

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

Singh Charanjeet*, Ahuja Dharmendra, Mehta S C

Faculty of Pharmaceutical Science, Jayoti Vidyapeeth Women's University, Jaipur

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ABSTRACT

The investigation aimed to evaluate the anti-cancer properties of alcoholic extracts and the fractions *Cleome viscosa* 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 fractions 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 viscosa* 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 viscosa* Linn. Which are largely attributable to the improve or synergies effect of their constituents.

Keywords: *Cleome viscosa*, 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 viscosa 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 viscosa* Linn. This plant

*Author for Correspondence: charanjeet2004@gmail.com

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

Treatment	Dose (mg/kg) (p.o.)	% Increase in Body weight as compared to day "0"		
		Day 3	Day 5	Day 7
EAC Control	0.25% Sod. CMC	28.46 ± 0.21	29.56 ± 0.57	31.51 ± 0.31
Cisplatin	3.5	30.91 ± 1.10	29.94 ± 0.96	30.09 ± 0.85 ^a
	100	30.64 ± 1.64	31.61 ± 1.58	33.54 ± 1.88
CVA	200	30.35 ± 1.54	31.28 ± 1.52	31.74 ± 1.56
	100	29.19 ± 1.34	30.47 ± 1.45	31.98 ± 1.51 ^a
CVP	200	30.13 ± 1.16	31.44 ± 1.47	32.71 ± 1.59
	100	30.61 ± 1.09	31.88 ± 1.18	33.65 ± 1.26
CVD	200	28.94 ± 1.48	30.15 ± 1.59	31.94 ± 1.68
	100	29.16 ± 1.81	29.44 ± 1.73	31.11 ± 1.48
CVE	200	26.87 ± 1.54	27.10 ± 1.81	28.54 ± 1.91

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.

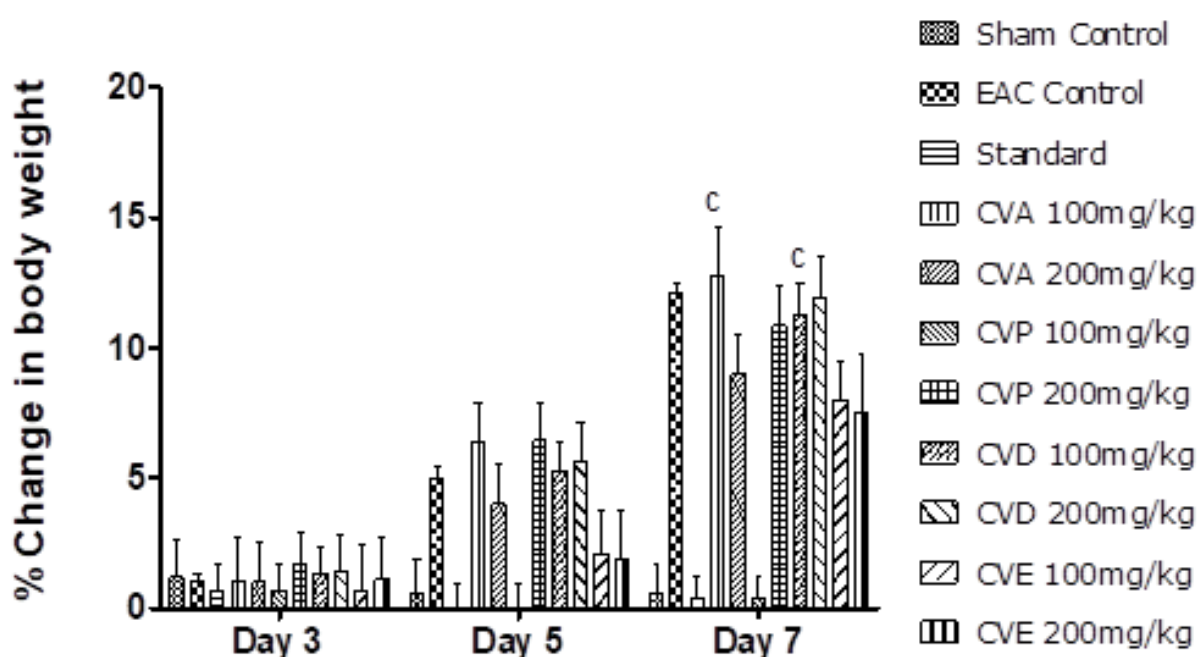


Figure 1: Effect of different extracts/fractions of CV on body weight changes in EAC inoculated mice (Day wise) 3th, 5th, and 7th Day

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 diabetes¹³. 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 vina alkaloids, taxanes, camptothecins and

epipodophyllotoxins are currently part of many standard plant-derived utilized as anti-cancer agent¹.

Considered chemotherapy an important method of treatment for cancer, he provided some plants such as *Catharanthus roseus*, *Podophyllum peltatum*, *Podophyllum emodii*, *Taxus brevifolia*, *Ochrosia elliptica* and *Campototheca 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 2: Effect of different extracts/fractions of CV on body weight changes in EAC inoculated mice (Day wise) 9th, 11th, and 13th Day.

Treatment	Dose (mg/kg) (p.o.)	% Increase in Body weight as compared to day "0"		
		Day 9	Day 11	Day 13
EAC Control	0.25% Sod. CMC	33.00 ± 0.57	36.78 ± 0.98	37.13 ± 0.25
Cisplatin	3.5	30.34 ± 1.09 ^c	32.44 ± 1.43 ^c	32.63 ± 1.65 ^c
	100	35.83 ± 2.46 ^c	36.27 ± 2.56 ^c	34.70 ± 2.51 ^c
CVA	200	36.03 ± 1.77	36.23 ± 1.69 ^b	36.64 ± 2.41 ^c
	100	33.79 ± 1.41 ^c	35.73 ± 1.58 ^c	34.80 ± 1.48 ^c
CVP	200	37.09 ± 1.60 ^b	37.96 ± 1.51	36.72 ± 0.52
	100	36.68 ± 1.65	35.21 ± 2.64 ^c	35.13 ± 1.71 ^c
CVD	200	36.12 ± 1.51 ^c	37.76 ± 1.24	37.29 ± 1.38
	100	34.71 ± 1.06	35.81 ± 2.68	36.37 ± 3.20
CVE	200	30.23 ± 1.58	30.66 ± 1.56 ^c	30.18 ± 1.53 ^c

All the values are mean ± SEM of six samples, ^ap < 0.05, ^bp < 0.01 & ^cp < 0.001, compared to EAC Control

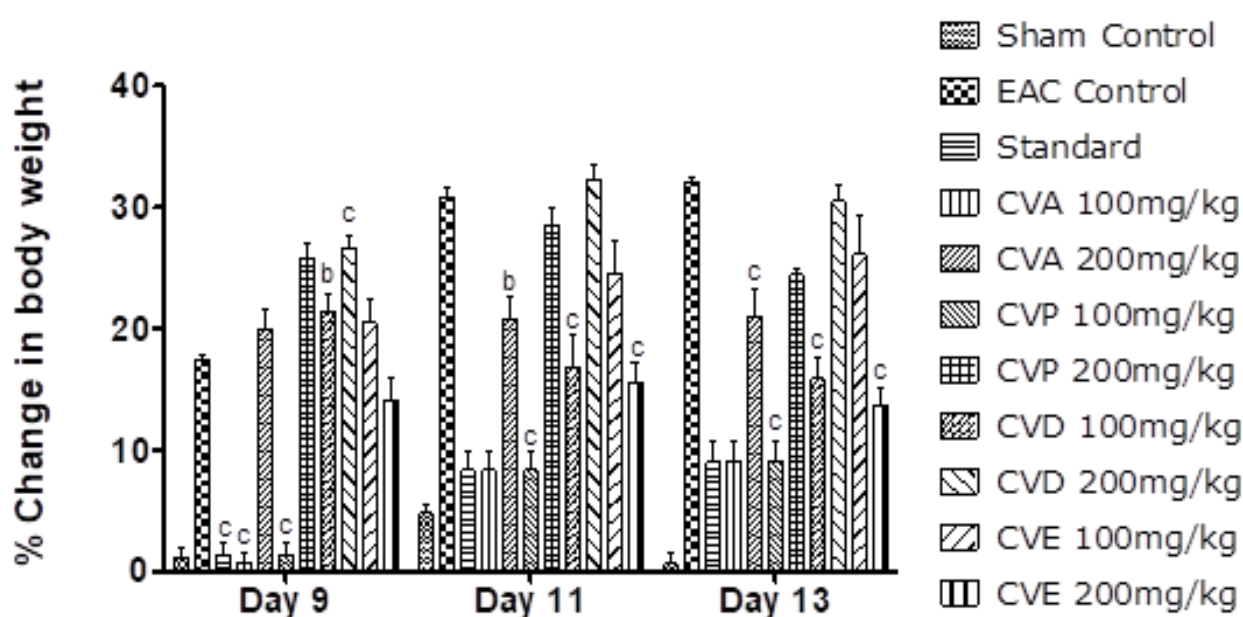


Figure 2: Effect of different extracts/fractions of CV on body weight changes in EAC inoculated mice (Day wise) 9th, 11th, and 13th Day

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 viscosa* 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 viscosa* 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. The percentage yield of various extracts/fractions was ethanol (CVE) [24.61% w/v], petroleum ether (CVP) [3.19 % w/v] and dichloromethane (CVD) [9.04 % w/v]

Aqueous Extract

1000 g of the coarsely powdered root of *Cleome viscosa* 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

Table 3: Effects of different extract/fractions of CV on MST and %ILS in EAC inoculated mice.

Treatment	Dose (mg/kg) (p.o)	MST	% ILS
EAC Control	0.25% Sod. CMC	12.23 ± 6.21	-
Cisplatin	3.5	22.28 ± 11.20	82.71 ± 22.34 ^c
	100	17.14 ± 9.81	40.19 ± 20.87 ^c
CVA	200	17.46 ± 9.35	43.48 ± 19.88 ^c
	100	16.49 ± 7.61	35.28 ± 10.26 ^c
CVP	200	18.63 ± 9.37	53.21 ± 20.19 ^c
	100	15.64 ± 7.61	28.62 ± 7.18 ^c
CVD	200	17.89 ± 8.42	40.17 ± 10.15 ^c
	100	16.61 ± 8.46	36.81 ± 9.94 ^c
CVE	200	16.47 ± 8.44	35.21 ± 10.58 ^c

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

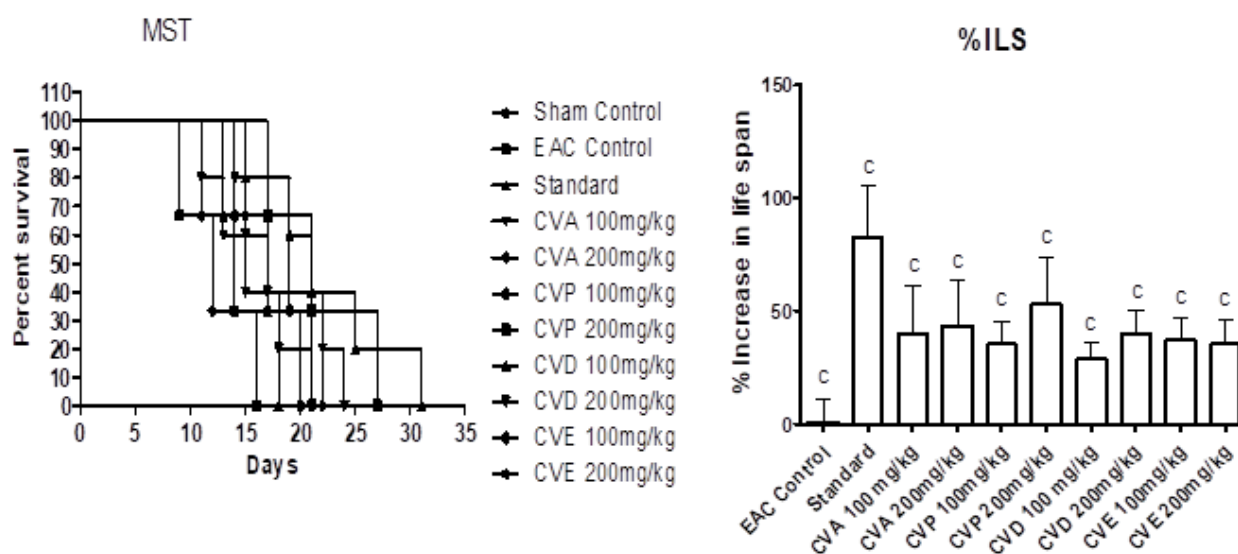


Figure 3: Effects of different extract/fractions of CV on MST and %ILS in EAC inoculated mice.

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 viscosa* Linn. in Swiss albino mice was performed as per OECD guidelines-425¹⁶. 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

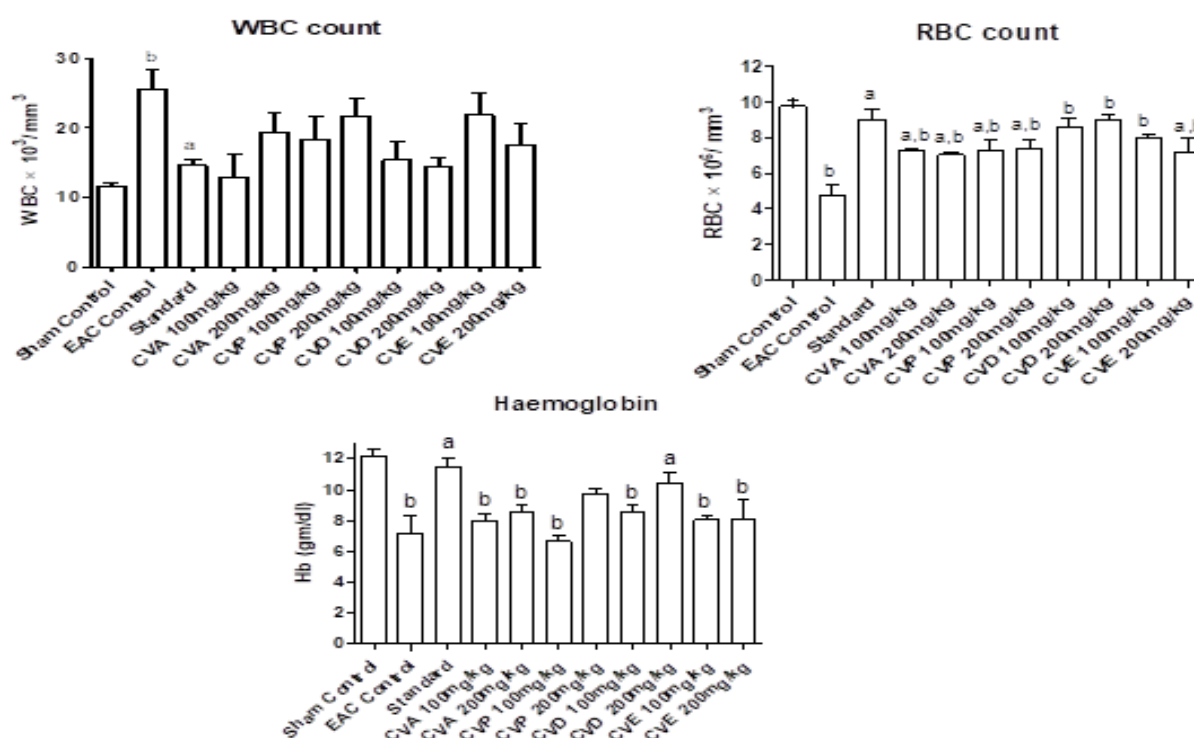
Ehrlich ascites carcinoma (EAC) Model

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 1x10⁷ (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

Table 4: Effect of different extracts/fractions of CV on various haematological parameters (WBC count, RBC count and Haemoglobin content).

Treatment	Dose (mg/kg) (p.o)	WBC (1×10^3 cells/mm ³)	RBC (1×10^6 cells/mm ³)	Haemoglobin (gm %)
Sham Control	-	11.67 ± 0.55	9.71 ± 0.47	12.28 ± 0.48
EAC Control	0.25% Sod. CMC	25.48 ± 2.71 ^b	4.78 ± 0.68	7.26 ± 1.19 ^b
Cisplatin	3.5	14.61 ± 0.80	9.17 ± 0.65	11.48 ± 0.68 ^a
CVA	100	12.91 ± 3.351 ^a	7.28 ± 0.18 ^b	7.97 ± 0.54 ^b
	200	19.35 ± 2.90	7.43 ± 0.18	8.58 ± 0.32 ^b
CVP	100	18.34 ± 3.38 ^b	7.38 ± 0.58 ^b	6.65 ± 0.48 ^b
	200	21.68 ± 2.54 ^b	7.45 ± 0.48 ^b	9.74 ± 0.28 ^b
CVD	100	15.40 ± 2.68	8.68 ± 0.49 ^b	8.61 ± 0.31 ^b
	200	14.39 ± 1.37	9.17 ± 0.28	10.46 ± 0.68 ^b
CVE	100	21.84 ± 3.11 ^{a,b}	8.08 ± 0.18	8.38 ± 0.38 ^b
	200	17.59 ± 3.17 ^{a,b}	7.17 ± 0.88 ^b	8.42 ± 1.28 ^b

All the values are mean ± SEM of six samples, ^a p < 0.05, compared to EAC Control and ^b p < 0.05, compared to sham control



All the values are mean ± SEM of six samples, ^a p < 0.05, compared to EAC Control and ^b p < 0.05, compared to sham control

Figure 4: Effect of different extracts/fractions of CV on various haematological parameters (WBC count, RBC count and Haemoglobin content).

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.¹⁷. 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)^{18,22}, tumor growth parameters (tumor weight, ascitic fluid volume,

packed cell volume, viability and non-viability)¹⁹ and organ weights²⁰. The remaining animals were kept for monitoring the mean survival time and the percentage increase in life span²¹

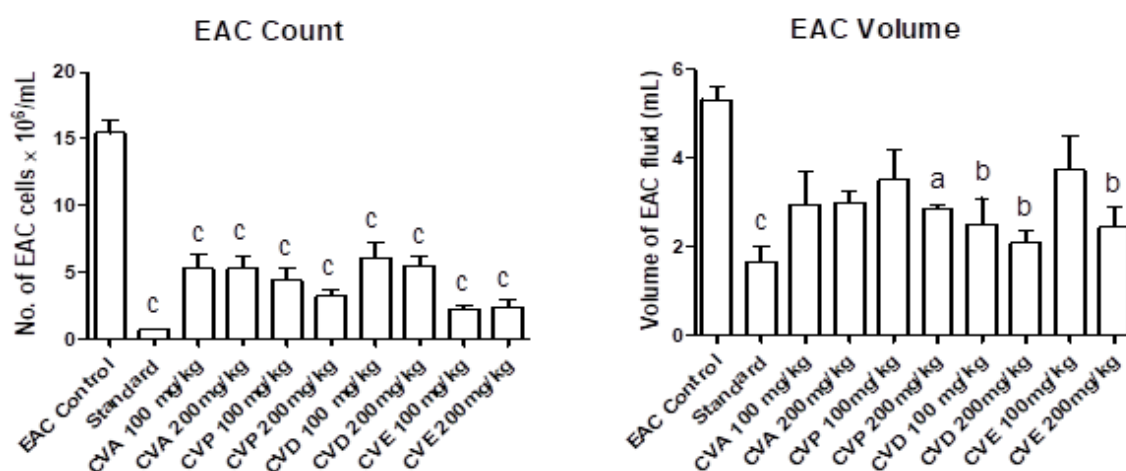
Statistical Analysis

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.

Table 5: Effect of different extracts/fractions of CV on Ascitic fluid count, Ascitic fluid volume, Spleen weight and Angiogenesis.

Treatment	Dose (mg/kg) (p.o.)	Ascitic fluid count (1×10 ⁶ cells/ml)	Ascitic fluid volume (ml)	Spleen weight	Angiogenesis
Sham Control	-	-	-	0.13 ± 0.08	8.44 ± 0.31
EAC Control	0.25% Sod. CMC	15.47 ± 0.93	5.37 ± 0.30	0.27 ± 0.03	20.28 ± 0.89
Cisplatin	3.5	0.61 ± 0.18 ^c	1.61 ± 0.36 ^c	0.15 ± 0.04	10.19 ± 0.78 ^c
CVA	100	5.23 ± 1.24 ^c	2.93 ± 0.79	0.14 ± 0.04	14.57 ± 1.28 ^c
	200	5.28 ± 1.08 ^c	3.38 ± 0.21	0.15 ± 0.02 ^a	12.58 ± 1.17 ^c
CVP	100	4.32 ± 0.96 ^c	3.53 ± 0.68	0.16 ± 0.01	15.78 ± 0.88 ^a
	200	3.18 ± 0.41 ^c	2.99 ± 0.18 ^a	0.15 ± 0.05	16.38 ± 0.89
CVD	100	6.18 ± 1.15 ^c	2.74 ± 0.57 ^b	0.11 ± 0.01	13.34 ± 0.36 ^c
	200	5.44 ± 0.63 ^c	2.88 ± 0.21 ^b	0.12 ± 0.02	12.69 ± 0.68 ^c
CVE	100	2.27 ± 0.24 ^c	3.74 ± 0.66	0.15 ± 0.01	15.68 ± 0.89 ^a
	200	2.32 ± 0.51 ^c	3.43 ± 0.48 ^b	0.12 ± 0.01	14.55 ± 1.38 ^c

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



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

Figure 5: Effect of different extracts and fractions of CV on Ascitic fluid count and Ascitic fluid volume.

RESULT AND DISSICUSSION

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 in bodyweight from 9th day onward. CVP (200mg/kg) was effective (p < 0.01) in reducing body weight. CVA (200mg/kg) and CVD (100mg/kg) showed significant (p < 0.001) effect on day 11th. CVE (100mg/kg) showed no significant reduction in bodyweight even after 13th day. (Table 1 & 2, Fig. 1 & 2)

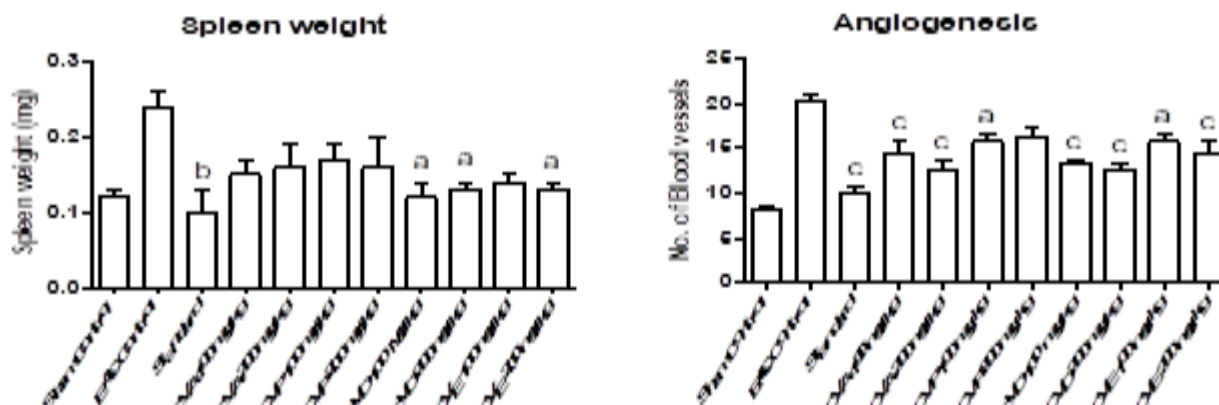
Effect of treatment on mean survival time (MST) and % increase in life span (%ILS) in EAC inoculated mice

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, 16.47 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).



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

Figure 6: Effect of different extract and fractions of CV on Spleen weight and Angiogenesis.

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 viscosa* 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 viscosa* Linn.

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