

Anticancer activity of *Cissampelos pareira* against dalton's lymphoma ascites bearing mice

B. Samuel Thavamani, Molly Mathew¹, S. P. Dhanabal²

Department of Pharmacognosy, PSG College of Pharmacy, Peelamedu, Coimbatore, ¹Malik Deenar College of Pharmacy, Kasargod, Kerala, ²Department of Phytopharmacy and Phytomedicine, JSS College of Pharmacy (JSS University, Mysore), Ooty, Tamil Nadu, India

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ABSTRACT

Background: *Cissampelos pareira* (Menispermaceae) is used in folk Indian system of alternative medicine, for its analgesic, antipyretic, diuretic, antilithic, and emmenagogue properties. **Objective:** To evaluate *Cissampelos pareira* (*C. pareira*) for *in vitro* cytotoxicity and *in vivo* antitumor activity against Dalton's Lymphoma Ascites (DLA) cells in Swiss mice. **Materials and Methods:** *Cissampelos pareira* was successively extracted using different solvents. *In vitro* cytotoxicity was assessed by the MTT assay. An *in vivo* study was carried out in methanol extract. Twenty-four hours after intraperitoneal inoculation of the DLA cells in mice, the methanol extract of *C. pariera* (MECP) was administered at 200 and 400 mg/kg body weight for 14 consecutive days. On day 14, six mice were sacrificed and the rest were kept alive for assessment of increase in life-span. The antitumor effect was assessed by evaluating the packed cell volume, viable tumor cell count, increase in body weight, and increase in life-span. The hematological and serum biochemical parameters and anti-oxidant properties were assessed by estimating the superoxide dismutase (SOD), catalase (CAT), and lipid peroxidation. **Results:** Methanol Extract of *Cissampelos pariera* (MECP) showed a potent cytotoxic activity, with an IC₅₀ value of 95.5 µg/ml and a significant ($P < 0.001$) decrease in packed cell volume, viable cell count, and an increased lifespan (54 and 72%). The hematological and serum biochemical profiles were restored to normal levels in MECP-treated mice. The MECP-treated group significantly ($P < 0.001$) decreased SOD, lipid peroxidation, and CAT to normal. **Conclusion:** This study demonstrated that *C. pariera* exhibited significant *in vitro* and *in vivo* anti-tumor activities and that it was reasonably imputable to its increasing endogenous mechanism of antioxidant property.

Key words: Antioxidant, antitumor, *Cissampelos pariera*, cytotoxicity, Menispermaceae

INTRODUCTION

Cancer is a major health issue, despite the advancement in the healthcare sector. The present day research is aimed at identifying synthetic and natural molecules to be used in the prevention or treatment of cancer. Chemotherapy is considered as the most effective method, among many other methods prevalent, to treat cancer. However, chemotherapeutic agents affect the host cells, especially the bone marrow, epithelial tissues, reticuloendothelial system, and gonads.^[1] There are several chemotherapeutic agents that produce serious chronic or delayed toxicities that may be irreversible, particularly in the heart, lungs, and kidneys.^[2] Therefore, a novel approach to reduce

unwanted toxicity is to employ newer natural products that may act with different and distinct mechanism (s) and/or reduce the side effects. Hence, at present, natural products have been contemplated to be of exceptional value in the development of effective anticancer drugs, with minimum host cell toxicity, possessing good antioxidant potential.^[3]

Cissampelos pariera Linn. (Menispermaceae) is a climbing shrub that grows in the tropical and subtropical regions of India. *C. pareira* is also called as the Midwives's herb, as it is used in the treatment of the female reproductive system. It is used as an astringent, antispasmodic, analgesic, antipyretic, diuretic, antilithic, and emmenagogue.^[4] *Cissampelos pareira* is reported to have cardioprotective,^[5] hepatoprotective,^[6] antioxidant and immunomodulatory,^[7] antifertility,^[8] antiarthritic,^[9] and anti-inflammatory activities.^[10] The plant is reported to have phytoconstituents like cissampeloflavone,^[11] cissamparine,^[12] pareirubines A and B,^[13] hayatinin,^[14] and protoberberine alkaloids.^[15] No pharmacological investigation in the perspective of

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Address for correspondence:

Assoc. Prof. Samuel B. Thavamani, Department of Pharmacognosy, PSG College of Pharmacy, Peelamedu, Coimbatore - 641 004, Tamil Nadu, India.
E-mail: samtmani78@rediffmail.com

antitumor activity has yet been reported on *C. pariera*. Therefore, the present study evaluates the *in vitro* and *in vivo* anticancer properties of *C. pariera*.

MATERIALS AND METHODS

Collection and extraction of plant materials

The whole plant was collected in October 2011, from Kalakkad, Tirunelveli District, in South India. The specimen was identified by Prof. V. Chelladurai, Research Officer - Botany, C.C.R.A.S. Govt. of India (Retired). A voucher specimen was prepared in our research laboratory and maintained with voucher no. PSGCP/DPC/02, for further reference. Immediately after collection, the whole plant was washed thoroughly with water and shade-dried at room temperature. The shade dried plant was then pulverized to form a coarse powder and used for extraction. The crude drug was successively extracted in a Soxhlet apparatus, using hexane, petroleum ether, chloroform, methanol, ethyl acetate, and water. The solvents were removed by distillation on a water bath at atmospheric pressure. The final traces of solvents were removed with the help of a rotary evaporator under reduced pressure^[16] and the residual solvents were found to be within the limits of the International Conference on Harmonization (ICH) guidelines 2012. All the extracts were subjected to *in vitro* anti-proliferative activity against the MCF-7 cancer cell line. The methanol extract of *C. pariera* (MECP) was used for *in vivo* antitumor activities.

Estimation of the total alkaloid, phenolic, and flavonoid content

The total amount of alkaloids present in the crude extract was assayed as per the protocol reported earlier.^[17] Total phenolic and flavonoid contents were determined in the MECP extract using the Folin-Ciocalteu reagent and Dowd method, respectively.^[18,19]

In vitro cytotoxic studies

The human breast cancer cell line (MCF-7) was obtained from the National Center for Cell Science (NCCS), Pune, and grown in the Eagles Minimum Essential Medium containing 10% fetal bovine serum (FBS). All the cells were maintained at 37°C, 5% CO₂, 95% O₂ air, and 100% relative humidity. The maintenance cultures were passaged weekly and the culture medium was changed twice a week.

Cell treatment procedure

The monolayered cells were detached with trypsin-ethylene diamine tetra acetic acid (EDTA) to make a single cell suspension. The viable cells were counted using a hemocytometer and diluted with a medium with 5% FBS, to give a final density of 1 × 10⁵ cells/ml. The cell

suspension (100 µL per well) was seeded into 96-well plates at a plating density of 10,000 cells/well, and incubated to allow for cell attachment at 37°C, 5% CO₂, 95% O₂, and 100% relative humidity. After 24 hours the cells were treated with serial concentrations of the extracts. They were initially dissolved in dimethylsulfoxide (DMSO) and further diluted in a serum-free medium, to produce five concentrations. Each concentration (100 µl per well) was added to the plates, to obtain final concentrations of 250, 125, 62.5, 31.25, and 15.62 µg/ml. The final volume in each well was 200 µl and the plates were incubated at 37°C, 5% CO₂, 95% air, and 100% relative humidity for 48 hours. The medium without samples served as the control. Triplicates were maintained for all concentrations. After 48 hours of incubation, 15 µl of MTT (5 mg/ml) in phosphate-buffered saline (PBS) was added to each well and incubated at 37°C for four hours. The medium with MTT was then flicked off and the formed formazan crystals were solubilized in 100 µl of DMSO and then the absorbance was measured at 570 nm using a microplate reader. The percent cell inhibition was determined using the following formula.

$$\text{Percent cell inhibition} = 100 - \frac{\text{Absorbance (sample)}}{\text{Absorbance (control)}} \times 100$$

The non-linear regression graph was plotted between percent cell inhibition and Log₁₀ concentration, and IC₅₀ was determined using the GraphPad Prism 6 software.

Acute toxicity

The acute toxicity in male Swiss albino mice was studied as per the Organization for Economic Cooperation and Development (OECD) guideline 425. The LD₅₀ value of MECP was determined using the method of maximum likelihood.

Induction of cancer using Dalton's lymphoma ascites cells

Dalton's Lymphoma ascites (DLA) cells were obtained under the courtesy of the Amala Cancer Research Center, Thrissur, Kerala, India. The cells were maintained *in vivo* in Swiss albino mice by intraperitoneal administration. When transforming the tumor cells to the grouped animals, the DLA cells were aspirated from the peritoneal cavity of the mice by using saline. The cells were counted and further diluted, so that the total cells would be 1 × 10⁶/mouse. This dilution was given intraperitoneally.

Animals

Male and female adult Swiss Albino mice, about 8 weeks old, with an average body weight of 23 ± 2 g were procured from the KM College of Pharmacy, Madurai,

Tamil Nadu. They were housed in microloan boxes, in a controlled environment, with a temperature of $25 \pm 2^\circ\text{C}$ and a 12 hour dark/light cycle, with a standard laboratory diet, and water *ad libitum*. The study was conducted after obtaining clearance from the Institutional Animal Ethical Committee (Protocol. No. IAEC/KMCP/92/2013). The mice were segregated based on their gender and acclimatized for 15 days before commencement of the experiment.

Treatment protocol

The animals were divided into five groups ($n = 12$). All the groups, except the first group, received 0.1 ml cell suspension of the DLA tumor cell line (1×10^6 cells/mouse, i.p.). This was considered as day '0'. The first group served as the normal saline control, which received normal saline of 5 mL/kg body weight, orally (p.o). The second group served as the DLA tumor cell line control. The third group received the standard drug 5-fluorouracil (20 mg/kg body weight, i.p.) for nine consecutive days. After 24 hours of tumor inoculation, the fourth and fifth groups received MECP at doses of 200 and 400 mg/kg body weight, p.o. On day 15, blood was withdrawn by the retro-orbital plexus method, for estimation of the hematological and serum biochemical parameters. The mice were sacrificed for a study of the antitumor parameters. The remaining animals of each group were kept alive with food and water *ad libitum*, to check for increase in lifespan of the mice.^[20] The effect of MECP on tumor growth and the host's survival time was assessed by observation of body weight, packed cell volume, viable cell count, and percent increase in lifespan.

Cancer cell count

The fluid (0.1 ml) from the peritoneal cavity of each mouse was withdrawn by sterile syringe and diluted with 0.8 ml of sterile Phosphate Buffer Solution and 0.1 ml of trypan blue (0.1 mg/ml) and the total number of the living cells was counted using a hemocytometer. The cell count was calculated by the formula:

$$\text{Cell Count} = (\text{No. of cells in dilution}) / (\text{Area} \times \text{thickness of liquid film}).$$

Hematological parameters

Various hematological parameters including white blood cell (WBC) count, red blood cell (RBC) count, platelet count, hemoglobin, and packed cell volume, were determined.

Body weight

All the mice were weighed from the beginning to the fifteenth day of the study. An average increase in body weight on the fifteenth day was determined.

Percentage increase in lifespan

Percentage increase in lifespan (ILS) was calculated by the formula:

$$\% \text{ ILS} = (\text{Lifespan of treated group}) / (\text{Lifespan of control group}) - 1 \times 100.$$

Serum enzyme and lipid profile

The serum was analyzed for aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase (ALP), total cholesterol (TC), and triglycerides (TG). All biochemical investigations were done by using COBAS MIRA PLUS-S autoanalyzer from Roche, Switzerland. The hematological tests were carried out in COBAS MICROS OT 18 from Roche.

Estimation of Lipid peroxidation, Superoxide dismutase, Catalase

The thiobarbituric acid reactive substances (TBARS) in the cell lysate tissues were measured as per the method reported earlier.^[21] The TBARS content was expressed in micromole/milligram ($\mu\text{moles/mg}$) of protein. The SOD activity in cell lysate was determined as per the method followed earlier.^[22] Enzyme activity was expressed as 1 Unit = 50% inhibition/minute/milligram of protein. The CAT activity in cell lysate was assayed as per the method reported earlier.^[23] CAT was expressed in terms of micromoles of hydrogen peroxide decomposed/minute/milligram of protein.

Statistical analysis

The values are represented as mean \pm SD. The experimental data were assessed by the One-way Analysis of Variance (ANOVA) method followed by Tukey's multiple comparison. The results were considered to be statistically significant when the $P < 0.05$.

RESULTS

Acute toxicity

The MECP did not show any toxic symptoms in animals over a period of 24 hours. The oral extract was non-lethal even at the single dose of 2000 mg/kg.

Secondary metabolites

Estimation of the secondary metabolites showed a significant content of total alkaloid (135.4 mg/100 g), total phenolic (39.5 GAE mg/g), and total flavanoid (4.47 RE mg/g) in MECP [Table 1].

In vitro anti-proliferative study

The MECP exhibited significant ($P < 0.001$) anti-proliferative activity against the breast cancer cell line MCF-7, with a mean IC_{50} value of $95.5 \pm 4.9 \mu\text{g/ml}$.

The IC₅₀ values of hexane, petroleum ether, chloroform, ethyl acetate, and aqueous extract were found to be 255 ± 17.88, 221.5 ± 16.68, 147 ± 7.9, 213.5 ± 8.4, and 224 ± 14.15 µg/ml, respectively [Figure 1].

Effect on tumor growth

The average lifespan of animals in the DLA tumor control group was found to be 48% [Table 2]. The average lifespan of animals treated with MECP at the doses of 200 and 400 mg/kg body weight was found to be 54 and 72%, respectively, whereas the average lifespan of the 5-FU treated group was found to be 92%. An increase in packed cell volume was observed in the DLA control mice when compared with the extract-treated group. The MECP-treated groups showed a significant ($P < 0.001$) reduction in packed cell volume. Similarly, the MECP-treated group exhibited a significant ($P < 0.001$) decrease in the viable cell count at a dose of 400 mg/kg when compared with the MECP-treated group ($P < 0.01$) at the dose of 200 mg/kg. Moreover the antitumor nature of MECP at a doses of 200 and 400 mg/kg was also evident by the significant ($P < 0.001$) reduction in percent increase in body weight of animals treated with MECP at doses of 200 and 400 mg/kg body weight, as compared to DLA tumor-bearing mice. All these results indicated that MECP had significant ($P < 0.001$) activity to inhibit tumor growth induced by the DLA cell line.

Effect on hematological parameters

The hematological parameters were significantly ($P < 0.001$) altered after 14 days of treatment when compared with

the DLA control group. The total WBC count had increased in the DLA control cell whereas, the RBC count, hemoglobin, and platelets decreased in the DLA control cell. After treating for 14 days with MECP, at doses of 200 and 400 mg/kg, the body weight and hematological parameters had normalized, close to the normal group. The WBC significantly ($P < 0.001$) decreased in both the MECP-treated groups, and the RBC significantly ($P < 0.01$) increased in both the MECP-treated groups. Similarly, hemoglobin significantly ($P < 0.001$) increased in both the MECP-treated groups. Platelets significantly ($P < 0.01$) increased in both the MECP-treated groups. All these results suggest the anticancer nature of MECP at a dose of 200 and 400 mg/kg body weight. However, the reference drug 5-FU at the dose of 20 mg/kg body weight produced significant ($P < 0.001$) results in all these parameters [Table 3].

Effect on biochemical parameters

Biochemical parameters like aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglycerides (TGL), and serum cholesterol in the DAC control group were significantly ($P < 0.001$) elevated as compared to the normal saline group [Table 4].

Table 1: Estimation of total alkaloid, phenolic, and flavanoid content of the extracts

Name of the extract	Total alkaloid (mg/100 g)	Total phenolics GAE mg/g	Total flavanoids RE mg/g
Hexane extract	42.7	36.4	1.54
Petroleum ether extract	53.5	34.5	1.74
Chloroform extract	155.3	22.5	3.72
Ethyl acetate extract	60.5	29.4	3.47
Methanol extract	135.4	39.5	4.47
Aqueous extract	33.8	20.1	1.24

GAE: Gallic acid equivalent; RE: Rutin equivalent (at column width)

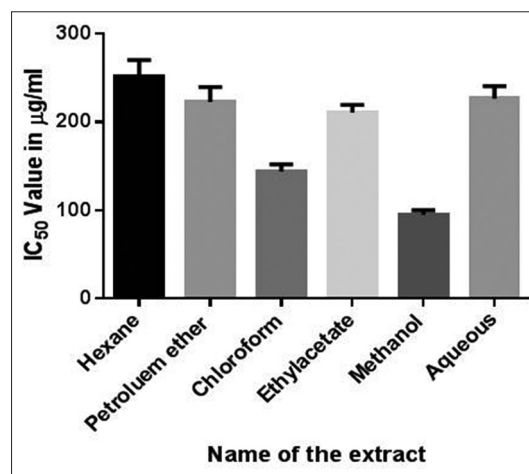


Figure 1: Anti-proliferative studies of hexane, petroleum ether, chloroform, ethyl acetate, methanol, and aqueous extracts against the MCF-7 cancer cell line

Table 2: Effect of MECP on body weight, packed cell volume, viable cell count, and percent increased lifespan of tumor-induced mice

Groups	Increase in body weight (g)	Packed cell volume (ml)	Viable cell count (cells × 10 ⁶ /mL)	Percent ILS Lifespan
Normal saline (5 mL/kg)	2.32±0.51	-	-	>>30 days
DLA control (1×10 ⁶ cells/mouse)	7.72±1.13 ^{#a}	32.27±0.87	2.69±0.26	48%
DLA+5F uracil (20 mg/kg)	3.19±0.36 ^{*a}	18.56±0.62 ^{*a}	1.46±0.21 ^{*a}	92%
DLA+MECP (200 mg/kg)	4.13±0.38 ^{*a}	22.70±0.95 ^{*a}	2.03±0.33 ^{*b}	54%
DLA+MECP (400 mg/kg)	4.33±0.33 ^{*a}	21.58±0.50 ^{*a}	1.71±0.28 ^{*a}	72%

Values are mean±SEM (n=12), [#]DLA control group versus normal saline group, ^{*}All treated groups versus DLA control group, ^aP<0.001; ^bP<0.01, Data were assessed by the one-way ANOVA method followed by Tukey's multiple comparison

Table 3: Effect of MECP on the hematological parameters

Groups	Total WBC cells/ml $\times 10^3$	RBC count mill/cumm	Hb gm/dl	Platelets lakhs/cumm
Normal saline (5 mL/kg)	10.78 \pm 0.28	4.55 \pm 0.39	12.52 \pm 0.57	3.45 \pm 0.43
DLA control (1×10^6 cells/mouse)	14.22 \pm 0.37 ^{#a}	2.48 \pm 0.33 ^{#a}	7.32 \pm 0.40 ^{#a}	1.79 \pm 0.25 ^{#a}
DLA+5F uracil (20 mg/kg)	11.24 \pm 0.32 ^{*a}	4.12 \pm 0.35 ^{*a}	11.49 \pm 0.47 ^{*a}	2.89 \pm 0.31 ^{*a}
DLA+MECP (200 mg/kg)	12.69 \pm 0.44 ^{*a}	3.38 \pm 0.22 ^{*b}	10.20 \pm 0.34 ^{*a}	2.45 \pm 0.46 ^{*b}
DLA+MECP (400 mg/kg)	12.09 \pm 0.35 ^{*a}	3.27 \pm 0.23 ^{*b}	10.29 \pm 0.60 ^{*a}	2.60 \pm 0.40 ^{*b}

Values are mean \pm SEM ($n=12$), [#]DLA control group versus normal saline group, ^{*}All treated groups versus DLA control group, ^a $P < 0.001$; ^b $P < 0.01$, Data were assessed by one-way ANOVA method followed by Tukey's multiple comparison, WBC: White blood cell; RBC: Red blood cell; MECP: Methanol extract of *C. pariera*; DLA: Dalton's lymphoma ascites

Table 4: Effect of MECP on serum biochemical parameters

Groups	Cholesterol (mg/dl)	TGL (mg/dl)	AST (U/L)	ALT (U/L)	ALP (U/L)
Normal saline (5 mL/kg)	116.8 \pm 1.81	135.18 \pm 3.36	43.09 \pm 1.41	35.55 \pm 1.74	128.99 \pm 1.42
DLA control [@]	141.67 \pm 1.99 ^{#a}	223.08 \pm 4.25 ^{#a}	86.7 \pm 1.59 ^{#a}	62.95 \pm 1.47 ^{#a}	242.84 \pm 2.72 ^{#a}
DLA+5F uracil (20 mg/kg)	120.96 \pm 2.05 ^{*a}	170.04 \pm 2.82 ^{*a}	58.87 \pm 2.03 ^{*a}	44.39 \pm 2.42 ^{*a}	162.17 \pm 3.99 ^{*a}
DLA+MECP (200 mg/kg)	123.41 \pm 5.01 ^{*a}	184.09 \pm 4.70 ^{*a}	67.25 \pm 2.32 ^{*a}	51.60 \pm 1.23 ^{*a}	198.58 \pm 2.58 ^{*a}
DLA+MECP (400 mg/kg)	124.50 \pm 2.31 ^{*a}	179.09 \pm 3.20 ^{*a}	64.04 \pm 1.35 ^{*a}	47.61 \pm 2.75 ^{*a}	191.01 \pm 2.14 ^{*a}

[@](1×10^6 Cells/mouse), Values are mean \pm SEM ($n=12$); [#]DLA control group versus normal saline group, ^{*}All treated groups versus DLA control group, ^a $P < 0.001$, Data were assessed by one-way ANOVA method followed by Tukey's multiple comparison; MECP: Methanol extract of *C. pariera*; DLA: Dalton's lymphoma ascites; TGL: Triglycerides; ALT: Alanine aminotransferase; AST: Aspartate amino transferase; ALP: Alkaline phosphatase

Treatment with MECP at doses of 200 and 400 mg/kg body weight significantly ($P < 0.001$) reduced AST, ALT, ALP, TGL, and serum cholesterol to the normal values. The treatment with standard 5- FU also gave similar results.

Effect on superoxide dismutase, catalase, and lipid peroxidation

Superoxide dismutase and catalase activities were reduced significantly ($P < 0.001$) in the DLA control groups compared to that of the normal group. Treatment of both the MECP group significantly ($P < 0.001$) restored the SOD level to the normal value, when compared with the DLA control group. Similarly, administration of MECP at 200 and 400 mg/kg significantly ($P < 0.001$) recovered the CAT level to the normal value when compared with the DLA control group [Table 5].

The TBARS levels expressed as malondialdehyde (MDA) were significantly ($P < 0.001$) increased in the DLA control animals when compared to that of the normal control group. Treatment with MECP at 200 and 400 mg/kg body weight significantly ($P < 0.001$) reduced the MDA levels when compared with the DLA control group.

DISCUSSION

The present study was aimed at analyzing the *in vitro* antitumor activity of various *Cissampelos pariera* extracts against the MCF-7 cancer cell line and *in vivo* antitumor activity of the active extract in DLA tumor-bearing mice. The MECP showed a significant ($P < 0.001$) cytotoxic activity with an IC_{50} value of 95.5 μ g/ml when compared

Table 5: Effect of MECP on lipid peroxidation, SOD, and CAT in DLA bearing mice

Treatment	MDA (μ M/g of protein)	SOD (IU/mg of protein)	CAT (IU/min/mg of protein)
Normal saline (5 mL/kg)	161 \pm 2.66	7.5 \pm 0.43	34.5 \pm 0.11
DLA control (1×10^6 cells/mouse)	500 \pm 5.91 ^{#,a}	3.8 \pm 0.36 ^{#a}	9.5 \pm 0.25 ^{#a}
DLA+5-FU (20 mg/kg)	284 \pm 2.66 ^{*a}	6.3 \pm 0.49 ^{*a}	29 \pm 0.17 ^{*a}
DLA+MECP (200 mg/kg)	313 \pm 8.7 ^{*a}	6.9 \pm 0.29 ^{*a}	19 \pm 2.42 ^{*a}
DLA+MECP (400 mg/kg)	257 \pm 2.95 ^{*a}	6.2 \pm 0.67 ^{*a}	26 \pm 2.48 ^{*a}

Values are mean \pm SEM ($n=6$); [#]DLA control group versus normal saline group, ^{*}all treated groups versus DLA control group, ^a $P < 0.001$ Data were assessed by One way Anova method followed by Tukey's multiple comparison, MECP: Methanol extract of *C. pariera*; DLA: Dalton's lymphoma ascites; MDA: Malondialdehyde; SOD: Superoxide dismutase; CAT: Catalase; FU: 5 Fluoro Uracil

with other extracts. The results of the *in vivo* study revealed that MECP with doses of 200 and 400 mg/kg body weight significantly ($P < 0.001$) reduced the packed cell volume and tumor cell count, and restored the hematological and serum biochemical parameters to the normal values.

Ascetic fluid is the direct nutritional source for tumor cells, and therefore, a rapid increase in ascetic fluid with tumor growth would be a means to meet the nutritional requirement of tumor cells.^[24,25] An enormous increase in ascetic fluid volume was observed in the DLA tumor-bearing hosts, but the group treated with MECP showed a decrease in packed cell volume and viable tumor cell count, and an increased lifespan in the DLA tumor-bearing mice. The reliable measure for assessing the value of an anticancer drug was

increasing the lifespan of the tumor-bearing animal.^[26] It could, therefore, be understood that MECP increased the lifespan of DLA-bearing mice, which was due to the prevention of tumor development. Thus, it can be seen that MECP has antitumor activity against DLA-bearing mice at a dose of 200 mg/kg and 400 mg/kg body weight.

Myelosuppression and anemia are the major problems that are encountered in the treatment of cancer chemotherapy.^[27,28] Anemia occurring in tumor-bearing mice is mainly due to a reduction in erythrocytes or hemoglobin and this may happen either due to iron deficiency or due to hemolytic or myelopathic conditions.^[29] In the present study, the results indicate that MECP significantly increased the erythrocyte count and hemoglobin level when compared to those of the DLA control mice. Moreover, the WBC count had decreased when compared to that of the DLA control mice. These parameters showed that MECP showed a lesser toxic effect to the hemopoietic system and had a reasonably selective affinity to the tumor cell, and hence, it could maintain the normal hematological profile.

Increased levels of AST, ALT, ALP, and serum bilirubin are indicative of impaired liver functions due to cancer.^[30,31] The significantly increased levels of total AST, ALT, ALP, TGL, and cholesterol in the serum of tumor-inoculated animals indicated liver damage and loss of functional integrity of the cell membrane. Treatment with MECP at a dose of 200 and 400 mg/kg body weight restored the above-mentioned parameters to a normal level.

In the present study, the biochemical examination of DLA-inoculated animals showed marked changes indicating the toxic effect of the tumor. MECP significantly reduced the viability of tumor cells and packed cell volume, and normalized the hematological profile and serum biochemical parameters, raising the lifespan of the treated group as compared with those of the DLA control mice. Also the group treated with MECP improved the enzymatic and non-enzymatic antioxidant systems. Decrease of lipid peroxidation and augmentation of SOD and CAT in MECP-treated mice showed its potential as an inhibitor of DLA-induced intracellular oxidative stress. Thus, MECP demonstrated a remarkable *in vitro* and *in vivo* antitumor activity against DLA in mice, probably by attributing lipid peroxidation and increasing endogenous antioxidant systems.

CONCLUSION

The methanol extract of *Cissampelos pariera* contains high amounts of alkaloids, phenolic compounds, and flavonoids, and exhibits significant *in vitro* and *in vivo* antitumor activity.

The antitumor activity may be due to its marked antioxidant potential.

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