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PHARMACEUTICO-ANALYTICAL AND AN IN-VIVO ANTI-CANCER ACTIVITY USING RAUDRA RASA AGAINST SUBCUTANEOUSLY INDUCED EHRLICH ASCITES CARCINOMA CELLS

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ABSTRACT

Background & Objective: Cancer remains a global health challenge, with current therapies often limited. *Ayurveda* describes *Rasaushadis* as a potent mediicne effective even in small doses. *Raudra Rasa* is a a classical *Ayurvedic* formulation which is specifically mentioned under *Arbuda Chikitsa* in various *Granthas*. The present study was undertaken to evaluate *Raudra Rasa*[1] with respect to its Pharmaceutical preparation, Analytical study, Safety and Anti-cancer potential. **Methods:** *Raudra Rasa*[1] was prepared as per the classical reference. For this *Kajjali*[2] was prepared by taking *Samaguna* of *Shuddha Parada* and *Shuddha Gandhaka*. This *Kajjali*[2] was subjected to *Bhavana* with *Swarasa* of *Nagavalli, Meghanada, Punarnava* and *Gomutra-Pippali Kashaya* which was subjected to *Laghuputa*.[3] After successfully obtaining the final product, it was sent for analytical and physio-chemical analysis. **Results:** The results showed that the prepared formulation was well tolerated at therapeutic dose and showed significant improvements in hematological parameters, reduction in tumor volume, and increased survival time in Acute toxicity study and animal experimental study in EAC[4] model respectively. **Interpretation & Conclusion:** Considering all the reports it can be concluded that the study provides scientific evidence to support the classical claims of *Raudra Rasa* having potential to be prescribed in *Arbuda* condition.

KEYWORDS: *Raudra Rasa; Arbuda*; EAC study; Acute Toxicity study; Anti-cancer.

INTRODUCTION

Tumour / neoplasm is a mass of tissue formed as a result of abnormal, excessive, uncoordinated, autonomus and purposeless proliferation of cells.[5] When the tumour mass proliferate rapidly, spread throughout the body and may eventually cause death of the host, it is termed as "Cancer", which literally means crab thus reflecting the true nature of cancer since it sticks to the part stubbornly. Cancer can occur anywhere in the body most common ones being Lung cancer, Prostate cancer in men, Breast cancer in women.[6]

Cancer treatment include early detection and treatment varies on type, stage etc of cancer. The common treatment in allied sciences include Surgery, Chemotherapy, Radiotherapy, Immunotherapy etc.

Cancer research is going on in various system of medicines, one among them is Ehrlich Ascites Carcinoma Model in which there will be 100% malignancy and is referred to as an undifferentiated carcinoma & is hyperdiploid with rapid proliferation and shorter life span.

EAC resembles human tumors with respect to formation of ascites & pleural effusion. If not treated, it leads to advanced stages of cancer with severe symptoms such as abdominal distention, shortness of breath & fatigue.

Ayurveda views *Arbuda* as a result of imbalances in *Tridoshas* with predominance of *Kapha* and *Medas*, which invade *Mamsa* and *Asrk* disrupting the normal functioning of tissues and organs. The main goal of *Arbuda Chikitsa* is to restore balance and harmony within the body.

Ayurveda has explored the potential benefits of certains herbs and formulations, including *Rasaushadhis* in cancer management. One such formulation mentioned in classics for *Arbuda* is "*Raudra Rasa*".[1] It is a herbo mineral formulation prepared by subjecting *Kajjali* to sequential *Bhavana* with *Swarasas* and *Kashayas* followed by *Laghu Puta* and is indicated in *Arbuda*.

Although there is a clear mention about *Raudra Rasa* in various classics, this formulation is not much explored/utilized in *Ayurveda* as the mode of action of this formulation is unaware to us. The proficiency of this particular formulation & its effectiveness in managing above said symptoms in advanced stages need to be studied and explored inorder to make use of it for the treatment of *Arbuda*. Hence study is to be carried out to test *Raudra Rasa's* anti cancer potential on Swiss albino mice using EAC induced model and its beneficial effect in increase of survival time, decrease in proliferation of cells with reduction in the increased body weight.

MATERIALS AND METHODS

❖ **Review of Literature**

I. Disease Review

According to *Acharya Sushruta*, *Arbuda* is having *Lakshnas* such as *Vrittham, Sthiram, Manda Rujam, Mahaanta, Analpamulam, Chira Vriddhi* and *Apakam is* caused by aggravated *Doshas leading to vitiation of Mamsa producing muscular swelling. Arbuda* is of 6 types *Vataja, Pittaja, Kaphaja, Raktaja, Mamsaja and Medaja.* Among these *Raktaja* and *Mamsaja* are said to be *Asadya.*

Arbuda/ cancer which is leading cause of death was chosen for thesis because of its high mortality rate, its growing global impact and its urgent need for continued research for better results.

II. Formulation Review

Formulation - *RAUDRA RASA*[1]
Reference - *RASENDRA CHINTAMANI*
Raudra Rasa is one of the Herbo-Mineral formulation mentioned in the most of the *Rasa Granthas*under *Arbuda Chikitsa*.

Table no. 1: Reference of Raudra Rasa.

Sl. No	Texts/ Reference	Ingredients
1.	*Brihat Rasa Raja Sundhara* [7]	*Parada* - 1 part ＋ *Gandhaka* - 1 part → *Kajjali, Nagavalli Swarasa, Megahanada Swarasa, Punarnava Swarasa, Gomutra* ＋ *Pippali Kwatha→Bhavana* with each followed by *Laghu Puta*
2.	*Rasendra Sara Sangraha*[8]	
3.	*Rasendra Chintamani*[1]	
4.	*Rasa Chikitsa*[9]	
5.	*Bhaishajya Ratnavali*[10]	
6.	*Rasa Manjari*[11]	
7.	*Basavarajiyam*[12]	Addition of *Bala Swarasa* as a *Bhavana Dravya.*
8.	*Vaidhya Chintamani*[13]	

II. Drug Review

The ingredients of *Raudra Rasa* are.

Table no. 02: Ingredients of Raudra Rasa.

Sl.No	Ingredient	Botanical Name/ Chemical Name	Family	Guna-Karma
1.	*Parada*	Hydragyrum	-	*Tridosha Shamaka, Yogavahi, Rasayana, Vrushya, Balya, Vaystambaka, Pustikaraka, Vrana Shodhaka* and *Ropaka, Krimighna*
2.	*Gandhaka*	Sulphur	-	*Kaphavatahara, Pittavardhaka, Rasayana, Vrushya, Kandu, Kustha, Vishahara, Deepaka, Pacaka*.
3.	*Nagavalli*	*Piper betel* Linn.	*Piperaceae*	*Vishada, Ruchya, Teekshna, Ushna, Vrshya, Tikta, Katu, Kshara, Raktapittakara, Laghu, Balya, Kaphahara, Sramahara* etc.
4.	*Meghanada*	*Amaranthus spinosus* Linn.	Amaranthaceae	*Laghu, Sheeta, Ruksha, Pitta-Kapha - Raktajit, Srstamutra mala, Ruchya, Deepana, Vishahara, Madhura, Mada*.
5.	*Punarnava*	*Boerhavia diffusa* Linn.	Nyctaginaceae	*Tikta, Katu Vipaka, Sheeta, Laghu, Vatala, Grahanikaaraka, Madhura, Kashaya, Saraka, Ruksha, Hrdya* etc.

6.	*Gomutra*	Cow's Urine	-	*Kapha-Vata Shamaka Shoolaghna, Gulmahara, Udararogahara, Anahahara, Kandughna, Mutrarogahara, Kushtaghna, Vishaghna*
7.	*Pippali*	*Piper longum* Linn.	Piperaceae	*Vatahara, Kaphahara, Deepana, Ruchya, Rasayana, Hrdya, Vrsya, Tridosahara, Rechana*

❖ **PHARMACEUTICAL STUDY**

i. The pharmaceutical study started with the *Shodhana* of *Parada* and *Gandhaka*.
ii. *Parada Shodhana*[14] was done as per the reference from *Rasendra Sara Sangraha*. *Ashuddha Parada* was subjected to *Bhavana* with *Haridra Churna* and *Kumari Swarasa* for 24 hours followed by *Urdwapatana* for 12 hours.
iii. *Gandhaka Shodhana*[15] was done as per reference from *Rasa Ratna Sammucchaya. Ashuddha Gandhaka* was subjected to *Dhalana* in *Bringaraja Swarasa* for 7 times.
iv. *Shuddha Parada* and *Gandhaka* was subjected to *Mardhana* for 63 hours untill it attained *Kajjali Siddha Lakshanas.*[2]
v. *Kajjali* was further subjected to *Bhavana* with *Nagavalli Swarasa, Meghanada Swarasa, Punarnava Swarasa* and *Gomutra Pippali Kashaya* for 3 hours each.
vi. The *Bhavitha Kajjali* was made into *Chakrikas*, dried and placed in a *Sharava* and sealed.
vii. This *Sharava Samputa* was subjected to *Laghuputa.*[3]
viii. The prepared *Raudra Rasa*[1] was collected and weighed.

✧ Final weight of *Raudra Rasa* : 18.8 g (Yield of 1.2 %)

❖ **ACUTE TOXICITY STUDY**

PROCEDURE

a) 1 Female Swiss Albino Mouse was selected randomly and weighed (Wt - 38.38 g)

Dose Considered - 2000mg / Kg
If 2000 mg → Kg
Then for 38.38 g → ?
$2000 \times 38.38 = 76.76$ mg i.e 0.076 g
1000

Volume -
If 2000 mg → 1 ml
Then 76.76 mg → ?
$76.76 \times 1 = 0.038$ ml
2000

As the volume dose was too less, 0.1 ml was considered.

b) 0.09 g (Trial Drug) + Acacia Gum Powder + 1 ml of Distilled Water was dissolved for proper solubility of the trial drug.
c) The mouse was held in scruffing technique and was administered with the prepared Trial Drug per orally. Dose - 0.3 ml.
d) As no Signs of Mortality of Morbidity was shown by the mouse afer 24 hours of dosing, Toxicity study was decided to be continued for higher dosage levels. Another mouse was selected and the same procedure was repeated.
e) Obs - Both the mice were Alive, Active with normal Behaviour, Study was further continued for higher dosage (500 mg / Kg).
f) 4 Female Swiss Albino Mouse was selected randomly, weighed and marked and same procedure repeated.
g) The mice were kept under observation for 14 days each and showed no behavioral changes, body weight changes, nature, severity or mortality.
h) Hence considering the outcome of the study the dosage required for the EAC Experimental study of the Trial Drug was decided as follows
➢ LOW DOSE - 2000 mg / Kg
➢ HIGH DOSE - 5000 mg / Kg.
i) According to the Study conducted, it was concluded that the trial drug was not toxic even on high dosage(500 mg/ Kg).
j) These decided toxicity doses were converted into Therapeutic dose and used for the experimental study.

❖ **ANIMAL EXPERIMENTAL STUDY[4]**

Animal - Mice
Strain - Swiss Albino Mice
Study - Anti Cancer Activity of *Raudra Rasa*
Trial Drug - *Raudra Rasa*[1]
Standard Drug - 5-Flurouracil

PROCEDURE

1. The mice were divided into 4 groups (Cancer control, Trial Group 1, Trial Group 2, Standard group) with 6 mice in each group, marked and housed for acclimataisation providing pellet feed and purified water.
2. EAC Cancer cells which were developed in the abdominal cavity of Swiss Albino Mice was extracted via 1ml syringe.
3. This extracted cancer looked like a thick viscous liquid of creamish - white colour.
4. Each animal receives 1×10^7 cells which was adjusted to 0.2 ml using PBS solution which is to be inoculated to each mouse.
5. After scruffing and holding the mouse in position, it was inoculated with 0.2 ml of freshly extracted EAC cancer cells.
6. Haematological parameters were checked on the day of inoculation.

DAY 1 - DOSING

1. 1 mouse each from Trial group 1 and Trial group 2 were found dead after 24 hrs of cancer innoculation.
2. Dose and volume of the Trial and standrad drug to be given to the animals were calculated according the body weight and resuts of the Acute Toxicity study.
3. A dosing syringe was attached to a 1ml syringe tube.
4. After making sure to eliminate all air bubbles, the drug was taken in the dosing tube.
5. As per the dose calculated, the required amount of drug was taken and fed to the mice.
6. After feeding the mice were caged again and left undisturbed.

DOSE CALCULATION

EXAMPLE

I. STANDARD DRUG - 5-FLUROURACIL.

Dose - 20mg / Kg

Volume - 2 mg / ml

1. Marking - HEAD (Wt - 36.73 g)

If 1000 g → 20 mg

Then 36.73 g → ?

$36.73 \times 20 = 0.73$ mg (drug dosage)

1000

Volume =

If 2mg → 1 ml

Then 0.73 mg → ?

$0.73 \times 1 = 0.365$ ml. (Volume)

2

- As per the dose calculated, the required amount of drug was taken and fed to all the mice for 14 days.
- Haematological parameters were checked on 7th and 14th day. Animals were weighed and dose calculated and changed on 7th and 14th day too.
- Mortality rate of mice was high due to various reasons like inter-animal agression, tumour burden etc.
- Cancer cells were extracted via 1 ml syringe and measured for the volume from all the alive animals on the 14th day.
- The died mice from cancer control group was dissected to visualise the tumour.
- On dissection, the tumour was found to be liquid in nature and no solid mass was found.
- The liquid was collected and measured.
- Animals survived at the end of study - 2 from trial group 1, 1 from trial group 2.

DISCUSSION

The raw drugs needed for the study was procured from Amrit Kesari Depot, Bengaluru and authenticated from *Rasashastra and Bhaishajya Kalpana* Department of Sri Sri College of Ayurvedic Science and Research (SSCASR), Bengaluru. The *Bhavana Dravyas* were freshly collected from Garden of SSCASR and authenticated from *Dravyaguna* department.

The Pharmaceutical Study was carried out in the Teaching Pharmacy of Department of *Rasashastra and Bhaishajya Kalpana*, SSCASR. Analyticals tests were conducted in Poornayu Research Centre, Bengaluru. Animal Experimental study was carried out in Acharya and B.M Reddy College of Pharmacy after clearance of the Animal Ethical Committee (Ref: IAEC/ABMRCP/2024-2025/32).

ANALYTICAL STUDY OF RAUDRA RASA

1. ORGANOLEPTIC PARAMETERS

a) Colour - Jet Black
b) Odour - No specific odour appreciated
c) Taste - No specific taste appreciated
d) Appearance / Touch - *Bhasma* form (fine powder)

BHASMA PAREEKSHAS

Table no. 03: *Bhasma Pareekshas* of *Raudra Rasa.*

Sl.No	*Bhasma Pareeksha*	Result	Interpretaion
1.	*Rekhapurnatva*	Passed	Indicates its fineness and reduced particle Size
2.	*Slakshnatva*	Passed	Fineness and uniform distribution of particles.
3.	*Varitara*	Floated	Lightness and fineness due to expanded surface area.
4.	*Unnama*	Floated	Extreme fineness and lightness of *Bhasma*
5.	*Nischandratva*	Positive	Absence of free metal or mineral

Table no. 04': Analytical Study Results.

Sl.No	Analytical Study	Result	Interpretaion
1)	pH Value	9.52	Mildly alkaline nature
2)	Loss on Drying	1.6182%	Very low moisture content
3)	Acid Value	7.692	Presence of a small proportion of free acidic constituents
4)	Ash value	48.621%	High proportion of inorganic mineral matter
5)	Acid Insoluble Ash	2.453%	Small fraction of inorganic matter
6)	Water Soluble Ash	3.394%	proportion of inorganic salts soluble in water

INSTRUMENTAL ANALYSIS

❖ **SEM-EDX**

The images shows the size of both nanometer and micrometer.

The photos as observed is having particles crystalline nature, equant shaped segregated particles which are radically symmetrical.

The SEM analysis of *Raudra Rasa* at varying magnifications (1500× to 4000×) revealed a heterogeneous microstructure composed of both nano-sized particles (as small as ~333–378 nm) and micron-sized particles (up to ~1.6 µm), aggregated into larger porous clusters.

The particles exhibited irregular shapes, with some showing smooth, dense surfaces while others displayed rough, flaky, or layered textures. This variation in morphology and size indicates thorough incineration (puta) and trituration (bhavana) processes, resulting in ultrafine particles with high surface area.

The porous architecture and micro–nano distribution are significant, as they can enhance dissolution, bioavailability, and cellular uptake of active constituents. Such structural characteristics are particularly relevant to the therapeutic efficacy of *Raudra Rasa*, as they may facilitate improved tissue penetration and sustained release of active components, thereby supporting its potential anticancer activity.

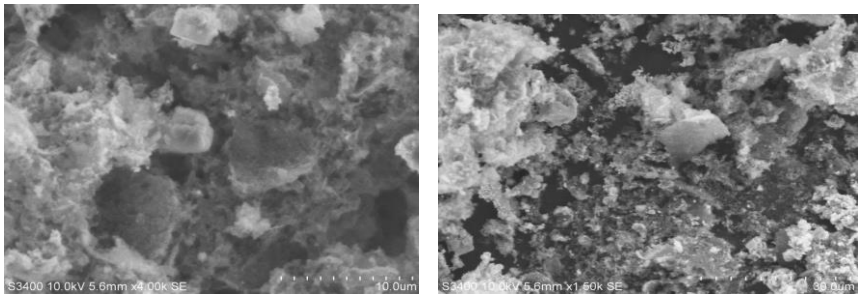


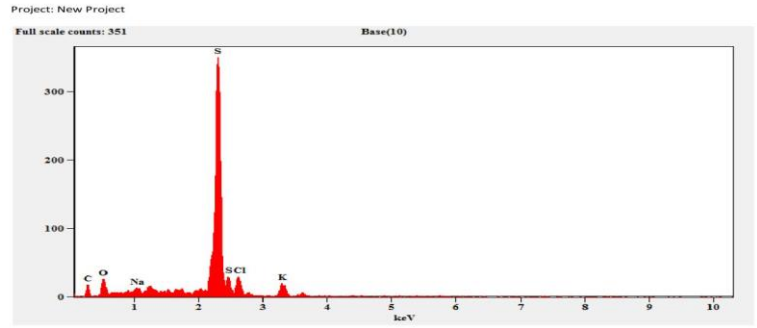
Fig 01: SEM Images of Raudra Rasa.

❖ **EDS/EDX**

The major peak corresponds to sulfur (S), indicating it is the most abundant element present. Smaller peaks for carbon (C), oxygen (O), sodium (Na), chlorine (Cl), and potassium (K) suggest the presence of organic matter, oxides, and possible salt or mineral components.

Sulphur being the major component in the given sample with a % of 37.40 indicating the given research sample has highest amount of sulphur followed by carbon (36.70%), oxygen (15.4%). Residual amount of chloride (5.08%), Potassium (4.22%) and sodium (1.19%) was also identified in the given research sample.

Sulphur-containing formulations are known for their detoxifying, antimicrobial, and cytotoxic properties, which can inhibit tumor growth. The presence of elements like potassium and sodium can help maintain cellular electrolyte balance, supporting systemic health during treatment. In the context of cancer medicine, a sulfur-dominant composition suggests the formulation may exert its effect through oxidative stress modulation, apoptosis induction in malignant cells, and immune stimulation.



Quantitative Results for: Base(10)

Element Line	Weight %	Weight % Error	Atom %
C K	36.70	± 3.67	55.68
O K	15.40	± 2.75	17.54
Na K	1.19	± 0.36	0.94
S K	37.40	± 0.96	21.26
S L	---	---	---
Cl K	5.08	± 0.42	2.61
Cl L	---	---	---
K K	4.22	± 0.30	1.97
K L	---	---	---
Total	100.00		100.00

Fig 02: EDX image of Raudra Rasa.

❖ **FTIR/ATR- Bruker II Alpha**

The FTIR spectrum of *Raudra Rasa* shows prominent peaks corresponding to both organic and inorganic components, indicating successful integration of herbal and mineral ingredients. Broad O–H stretching (~3400 cm^{-1}) and aromatic C=C bands (~1600–1500 cm^{-1}) reflect the presence of phytoconstituents from *Bhavana Dravyas*, while sharp peaks in the lower wavenumber region (894–535 cm^{-1}) correspond to metal–oxygen and metal–sulphur bonds typical of a *Rasaushadhi* minerals.

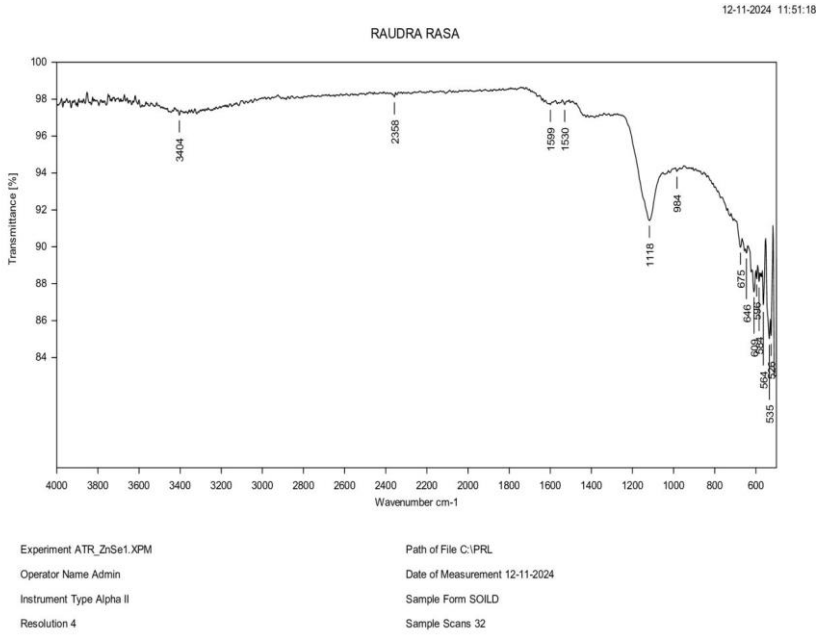


Fig 03: FTIR Image of Raudra Rasa.

RESULTS OF ANIMAL EXPERIMENTAL STUDY

In the present study, hematological parameters, tumor volume, and survival rates were evaluated on Day 14 to assess the therapeutic efficacy of *Raudra Rasa* in EAC-induced mice compared with control and standard treatment groups.

Table no. 05: Comparison between Study Groups on Day 14.

Parameters	Control group	Trial Group 1	Trial group 2	Standard Group
Body Weight	46.54± 0.001	41.21± 0.21	37.33±0.11	23.1± 0.011
RBC	5.72±0.01	10.19±0.69	11.36±0.01	10.5±0.01
WBC	28.6±0.77	10.8±0.3	9±0.01	10.5±0.001
Platelet	255±10.6	1119±100	1892±122	1255±10
Haemoglobin	8.7±0.01	16.05±0.8	18.8±0.01	17.7±0.02
Tumor Volume	2.2 ml	0.7 ml	0.6 ml	0.05 ml
Life Span	8 days	10 days	11 days	10 days

The comparative analysis of study groups on Day 14 in the EAC-induced mice model shows significant variations in hematological and tumor parameters.

The control group exhibited high tumor volume (2.2 ml), elevated WBC count, reduced RBC and haemoglobin levels, and a short lifespan (8 days), indicating aggressive tumor progression.

Both trial groups treated with *Raudra Rasa* demonstrated reduced tumor volumes (0.7 ml and 0.6 ml, respectively), improved RBC and haemoglobin levels, and extended lifespan (10–11 days), suggesting suppression of tumor growth and partial restoration of hematological balance.

The standard group showed the greatest tumor reduction (0.05 ml) with notable improvement in hematological parameters, serving as a positive reference.

Overall, the data suggest that *Raudra Rasa* exerts measurable anticancer activity in EAC, though slightly less potent than the standard drug.

Reasoning

The observed changes in hematological parameters, tumor volume, and lifespan could be attributed to the cytotoxic and tumor growth–inhibiting properties of *Raudra Rasa*.

Its herbo-mineral constituents, processed through *Shodhana* and *Bhavana*, may enhance bioavailability and facilitate targeted action at the cellular level, leading to apoptosis induction, inhibition of angiogenesis, and suppression of tumor cell proliferation.

Additionally, the antioxidant and immunomodulatory effects of the formulation could help restore RBC, platelet, and haemoglobin levels by reducing oxidative damage and improving bone marrow function. Together, these mechanisms likely contributed to reduced tumor burden and improved survival in the treated groups.

Discussion on the parameters considered for study

The selected parameters—RBC, WBC, Haemoglobin, Body Weight, Platelet Count, Tumor Volume and Survival Time—provided a comprehensive assessment of the therapeutic response to *Raudra Rasa* in EAC-induced mice.

- **RBC Count** – EAC progression often leads to anemia due to bone marrow suppression, hemolysis, and nutritional depletion. Restoration or maintenance of RBC levels in treated groups indicated hematopoietic protection and reduced tumor induced destruction.
- **WBC Count** – Tumor growth and associated inflammatory responses can elevate WBC counts. Observing WBC trends helped evaluate whether *Raudra Rasa* controlled tumor-related inflammation without causing significant immunosuppression.
- **Haemoglobin** – A direct measure of oxygen-carrying capacity, haemoglobin levels typically fall in advanced cancer due to anemia of chronic disease and nutritional deficits. Improvement in treated groups indicated mitigation of tumor-related anemia.
- **Body Weight** – In ascitic tumor models, body weight changes reflect both tumor burden (increased due to ascitic fluid) and general health status (decreased in cachexia). Reduction in excessive weight gain from ascites in treated groups suggested tumor suppression, while prevention of severe weight loss indicated improved systemic health.
- **Platelet Count** – Thrombocytopenia is common in advanced cancer due to marrow infiltration or drug toxicity. Preservation of platelet levels in treatment groups implied bone marrow protection and lower systemic toxicity.
- **Tumor Volume** – A direct indicator of anticancer activity, tumor volume assessment quantified the efficacy of *Raudra Rasa* in restricting malignant cell proliferation.
- **Survival Time** – Prolongation of survival in treated mice indicated that the formulation not only slowed tumor progression but also improved overall physiological resilience.

Collectively these parameters showed assesment of both Anti-cancer activity and Systemic safety, strengthening the evidence for *Raudra Rasa* as a potential supportive therapy in Cancer management.

Fig 04 - Raudra Rasa.

Fig 05, 06 - Bhasma Pareekshas of Raudra Rasa.

Fig 07 - Feeding trial drug to cancer infected mouse.
Fig 08 - Collecting blood from retro orbital plexus.
Fig 09 - Extracting tumour from the peritoneal cavity of cancer infected mouse.

CONCLUSION

Raudra Rasa[1] contains potent mineral preparations processed with specific herbal media, which are believed to possess properties like *Deepana, Pachana, Rasayana and Shothahara* properties - all releveant in managing cancer pathology as explained in *Ayurveda* terms.

Bhasma Pareekshas and organoleptic study conducted showed perfect *Paka* of *Bhasma.* Analytical evaluations including physio-chemical tests, SEM-EDX and FTIR confirmed the presence of desired characters thereby supporting the authenticity and quality of the prepared formulation.

The Acute Toxicity study demonstrated that the prepared *Raudra Rasa*[1] was well tolerated at therapeutic doses with no significant toxic symptoms, thereby validating its safety.

The animal experimental study conducted on the EAC[4] (Ehrlich Ascites Carcinoma) model showed significant improvements in hematological parameters, reduction in tumor volume, and increased survival time, suggesting a promising anticancer potential of the formulation. (Hence H_1 is accepted - Alternate Hypothesis)

Overall the study provides scientific evidence to support the classical claims of *Raudra Rasa*.[1] Further studies are suggested to substantiate its therapeutic application.

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