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TOXICOLOGICAL STUDY OF UDAYABHASKARA RASA

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ABSTRACT

Udayabhaskara rasa is a herbomineral preparation that contains *Gandhakamarita Tamra Bhasma, Shu. Vatsanabha and Maricha* explained in *Rasendra Sara Sangraha* in *Kushtaroga Chikitsa adhyaya*[1] Despite having therapeutic utility there is hesitation in accepting the usage of heavy metals like Copper (*Tamra*), a poisonous drug like Aconitum ferox (*Vatsanabha*) and Sulphur (*Gandhaka*)also can cause poisonous effects if it is used in more quantity. As *Rasashastra* holds a valid response of drug-designing and analysing with a vision to produce safe drugs. This yoga also has the necessity of proving its safety in the global scenario of recent times. So, this research work is an attempt to perform an acute and sub-acute toxicity study of *Udayabhaskara rasa*. An acute toxicity study of the test drug was carried out at the dose of 2000mg/kg,550mg/kg.175mg/kh bw po orally in albino mice. For sub-acute toxicity *udayabhaskara rasa* was administered at therapeutic equivalent dose (TED) (0.35mg/kg bw po), TEDx 2(0.70mg/kg bw po), TEDx5(1.75mg/kg bw po) for 28 days. Acute toxicity results showed that the drug produces signs and symptoms of toxicity and mortality up to an oral dose of 550mg/kg BW Po. The data generated during the sub-acute oral toxicity study indicated that *udayabhaskara rasa* is mildly toxic in the administration of the TED dose level.

Keywords: *Udayabhaskara rasa*, Acute toxicity study, Sub Acute study

INTRODUCTION

Udayabhaskara rasa is one among the *Kharaliya rasayana* explained in *Rasendra sara sangraha* in *kushtaroga chikitsa adhyaya* indicated in *sarva kushta* of different stages like *galitha, sphutitha, vipula*, etc and also in *dadru, pama, vicharchika* type of *kushta*. which contains 10 parts of *gandhaka marita tamra bhasma,* and 2 parts of *shu*. *Vatsanabha*,5 parts of *Maricha* and the dose is 1*ratti*(125mg). For the preparation of *Udayabhaskara rasa*. *Tamra bhasma* was prepared first then *Shoditha Vatsanabha churna* and *maricha churna* were added and triturated well. For *Samanya shodhana*[2] of *tamra, Tamra Pathra* is heated and quenched seven times in *Taila, Takra, Gomutra, Aranala*, and *Kulatha Kwatha* in order. For *Vishesha shodhana*[3] *pachana* in *Gomutra* for 3hrs. Then *vishesha Shodhita tamra* is taken for *marana*[4] along with the equal quantity of *Shu*. *gandhaka* and added sufficient quantity of *nimbu rasa, mardana* given and *chakrikas* prepared then subjected for *puta* later Amrutikarana[5] done with *Panchamritha. Vatsanabha shodhana*[6] was done by immersing *Vatsanabha* in *Gomutra* for 7 days and kept in sunlight. everyday *Gomutra* was changed. *Gandhaka shodhana*[7] was done by *Galana* method with *ghrita* and *ksheera* 7 times.

Shodhana is done not only to remove impurities but also to enhance the properties of the drug. *Gandhaka* and *tamra* are extracted from ores, *Vatsanabha* contains toxic substances hence to eradicate the impurities from them and make them suitable for the further pharmaceutical process, *shodhana* is required.

Putikarana is a procedure adopted to convert heterogeneous materials into a homogeneous substance with reduced particle size so that it can be easily absorbed into the body systems, during Putikarana change in physical as well as the chemical structure takes place. For the preparation of Gandhakamarita tamra bhasma total of 13 Gaja puta has been given.

Amruthikarana- In Rasaratna Samucchaya a special samskara was adopted as a continuation of *Tamra marana*. Later authors coined the term '*Amruthikarana'* which literally means 'changing into nector' this mainly helps to remove shishta doshas from *Mruta* to bring about guna vrudhi in the *bhasma***.** For *tamra Amruthikarana* 3 *Gajaputa* has been given.

MATERIALS AND METHODS

Tamra, Gandhaka, Vatsanabha, and Maricha were procured from authenticated sources. Instrumental analysis like XRD, SEM, EDX, and FTIR of ingredients and the final product was carried out at the Indian Institute of Science, C V Raman Road, Bengaluru. Physico-chemical analysis was carried out at Drug testing Laboratory, Government central Pharmacy, Jayanagar, Ashok pillar, Bengaluru. A toxicological study was carried out at PES college of pharmacy Hanumanth Nagar.

INSTRUMENTAL ANALYSIS

Physico-chemical Analysis: pH- 5.02Total, ash-36%, Acid insoluble ash-12%, Loss on drying-1.42%, Copper assay-98.48%. Organoleptic characters: Colour-black, Form-fine powder, Touch-smooth, and soft, Odour- the smell of *Gomutra* and *maricha*. XRD: A total of 21 peaks were identified in *Udayabhaskara Rasa* at different angels (2θ) from 12.5093 to 85.8060. 5 strong peaks were chosen as strong with their relative intensity and compared to a standard X-ray power diffraction file (XPDF). 4 strong peaks of Cupric Sulphide and 1 strong peak of Silicon di oxide were identified. SEM-EDX**:** The major elements found in the sample of *Udayabhaskara rasa* are Carbon33.3%, Copper35.56%, Oxygen21.15%, and Sulphur 7.54%. The minor elements found in the sample are Potassium1.67% and calcium0.32%. Particle size ranges from 300nm to 900nm.FTIR: *Udayabhaskara rasa* showed C-H bonding present in formulation suggesting alkanes and C=C bonding suggesting the presence of alkynes.

Experimental Animals

Animal ethical committee proposal no: PESCP/IAEC/166/2020.

Female albino Swiss mice having a weight range of 20-25g were taken for acute toxicity study and healthy rats of either sex weighing 150-200g were taken for sub-acute toxicity study. Animals were al-

lowed a 1-week acclimatization period prior to the study. Animals were housed under a temperature of 22±3°C, relative humidity of 50-70%, and 12hours light and 12 hours dark cycle. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding and changed every day. The animals were free to access standard food pellets and water. Animals were provided a normal chow diet to all groups of animals' throughout the experimental period.

Dose selection[8]:

Acute Toxicity Study:

SN	Name	Treatment
1	Normal control	1 % CMC 2ml/kg bw
2	Udayabhaskara Rasa	175mg/kg bw po
3	Udayabhaskara Rasa	550mg/kg bw po
4	Udayabhaskara Rasa	2000 mg/kg bw po

Sub-Acute Toxicity Study:

G p No n=10 Male 5, female 5	**Group Name**	**Dose and route**	**Treatment days**
I	Vehicle Control	1%w/v CMC 10ml/kg bw po	Daily 28days
II	Udayabhaskara Rasa High dose	0.35mg/kg bw po	Daily 28days
III	Udayabhaskara Rasa Medium dose	0.70mg/kg bw po	Daily 28days
IV	Udayabhaskara Rasa Min dose	1.75mg/kg bw po	Daily 28days

Acute oral Toxicity study[9]:

Three healthy mice (overnight fasted) were used in the study. The dose was calculated according to the body weight. Rat No1 was treated as normal control. The sample (dose 175mg/kg b. w P. O) was administered orally (oral gavage) to Rat No 2 and 3 using a rat 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 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 treatment. After the administration of the sample feed was withheld for a further period of 3-4h.

Sub-acute oral Toxicity study[10]:

It was carried out according to OECD 407 guidelines. 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 *udayabhaskara rasa* at the doses of 0.35mg, 0.70mg and 1.75mg /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 of rats and the time of onset, and duration of these symptoms.

The mortality and morbidity till the 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 was expressed as 7-day cumulative value. At the end of the experiment (on the 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 an overdose of ketamine (150mg/kg Ip) and the liver, heart, 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 the 'p-value was=05 or less.

RESULT AND DISCUSSION:

An acute toxicity study of the test drug was carried out to record immediate adverse signs and symptoms of the drug in female Swiss mice at dose levels that are several folds higher than the therapeutic equivalent dose. After Administration of udayabhaskara rasa. Behavioral changes (difficulty in defecation) were seen in mice and mortality was observed at *udayabhaskara rasa* 2000mg and 550mg/kg bwpo.175mg/kgb wpo dose given to mice is active and alive until completion of the study. which suggests that the LD_{50} value may be higher than 175mg/kg by oral route.

Sub-acute oral toxicity study examines toxicity caused by repeated dosing over an extended period of 28days 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 ten is used as the basis for the determination of the observed effects level (NOEL). In the present sub-acute study, the rats were treated with *udayabhaskara rasa* at doses of 0.35mg/kgbwpo,0.70mg/kgbwpo, and 1.75mg/kgbwpo and showed no signs of morbidity and mortality.

Table 01: Effects of Suryashekhara rasa on haematological parameters recorded in the sub-acute oral toxicity study

Biological parameters	Control group	TED	TED×2	TED× 5
Hb%	14.40±1.56	15.20±1.32	14.97±1.28	14.45±1.09
RBC Count	5.34±0.51	5.75±0.49	5.40±0.34	5.76±0.59
WBC Count	9000±588.78	6.294±2.272	4.778±0.7558	11.994±7.922
Platelet Count	2.55±0.72	3.55±0.73	3.33±0.81	3.1±0.71

Analysis of effects on haematological parameters observed that Hb% and RBC showed a non-significant increase in TED, TED×2, and TED×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 the 11and111 group and a significant increase in group 1V, but values are in the normal range when compared to the control group.

Table 02: Effects of *udayabhaskara rasa* on biological parameters recorded in the sub-acute oral toxicity study

Biological Parameters	Control group	TED	TED×2	TED×5
Total cholesterol	26.875±1.46	24.84±2.53	30.50±6.27	34.26±4.17
Triglycerides	95.932±20.21	96.538±9.58	97.681±11.86	150.4±46.55
Sr, Creatinine	0.18±0.06	0.21±0.09	0.35±0.11	0.68±0.03
Serum bilirubin	0.27±0.12	0.30±0.10	0.53±0.08	0.60±0.16
Serum total protein	2.51±0.28	2.32±0.21	2.84±0.21	2.88±0.24

In the present study group, TED has shown a non- rum creatinine value However in histopathology study of significant decrease in total cholesterol value, whereas the shown glomerulus- normal cellularity with mild conges- TEDx2andTEDx5 group has shown a statistically signifi- tion, Tubules- focal loss of brush border in epithelial cells, cant increase compared to the control group, but the values blood vessel mild congestion with a normal interstitial. and are normal in range. But in histopathology of the heart. The in group TED×5 has shown Tubules-Necrosis with exfoli- interstitial space shows mild to moderate edema chronic atedcells.
inflammation and congested vascular space in places. In Bilirubin was found to be a statistically significant the present study groups, TEDandTEDx2 have shown a increase in TEDandTEDx2 groups und a Highly sig- non-significant decrease compared to the control group. In nificant increase in the TEDx5 group compared to the group, TEDx5 has shown a highly significant increase control groups Increased bilirubin may be due to he- in triglyceride value may be due to changes in the histo- patic disorders, bile duct problems, and destruction of pathology of the heart. In the present study, TE- erythrocytes. However, histopathology supports drug DandTEDx2 shows statistically and moderately significant toxicity at higher doses.
increase TEDx5 shows a highly significant increase in se-

Table 03: Effects of *Udayabhaskara rasa* on Urine Parameters in the sub-acute Toxicity study

Urine Parameters	Colour	Turbidity	pH	Protein	Glucose	Ketone bodies	Bilirubin
Control Group	Pale yellow	-ve	7.13±0.06	-ve	-ve	-ve	-ve
TED	Pale yellow	-ve	7.11±0.05	+ve	+ve	-ve	-ve
TEDx2	Pale yellow	-ve	7.08±0.04	+ve	+ve	-ve	-ve
TEDx5	Pale yellow	-ve	7.03±0.05	+ve	+ve	-ve	-ve

The Colour of urine was pale yellow in all groups. Turbidity, Sedimentation, Ketone bodies, and bilirubin have shown negative, whereas protein and glucose are positive. These elevated levels of glucose in the urine may also be a result of renal glycosuria. Protein maybe excreted in the urine when the kidneys aren't working properly since there were changes in the histopathology study of the kidney.

Table 04: Effects of *Udayabhaskara rasa* on Physical parameters in the sub-acute oral toxicity study

Physical parameters	Control group	TED	TEDx2	TEDx5
Body weight	1.356±1.05	1.211±0.42	1.388±0.44	1.345±1.02
Feed intake	560.3±11.72	544.3±6.22	536±3.47	528±5.35
Water intake	1566.9±40.08	1443.2±46.2	1422.1±40.26	1418.1±65.74

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

Histopathology Evaluation:

Kidney: In kidney section from group TED and TED ×2 has shown glomerulus- normal cellularity with mild congestion, Tubules- focal loss of brush border in epithelial cells, blood vessel mild congestion with normal interstitium. and in group TED×5 has shown Tubules-Necrosis with exfoliated cells.

Liver: In liver section from group TED and TED×2 has shown the periportal zone exhibits moderate inflammation. The central veins appear congested in places and sinusoids show mild congestion in group TED×5 the periportal zone shows mild to moderate inflamation. The central veins and sinusoids appear congested.

Heart: In the heart section from group TED and TED×2 has shown the cardiac muscle fibers shows

necrosis comprising of loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations, The interstitial space shows mild edema mild inflammation, and congested vascular spaces at places and in the group TED×5 has shown the cardiac muscle fibers shows focal loss of integrity of myocardial cell membrane, myofibrillar structure with loss of striations and loss of continuity with adjacent myofibrils. The interstitial space shows mild to moderate edema chronic inflammation and congested vascular space in places.

Figure 1: Histopathological observation of Liver, Heart, and kidney of the control group.
Liver Heart Kidney

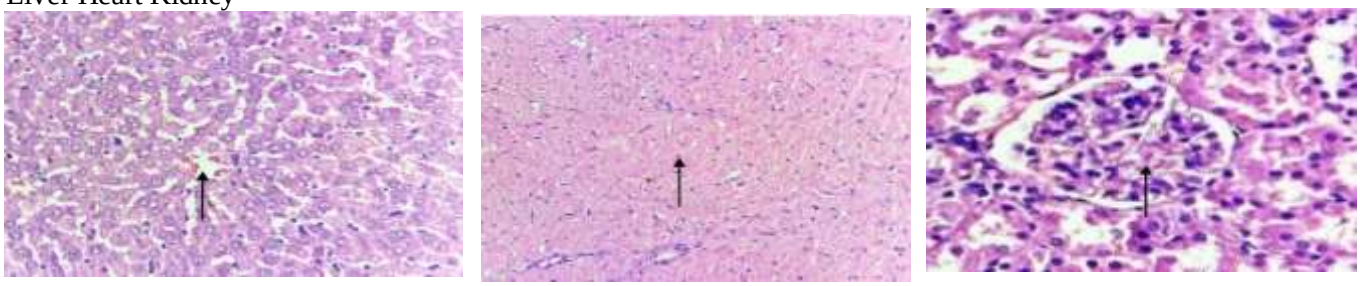


Figure 2: Histopathological observation of Liver, Heart, Kidney of Group 2(TED) Liver Heart Kidney

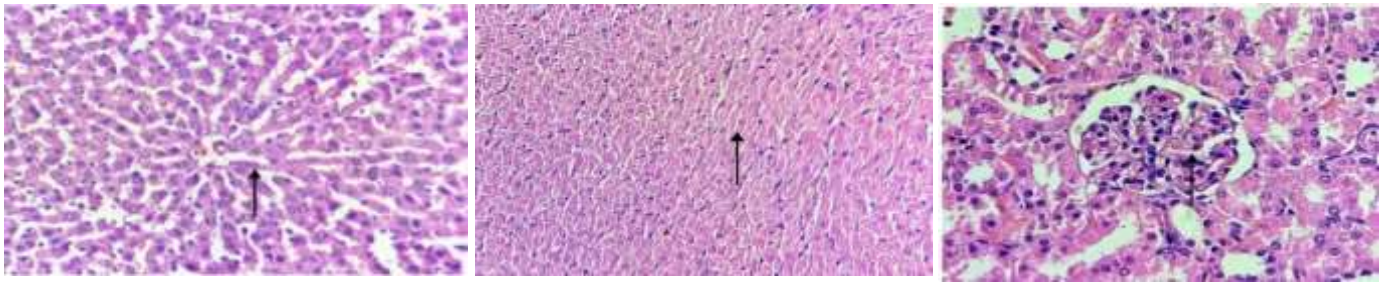


Figure 3: Histopathological observation of Liver, Heart, Kidney Group 3(TEDx2) Liver Heart Kidney

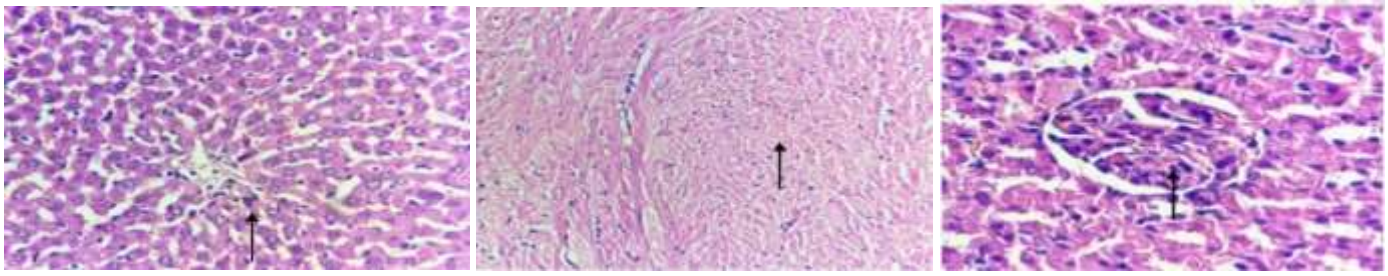
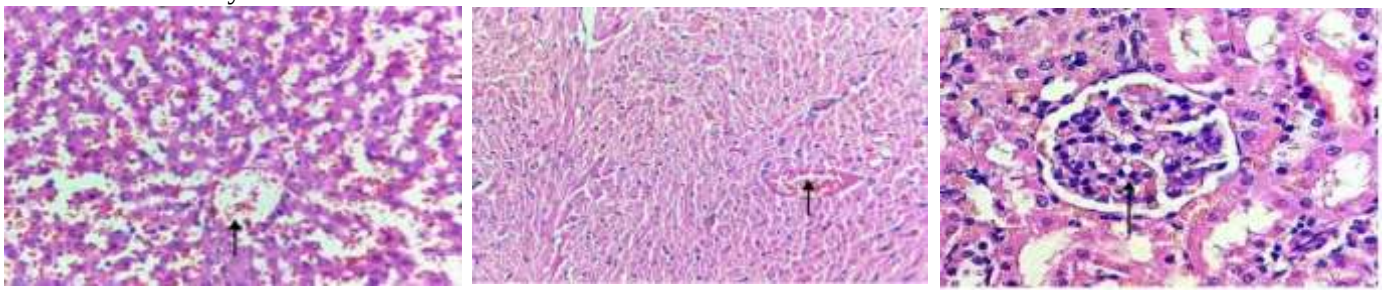


Figure 4: Histopathology observation of Liver, Heart, Kidney of Group 4(TEDx5)
Liver Heart Kidney



CONCLUSION

With the factual evidence obtained from all experimental study data, it has been concluded that Udayabhaskara rasa LD_{50} is more than 175mg/kg bw po and it is mildly toxic in therapeutic equivalent dose may be because of the presence of alkanes and alkynes in both *Vatsanabha* and *Gandhaka marita tamra bhasma*

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