Anti-cancer Activity and Toxicity of Ayurvedic Compound W.S.R to Myeloid Leukaemia-In Vivo Study

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ABSTRACT

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. Cancer is a leading cause of death and disability globally, impacting more than 14 million people each year. Leukaemia is a type of cancer of blood which is caused by the rapid production of abnormal WBC. In Ayurveda the reference of the cancer and blood cancer found indirectly under the heading of **arbuda** and **rakta arbuda** respectively. General leukemia’s are classified on the basis of cell type predominately involved, into myeloid and lymphoid, and on the basis of natural history of the disease into acute and chronic.

In Ayurveda there are so many herbo-minerals drugs have described for cancer. The study herbominerals drugs has prepared by using purified Arsenic, *Vinca rosea* and Urgenia indica to study in vivo myeloid leukaemic activity and toxicity. This study was conducted according to OECD guidelines 423 and the anti leukaemic activity was done by benzene induced myeloid leukaemia in albino mice after animal ethical clearance. The highest dose of the test drug (2000mg/kg) in acute toxicity study shows minimal adverse effect of toxicity on liver and no adverse effect was found kidney and spleen. The effect of study drug shows good anti myeloid leukaemic activity though standard drug was found better than study drug. Overall study was found safe and effective on myeloid leukaemia.

Keywords: **Rakta Arbuda**, Leukaemia, Myelocytic leukaemia

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Introduction

Cancer is when abnormal cells divide in an uncontrolled way. Some cancer may eventually spread into other tissues.[1] Leukaemia is a cancer which starts in blood forming tissue, usually the bone marrow. It leads to the over – production of abnormal WBC, the part of the immune system which defends the body against infection[2]
Leukemia accounts for 4% of all cancer deaths. Leukemia was the 12th most common class of neoplastic disease, and the 11th most common cause of cancer-related death. AML accounts for approximately 20% of acute leukemia in children and 80% of acute leukemia in adults. AML is the second most common leukemia in children. Acute myeloid leukemia (AML) an annual incidence in India varying from 0.9 to 1.5 per 100,000 children the annual incidence of CML in India was originally reported to be 0.8 to 2.2 per 100,000 populations. A recent study from the Mumbai Cancer Registry specifically examined CML and reported an age-adjusted rate (AAR; per 100,000) of 0.71 in males and 0.53 in females. The incidence varied across age groups, with an increased incidence in older individuals.

In general leukemias are classified on the basis of cell type predominately involved, into myeloid and lymphoid and on the basis of natural history of the disease into acute and chronic. Leukemias are traditionally classified into four main groups; Acute lymphoblastic leukaemia (ALL), Acute myeloid leukaemia (AML), Chronic lymphocytic leukaemia (CLL), Chronic myeloid leukaemia (CML). Hairy cell leukaemia (HCL) is an unusual variant of lymphoid neoplasia. Acute myeloid leukaemia (AML), also known as acute non-lymphoblastic leukaemia or acute myelogenous leukaemia, is a group of different malignant disorders which is characterized by rapid growth of abnormal white blood cells and accumulation of leukaemia immature cells in the bone marrow and finally in blood stream (Smith et al., 2004).

Though the treatment of leukaemia in form of chemotherapy and radiotherapy is available in Modern science the morbidity and mortality is still high. In Ayurveda there is so many herbal, herbomineral, and mineral drugs are described for cancer (Arbuda), but there is need to evaluate the anticancer effect of such drug on scientific parameter.

Injection Arsenic tri oxide (Trisonex) is proved chemotherapeutic agent used for leukaemia. The Purified Arsenic (Sudh Sankheya) is a Sthavar dhatu visha which can be shows anti-cancer effect on leukaemia. If it used in therapeutic doses and may be shows less toxic effect than injection arsenic trioxide. The various alkaloids of Vinca rosea are using as an anti-cancerous agent in leukaemia. Hence the hypothetical Ayurvedic compound prepared from Purified Arsenic powder (Sudha Somala Bhasm), aqueous extract of Vinca rosea and Urgenia indica has selected for this Anti-cancer study W.S.R to myeloid leukaemia.

2. Material & Methods

Material: The items listed below had been taken for experimental study.

Test Sample - Preparation of test sample (Ayurvedic compound) 4 mg arsenic mixed with 10 gm of aqueous extract of Vinca rosea and 200 mg of aqueous extract of Urgenia indica were mixed properly by using pastel motar.

Chemicals - Picric acid, distilled water, Normal saline, Ethanol & Methanol, N-Butanol, Hematoxylene, Benzine.

Equipment- Polypropylene cages, Water bottle, Anesthetic chambers, Syringe, Oral feeding needle, Sterile blood sample collection vial, Weighing machine, Glass slides, Beaker and funnel, Test tubes and Dissection box.

Experimental Animal - Number: 33, Strain: Swiss Albino Mice

Feed Material and Water: Pallated feed, R.O. Water

Methods:

A) Oral acute toxicity study - Housing and feeding conditions was done according to OECD guideline 423.

Preparation of animals: The animals were randomly selected, marked with Picric acid H, B T for individual identification, and kept in their cages for at least 5 days.

Number of animals and dose levels: Three animals are used for each group. Group 1 had been received 50 mg/kg test sample, Group 2 had been received 300 mg/kg test sample and Group 3 had been received 2000 mg/kg test sample.

Administration of doses: The test substance had been
administered in a single dose by gavage using an oral feeding needle.

**Observations:** Animals were observed individually after dosing at least once during the first 4 hour, 24 hour, 30 hour, 48 hour, one week and second week. Changes in skin and fur, eyes, mucous membranes, salivation, Lethargy, sleep, coma, convulsions, tremors, diarrhoea, morbidity, mortality was observed. Pathology: All test animals (including those that die during the test or are removed from the study for animal welfare reasons) were subjected to gross necropsy. All gross pathological changes are recorded for each animal. Histopathological studies: At the end of experimental period, one animal of each group was sacrificed and observed for gross lesions of internal organs.

**B) Benzene Induced Myeloid Leukaemia In Albino Mice.**

**Test drug dose Calculation:** Dose fixation of test drug for Mice was calculated on the base of body surface area ratio by referring to table of Paget & Barnes.

- **Human dose** - Arsenic - 4 mg, Vinca rosea - 10gm, Urgenia indica-200mg
- **Human equivalent dose** = 10204mg
- **Dose of study drug in mice**
  
  Human equivalent dose x Conversion factor [0.007]
  
  $10204 \times 0.007 = 71.428mg$

- **Route of drug administration** – oral, once a day
- **Duration of administration** - 21 days
- **Housing and feeding conditions was done according to OECD guideline 423.**

**Marking of Swiss albino Mice for identification**-

The albino Swiss albino mice were marked with Picric acid in each group as Head, Back, Tail, Head and Back, Back and Tail and Head and Tail.

**3. Group design -**

12 adult Swiss albino Mice were divided into two groups having six Swiss albino mice in each. These groups received different treatment in following manner:

- **Study group 1**- In this group the myelocytic leukaemia was produced by giving carcinogenic agent and then Ayurvedic compound was given as per schedule.

- **Standard group 1**- Injection Trisonex [arsenic tri oxide] was given after developing the myelocyte leukaemia by carcinogenic agent in this group.

**Experimental procedure:**

12 adult Swiss albino mice were used to induce myelocytic leukaemia.

**Induction of myelocytic leukemia by benzene:**

12 albino Swiss mice were weighted before starting the experimental, benzene was used to induced leukemia in female Swiss albino mice by using two doses (0.2ml/kg) for (4 months) by two (I.P.) injections /week..

**Evaluation of myelocytic leukaemia after experimental study**

**Blood collection:** After the end of the experimental period the animals sacrificed and blood was collected by using of (5ml) disposable syringe, then (1ml) of blood put in EDTA tube for measuring of hematological parameters which included total white blood cells (WBC), neutrophils, basophils, lymphocytes monocytes, RBC and Hb by using of haematology analyzer.

**Collection of Bone Marrow:** Bone marrow of mice had been collected form spinal cord after anestization with ketamine and xylazine.

**Statistical analysis** - The results are expressed as mean ± SE. Comparison between before and after treatment were performed Student t test paired and in Comparison between the treatment groups and control were performed by analysis of variance (ANOVA) followed by Dunnet’s multiple test. In all tests the criterion for statistical significance was $P < 0.05$.

**4. Observation and Results**

1. **Oral Acute Toxicity study According to OECD Guideline 423**
Haematological Toxicity study of Ayurvedic compound:

Table no I: Haematological Observations on 14th day at dose 50 mg/kg, 300 mg/kg and 2000mg/kg

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Haematological Parameters</th>
<th>50 mg/kg (Mean)</th>
<th>300 mg/kg (Mean)</th>
<th>2000 mg/kg (Mean)</th>
<th>Normal Range</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>14th day</td>
<td>14th day</td>
<td>14th day</td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>Haemoglobin</td>
<td>11.87</td>
<td>12.56</td>
<td>12.96</td>
<td>11.5-16.1 grams per deciliter</td>
</tr>
<tr>
<td>2.</td>
<td>WBC</td>
<td>7.43</td>
<td>8.76</td>
<td>8.43</td>
<td>6.6-12.6 x 10^3/mm^3</td>
</tr>
<tr>
<td>3.</td>
<td>RBC</td>
<td>7.3</td>
<td>8.5</td>
<td>7.25</td>
<td>6.76-9.75 x 10^6/mm^3</td>
</tr>
<tr>
<td>4.</td>
<td>Neutrophils</td>
<td>2.57</td>
<td>5.76</td>
<td>4.46</td>
<td>1.77-3.38 x10^3/mm^3</td>
</tr>
<tr>
<td>5.</td>
<td>Lymphocytes</td>
<td>6.43</td>
<td>8.72</td>
<td>8.38</td>
<td>4.78-9.12 x 10^3/mm^3</td>
</tr>
<tr>
<td>6.</td>
<td>Eosinophils</td>
<td>0.05</td>
<td>0.08</td>
<td>0.07</td>
<td>0.03-0.08 x 10^3/mm^3</td>
</tr>
<tr>
<td>7.</td>
<td>Monocytes</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
<td>0.01-0.04 x 10^3/mm^3</td>
</tr>
<tr>
<td>8.</td>
<td>Basophil’s</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00-0.03 x 10^3/mm^3</td>
</tr>
</tbody>
</table>

Histopathology Study of Acute toxicity of Ayurvedic compound-50 mg/kg

Liver: minimal inflammatory cellular infiltration and almost near normal liver architecture

Kidney: The renal glomeruli, the proximal and with distal convoluted tubules show normal structure.

**Histopathology Study of Acute toxicity of Ayurvedic compound-50 mg/kg**

Liver: normal lobular architecture with central vein and radiating hepatic cords

Kidney: shows severe degenerative alterations in the tubules

**Histopathology Study of Acute toxicity of Ayurvedic compound-2000 mg/kg**

Liver: normal arrangement of hepatocytes with little evidence of fatty vacuoles and cellular necrosis

Kidney: The renal glomeruli (G), the proximal (X) and with distal (D) convoluted tubules show normal structure.

**Result**

- Effect of Anti-cancer drugs on hematological parameter

**Table No II: The mean WBC level before and after treatment in study and standard groups**

<table>
<thead>
<tr>
<th>Marking</th>
<th>Study group 1</th>
<th>Standard group 1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>BT</td>
<td>AT</td>
</tr>
<tr>
<td>H</td>
<td>15.2</td>
<td>14.6</td>
</tr>
<tr>
<td>B</td>
<td>16.8</td>
<td>16.5</td>
</tr>
<tr>
<td>T</td>
<td>16.1</td>
<td>15.6</td>
</tr>
<tr>
<td>HB</td>
<td>15.3</td>
<td>15.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>BT</th>
<th>15.2</th>
<th>14.9</th>
<th>15.8</th>
<th>13.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>HT</td>
<td>15.2</td>
<td>16.4</td>
<td>17.1</td>
<td>15.6</td>
<td></td>
</tr>
</tbody>
</table>

Table No III: Effect of test and standard drug on myelocytic leukaemia

<table>
<thead>
<tr>
<th>Evaluation</th>
<th>Study GP 1 Mean ± SEM</th>
<th>Standard GP 1 Mean ± SEM</th>
<th>Diff</th>
<th>Diff %</th>
<th>P value</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC Total (10³/mm³)</td>
<td>15.62±0.313</td>
<td>14.52±0.359</td>
<td>1.10</td>
<td>7.04</td>
<td>0.0437</td>
<td>Yes</td>
</tr>
<tr>
<td>Neutrophils</td>
<td>23.90±0.244</td>
<td>19.67±0.433</td>
<td>4.23</td>
<td>17.71</td>
<td>0.0001</td>
<td>Yes</td>
</tr>
<tr>
<td>Basophils</td>
<td>0.51±0.026</td>
<td>0.40±0.017</td>
<td>0.11</td>
<td>20.98</td>
<td>0.0065</td>
<td>Yes</td>
</tr>
<tr>
<td>Lymphocytes</td>
<td>71.03±2.263</td>
<td>60.64±2.135</td>
<td>10.38</td>
<td>14.62</td>
<td>0.0075</td>
<td>Yes</td>
</tr>
<tr>
<td>Monocytes</td>
<td>8.66±0.271</td>
<td>6.15±0.412</td>
<td>2.51</td>
<td>28.96</td>
<td>0.0005</td>
<td>Yes</td>
</tr>
<tr>
<td>RBC</td>
<td>5.05±0.212</td>
<td>6.15±0.129</td>
<td>1.10</td>
<td>21.84</td>
<td>0.0012</td>
<td>Yes</td>
</tr>
<tr>
<td>Hb</td>
<td>10.07±0.359</td>
<td>12.04±0.269</td>
<td>1.97</td>
<td>19.56</td>
<td>0.0013</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table no. IV: Effect of test and Standard drug on myelocytic leukaemia on Bone marrow Pattern (Study GP 1 and Standard GP 1)
5. Discussion

Cancer is a leading cause of death and disability globally, impacting more than 14 million people each year. Leukaemia accounts for 4% of all cancer deaths. The leukaemia is a group of disorders characterized by malignant transformation of blood-forming cells. Treatment of the newly diagnosed patient with AML is usually divided into two phases, induction and post remission management.[6] The Cancer and blood cancer is not new for Ayurveda and described under the heading of Arbuda and Rakta Arbuda. Most of the Acharya like Shusruta, Vagbhatt, Madhav, Bhavprakash and Yoga ratnakar has supposed six types of Arbuda including vata, pitta, kapha, raktaj, Mansaj and Medaj. According to Acharya Sushruta Vataja, Pittaja, Kaphaja, and Medoja Arbuda are Sadhya (curable), where as Raktajarbuda and Mamsarbuda are Asadhya (incurable).[7] According to Vagbhatt Dosas getting aggravated vitiated the blood present inside the veins, causing contraction, pain and ripening, produce a growth of tumour, bleeding constantly; the tumour develops fast and discharges vitiated blood in large quantities. All most all the Acharya has not mentioned the any treatment of Rakta Arbuda as its asadhya (incurable). Thus Rakta Arbuda described in Ayurveda is nearby similar with leukaemia clinically. The Ayurvedic compound was selected for these studies have total three ingredient including pure Arsenic tri oxide, Vinca rosea and Urgenia indica. Injection Arsenic tri oxide (Trisonex) is proved chemotherapic agent used for leukaemia. Hence the hypothetical Ayurvedic compound prepared from Purified Arsenic powder (Sudha Somala Bhasm), aqueous extract of Vinca rosea and Urgenia indica has selected for this Anti-cancer study W.S.R to myeloid leukaemia to evaluate the in vivo anti-cancer activity and toxicity study of Ayurvedic compound and to compare in vivo anti-cancer effect of Ayurvedic compound with trisonex injection. The entire in vivo experiments were studied in to two parts acute toxicity study and anti-cancer activity W.S.R to leukaemia. The acute toxicity of Ayurvedic compound was done according to OECD guidelines 423. The experiment was studied in three groups having three animals for each group. Group 1 had been received 50 mg/kg test sample, group 2 had been received 300 mg/kg test sample and group 3 had been received 2000 mg/kg test sample. In study group 1, six mice of myelocytic leukaemia from leukaemia model were selected and then Ayurvedic compound will be given as per schedule.

The bone marrow of experimental mice in study group 1 and standard group 1 after treatment was collected from spinal cord after anestintization with ketamine and xylazine and histopathology of bone marrow was observed for any leukaemic changes after treatment in all groups. The acute toxicity was done as per OECD guidelines 423 and the experimental mice were divided into three groups. In first group test sample was given in 50 mg/kg in second group the test dose was given in 300 mg/kg and in third group the test dose was given in 2000 mg/kg. None of the toxicity was found in group 1 (50 mg/kg). In second group of test dose 300 mg/kg and the toxicity was found
till 24 hours of observation then after 48 hours one mouse found morbid conditions which also died after 48 hours of observation. In third group of test dose 2000 mg/kg and one experimental mice was found morbid condition which also died after 48 hours of observation, whereas two experimental was found morbid and also died after one week of observation.

All gross pathological changes were recorded for each animal after sacrifices and samples were send for Histopathological studies for this purpose the organ were excised immediately after sacrificing and processing of tissue were done by using solvents after the section cutting and staining were done.

Minimal inflammatory cellular infiltration was found in histology of hepatic cells in group 1 (50 mg/kg) of acute toxicity study which shows almost near normal hepatic architecture. The renal glomeruli, the proximal and with distal convoluted tubules has shown normal structure of renal tissue in group 1 (50 mg/kg) of Acute toxicity study. Its mean that both hepatic tissue and renal tissue was safe in group 1 having dose 50 mg/kg of test drug. Normal lobular architecture with central vein and radiating hepatic cords of hepatic tissues was shows in group 2 (300 mg/kg) of acute toxicity while severe degenerative alterations in the tubules was found in group 2 (300 mg/kg) of acute toxicity of test drug. Its mean that the hepatic tissue was not found any adverse effect of hepatic toxicity while renal tissue show severe adverse effect of toxicity in this group. Little evidence of fatty vacuoles and cellular necrosis along with normal arrangement of hepatic tissue was found in group 3 (2000 mg/kg) of acute toxicity of test drugs. The renal glomeruli, the proximal and with distal convoluted tubules has shown normal structure of renal tissues in group 3 (2000 mg/kg) of acute toxicity of test drugs. Its means that highest dose of the test drug in acute toxicity study as per OECD guidelines also shows either none or minimum adverse effect of toxicity on liver and none of adverse effect toxicity in renal cell. At the highest dose (2000 mg/kg) of test drug in acute toxicity the minimum toxicity was found but in medium dose (300 mg/kg) of test drug in acute toxicity the severe degenerative changes in the renal tubules was found. It may be due to idiosyncrasy effect of test drug on those particular mice used in group 2 (300 mg/kg). Where sometimes a small or otherwise indulge dose of substance may result in severe toxicity. This phenomena may be explain as an abnormal response of the living body and it is allergic response to that particular substance with that particular living body.

The mean level of WBC has been substantially was found decreased in study group of after treatment than before treatment in each experimental mice of marked H, B, T and BT but experimental mice of marking HB and HT it is increased rather than decreased. It means that the leukaemia has been calm down in study group 1 of experimental mice of all marking except HB and HT. Among all the marking in study group 1 having positive effect on leukaemia the experimental mice marking as a H have highest result followed by experimental as T, B and BT. Though as compare to standard group 1 and anti-leukemic effect of study group 1 was found less but the test drugs shows some positive effect on leukaemia overall. The effect of standard drugs on WBC, neutrophil, monocyte and basophil was found better than the test drugs although study drug was also showed somewhat significant results. The bone marrow biopsy in myelocytic leukaemia of both test as well as standard group showed markedly hyper cellular marrow with cellularity. Myelogenous leukaemia with >25% myeloblast in the marrow and >15% mature myeloid at blast stage was seen.

6. Conclusion

Cancer is the uncontrolled growth of cells, which can invade and spread to distant sites of the body. It is a leading cause of death and disability globally, impacting more than 14 million people each year. The Cancer and blood cancer is not new for Ayurveda and described under the heading of Arbuda and Rakta Arbuda. The vitiated Dosha compressing and contracting the blood (Shonita) and blood vessels without undergoing suppuration and along with the discharge make the muscular lump prominent is called as Rakta-Arbuda. As the Rakta arbuda has included in asdhaya (incurable)
categories according to most of Acharyas including Shrusruta, Vagbhhatt, Madhav, Bhaw prakash, Yoga ratnakar, vagbhat etc. Hence, this study was done with entitle “Anti-cancer Activity and Toxicity of Ayurvedic Compound W.S.R to Myeloid Leukaemia-In Vivo Study” The Ayurvedic compound was selected for these studies containing pure Arsenic tri oxide, Vincia rosea and Urgenia indica. Injection Arsenic tri oxide (Trisonex) was selected as a standard to compare anti-cancer effect of study drug. The overall experimental study the study drugs was found safe on physiological and hematological parameter in all the doses form. The effect of Standard drugs was found just better than study drugs on leucocyte count though the Study drugs also shows perform better on leukaemia.

References

2. (https://www.leukaemiacare.org.uk/support-and-information/information-about-blood-cancer/blood-cancer-information/leukaemia/) Downloaded on 13/03/2019