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Research Article

IN VIVO STUDY OF EFFICACY OF *TRAILOKYASUNDAR RASA* IN CHRONIC MYELOID LEUKEMIA

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ABSTRACT

Trailokyasundar rasa is one of the *Rasakalpa* which is explained in *Rasaratnasamucchaya, and is* suggestive of its efficacy in *Pandu roga. Pandu roga is* a *Pitta pradhanavyadhi* in which *Rasadhatu* and *Raktadhatu* are mainly affected. According to modern texts, all disorders of vitiated digestion, absorption and assimilation can be considered under *Pandu.* According to modern texts, all disorders of vitiated digestion, absorption and assimilation can be considered under *Pandu*. Therefore, it can be said that *Pandu* is a very broad term comprising various *Rasa* and *Raktpradoshajvikara* such as anaemia, megaloblastic anaemia, blood disorders, leukemia's etc.

Material and method: On the basis of this information animal models were planned to evaluate the effect of self-prepared test drug. Study duration -1 month.

Discussion and results: The activity of *Trailokyasundar rasa* is highly significant by reducing tumour volume and growth inhibition in animal models of chronic myeloid leukemia. On the 29thday of the experiment tumour size of the control group was 20.05 mm, comparatively in the study group, mean tumour size was of 5.26 mm. Also it prevented decrease in the platelet count and Hb% as compared to untreated mice of CML. Rate of increment of WBC's was also comparatively reduced in *Trailokyasundar rasa* treated mice.

KEYWORDS: *Rasa aushadhis, Trailokyasundar rasa, Panduroga,* Chronic Myeloid Leukemia.

INTRODUCTION

Any scientific system can be improvised and can achieve fulfillment with the help of constant research and inventions. Even in the ancient and ageless Ayurvedic classics, a strong emphasis has been given on continuous updating and revalidation of acquired knowledge. Continuous research work goes on to find out the cause and effect relationship as well as the corresponding cures on various ailments in living being.

Rasa Shastra, with its blend of mystical and medical insights, is source of valuable knowledge. Medical alchemy and mercurial medicines have been widely accepted and utilized for over a thousand years on the Indian subcontinent. *Rasa aushadhis* are appreciated for their smaller dosages, quicker effectiveness, long durability etc. Apart from their therapeutic efficacy in minute doses, *Rasa aushadhis* were found very effective for the preservation and promotion of positive health and prevention of diseases which is the primary aim of Ayurveda.

Trailokyasundar rasa is one of the *Rasakalpa* which is explained in *Rasaratnasamucchaya,* the very basic *Grantha* of *Rasashastra.* The reference of the *Trailokyasundar rasa* is suggestive of its efficacy in *Pandu roga.*[1] *Pandu roga* is one of the most conspicuous diseases as per the classics, as it encompasses a wide spectrum of pathological events and so also the symptoms. It is a *Pitta pradhana vyadhi* in which *Rasadhatu* and *Raktadhatu* are mainly affected.[2]

According to modern texts, all disorders of vitiated digestion, absorption and assimilation can be considered under *Pandu*.[3] Therefore, it can be said that *Pandu* is a very broad term comprising various *rasa* and *Raktpradoshajvikara* such as anaemia, megaloblastic anaemia, blood disorders, Leukemia's etc., Leukemia implies blood cancer and is characterised by clonal, neoplastic proliferation of immature cells of haemopoetic system, which cause aberrant or arrested differentiation.[4] Among the various types of leukemia, chronic myeloid leukemia accounts for about 2.8% of the total cancer affected population. CML is one of the commonest adult leukemia in Indian population accounting for 30% to 60% of all adult leukemias.[5]

Cancer has been recognized and characterized by the science of Ayurveda. Numerous drugs have been mentioned for the management of this condition. If evaluated methodically, they may generate some curative or supportive remedy for the sufferers of this disease.

For the rational treatment of any disease knowledge on the mode of action of a drug, its effects on various body systems and the probable adverse effects is important. Comparing out pharmacological study provides such a basis.

Thus a sincere attempt has been taken to assess efficacy of *Trailokyasundar rasa* which is exclusively mentioned in *Rasaratnasamuchhaya Pandurogadhikara*, in chronic myeloid leukemia by in vivo study, as relation between *Panduroga* and CML can be successfully interpreted.

Material and Methods

On the basis of this information, animal models were planned to evaluate the effect of test drug.

The measures of anticancer activity are primarily the:

(a) Reduction of tumour size and

(b) Increase in the life span of the mice.

In addition to the anticancer activity, the in vivo screen provides information on potential toxicities, tolerated doses and dosage regimens, and the spectrum of activity.

In vivo study material

i. SCID mice, 6–10 weeks old (Taconic Farms, mod. no. CB17SC-M) Medium 199 with Earle's salts, L-glutamine and sodium bicarbonate (Invitrogen, cat. no. 11150-059)
ii. Penicillin/streptomycin solution 100X (Sigma, cat. no. P4333)
iii. A-33 disinfectant (Ecolab Professional Products, cat. no. 61122362)
iv. 3 M Vet bond tissue adhesive (Webster Veterinary Supply, cat. no. 480200) v.70% (vol/vol) ethanol.
v. Isoflurane, USP (Baxter).
vi. Gauze squares.

Study design

i. **Study centre:** Tata memorial centre, Advanced Centre for Treatment, Research and Education in Cancer, Kharghar, Navi Mumbai.
ii. **Type of study:** In vivo efficacy testing.
iii. **Selected animal model for study:** Xenograft SCID mice.
iv. **Total no of SCID mice:** total 12 SCID mice were selected for study. 6 mice for each induced selected chronic myeloid leukemia Cells-K562. These 12 mice were divided into 2 groups.
v. **Skeleton of study**

Groups: 2 groups each consisting of 6 SCID mice of both sex.

a) Study group -The test drug i.e. TSR was administered.

b) Placebo group-No drug was given.

vi. **Dose Calculation**

Suitable mouse dose was calculated by referring to the Paget and Barnes table.

= Human adult dose x body surface area ratio convertible factor

= 375 mg x 0.0026

= 0.975/20 g body weight of mouse

= 0.975 x 50 (converting to mg/kg body weight by multiplying with suitable factor 50)

= **48.75 mg/kg body weight of mouse**

vii. **Dose of TSR (*Trailokysundar rasa*)**

48.75mg/kg weight of mouse.

viii. **Tumour model: Human Xenograft** K562–Chronic Myeloid Leukemia.

ix. **Tumour size checking:** Daily by digital Vernier caliper.

x. **Total duration of the Study:** 1 month.

xi. **Parameters for efficacy assessment:** Tumour volume, weight loss and mortality of mice.

Methods: Animal models preparation

Steps 1: Preparation of cell lines

Harvest cells were grown in monolayer culture during the exponential growth phase using trypsin or appropriate enzymes for the specific cell line. Suspend cells in medium plus serum. Quantitated the number of cells present using a haemocytometer. Cells were centrifuged at 225g and 20°C for 5 min. the medium was removed and resuspended the cells in medium without serum at a concentration of 1 × 108 cells per ml of medium.

Steps 2: Tumour Transplantation

i) Anaesthesia was given to mice. After the mouse reached sufficient aesthetic depth, it was removed from the chamber and placed (ventral side down) in a properly sized nose cone.

ii) Using gauze square saturated with 70% (vol/vol) ethanol, wiped the area from the mid-spine to the base of the tail to prepare for the incision.

iii) Made a small, horizontal incision of 5mm in length, 10mm above the base of the tail, using small surgical scissors.

iv) The tip of the scissors is inserted into the incision, directly over the flank, and opened the scissors to introduce a pocket in the subcutaneous space.

v) Inserted one individual piece of tumour into the pocket created using forceps.

vi) While the pocket was still open, placed one drop of 100X penicillin/streptomycin solution into the opening.

vii) Closed the incision site with one drop of Vet bond tissue adhesive. Held the tissue together for 3–5sec with forceps to allow for drying.

viii) Then the cell suspension was Agitate to prevent the cells from settling, and withdrew from the sterile tube into a 1-cc TB syringe with the needle removed.

ix) Skin of the mouse was lifted to separate it from the underlying muscle and injected with a 21G needle, 0.1ml of the cell suspension (1 × 107cells) subcutaneously.

x) Animal was placed in a clean cage and observed for 10–15 min to ensure recovery from the anesthetic.

Preclinical study method

Tumour transplantation: 21/10/2015

Experiment started: 09/11/2015

Experiment ended: 08/12/2015

The xenograft model mice were prepared

Total 12 severe combined immune defiant SCID model mice were taken for study.

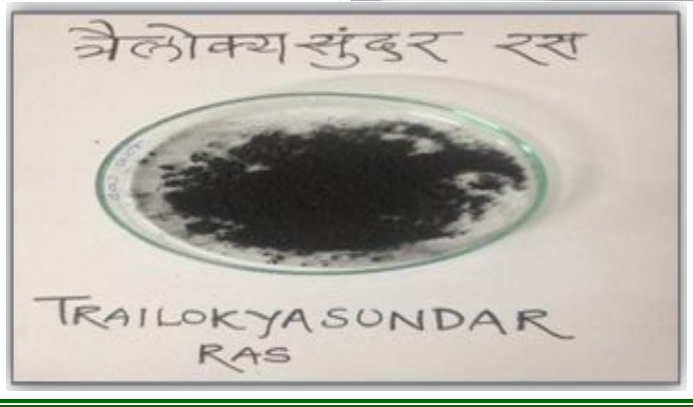
Step I- The animals were divided into 4 groups, each group consisting of 6 animals.

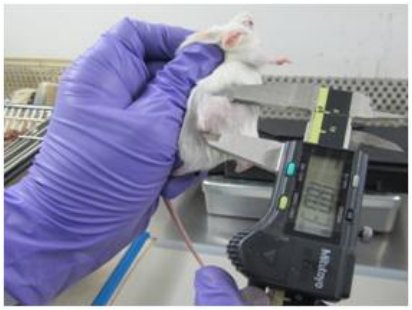
Step II- One group was kept as control and the other groups were kept as treated groups. In each group 6 rats were taken and weighed (B.T.) and numbering was done.

Step III- Drug dose for human was fixed and the dose was converted from human dose to animal dose. Oral dose of *Trailokyasundar rasa* was given to study group with the help of distilled water daily. For control group only distilled water was administered.

Step IV- The animals were carefully observed daily for tumour size and any overt changes.

Step V -Drug suspension was given for 30 days. On the completion of the experiment, blood samples of mice of control group, study group and normal SCID mice i.e. of negative control group were collected for conducting haematological study like Hb%, total WBC count and Platelet count.



Observations and Results

Table II.1: Tumour volume of control group

Week	Days	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6
0.00	1	0.08	0.09	0.10	0.12	0.10	0.15
0.71	5	0.20	0.18	0.13	0.26	0.18	0.23
1.29	9	0.29	0.39	0.19	0.61	0.11	0.24
1.71	12	0.46	0.36	0.27	1.68	0.31	0.36
2.14	15	0.74	0.81	0.30	2.12	0.59	0.56
2.57	18	0.87	1.14	0.31	2.59	0.86	0.77
3.00	21	1.29	1.45	0.38	3.53	0.87	0.74
3.57	25	1.92	2.01	0.48	4.21	1.22	1.10
4.14	29	2.10	2.28	0.58	4.77	1.45	1.37

Table II.2: Tumour Volume Study group

Week	Days	Mouse 1	Mouse 2	Mouse 3	Mouse 4	Mouse 5	Mouse 6
0.00	1	0.05	0.09	0.07	0.07	0.05	0.09
0.71	5	0.05	0.09	0.07	0.07	0.05	0.09
1.29	9	0.04	0.08	0.07	0.07	0.05	0.08
1.71	12	0.03	0.07	0.07	0.06	0.04	0.04
2.14	15	0.05	0.08	0.14	0.18	0.05	0.08
2.57	18	0.11	0.06	0.22	0.19	0.07	0.08
3.00	21	0.16	0.09	0.24	0.17	0.15	0.08
3.57	25	0.29	0.18	0.36	0.31	0.24	
4.14	29	0.33	0.21	0.43	0.34	0.26	

Table II.3: Relative Tumour Volume

Weeks	Days	Control group	Study group
0.00	1	1.00	1.00
0.71	5	1.86	0.98
1.29	9	2.95	0.92
1.71	12	5.29	0.80
2.14	15	8.07	1.45
2.57	18	10.30	1.87
3.00	21	13.12	2.45
3.57	25	17.58	4.65
4.14	29	20.05	5.26

Table II.4: Percentage of survival

Weeks	Days	A	B
0.00	1	100	100
0.71	5	100	100
1.29	9	100	100
1.71	12	100	100
2.14	15	100	100
2.57	18	100	100
3.00	21	100	100
3.57	25	100	83.3
4.14	29	100	83.3

Table II.5: Animal body weight

Weeks	Days	Control group	Study group
0.0	1	22.0	22.4
0.7	5	22.1	22.2
1.3	9	22.3	22.1
1.7	12	22.0	22.3
2.1	15	21.5	21.9
2.6	18	20.7	21.7
3.0	21	20.1	21.2
3.6	25	20.2	18.2
4.1	29	20.4	21.7

Table II.6: T/C from RTV Data

Weeks	Days	A /B
0.00	1	1.00
0.71	5	0.53
1.29	9	0.31
1.71	12	0.15
2.14	15	0.18
2.57	18	0.18
3.00	21	0.19
3.57	25	0.26
4.14	29	0.26

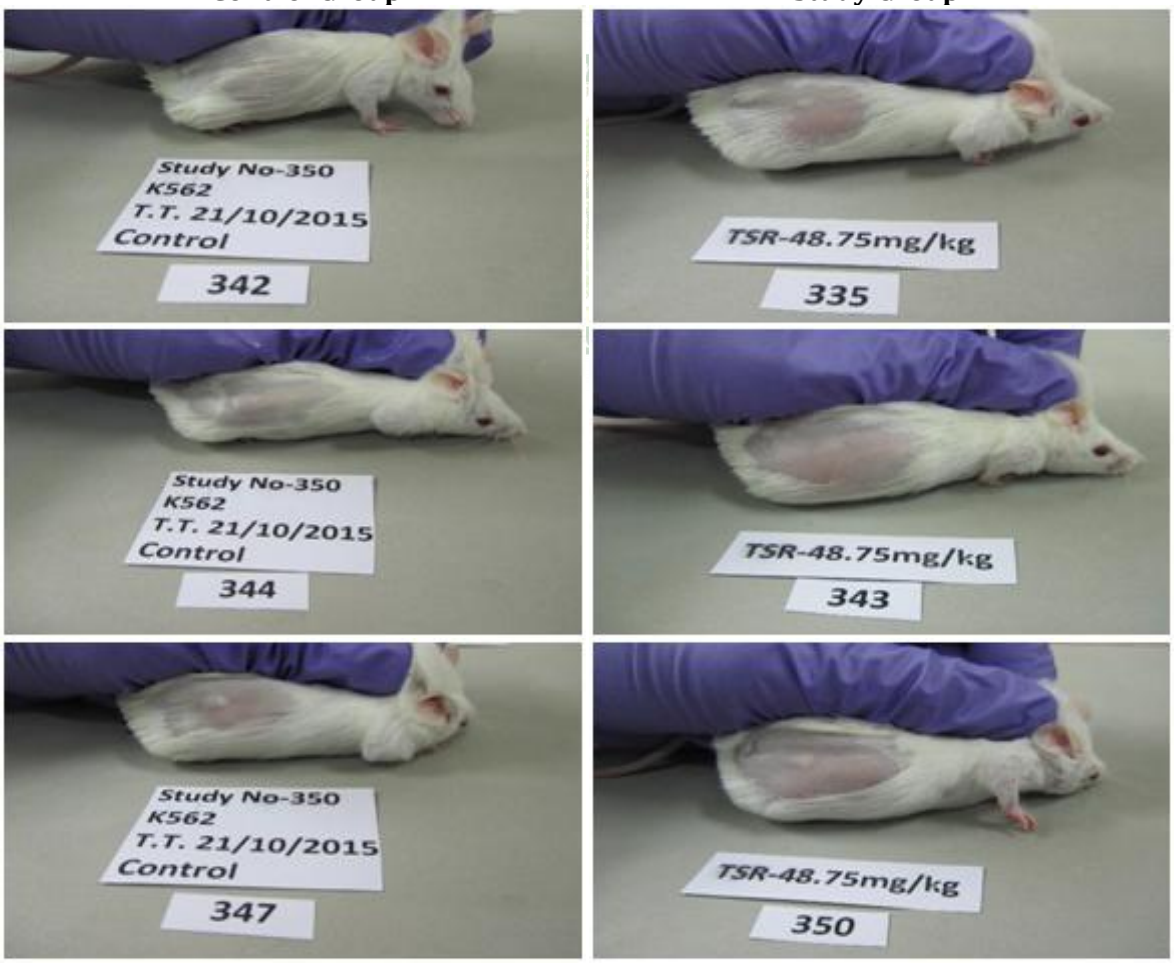
Activity Criteria: T/C ≤ 0.2 is considered to demonstrate significant activity. T/C ≤ 0.42 is considered to demonstrate moderate activity.

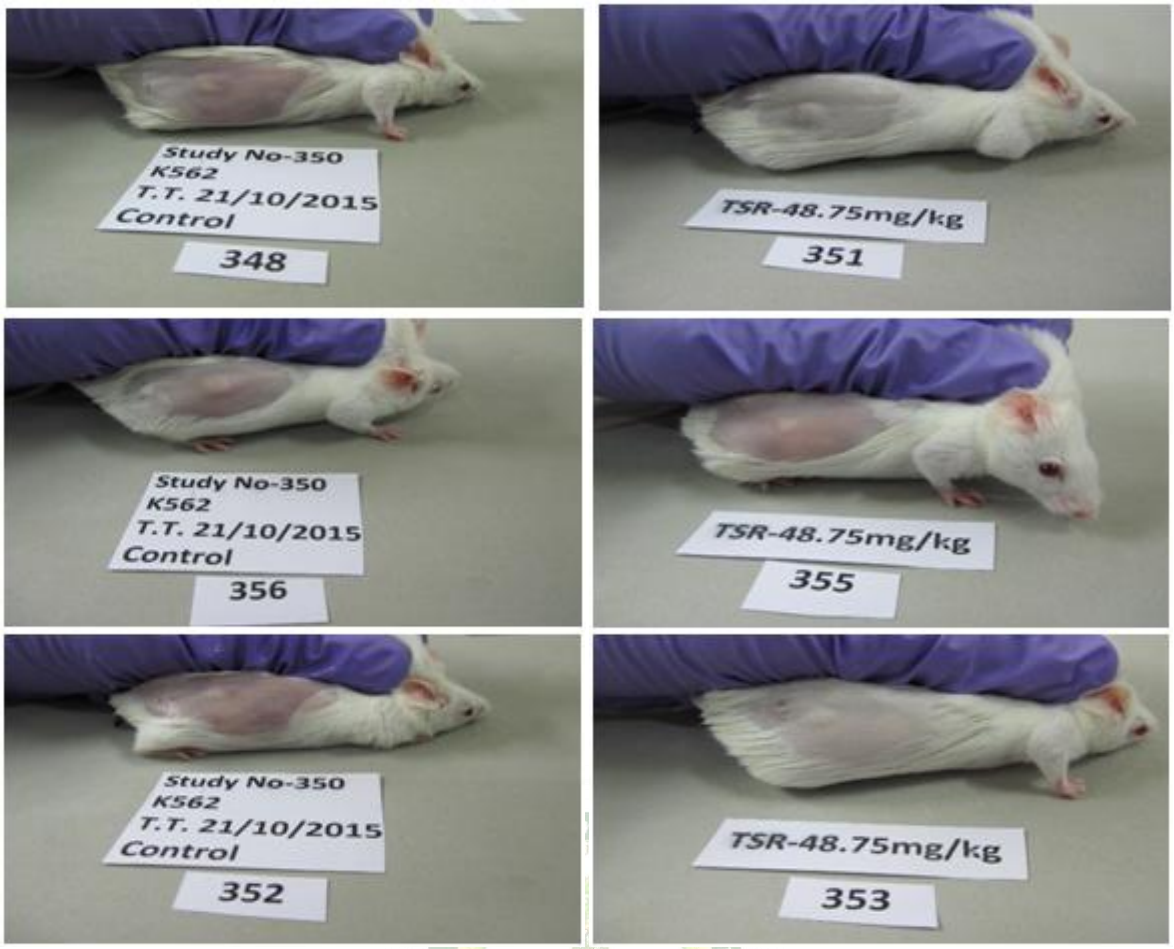
Table II 6: Haematological Parameters

Normal SCID mice		Control group	Study group
Haemoglobin %	15.4	14.3	**14.8**
Total WBC count/cm3	1900	5800	**5100**
Platelet count (Lakhs/mm3)	5.76	4.10	**5.27**

1st day of the experimental study

Control Group **Study Group**



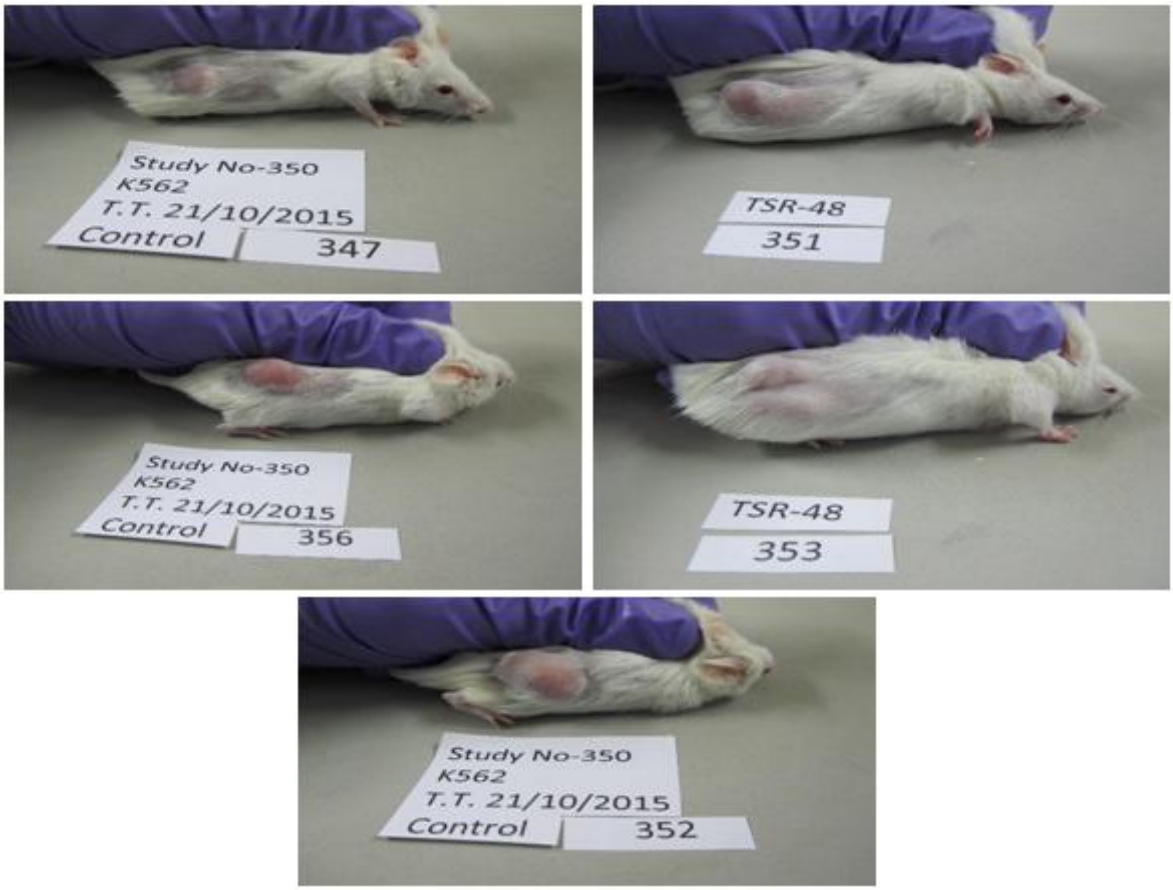


15th day of the experimental study

Control Group **Study Group**

Last day of the experimental study

Study Group **Control Group**



DISCUSSION

Animal models have been used as the front line in predicting efficacy and finding toxicities for cancer chemotherapeutic agents before entering the clinic. This has resulted in the developing various in vivo models exhibiting the traits of neoplastic diseases.

Discovery of nude athymic mice that were T-cell deficient[6] and later B-cell-deficient and T-cell-deficient severe combined immunodeficient (SCID/SCID) mice allowed the routine and efficient transplantation and propagation of human tumour tissues (xenografts) in mice. These mouse strains allow pre cultured in vitro human cell lines to be propagated subcutaneously, reconstituting solid tumours. Human tumour tissue explants obtained from biopsy or autopsy could be transplanted directly into these strains of mice.

Relative tumour volume

In K562 cancer cell line study 12 xenograft model mice were taken out of which 6 were in control group, 6 in study group. In control group and study group, the tumour size was observed 4-5mm at 7 days after tumour transplantation. On first day of the study, for ease of calculation, tumour size of all the mice was taken to be 1 mm.

The test drug was administered orally, daily for up to one month to the study group. On fifth day of the study, increase in mean tumour size of mice in control group was observed from 1mm to 1.86mm. whereas in mice of study group the tumour size reduction was notable. Tumour size was reduced from 1mm to 0.98mm and further reduction in tumour size was seen up to 12th day of the study. Tumour was reduced up to 0.80mm. concurrently in control group, significant increase in size was observed i.e. 5.29mm on 12th day. Progressive increase in the tumour size of control group was observed. On the 29th day of the experiment tumour size of the control group was 20.05mm. comparatively in the study group, mean tumour size was of 5.26mm. (Table II.3)

In chart no II.3, the difference between relative tumour volumes of both the groups was found to be significant.

Animal body weight and survival

No noticeable change in body weight of mice in both the groups (i.e. control group and study group) was observed. 100% survival was seen in control group whereas due to death of one mouse on 25th day of the study, the survival rate in study group was observed to be 83.3% (Table II.4 and Table II.5). Cause of the death of the single mouse in study group remained unidentified.

Haematological investigations

Blood samples of SCID mice were collected on 29th day of the study. For comparison blood samples

from normal SCID mice i.e. negative control were also collected. Haemoglobin percentage, total WBC count and platelet count were calculated.

Significant fall in the haemoglobin percentage of control group was observed as compared to that of normal mice. In the mice of study group, Hb was 14.8% which is closer to that of normal standard value in SCID mice. (Chart no.II.)

Conspicuous increase in WBC count of the control group i.e. up to 5800, shows prognosis of CML in mice, as WBC count of normal SCID mice was 1900. It was slightly controlled in study group mice as the WBC count was 5100. (Chart no.II.7.2)

Platelet count of normal SCID mice was 5.75 lakhs/mm^3, whereas it was reduced in control group mice. Platelet count was 4.10 lakhs/mm^3 in control group and in study group, it was maintained at 5.27 lakhs/mm^3. (Chart no.II.7.3)

Hb, WBC and platelet count demonstrates that prognosis of CML was controlled in the drug treated group as compared to mice in control group.

T/C values from RTV data

As per the international norms of cancer research, if the treatment/control from RTV data is ≤0.2 then the activity of drug or element is considered as highly significant and if it is > 0.2 to ≤ 0.4 then is considered to demonstrate moderate activity. In Table no.II.6, the highlighted area shows T/C value from RTV to be below 0.2. So it is highly suggestive of significant activity of TSR on CML induced Xenograft SCID mice. This is in accordance with the criteria for assessment of herbo-mineral compound. For herbal drug it is T/C ≤ 0.4. But, herbo-mineral drug formulation there is no such criteria available yet. So in the current study, results were determined as per the norms for chemical element.

From the obtained results it can be said that, the activity of *Trailokyasundar rasa* is highly significant by reducing tumour volume and growth inhibition in animal models of chronic myeloid leukemia. Also it prevented decrease in the platelet count and Hb% as compared to untreated mice of CML. Rate of increment of WBC's was also comparatively reduced in *Trailokyasundar rasa* treated mice.

CONCLUSION

Trailokyasundar rasa is found highly significant in reducing tumour volume and inhibiting the growth of CML cells. Also it restores the haematological components as compared to control group.

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