In Vivo Anticancer Activity of *Cleome viscose* Linn. alcoholic extract and its fractions against Ehrlich’s Ascites Carcinoma (EAC) Cell Line

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**ABSTRACT**

The investigation aimed to evaluate the anti-cancer properties of alcoholic extracts and the fractions Cleome viscose Linn. against Ehrlich Ascites Carcinoma (EAC) cell lines in Swiss albino mice. In the case of EAC tumor, 24 hours after tumor inoculation, the extract and fractions are administered every day within 14 days. On the fifteenth day the mice were sacrificed to monitor antitumor activity. The effect of alcoholic extracts and their fraction in terms of mean survival time, percentage increase in life span, spleen weight, ascitic fluid volume, ascitic fluid volume and angiogenesis of EAC-bearing mice and simultaneous alteration in hematological profile were estimated. Alcoholic extracts and their fractures exhibited an impact on mean survival time, percentage increase in life span, spleen weight, ascitic fluid volume, ascitic fluid volume, angiogenesis and haematological parameters in EAC tumor bearing mice. Hematological profile was reverted to normal level in the extracts and their fractions treated mice. From the current study of alcoholic extracts and its fraction of *Cleome viscose* Linn. exhibited antitumor action in a dose dependent manner comparable to that of standard drug. So, the current research provides a scientific basis for the curative use of *Cleome viscose* Linn. Which are largely attributable to the improver or synergies effect of their constituents.

**Keywords:** *Cleome viscose*, Ehrlich’s ascites

**INTRODUCTION**

Natural products, especially plants, are utilized to treat various diseases for thousands of years. Land plants have been used as medicines in Egypt, China, India and Greece since ancient times and many modern medicines have been developed. The first written testimony on the use of healing plants appeared in about 2600 BC1. Cancer is one of the leading causes of death worldwide and the problem grows every day2.

Considered blood, lung cancer, breast cancer, prostate cancer, cervical cancer and cancer of the bones and cancer of the most common types of cancer worldwide and may have all these cancers are the cause of death3. It is a group of diseases caused by the loss of control of the cell cycle, which leads to natural and uncontrolled cell growth4. Is linked to the development of cancer transformation genes carcinogens (oncogenes) and types of tumor inhibition and repair genes5; it is believed both by external factors such as tobacco, chemicals, radiation and infectious organisms and internal factors such as inherited mutations, hormonal conditions risk factors of immune system responsible for or are the causes of cancer6. imposes cancer a serious burden on health and public operations therapy is still scarce in science7. The ordinary methodologies cancer treatments are chemotherapy and radiation therapy and hormone therapy and surgical procedure. However, every one of these unit of conventional treatment units has a serious impact8. The high mortality and adverse effects of anticancer agents are key factors that stimulate researchers to find new drugs at low cost and more effective9. Because of these restrictions, scientists are constantly looking for natural compounds that can cure cancer10. Many natural compounds such as terpenoids, phenolic acids, peels, tannins, flavonoids, quinones, coumarins and alkaloids have been discovered from plant sources that contain important antioxidant activities and have an important role to play in the treatment of cancer11. Natural compounds with antioxidant activity they can prevent cell proliferation directly and stimulate the immune system12.

*Cleome viscose* Linn. (Capparidaceae) is an herb distributed in the tropics and plains of the world in India. The plant is an annual sticky herb with a strong smell, which is glandular and smooth hair. It grows about 30-90 cm in height and branching. The yellow, axillary flowers grow in a slippery run. The fruit is a capsule, compressed and poetic, while the bones are full of fine smoothness, under the cartilage, and dark brown when ripe. The biography identifies some names, such as wild mustard, dog mustard and sticky flowers. In India, the plant is known by many national names such as Hul-Hul, Kanphuti, Talwani, Pivala tilvana and Pashugandha. The plant is a common solution for a variety of diseases and is stored in ethnographic reviews and common treatment schemes, for example, Ayurveda and Unani. In the wake of people's popular claims of healing various diseases, the plant is explored in science to justify its potential as a therapeutic agent. The present research is investigating in vivo anticancer activity of *Cleome viscose* Linn. This plant

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traditionally is used for cooling, the stomach, diuretics, laxatives and anthelmintic action and is also useful in the treatment of fever due to malaria and fever due to indigestion, skin diseases, leprosy, blood disorders and disorders of the uterus. The leaves are used to treat ear disorders, headaches, swellings, ulcers and ulcers. The seeds are documented as useful for worm infections, fever, diarrhea, convulsions and skin diseases. In Sri Lanka, the roots and seeds are heart stimulant and are treatment in case of snake bites. The leaves are used by Australian natives in headaches. In Israel, plants are used to treat diabetes 13. Although today we have many anti-cancer agents, there is still a lack of adequate cancer control. So, there is a constant need to develop newer and more effective anticancer drugs that will help address this problem. The main groups of anticancer drugs such as vinca alkaloids, taxanes, camptothecins and epipodophyllotoxins are currently part of many standard plant-derived utilized as anti-cancer agent 1.

Considered chemotherapy an important method of treatment for cancer, he provided some plants such as Catharanthus roseus, Podophyllum peltatum, Podophyllum emodi, Taxus brevifolia, Ochrosia elliptica and Campothecia acuminata, effective principles, which are used for the advanced control of malignant tumors in the clinical level 14,15. However, most chemical reagents exhibit severe natural toxicity and cause side effects. Many powerful drugs are expensive, mutagenic and carcinogenic. Therefore, there is a need to find alternative medicines, which are highly effective in harmlessness, cheap and available to the average person. This can be achieved by examining new molecules or plant products, which can be effective at non-toxic dose levels. In Ayurvedic treatment systems, dry powders or raw extracts are used by plants to treat various diseases including

### Table 1: Effect of different extracts/fractions of CV on body weight changes in EAC inoculated mice (Day wise) 3th, 5th, and 7th Day

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) (p.o.)</th>
<th>% Increase in Body weight as compared to day “0”</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 3</td>
</tr>
<tr>
<td>EAC Control</td>
<td>0.25% Sod. CMC</td>
<td>28.46 ± 0.21</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>3.5</td>
<td>30.91 ± 1.10</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30.64 ± 1.64</td>
</tr>
<tr>
<td>CVA</td>
<td>200</td>
<td>30.35 ± 1.54</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>29.19 ± 1.34</td>
</tr>
<tr>
<td>CVP</td>
<td>200</td>
<td>30.13 ± 1.16</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>30.61 ± 1.09</td>
</tr>
<tr>
<td>CVD</td>
<td>200</td>
<td>28.94 ± 1.48</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>29.16 ± 1.81</td>
</tr>
<tr>
<td>CVE</td>
<td>200</td>
<td>26.87 ± 1.54</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM of six samples, *p < 0.05, **p < 0.01 & ***p < 0.001, compared to EAC Control.

![Figure 1: Effect of different extracts/fractions of CV on body weight changes in EAC inoculated mice (Day wise) 3th, 5th, and 7th Day](image-url)
cancer. No disposition is attributed to a compound only influence, but also to the other components found in the extracts / fractions of crude extract. The rationale for this type of treatment is that the toxicity of the active substance can be detected elsewhere which may not have the desired therapeutic properties.

**MATERIAL AND METHODS**

**Collection of Plant Material**
The plant material (*Cleome viscose* Linn.) was collected by Smriti van Jaipur (Jaipur, Rajasthan, India) in September month and was authenticated by Herbarium, department of botany, University of Rajasthan, Jaipur, Rajasthan, India. The plant was deposited in the herbarium at Department of Botany (University of Rajasthan, Jaipur, Rajasthan, India).

**Preparation of Extract**
Alcoholic extract: - The coarsely powdered (1000 g) oven-dried *Cleome viscose* Linn. was extracted with alcohol by using Soxhlet apparatus for 72 h. After completion of extraction, the solvent was removed by distillation and concentrated. The yield obtained was 18.96 % w/v.

**Fractionation of Crude Extract**
Fractionation of alcoholic extract completely dried ethanolic extract was suspended in distilled water and extracted successively and exhaustively with solvents of increasing polarity like petroleum ether, dichloromethane, n-butanol, ethyl acetate. Each fraction was concentrated using rotary evaporator (Rotavapor, R-210, BUCHI Laborte, Switzerland) and stored in vacuum desiccator.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) (p.o.)</th>
<th>% Increase in Body weight as compared to day “0”</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Day 9</td>
</tr>
<tr>
<td>EAC Control</td>
<td>0.25% Sod. CMC</td>
<td>33.00 ± 0.57</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>3.5</td>
<td>30.34 ± 1.09a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>35.83 ± 2.46a</td>
</tr>
<tr>
<td>CVA</td>
<td>200</td>
<td>36.03 ± 1.77b</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>33.79 ± 1.41c</td>
</tr>
<tr>
<td>CVP</td>
<td>200</td>
<td>37.09 ± 1.60a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>36.68 ± 1.65a</td>
</tr>
<tr>
<td>CVD</td>
<td>200</td>
<td>36.12 ± 1.51a</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>34.71 ± 1.06b</td>
</tr>
<tr>
<td>CVE</td>
<td>200</td>
<td>30.23 ± 1.58c</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM of six samples. a p < 0.05, b p< 0.01 & c p< 0.001, compared to EAC Control.

**Aqueous Extract**
1000 g of the coarsely powdered root of *Cleome viscose* Linn. was extracted by chloroform water (1:99) by cold maceration process for 7 days. After completion of extraction, the marc was filtered through muslin cloth and concentrated. The yield of CVA was obtained 18.43 %.

**Experimental Animals**

Figure 2: Effect of different extracts/fractions of CV on body weight changes in EAC inoculated mice (Day wise) 9th, 11th, and 13th Day.
Swiss albino mice were obtained from the animal house of Jaipur College of Pharmacy, Jaipur, Rajasthan, India and they were maintained under standard laboratory conditions throughout the study. The animals were maintained under standard laboratory conditions (temperature 25±2°C and 55±5% relative humidity with dark/light cycle 14/10 h) and were allowed free access to standard dry pellet diet and water ad libitum. Twelve to sixteen-week old Swiss albino mice weighing 27-30 g were used for the experiments. The study protocol was approved by the Institutional Animal Ethics Committee (931/PO/Re/S/06/CPCSEA) and all the animal experiments were carried out in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India.

Acute Toxicity
The acute oral toxicity of Cleome viscose Linn, in Swiss albino mice was performed as per OECD guidelines-425.16. The extract was safe up to the dose of 2g/kg b.w. P.O. for mice. No mortality or toxicities were observed in any of the treatments.

Selection of Doses and Grouping of Animals
The doses selected for the fractions were about 1/10th and 1/20th of the maximum tolerated safe dose found from acute toxicity studies. They were administered once daily by p.o. route. The dose of standard drug (Cisplatin) selected was 3.5 mg/kg. This was calculated by computing the minimum human dose to the mice and from past experience. Animals were grouped by taking 06 animals per group. One each group for normal control, EAC control and standard whereas two each group for four fractions at two different doses (100mg/kg and 200mg/kg) were taken.

In-vitro Anticancer Activity
Ehrlich ascites carcinoma (EAC) Model
The EAC cells originally obtained from National Centre for cell science, Pune, India, (NCCS), were maintained and propagated by serial i.p transplantation of EAC cells in an aseptic environment. The EAC cells propagated for 12-14 days were used in experiment. The tumor cell cultures for EAC were started from mice Ehrlich Ascites with at least one passage in vitro prior to use. The ascitic fluid is drawn using an 18-gauge needle into sterile syringe. Tumors viability was determined by Trypan blue exclusion assay and cells were counted using hemocytometer. The Ascitic fluid was suitably diluted in normal saline to get a concentration of 1x10^7 (ten million) cells /ml. From this stock suspension 0.25ml (2.5 million cell/mice) was injected i.p to obtain ascitic tumor. The mice were weighed on the day of tumor inoculation and then for every three days.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg) (p.o)</th>
<th>MST</th>
<th>% ILS</th>
</tr>
</thead>
<tbody>
<tr>
<td>EAC Control</td>
<td>0.25% Sod. CMC</td>
<td>12.23 ± 6.21</td>
<td>82.71 ± 22.34c</td>
</tr>
<tr>
<td>Cisplatin</td>
<td>3.5</td>
<td>22.28 ± 11.20</td>
<td>40.19 ± 20.87c</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>17.14 ± 9.81</td>
<td>43.48 ± 19.88c</td>
</tr>
<tr>
<td>CVA</td>
<td>200</td>
<td>17.46 ± 9.35</td>
<td>35.28 ± 10.26c</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>16.49 ± 7.61</td>
<td>35.28 ± 10.26c</td>
</tr>
<tr>
<td>CVP</td>
<td>200</td>
<td>18.63 ± 9.37</td>
<td>53.21 ± 20.19c</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>16.64 ± 7.61</td>
<td>28.62 ± 7.18c</td>
</tr>
<tr>
<td>CVD</td>
<td>200</td>
<td>17.89 ± 8.42</td>
<td>40.17 ± 10.15c</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>16.61 ± 8.46</td>
<td>36.81 ± 9.94c</td>
</tr>
<tr>
<td>CVE</td>
<td>200</td>
<td>16.47 ± 8.44</td>
<td>35.21 ± 10.58c</td>
</tr>
</tbody>
</table>

All the values are mean ± SEM of five samples, a p < 0.05, b p< 0.01 & c p< 0.001, compared to EAC Control

![Graph](image-url)
days. The animal care and handling were carried out in accordance to guidelines issued by CPCSEA. Out of six different fractions four have shown potent cytotoxic activity in in-vitro study so they were taken up for further evaluation in EAC Model. Treatment was given on 3rd, 5th, 7th, 9th, 11th, and 13th day of tumor inoculation p.o.

Cisplatin (one dose) was injected on 1st day only.

**Experimental observations**

The total body weight gain of the animals was recorded every three days throughout the duration of experiment. On day 15 six animals were sacrificed from each group for evaluating the hematological (RBC, WBC, Hb)\(^1\), tumor growth parameters (tumor weight, ascitic fluid volume, packed cell volume, viability and non-viability)\(^1\) and organ weights\(^2\). The remaining animals were kept for monitoring the mean survival time and the percentage increase in life span\(^3\).

**Statistical Analysis**

The experimental results were expressed as mean ± S.E.M (n=6 mice per group). Results were analyzed by the one-way ANOVA followed by Tukey-Kramer post hoc multiple comparison test using Graph pad 5. Where \(^a\) \(p<0.05\), \(^b\) \(p<0.01\) and \(^c\) \(p<0.001\) considered being significant, very significant and highly significant, respectively.
RESULT AND DISCUSSION

Effect of treatment on change in body weight in EAC inoculated mice

The EAC inoculated mice were found to gain body weight progressively. The maximum gain in tumour weight (37.13%) was observed on day 13th of tumour inoculation. The standard drug, Cisplatin administered on day 1st significantly (p< 0.05) reduced the elevated body weight as compared to control from 7th day onwards. CVP (100mg/kg) showed significant (p<0.05) reduction in elevated body weight as compared to EAC control from 7th day onward. CVA (100mg/kg) and CVD (200mg/kg) showed significant (p<0.001) reduction from 9th day onward. CVP (200mg/kg) was effective (p<0.01) in reducing body weight. CVA (200mg/kg) and CVD (100mg/kg) showed no significant reduction in bodyweight even after 13th day. (Table 1 & 2, Fig. 1 & 2)

Effect of treatment on mean survival time (MST) and % increase in life span (%ILS) in EAC inoculated mice

Mean survival time in EAC inoculated mice was found to decrease significantly when compared to normal as well as with extracts treated mice. In EAC inoculated control mice first mortality was observed on day 9 and all animals were dead by day 16. The MST of EAC control mice (12.23) was significantly (p<0.001) improved by the Cisplatin treatment (22.28). The MST of CVA fraction treatment at 200 and 100 mg/kg was found to be 17.46 and 17.14 respectively. On the other hand, CVP at 200 mg/kg and 100 mg/kg increased the MST to 18.63 and 16.49 respectively. CVD (200 and 100 mg/kg) as well as CVE (200 and 100mg/kg) increased MST to 17.89, 15.64 and 16.61 respectively. (Table 3; Fig. 3). CVP (200mg/kg), CVD (200mg/kg) and CVA (200 and 100 mg/kg) and were significantly increased life span by 53%, 40% and 43%, 40% respectively.

Effect of treatment on Haematological parameters in EAC inoculated mice

Compared to sham control, WBC count was found to increase two folds in EAC inoculated mice. Cisplatin administration was found to significantly (p<0.001) reverse the elevated WBC count in EAC inoculated mice. In case of fractions treated mice, WBC count was found to be significantly (p<0.05) decreased at both doses. CVA and CVD were more effective in reducing elevated WBC count (Table 4 and Fig. 4).

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RBC count was significantly reduced in EAC inoculated mice as compared to sham control. Cisplatin treatment was found to be significantly (p<0.05) preventing reduction in RBC count. CVA and CVD at both the doses significantly (p<0.05) improve the RBC count as compared to EAC control (Table 4 and Fig. 4). Haemoglobin content was found to be reduced significantly (p<0.05) compared to sham control. Cisplatin treatment was found to restore the haemoglobin at normal level. CVD (200mg/kg) significantly improve the haemoglobin level compared to EAC control (Table 4 and Fig. 4).

Effect of treatment on ascitic fluid volume and count in EAC inoculated mice
Ascitic cell count was found to be 15.42 million cells/ml. treatment with Cisplatin significantly (p<0.001) reduced EAC count to 0.6 million cells/ml. All the selected fractions at both the doses significantly (p<0.001) reduced ascitic cell count compared to EAC control (Table 5 and Fig. 5).
Ascitic fluid volume was found to be 4.51 ml which significantly reduced with treatment of Cisplatin up to 1.65 ml. All the fractions at both tested doses showed considerable reduction in ascitic fluid volume compared to EAC control (Table 5 and Fig. 5).

Effect of treatment on spleen weight and angiogenesis in EAC inoculated mice
Spleen weight increases in case of increased degradation of RBC. Spleen weight was found to be increased significantly in EAC control group as compared to sham control. Cisplatin treatment caused significant (p<0.001) reduction in spleen weight compared to EAC control. CVD at both the doses and CVE (200mg/kg) reduced spleen weight significantly (p<0.05) compared to EAC control (Table 5 and Fig. 6).
The total number of blood vessels present on the ventral peritoneal skin layer which is in direct contact with the liquid tumour was counted. It was found to be very high in case of EAC control animals. Cisplatin as well as the fractions were found to significantly lower the blood vessel formation (p<0.001) compared to EAC control. CVD fraction showed dose-dependent anti-angiogenic activity (Table 5 and Fig. 6).

CONCLUSION
The present study asserting that the alcoholic extract and its fractions of Cleome viscose Linn. exhibited a significant in vivo anticancer activity against EAC cells. These significant and important preliminary outcomes can be taken as the basis upon which further studies should be carried out to delineate the detailed profile of these anticancer activities of Cleome viscose Linn.

REFERENCES