathological observations of Liver, Heart and Kidney of Group 3 (TEDx2)



Liver Heart Kidney

Figure 4: Histopathological observations of Liver, Herat and Kidney of Group 4 (TEDx5)



Liver Heart Kidney.

CONCLUSION

With thw factual evidence obtained by all experimental data, it has been concluded that Suryashekhara rasa LD50 is more than 2000mg/kg and it is mild toxic in therapeutic equivalent dose may be because of alkanes which are found in functional group of aconitum ferox.

REFERENCES

1. Acharya Anantha Deva Suri, Rasachinthamani, Siddhiprada Hindi Commentary by Prof. Siddhinandan Mishra Varanasi, Chaukambha Bharati Academy Publication, 1990, Stambhakha 4th, Verse 1-6. Pp.60
2. Sadananda Sharma, Rasa Tarangini, edited by Pandith Kashinath Shastry, Hindi commentary by

Dharmanandashastry, 11th Edition, New delhi, Motilal, banarasidas publication 2014, Taranga 5th Verse no 38-42. pp.82.
3. Vagbhata. Rasa Ratna Samuchhaya, translated in English by Ashok D Satpute Chaukhamba Sanskrit Prathishthan , 2010 3rd chapter Verse no 24-25.
4. Sadananda Sharma, Rasa Tarangini, edited by Pandith Kashinath Shastry, Hindi commentary by Dharmanandashastry, 11th Edition, New Delhi, Motilal, banarasidas publication 2014, Taranga 9th, Verse no 16-17. pp.202
5. Sadananda Sharma, Rasa Tarangini, edited by Pandith Kashinath Shastry, Hindi commentary by Dharmanandashastry, 11th Edition, New Delhi, Motilal, Banarasidas publication 2014, Taranga 24th Verse no 19-22. pp. 652
6. Wolters Kluwer-Med know journal of basic and clinical pharmacy March-May 2016
7. https://www.oecd.org/env/test-no-425-acute-oral-toxicity-up-and-down-proced
8. https://www.oecd-ilibrary.org/environment/test-no-407-repeated-dose-28-day-

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