



EVALUATION OF ANTI-TUMOUR ACTIVITY OF *CISSUS QUADRANGULARIS* LINN. AGAINST DALTON'S ASCITIC LYMPHOMA AND ERLICH ASCITIC INDUCED CARCINOMA IN MICE

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ABSTRACT

Cissus quadrangularis is a commonly used folklore medicine in India to hasten the fracture healing process, in the present study anti-tumour nature of plant extract was studied, Healthy Swiss albino mice is used, Group-1 served as a normal control, for which inoculation cell was not done. The remaining animals were inoculated with DLA (1×10^6 cells/mouse) and divided into 4 groups containing 6 animals in each group. Group-2 served as tumour control and Group-3 served as positive control, was treated with 5-Fluorouracil (dose of 20mg/kg body weight). Group-4 and 5 was treated with Ethanolic and aqueous extract of *Cissus quadrangularis* at the dose of 250mg/kg body weight. All the treatments were given orally after 24hrs of inoculation and continued once daily for 14 days. After the last dose, all mice from each group were sacrificed and blood was collected by retro-orbital plexus method and estimated for haematological parameters like WBC, RBC, Platelet and haemoglobin. The remaining blood was centrifuged and serum was used for the estimation of hepatoprotective parameters like AST, ALT, ALP, LD and TGL. The ascitic fluid was collected from the peritoneal cavity and measured for tumour cell packed volume and viable tumour cell count. Survival time was compared with control and Tumour mass also was measured from the 11th day of tumour induction. Diameter of tumour was measured on every 5th day for a period of 30days using vernier callipers. The average life span of DLA control group was found to be 48% where as EECQ and AECQ at the dose of 250mg/kg body weight increases the life span to 72% and 76% respectively however the average life span of 5-Fluorouracil treatment was found to be 94%. The preliminary phytochemical study of EECQ and AECQ indicate the presence of several amino acids, carbohydrate, alkaloids, glycosides, flavanoids, phenolic compounds and phytosterols. The observed antitumour activity may be due to the presence of any of these compounds.

Keywords: *Cissus quadrangularis*, 5-Fluorouracil, Dalton's ascitic lymphoma, Erlich ascitic carcinoma.

INTRODUCTION

Cancer is the abnormal growth of cells in our bodies. Cancer cell usually invade & destroy normal cells. Cancer may affect people at all ages, even fetuses, but the risk for most varieties increases with age.¹ Cancer causes about 13% of all human death.² Several chemotherapeutic agents are used to treat cancer, but they cause toxicity that prevent their usage.³ *Cissus quadrangularis* is known to be an ancient medicinal plant, with optimal healing in white tissue area of the body (tendon, ligament etc).⁴ Phytochemical analysis of *Cissus quadrangularis* indicate the presence of carotene, phytosterol, terpenoids, β -sitosterol, δ -amyrin, δ -amyrone and calcium. The plant *Vitis* or *Cissus quadrangularis* (sanskrit-Asthishrinkhala, ajravalli Hindi-Harjor) belong to the family Vitaceae and has been used as anthelmintic, dyspeptic, digestive tonic, analgesic in eye and ear disease, scurvy, irregular menstruation, asthma,^{4,5} fracture of bone and for complains of back and spine.^{6,7} *Cissus quadrangularis* is a commonly used folklore medicine in India to hasten the fracture healing process in cameroon, the whole plant is used for the treatment of oral dehydration, while in Africa & Asia, the leaves, stems & roots are utilized for the treatment of various ailments. However, so far no antitumour activity has been reported from this plant. The present study is focused to find out the anti-tumour nature of plant extract of *Cissus quadrangularis*.

MATERIALS AND METHODS

Plant Material

The fresh stem of *Cissus quadrangularis* were collected from Alagar kovil, Madurai, Tamil Nadu, India in month of July and the same were authenticated by botanist and it was shade dried, pulverized to get coarse powder (500g) which is then extracted with alcohol & distilled water using soxhlet apparatus for 72 hrs. Both extract were filtered, concentrated to dry mass by vacuum distillation, yielded a dark greenish residue.



Figure 1: *Cissus quadrangularis* Linn.



Animals

Healthy Swiss albino mice weighing between (20-40gm) were used throughout the study they were housed in micro nylon cages, maintained in standard lab conditions at $25 \pm 2^\circ\text{C}$ under a 12-h light/dark cycle. They were fed with standard pellet diet and water *ad libitum* and this study was approved by the Institutional Animal Ethical Committee (IAEC).

Tumour Cell line

Dalton's lymphoma ascites and Erlich ascitic carcinoma were obtained through the courtesy of Amla cancer research centre, Trissur, Kerala. They were maintained by weakly intra-peritoneal inoculation of 10^6 cells/ mouse.⁸

Effect of Ethanolic and Aqueous extracts of *Cissus quadrangularis* on DLA induced Anti-tumour studies

Group-1 containing 6 animals, served as a normal control, for which inoculation cell was not done. The remaining animals were inoculated with DLA (1×10^6 cells/mouse) and divided into 4 groups containing 6 animals in each group. Group-2 served as tumour control and Group 3 served as positive control, was treated with 5-Fu at the dose of 20mg/kg body weight.⁹ Group-4 and 5 was treated with ethanol and aqueous extract of *Cissus quadrangularis* at the dose of 250mg/kg body weight.¹⁰ All the treatments were given orally after 24hrs of inoculation and continued once daily for 14 days. After the last dose, all mice from each group were sacrificed and blood was collected by retro-orbital plexus method & estimated for hematological parameters like WBC, RBC, Platelet, hemoglobin.^{11, 12, 13} The remaining blood was centrifuged and serum was used for the estimation of hepatoprotective parameters like AST, ALT, ALP, LD and TGL. The ascitic fluid was collected from the peritoneal cavity and measured for tumour cell packed volume and viable tumour cell count.

Effect of Ethanolic and Aqueous extracts of *Cissus quadrangularis* on Survival time

Animals were inoculated with 1×10^6 cells/mouse on day 0 and treatment with Ethanolic extract of *Cissus quadrangularis* (EECQ) and Aqueous extract of *Cissus quadrangularis* (AECQ) started 24hrs after inoculation. The normal and tumour control group was treated with same volume of 0.9% NaCl solution. All treatments were carried out for 9 days. The antitumour efficacy of EECQ & AECQ was compared with that of 5-fluoro Uracil (20mg/kg i.p for 9 days). MST was noted with reference to control group. Survival time of treated group was compared with those of control using following calculation.

$$\text{Increase in life span} = T-C / C \times 100$$

Where T=number of days the treated animal survived.

C=number of days the control animal survived

Effect of EECQ and AECQ on Solid tumour

Mice were divided into 3 groups. Tumour cells (1×10^6 cells/mouse) were injected intramuscularly into right limb of all animals. The mice of group 1 were tumour control. Group 2 received EECQ (250mg/kg) orally for 5 alternative days. Tumour mass was measured from the 11th day of tumour induction. Diameter of tumour was measured on every 5th day for a period of 30days using vernier calipers. The volume of tumour mass was calculated using the formula $V=4/3\pi r^2$ where r is the mean of r_1 & r_2 which are 2 independent radii of tumour mass.¹⁴

Histopathological Changes

The architecture of Skeletal Muscle Bundle was completely intact in normal rats (Fig.2), Tumour Control Shows Skeletal Muscle Bundle with Sheets of Polygonal to Pleomorphic Cells with extensive Tumour and Necrosis (Fig.3), Positive Control was treated with 5-Fu at the dose of 20mg/kg body weight Shows Very mild area of Tumour and Necrosis (Fig.4),

Effect of EECQ in Skeletal Muscle Bundle shows minimal areas of Tumour and Necrosis (Fig.5) and Effect of AECQ in Skeletal Muscle Bundle shows mild areas of Tumour and Necrosis (Fig.6) The group treated with the Ethanolic extract alone was found to be effective when compared to aqueous extract treated group.



Figure 2: Normal Control Shows Clear Skeletal Muscle Bundle without any Tumour and Necrosis

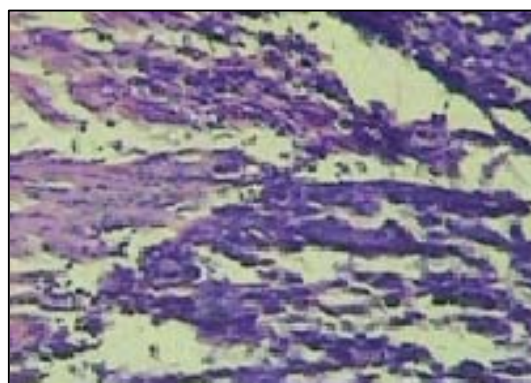


Figure 3: Tumour Control Shows Skeletal Muscle Bundle with Sheets of Polygonal to Pleomorphic Cells with extensive Tumour and Necrosis

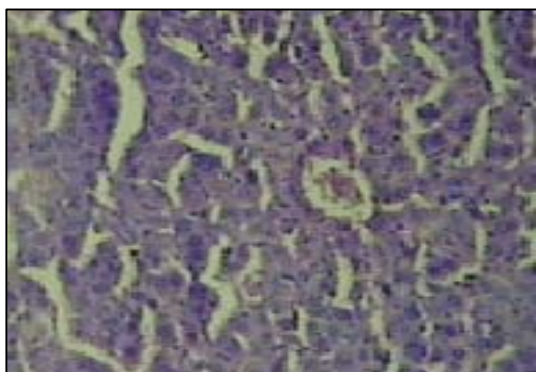


Figure 4: Positive Control was treated with 5-Fu at the dose of 20mg/kg body weight Shows Very mild area of Tumour and Necrosis

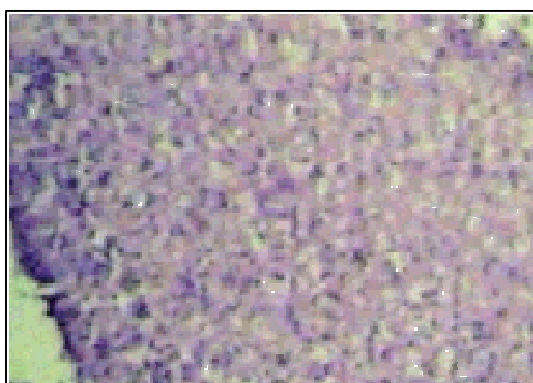


Figure 5: Effect of EECQ in Skeletal Muscle Bundle shows minimal areas of Tumour and Necrosis



Figure 6: Effect of AECQ in Skeletal Muscle Bundle shows mild areas of Tumour and Necrosis

Statistical analysis

The values are expressed as mean \pm SEM. The analysis of data was carried out with one-way ANOVA followed by Newman Keul's multiple range tests. P-Value < 0.05 were considered as significant.

RESULTS

The average life span of DLA control group was found to be 48% where as EECQ and AECQ at the dose of 250mg/kg body weight increases the life span to 72% & 76% respectively shown in Table 1. However the average life span of 5-Fluro Uracil treatment was found to be 94% indicating potent antitumour activity.

Effect of EECQ and AECQ on Hematological parameter

As shown in Table.2 the total WBC, PCV were found to be increased with a reduction in RBC, Hb and platelet in tumour control animals. At the same time treatment with EECQ & AECQ at the dose of 250mg/kg could change those altered parameter to near normal. However the Standard 5Fu at the dose of 20mg/kg body weight produce better results in all these parameters.

Effect on Biochemical parameter

The inoculation of DLA cells to tumour control animals caused significant increase in the level of AST, ALT, ALP, TGL and CE when compared to normal animals. The treatment with EECQ and AECQ at the dose of 250mg/kg body weight reversed the changes towards the normal values, which is shown in Table 3 Almost similar results were observed for 5Fu treatment.

Effect on Solid tumour

In tumour control animals the solid tumour volume induced by EAC, cells was found to be significantly increased from day 0 to day 30. Reduction in tumour volume was observed with EECQ and AECQ treatment from 15th day to end of experiment given in Table 4.

Table 1: Effect of *Cissus quadrangularis* extracts on survival time and viable cell count

Treatment	Number of animals	% ILS Life Span	Increase in Body Weight grams	Cancer Cell Count ml \times 10 ⁶
G1-Normal control	6	>>30 days	1.32 \pm 0.09	-
G2-Tumour control	6	48%	8.55 \pm 0.21	2.42 \pm 0.06
G3-Standard	6	94%	2.12 \pm 0.09	1.05 \pm 0.05
G4-Test (EECQ)	6	72%	7.32 \pm 0.12	2.10 \pm 0.10
G5-Test (AECQ)	6	76%	7.48 \pm 0.11	1.95 \pm 0.08

All values are expressed as Mean \pm SEM for 6 animals in each group

All values are found out by using one way ANNOVA followed by Newmann keul's multiple range tests.



Table 2: Effect of EECQ and AECQ on serum enzymes and lipid proteins

Treatment	Total WBC Cells/ml×10 ³	RBC Count Mill/cumm	Hb Gm/dl	PCV	Platelets Lakhs/cumm
G1-Normal control	10.36±0.10	4.20±0.12	11.5±0.08	14.35±0.13	2.59±0.2
G2-Tumour control	16.20±0.7	2.20±0.11	6.22±0.10	29.05±0.14	1.80±0.08
G3-Standard	11.20±0.21	3.91±0.04	10.40±0.12	18.55±0.20	2.40±0.08
G4-Test (EECQ)	15.11±0.10	2.60±0.10	6.95±0.10	27.80±0.32	2.01±0.10
G5-Test (AECQ)	14.90±0.12	2.96±0.11	7.20±0.15	26.80±0.21	2.10±0.11

All values are expressed as Mean ± SEM for 6 animals in each group

All values are found out by using one way ANNOVA followed by Newmann keul's multiple range tests.

Table 3: Effect of EECQ and AECQ on Hematological parameters

Treatment	Cholesterol mg/ml	TGL mg/dl	AST	ALT	ALP
G1-Normal control	112.20±0.42	131.20±0.23	37.50±0.52	30.50±0.11	123.60±1.9
G2-Tumour control	140.24±0.10	210.4±0.35	86.35±0.20	55.65±0.40	240.4±0.69
G3-Standard	120.50±0.18	140.96±0.40	54.60±0.12	40.28±0.40	160.41±0.58
G4-Test (EECQ)	137.25±0.30	181.32±0.21	83.22±0.42	53.20±0.32	235.06±3.5
G5-Test (AECQ)	138.30±0.35	160.21±0.21	84.36±0.30	54.10±0.12	233.10±3.22

All values are expressed as Mean ± SEM for 6 animals in each group

All values are found out by using one way ANNOVA followed by Newmann keul's multiple range tests.

Table 4: Effect of EECQ and AECQ on solid tumour volume

Treatment	Dose	Solid Tumour Volume(ml)			
		15 th Day	20 th Day	25 th Day	30 th Day
G1-Normal control	2ml/kg saline	2.84±0.04	3.65±0.06	4.62±0.09	5.70±0.10
G2- Test(EECQ)	250mg/kgEECQ	2.75±0.08	3.30±0.05	3.80±0.09	4.25±0.10
G3 -Test(AECQ)	250mg/kgAECQ	2.70±0.08	3.10±0.04	3.60±0.10	4.10±0.09

All values are expressed as Mean ± SEM for 6 animals in each group

All values are found out by using one way ANNOVA followed by Newmann keul's multiple range tests.

DISCUSSION

Plants have served as a good source of antitumour agents. Several studies have been conducted on herbs under a multitude of ethanobotanical grounds. A large number of plants possessing anticancer properties have been documented. Herbs of cassia quadrangularis was traditionally used in the treatment of tumours. The present investigation was carried out to evaluate the antitumour activity of EECQ and AECQ in DLA and EAC tumour bearing mice. The reliable criteria for judging the value of anticancer drug is the prolongation of life span of animal & decrease of WBC from blood.^{15,16} In DLA tumour bearing mice a regular rapid increase in an ascitic tumour volume was observed. The DLA bearing mice orally administered with EECQ and AECQ at the dose of 250mg/kg body weight showed significant change in the average life span to the animals of the tumour control group. However, the percent increase in the body weight, packed cell volume and number of viable tumour cells were found to be significantly less than the tumour control animal indicating the anticancer nature of the

extract. These results could indicate an indirect ideal effect which may involve macrophage activation and vascular permeability inhibition. Hence the observed antitumour nature of EECQ and AECQ may be due to the cytotoxic properties. The reversal of Hb content, RBC, platelet and WBC by the EECQ and AECQ treatment towards the value of the normal group clearly indicate that EECQ and AECQ possessed protective action on the haemopoietic systems.

CONCLUSION

It was reported that the presence of tumour in the human body or in the experimental animal is known to affect many function of the liver. The significantly elevated level of total cholesterol, TG, AST, ALT, ALP in serum of tumour inoculated animal indicated liver damage and loss of functional integrity of cell membrane. The significant reversal of these changes towards the normal by EECQ and AECQ treatments in the present study, the biochemical examination of DLA inoculated animal showed marked changes indicating the toxic effect of the



tumour. The normalization of these effects observed in the serum treated with EECQ and AECQ supported the potent antitumour and hepatoprotective effect of the extracts. The preliminary phytochemical study of EECQ & AECQ indicated the presence of several amino acid carbohydrate, alkaloids, glycosides, flavanoids, phenolic compounds and phytosterols. The observed antitumour activity may be due to the presence of the any of these compounds.

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