*ejpmr, 2023,10(10), 285-292*

SJIF Impact Factor 6.222

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

# IN VIVO - ACUTE ORAL TOXICITY STUDY OF RAS SINDOOR IN ALBINO RATS

**Mogal Bhushan B.[1]* and Patkar Abhay H.[2]**

[1]PhD Scholar & Assistant Professor - Department of Agadtantra, Ayurved Seva Sangh, Ayurved Mahavidyalaya, Nashik. (MS)

[2]Professor & HOD - Department of Agadtantra, Ayurved Seva Sangh, Ayurved Mahavidyalaya, Nashik. (MS)

***Corresponding Author: Mogal Bhushan B.**

PhD Scholar & Assistant Professor - Department of Agadtantra, Ayurved Seva Sangh, Ayurved Mahavidyalaya, Nashik. (MS)

**Article Received on 01/08/2023** **Article Revised on 21/08/2023** **Article Accepted on 11/09/2023**

## ABSTRACT

*Ras sindoor* is a mercury based Ayurvedic herbo-mineral preparation widely used very effectively in the treatment of different diseases. Regarding its safety, previous researchers did Acute and Chronic toxicity study only on self prepared *Ras sindoor*. But in society, people consume *Ras sindoor* as a therapeutic medicine available in market which is prepared by different pharmaceutical companies. Therefore, this investigation is an attempt to perform acute oral toxicity evaluation of marketed *Ras sindoor* sample Agad-B having maximum mercury percentage among three different reputed market samples of *Ras sindoor* to find out any adverse effect and to estimate the LD50 in albino rats by following OECD 423 guidelines. Body weight, clinical or toxicological signs & symptoms and changes observed in internal organs at necropsy were studied after administration of *Ras sindoor* sample Agad-B stepwise in the dose of 300 mg/kg and 2000mg/kg. *Ras sindoor* sample Agad-B was found to be safe as all study animals were survived for 14 days without producing any adverse effect and sign of toxicity up to an oral dose of 2000 mg/kg. Hence the LD50 of *Ras sindoor* sample Agad- B could be more than 2000 mg/kg body weight in the albino rats and thereby in human beings and is classified in Globally Harmonized Classification System (GHS) Category 5. This level of LD50 value shows that marketed *Ras sindoor* may not likely to produce pathological alterations at the therapeutic doses when administered in human beings for various clinical conditions.

**KEYWORDS:** *Ras sindoor*, Acute oral toxicity, LD50, OECD 423.

## INTRODUCTION

Ayurveda- the traditional healthcare and medicinal system prescribes herbal, mineral and herbo-mineral formulations for the treatment of various diseases. Use of purified and processed metals and minerals in the drug formulations and its use as medicament has been an integral part of Ayurvedic practice. The formulations having metallic content have unique process of preparation involving Shodhana (Purification or Detoxification) and Marana (incineration or calcinations). These processes detoxify the raw material by chemical transformations and thus modify the properties of materials to enhance therapeutic potential.[1, 2] But in the recent past, numerous allegations have surfaced in various contexts about the toxicity of metal or mineral based Ayurvedic medications. According to 'The Ayurvedic Formulary of India' mercury and lead are the most widely used heavy metals in the drug industry.[3] As they are potent nephrotoxic, hepatotoxic, neurotoxic and hematotoxic agents. The toxicity can be very much expected with the use of these metals in the drug if all the steps of traditional manufacturing methods were not followed properly. Pre-clinical studies of Ayurvedic formulations provide scientific basis for their traditional use to prove that they are safe and efficacious.[4]

**Mercury metal[5]:** A heavy metal having symbol – Hg. It is the only metallic element that is in liquid form at room temperature. It exists in three forms – Elmemtal mercury. inorganic salts of mercury and organic compounds of mercury. Elemental mercury is non-poisonou if swallowed but its vapour can give rise to acute toxicity whereas inorganic and organic salts are the toxic compounds of mercury. Inorganic salts of mercury are Mercuric Chloride (corrosive sublimate), Mercurous Chloride (calomel), Mercuric Sulphide (vermillion/cinnabar), Mercuric Cyanide, Mercuric Oxide, Mercuric Iodide, Mercuric Nitrate etc. organic salts of mercury are Methyl Mercury, Dimethyl Mercury, Ethyl Mercury and Phenyl Mercury.

Depending upon the route of administration, quantity consumed & period of exposure to the toxic mercurial compounds, they produces acute or/and chronic toxicity. Mercury & its compounds are used in various medicines. e.g. as in Disinfectant, dental amalgam, purgative, diuretic & earlier in the treatment of syphilis. Also

Elemental Mercury is commonly used in the manufacture of thermometer, barometer, calibration instruments, explosive and fireworks. In the modern Ayurvedic practice various mercury based drugs like *Ras sindoor, Makaradhwaja, Panchamruta Parppati* are use widely and very effectively in the treatment of various diseases by Ayurveda Physicians since long time because they are easily palatable, fast acting & its minute dose delivers a strong & long lasting effect as compare to the herbal medicines. Therefore these drugs are manufactured by number of pharmaceutical companies all over the India.

***Ras sindoor*** **-** A sublimated mercurial preparation prepared by a unique process called as *Kupipakwa Rasasyana*. Purified Mercury and Sulphur in the same ratio (1:1) were mixed together in mortar (*Khalva yantra*) and trituration started till the whole mixture was converted into a fine black, lustreless powder (*Kajjali*). This *Kajjali* was further levigated in decoction of *Vatankur* (aerial roots of *Ficus benghalensis* Linn.) for 3 times. After complete drying, it was filled in a glass bottle (*Kupi*), previously coated with 7 layers of mud smeared cloth and heated on a traditional furnace (*Valuka Yantra*) in a controlled intermittent manner with gradually increasing temperature *(Mrudu agni, Madhyam agni & Tivra agni)* till the blue flame emerging from the pot disappeared and the bottom of the bottle becomes red hot. A red hot iron rod was repeatedly inserted in the neck of the bottle so as to burn any accumulated Sulphur at the neck of the bottle. After adequate cooling, the bottle as broken and sublimate (*Ras sindoor*) deposited at the neck of the bottle was collected.[6]

Around 39 references of *Ras sindoor* are mentioned in classics of *Rasashastra*. According to the proportion of sulphur used in the process of Ras *sindoor*, there are different references available in classics i.e. *Chaturamsha*[7] (1/4part) to *Shadguna Balijarita*[8] (6 parts) *Ras sindoor*; amongs these, *Samagun Balijarita Ras sindoor* as per the reference of *Raastarangini* is a common and widely used *Ras sindoor* formulation in market. *Rasa-sindoor* is therapeutically very effective in *Kaphaja roga* (disease due to *kapha*), *Balakhasya* (loss of strength), *Dhatukhasya* (tissue wasting), *Hrud-daurbalya* (weakness of heart), *Prameha* (Diabetes), *Shula* (colicky pain)[9] and in different conditions like *Rajyayakshma* (Tuberculosis), *Rakta-pitta* (Bleeding disorders), *Rasayna* (Immuno-modulator), *Vrishya* (Aphrodisiac), *Pandu* (Anaemia) etc. with different *Anupana* (adjuvant or vehicle). The medicinal dose of *Ras sindoor* for adults or for the age more than 12 years is described in Ayurvedic text – *Rasatarangini* is 1 *Gunja* i.e. 125mg once a day.[10] There is increased number of case reports being published in National and International Journals of toxic metals poisoning after the use of Ayurveda remedies.[11, 12] As chronic Mercury poisoning is well known and established by research in modern toxicology and *Ras sindoor* being a mercurial preparation its safety profile is being questioned by allopathic physicians. Presently *Ras sindoor* is prepared and marketed by various pharmaceuticals companies like Baidyanath, Dabar, Dhutpapeshwar, Patanjali Divya pharmacy etc.

As a mercurial preparation, to study the characteristics and to find out the various compounds present with their proportionate in the formulation as well as to study the safety profile, previous researcher did analytical as well as in vivo acute and chronic toxicity study with or without adjutants only on self prepared *Ras sindoor*. **[13-21]** But in society, as a therapeutic medicine people consumes *Ras sindoor* available in market prepared by different pharmaceutical companies. After doing extensive Literature review of *Ras sindoor*, it is observed that there is no data available regarding Acute or repeated dose chronic toxicity evaluation of *Ras sindoor* manufactured by pharmaceutical companies. Therefore this research topic is undertaken to study the acute toxicity along with its relevant dimensions and to estimate the Lethal Dose-50 (LD50) of marketed *Ras sindoor* in albino rats. This study is followed by repeated dose chronic toxicity study of *Ras sindoor* in albino rats as per the OECD 408 guidelines to find out its safety profile for longer period.

## MATERIAL AND METHODS

Three samples of *Ras sindoor* which were manufactured by three different pharmaceutical companies were purchased from market after approval of Institutional Ethical Committee for proposed research. After observing the results of analytical study (Physico-chemical analysis, ICP-AES and XRD) of three *Ras sindoor* samples (Agad-A, Agad-B and Agad-C), the sample Agad-B was selected and finalize for further in vivo Acute toxicity study as it contained higher percentage of Mercury content as compare to the other *Ras sindoor* samples Agad-A and Agad-C. **[22]**

**Test article details -**

- Test Article: *Ras sindoor*
  Sample: Agad-B
- Physical State: Powder
- Colour: Reddish Brown
- Quantity: 5 gram


**Fig. No. 1: Study Drug - *Ras sindoor*: Sample Agad-B.**

**Table No. 1: Details of study Animals, Test system & Operation.**

| Sr. No. | Specification | Description |
|---|---|---|
| 1. | Species | Rats |
| 2. | Strain | Wistar albino rats |
| 3. | Source | APT Testing and Research Pvt. Ltd, Pune |
| 4. | Sex & Age | Female, 06-08 weeks |
| 5. | Body weight range | 150-200 gm |
| 6. | Inclusive Criteria | 1. Healthy albino wistar rats<br>2. Rats weighing 150-200 gms |
| 7. | Exclusive Criteria | 1. Less than weighing 150-200gms<br>2. Pregnant and or diseased rats.<br>3. Rats under trial of other experiments. |
| 8. | Identification | By unique identification number marked by writing on cage tag and by corresponding colour body markings. |
| 9. | No. of animals | Three animals were tested in per group. |
| 10. | Acclimatization | After veterinary examination, the rats were housed in their cages for 5 days prior to start of dosing in the experimental room. |
| | Husbandry | |
| 11. | Environmental Conditions | Room temperature conserve between 22+3$^0$C, relative humidity 50-60 % and illumination cycle set to 12 hours light and 12 hours dark. |
| 12. | Accommodation | 3 rats per cage housed in polypropylene cages withstainless steel grill top, bedding of clean paddy husk and facilities for food and water bottle. |
| 13. | Diet | Supplied Pelleted feed |
| 14. | Water | Potable water passed through Aquaguard‘ water filter was provided ad libitum in plastic bottleswith stainless steel sipper tubes. |

**Study design:**

Acute oral toxicity study by using OECD Guideline 423 at a limit dose of 2000 mg/kg body weight of *Ras sindoor* sample Agad-B orally to the wistar albino rats was conducted to estimate the LD50 of ‘*Ras sindoor* Agad-B’. The vehicle used for dose preparation was water. The test substance was introduced in the stomach with a single dose using stainless steel intubation needle fitted onto a suitably graduated syringe. Animals were fasted overnight prior to dosing (only food was withheld but not water) and 2 hours after dosing. All animals were weighted before administration *Ras sindoor* sample Agad-B. The study was performed in a stepwise manner.

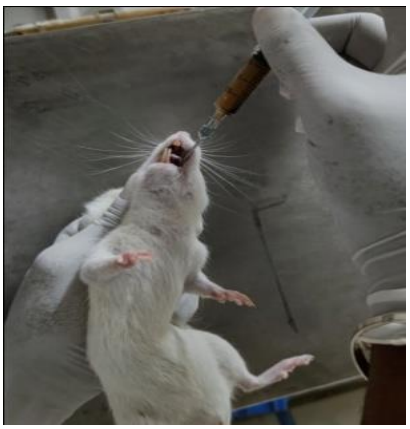
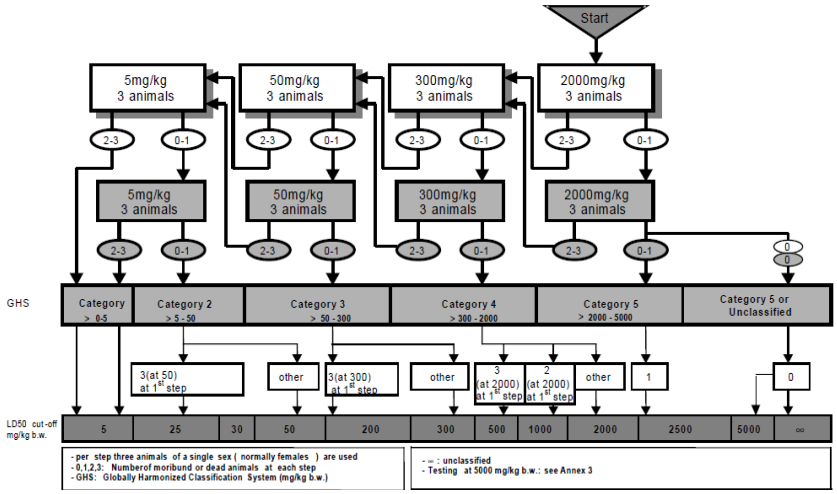


**Fig. No. 2: Oral dosing of test drug (*Ras sindoor* sample Agad-B) to Albino rat.**

ANNEX 2d: TEST PROCEDURE WITH A STARTING DOSE OF 2000 MG/KG BODY WEIGHT




[According to OECD, if the dose of a substance is unknown we can start the dose at 300 mg/kg if it shows mortality after 4-6 hrs that dose will be considered fatal dose otherwise lower or higher dose have to be studied.]

- In step 1 study, 3 female rats were administered orally test material '*Ras sindoor* sample Agad-B' of concentration of 300 mg/kg dose. All the animals were observed for 14 days after dosing.
- Then in step 2, repetition of dose 300 mg/kg of test material '*Ras sindoor* Agad-B' given to 3 female rats and all the animals were observed for 14 days after dosing.
- In step 3 study, after 1 week of step 1 and 2, another 3 female rats were administered with the test material '*Ras sindoor* Agad-B' of concentration of 2000mg/kg. All the animals in step 3 were observed for 14 days after dosing.
- Same procedure was carried out in step 4 repetition of dose (2000 mg/kg).

- Dose progression: The dosage volume administered to individual rat was as tabulated below and was adjusted according to most recently recorded body weight of respective rat.

**Table No. 2: Stepwise dosing pattern of *Ras sindoor* (RS) Sample: Agad-B in Acute oral toxicity study.**

| Steps | Test article | Test Dose (mg / 10 ml) | Test Concentration (mg / ml) |
|---|---|---|---|
| 1 | *Ras sindoor* sample Agad-B | 300 | 30.0 |
| 2 | *Ras sindoor* sample Agad-B | 300 | 30.0 |
| 3 | *Ras sindoor* sample Agad-B | 2000 | 200.00 |
| 4 | *Ras sindoor* sample Agad-B | 2000 | 200.00 |

All study animals were observed individually for their gross behavior, moribund status and mortality once during 30 minutes for first four hour with proper attentiveness, regularly during the first 24 hours and thereafter every day for 14 days.

**Body weight:**

Individual weights of animals were determined shortly before the test substance was administered (Pre-administration fasting weight) and at least weekly there after i.e. on Day 7$^{th}$ and Day 14$^{th}$ post treatment or at death. Weight changes were calculated and recorded.

**Clinical Signs and Symptoms:**

The cage side observation was carefully done without interrupting the animal's attention and after every hour animals were separately exposed to open area to note down the behavioral changes like elevated or declined motor activity, tremors, convulsions, spasm in muscle, straub's tail, catatonia, spasticity, anesthesia, lacrimation, salivation, diarrhea, writhing, arching and rolling, ophisthotonia, mode of respiration, Changes in skin and fur, eyes and mucous membranes, parameters of CNS expression like lethargy, hypo activity, relaxation, ataxic, narcosis, parameters of CNS stimulant like irritability, hyperactivity etc. All observations are meticulously recorded and separate record is preserved for every study animal.

**Necropsy:**

All the live animals were euthanized by $CO_2$ Asphyxia. Then the test animals were subjected to gross necropsy. All gross pathological changes were recorded for each animal.

**Statistical analysis:**
The obtained results from observations of experimental rats are presented as Mean± Standard Error of means (SEM) in each group. Weight parameter was subjected to one way ANOVA test followed by Bonferroni to determine the significance of deference within the groups at $p<0.05$.

**OBSERVATIONS AND RESULT**
Acute oral toxicity of *Ras sindoor* was performed as per OECD guidelines 423 in which *Ras sindoor* sample Agad-B was found safe.

- **Effect of *Ras sindoor* on Body weight**

**Table No. 3: Step 1 -Administration of *Ras sindoor* sampleAgad-B in the dose – 300 mg/kg.**

| Group | Animal No | Sex | 0 Day - Body weight | 07th Day - Body weight | 14th Day - Body weight |
|---|---|---|---|---|---|
| *Ras sindoor* sample Agad-B | 1 | F | 211.5 | 219.0 | 226.0 |
| | 2 | F | 200.0 | 215.0 | 217.5 |
| | 3 | F | 202.0 | 210.5 | 225.0 |
| Mean | | | 204.5 | 214.8 | 222.8 |
| SD | | | 6.1 | 4.3 | 4.6 |

**Table No. 4: Step 2 - Administration of *Ras sindoor* sampleAgad-B in the dose – 300 mg/kg.**

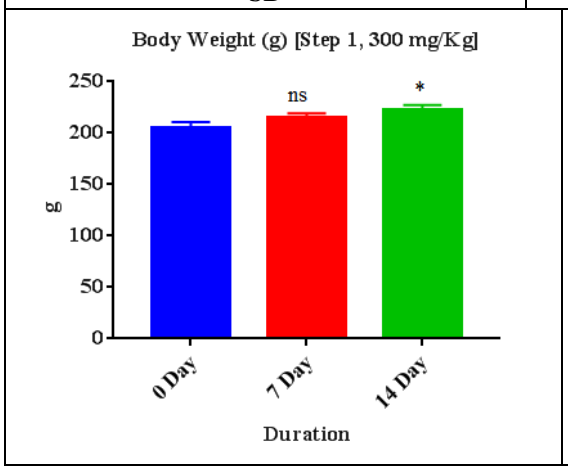
| Group | Animal No | Sex | 0 Day – Body weight | 7th Day – Body weight | 14th Day – Body weight |
|---|---|---|---|---|---|
| *Ras sindoor* sample Agad-B | 1 | F | 190.0 | 207.0 | 218.0 |
| | 2 | F | 187.5 | 214.0 | 235.0 |
| | 3 | F | 187.0 | 210.0 | 228.0 |
| Mean | | | 188.2 | 210.3 | 227.0 |
| SD | | | 1.6 | 3.5 | 8.5 |

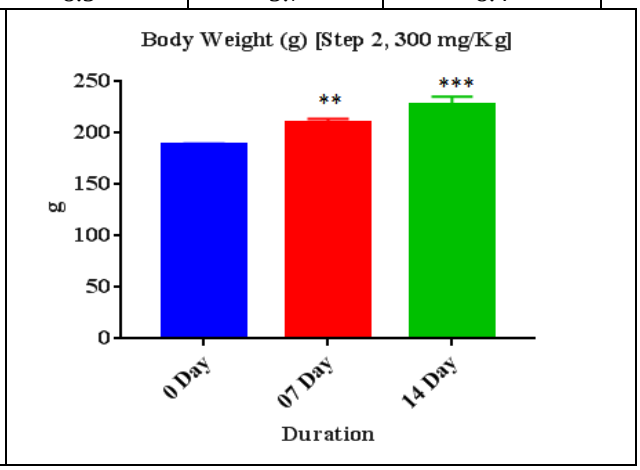
**Table No. 5: Step 3 - Administration of *Ras sindoor* sampleAgad-B in the dose – 2000 mg/kg.**

| Group | Animal No | Sex | 0 Day – Body weight | 7th Day – Body weight | 14th Day – Body weight |
|---|---|---|---|---|---|
| *Ras sindoor* sample Agad-B | 1 | F | 195.0 | 217.5 | 227.0 |
| | 2 | F | 202.0 | 222.5 | 234.0 |
| | 3 | F | 192.0 | 215.5 | 236.5 |
| Mean | | | 196.3 | 218.5 | 232.5 |
| SD | | | 5.1 | 3.6 | 4.9 |

**Table No. 6: Step 4 - Administration of *Ras sindoor* sampleAgad-B in the dose – 2000 mg/kg.**

| Group | Animal No | Sex | 0 Day – Body weight | 7th Day - Body weight | 14th Day - Body weight |
|---|---|---|---|---|---|
| *Ras sindoor* sample Agad-B | 1 | F | 199.0 | 220.0 | 235.0 |
| | 2 | F | 207.0 | 228.0 | 250.0 |
| | 3 | F | 190.0 | 217.0 | 236.0 |
| Mean | | | 198.7 | 221.7 | 240.3 |
| SD | | | 8.5 | 5.7 | 8.4 |



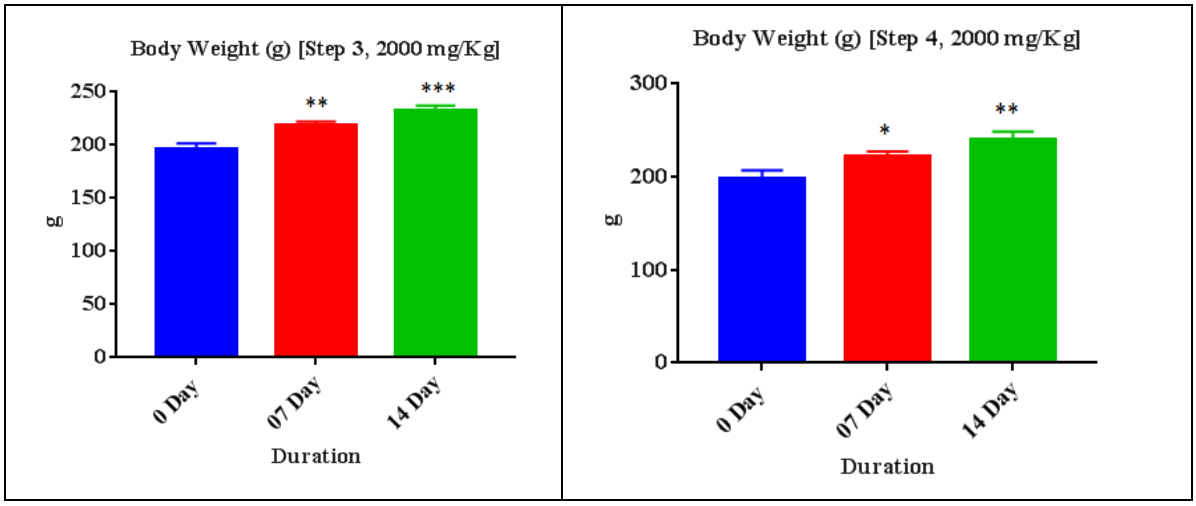



**Fig. No. 3: Effect of *Ras sindoor* sample - Agad-B on Body Weight (g) (n=3).**

**Data was expressed as mean ± SEM and was analyzed by One-way ANOVA followed by Bonferroni, ***P<0.001, **P<0.01, *P<0.05, ns= non significant.**

**Table No. 7: Stepwise and day wise changes in Mean Body weight of study animals After administration of *Ras sindoor* sample Agad-B.**

| Steps | Test drug | Study animal | Dose | Mean Body weight | | | 'P'- value |
|---|---|---|---|---|---|---|---|
| | | | | 0 day | 7th day | 14th day | |
| 1 | *Ras sindoor* sample Agad-B | 3 Female rats | 300 mg/kg | 204.5 | 214.8 | 222.8 | 14th day - **<0.05** |
| 2 | | 3 Female rats | 300 mg/kg | 188.2 | 210.3 | 227.0 | 7th day : **<0.01**<br>14th Day : < **0.001** |
| 3 | | 3 Female rats | 2000 mg/kg | 196.3 | 218.5 | 232.5 | 7th day : **<0.01**<br>14th Day **<0.001** |
| 4 | | 3 Female rats | 2000 mg/kg | 198.7 | 221.7 | 240.3 | 7th day : **<0.05**<br>14th Day : **<0.01** |

**Table No. 8: Observations regarding clinical toxic Signs, mortality and pathological changes in experimental rats at necropsy.**

| Steps | Test Drug | Study Animal | Dose | Signs of toxicity | Mortality | Pathological changes at necropsy |
|---|---|---|---|---|---|---|
| 1 | *Ras sindoor* sampleAgad-B | 3 Female rats | 300 mg/kg | Absent | NIL | NAD |
| 2 | | 3 Female rats | 300 mg/kg | Absent | NIL | NAD |
| 3 | | 3 Female rats | 2000 mg/kg | Absent | NIL | NAD |
| 4 | | 3 Female rats | 2000 mg/kg | Absent | NIL | NAD |

NAD: No Abnormality Detected

Gross behavior of all the study animals was found normal during the entire study period. No any clinical sign of toxicity and mortality was observed in any animal during the course of study (14 days). No gross pathological alterations were encountered in any of the female rats during their necropsy. From these observations, it can be deduced that the LD50 value of *Ras sindoor* sample Agad-B is greater than 2000 mg/kg body weight in the albino rats.

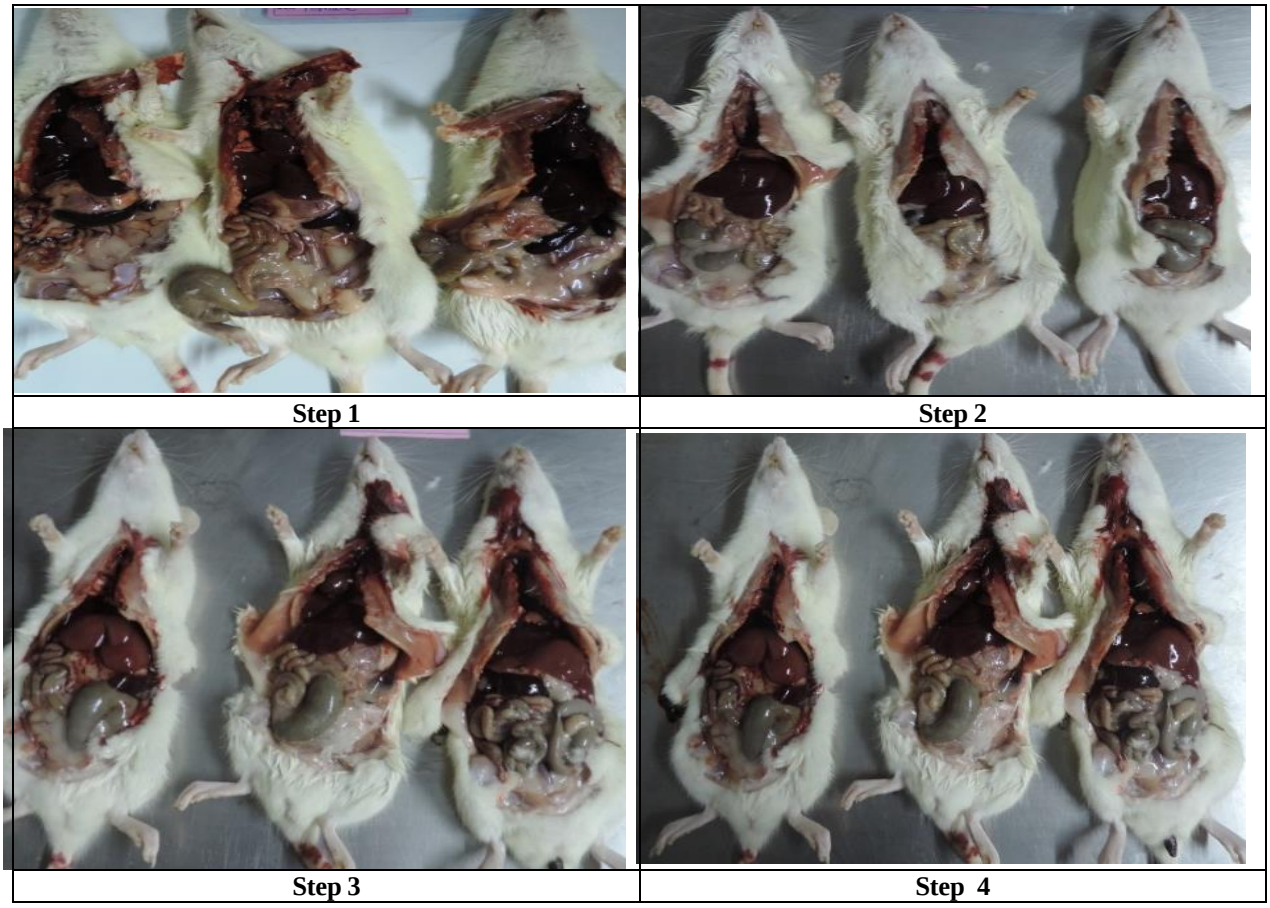



**Fig. No. 4: Photos of necropsy of experimental rats at each steps of acute toxicity study.**

## DISCUSSION

Acute oral toxicity studies are designed to determine the dose of drug that will produce the short term adverse effects or serious toxic effects or mortality and to evaluate the LD50 (lethal dose) of a test drug when administered in a single dose. LD50 is the subset of acute toxicity study. This is a standard measure of toxicity of a test drug that kills 50% of test animals upon exposure over a specified period of time. At the same time, adverse effects are observed that occur after short-term administration of single or multiple doses subjected during first 24 hours.

To evaluate the LD50 of *Ras sindoor,* Acute oral toxicity study was carried out as per the OECD - 423 guidelines at a limit dose of 2000 mg/kg. From the study it is observed that, *Ras sindoor* sample Agad- B was found to be safe as all study animals were survived for 14 days without producing any adverse effect sign of toxicity. Significant weight gain was observed in study rats in all 4 steps at the dose 300 mg/kg (for 2 groups) & 2000 mg/kg (for 2 groups) but, the gained body weight of rats were may or may not because of the treatment of *Ras sindoor*. Hence the LD50 of *Ras sindoor* sample Agad-B may be more than 2000 mg/kg and is classified in Globally Harmonized Classification System (GHS) Category 5.

## CONCLUSION

- *Ras sindoor* (sample Agad-B) was found to be safe in acute oral toxicity study at a limit dose of 2000 mg/kg body weight as per OECD guideline 423.
- From observations of acute study, it can be inferred that, LD50 value of *Ras sindoor* could be more than 2000 mg/kg body weight in the albino rats and in human beings. Hence, it is classified in Category 5 drug as per the Globally Harmonized Classification System (GHS). This level of LD50 value shows that *Ras sindoor* may not likely to produce pathological alterations at the therapeutic doses when administered in human beings for various clinical conditions.

Institutional Animal Ethical Committee (IAEC) approval – Received

Conflict of Interest – There are no conflict of interest.

As this study is the part of PhD research work registered under Maharashtra University of Health Sciences, Nashik. (M.S.)

Financial support & Sponsorship: Nil. All the expenses for the Acute toxicity study are bare by the researcher himself.

**REFERENCES**


1. Krishnamachary B, Rajendran N, Pemiah B, Krishnaswamy S, Krishnan UM, Sethuraman S, *et al.* Scientifi c validation of different purifi cation steps involved in the preparation of an Indian Ayurvedic medicine, *Lauha Bhasma*. J Ethnopharmacol, 2012; 142: 98-104.
2. Kohli KR. Ayurvedic medicines and heavy metals issue. Ayurveda Herit, 2005; 1: 5-6.
3. The Ayurvedic Formulary of India, Part-1, Edn-1, Ministry of Health and Family Planning, Govt. of India, 1978; 141-219.
4. http://apps.who.int/medicinedocs/en/d/Jh2946e/.
5. Gautam Biswas. Review of Forensic Medicine & Toxicology. The Health Sciences Publisher, New Delhi, 2015; 3: 494-496.
6. Sadanand Sharma, Rasa Tarangini, Kashinath Shastri, 11th edition, Motilal Banarsi Das publication, Varansi, 2009; 11, 135: 6, 162 – 167.
7. Rasa Hrudaya Tantra, Hindi Commentory Chaturbhuja Mishra, Chaukhamba publishers, Varanasi, 2005; 14, 2-6: 158-59.
8. Rasendra Purana, Hindi Commentory Ramaprasad Vaidya, Khemaraja ShriKrishnadas Publication, Mumbai, 2000; 3: 163-168, 89-90.
9. The Ayurvedic Formulatory of India,2nd ed. Vol.-I, Part-"B", New Delhi Department of AYUSH, Government of India, 2003; 112.
10. Sadanand Sharma, RasaTarangini, Pt. Kashinath Shastri, Motilal Banarsi Das publication, Varansi, 2009; 11, 135: 6 – 237.
11. Emma Lynch, Robin Braithwaite, A review of the clinical and toxicological aspects of traditional(herbal) medicines adulterated with heavy metals, Expert Opinion on Drug Safety, 2005; 4(4): 769-778.
12. Gogtay NJ, Bhatt HA, Dalvi SS, Kshirsagar NA, The use and safety of non-allopathic Indian medicines. Drug Saf, 2002; 25(14): 005-19.
13. Milan Dasoni 'A comparative Pharmaceutico-chemical study of Samaguna & Shadaguna Balijarita Ras sindoor with special reference to its toxicity & Bronchodilating effect';Dept. of Rasashastra, Jamnagar, 2002.
14. Sunil Kumar Singh, Anand Chadhari, DK Rai & SB Rai; 'Preparation & characterization of Mercury based Indian traditional Drug- Ras sindoor' – Indian Journal of Traditional Knowledge, 2009; 8(3): 346-351.
15. Arun A, Choudhary AK, Pharmaceutico-Analytical Profiles of Samaguna & ShadgunaBalijarita Rasasindura, M.Pharm. Thesis, Gujarat Ayurved University, Jamnagar, 2004.
16. Vyas YP, Dhundi M, Khedekar S, Patgiri BJ, Prajapati PK. 'A quality control parameters of Rasasindoora.'; International Journal of Ayurveda and Integrative Medicine, 2011; 2: 72-80.
17. Yadav P,Vyas M , Dhundi S, Khedekar S. Patgiri B.J., Prajapati P.K.;'Standard manufacturing procedure and characterisation of *Rasasindoora.'* International Journal of Ayurvedic Medicine, 2011; 2(2): 72-80.
18. Gaddamwar Shirish , Khaparde Prakash & Khiyani Rajkumar;' Physicochemical & Instrumental study of Samaguna Ras sindoor' Dept. of Rasashastra Govt.Ayurved College, Nagpur, 2012.
19. Rohit A. Gokarn, Dhiraj S. Rajput, and Biswajyoti Patgiri ;'Pharmaceutical standardization of *Samaguna Bali Jarita Rasasindura* prepared by conventional and modified method.' ;Ancient Sci ence of Life. Jan-Mar 2012; 31(3): 123–128.
20. Galib Ruknuddin; 'Review on Rasa sindhura - A Mercurial Preparation of Indian System of Medicine.'; International Journal of Pharmaceutical & Biological Archives 2012; 3(6):1360-1367.
21. Kanojia Anita, Sharma Anita & Gotecha Vinod Kumar 'Toxicological evaluation of Ras sindoor in Albino rats.' IAMJ Journal, 2013; 1, 4: 1 - 4.
22. Mogal B.B, Patkar A.H & Wadnerwar N.N, 'Analytical evaluation and Comparative assessment of three different market preparations of *Ras Sindoor* with special reference to its metallic Mercury concentration.' IJRSR, 2022; 13, 03: 496.