ANTITUMOR ACTIVITY OF CRATEVA MAGNA IN TRANSPLANTABLE TUMOR MODELS IN MICE

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Received: 28 August 2019, Revised and Accepted: 27 September 2019

ABSTRACT

Objective: The present study aims to evaluate the anticancer and immunomodulatory activity of both the alcoholic and aqueous extracts of Crateva magna (ALCM and AQCM) in solid tumor model and ascites tumor model in mice.

Methods: The study was divided into in vitro and in vivo sections. In vitro, antioxidant activity of both the extracts was evaluated by well-established antioxidant assays such as α, α-diphenyl-β-picrylhydrazyl radical scavenging, reducing power, and 3-ethylbenzothiazoline-6-sulfonic acid radical scavenging. Both the extracts were subjected for in vitro preliminary cytotoxicity screening by brine shrimp lethality assay and Trypan blue exclusion assay. In vivo antitumor activity of ALCM and AQCM was assessed at the doses of 100 mg/kg and 200 mg/kg bodyweight, respectively, by Dalton’s Lymphoma ascites induced robust tumor model and Ehrlich Ascites Carcinoma induced ascites tumor model in mice. Substantial tumor volume, tumor weight, and % inhibition of the tumor weight in treated and untreated groups were evaluated. In addition to the investigation of antitumor activity, the possible immunomodulatory activity was also assessed.

Results: The studies showed that the ALCM demonstrated more significant antioxidant activity. The results also showed more immunomodulatory activity when compared to AQCM.

Conclusion: The study proves that the plant extract is of higher therapeutic efficacy in cancer. However, the extracts require more exploration toward its usefulness in drug discovery.

Keywords: Crateva magna, Antitumor, Antioxidant, Immunomodulatory.

INTRODUCTION

Cancer is a disease ranked as the second leading cause of morbidity next to cardiovascular diseases [1]. In spite of extensive global research aimed at amending the miserable outcomes of cancer, no apparent decline in the overall mortality rate seen over the past 30 years. From the ancient period, plants are useful in the management of cancer [2]. Over 60% of the presently used anticancer agents obtained in one or the other from natural sources such as plants, marine organisms, and microorganisms [3]. About 13% of all human deaths occurred due to cancer. The American Cancer Society projected that around 7.6 million people die every year due to cancer [4]. Some of the new chemotherapeutic agents that are currently available for use in a clinical setting are plant-based components include Vinca alkaloids, taxane diterpenoids, epipodophyllotoxin ligands, camptothecin, and other derivatives of quinolone alkaloids.

Cravea magna (CM) belonging to the family Capparaceae commonly used in the treatment of urinary disorders that reoccur due to the progress of antibiotic resistance caused by the infecting organism. In traditional medicine, leaves have lithotriptic, diuretic, demulcent, and tonic properties. Externally, the paste of its leaves applied in cervical adenitis, abscess, and edematous wounds. The same adhesive is salutary in the rheumatic joint for relief of pain. In folk medicine, its stem pith used by the tribal people of Kandhamal district of Orissa known as Eastern Ghats of India. The bark of this plant used for lactation after childbirth, treatment of urinary disorders, bladder stones, fever, nausea, and gastrointestinal irritation. Earlier research was done on this plant reports the in vivo sedative and cytotoxic activities of the methanolic extract.

The alcoholic extract decreased the locomotor activity of mice in an elevated plus maze, open field, and hole cross tests. The alcoholic extract exhibited dose-dependent cytotoxicity in brine shrimp lethality assay with an IC50 of 59.67 µg/ml as compared to 0.45 µg/ml shown by standard vincristine sulfate [5] evaluated the bark of the plant for its antipyretic activity on TAB-VACCINE induced pyrexia in rabbits. The alcoholic extract of aerial parts of CM formed a significant decrease in the body temperature in hyperthermic rats. Remya et al. (2009) investigated the entire plant for the treatment of benign prostatic hyperplasia. Alam et al. (2006) evaluated the crude extract of leaves of CM for its antinociceptive activity in mice. The “acetic acid” analgesic test method in mice was used to assess the antinociceptive effect. Crude Ethanolic extracts of CM (250–500 mg/kg PO) showed dose-dependent, antinociceptive effect against chemically induced nociceptive pain stimuli in mice. In vivo and in vitro antitumor activity of leaves of CM has not reported so far. Hence, the current study emphases the antitumor activity of ethanolic and aqueous extract of the CM.

MATERIALS AND METHODS

Materials

Plant material and extraction

The plant material (fresh leaves of CM) was procured from Natural Remedies Pvt. Ltd., Bengaluru and was authenticated by Dr. M. N. Naganandini, Department of Pharmacognosy, JSS College of Pharmacy, Mysuru, India. The coarse powder was extracted with 95% ethanol in the Soxhlet extractor. The residue was dried over a water bath to obtain the alcoholic extract of CM (ALCM). The aqueous extract was prepared by cold maceration method. After completion of extraction, the marc was filtered through muslin cloth followed by filter paper and concentrated and dried on a water bath to obtain the aqueous extract of CM (AQCM).

Methods

The study was divided into in vitro and in vivo sections. In vitro, antioxidant activity of both the extracts was evaluated by well-established antioxidant assays such as α, α-diphenyl-β-picrylhydrazyl radical scavenging, reducing power, and 3-ethylbenzothiazoline-6-sulfonic acid radical scavenging. Both the extracts were subjected for in vitro preliminary cytotoxicity screening by brine shrimp lethality assay and Trypan blue exclusion assay. In vivo antitumor activity of ALCM and AQCM was assessed at the doses of 100 mg/kg and 200 mg/kg bodyweight, respectively, by Dalton’s Lymphoma ascites induced robust tumor model and Ehrlich Ascites Carcinoma induced ascites tumor model in mice. Substantial tumor volume, tumor weight, and % inhibition of the tumor weight in treated and untreated groups were evaluated. In addition to the investigation of antitumor activity, the possible immunomodulatory activity was also assessed.
Cell lines
Dalton’s lymphoma ascites (DLA) and Ehrlich Ascites Carcinoma (EAC) cell lines were procured from Amala Cancer Research Center, Thrissur, India.

Animals
All the animal experiments and handling of animals were approved by the Institutional Animal Ethics Committee (IAEC), JSS College of Pharmacy, Mysuru, India (IAEC approval number 134/2013). Swiss albino mice of eight to 10 weeks old weighing between 25 and 30 g were selected for the study. Animals were acclimatized to the experimental room for 1 week before the experiment. Animals were maintained under controlled conditions of temperature (27 ± 2°C) and were caged in sterile polypropylene cages containing sterile paddy husk as bedding material with a maximum of six animals in each cage. The mice were fed on a standard diet and water ad libitum.

Methods

In vitro antioxidant activity using mice
α, α-Diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging assay
Different concentrations of test extracts (ALCM and AQCM) (50, 100, 150, 200, 250, and 300 µg/ml) were reacted with 5 ml of fresh ABTS+ solution. Absorbance was measured against methanol as blank at 700 nm. The % scavenging activity was calculated [8].

3-Ethylbenzothiazoline-6-sulfonic acid (ABTS) – decolorizing assay
Different concentrations of test extracts (ALCM and AQCM) (50, 100, 150, 200, 250, and 300 µg/ml) were reacted with 5 ml of fresh ABTS+ solution. Absorbance was measured against blank after 15 min of incubation in the dark at 734 nm. L-Ascorbic acids were used as a standard. The % scavenging activity was determined, and IC50 was calculated [7].

In vitro cytotoxic activity
Trypan blue exclusion assay
EAC and DLA cells were aspirated from the peritoneal cavity of tumor-bearing mice. Two to three times, the cells were washed with PBS, and one million cells were incubated with different concentrations of ALCM and AQCM (20, 40, 60, 80, and 100 µg/ml) [10]. Cyclophosphamide (50 µg/ml) alone in a volume of 0.1 ml was used as a control, in 1 ml of PBS for 3 hat 37°C in sterile test tubes. 100 µL of Trypan blue dye (0.4% in PBS) was added after incubation, and the total number of dead (stained) and viable (unstained) cells were counted using a hemocytometer [11]:

% cytototoxicity = \( \frac{\text{No of stained cells}}{\text{Total no of cells}} \times 100 \)

Brine shrimp lethality assay
The test extracts of ALCM and AQCM were prepared using seawater. Ten Nauplli were transferred into sample vials and were treated with different concentrations of test compounds (100, 200, 300, 400, and 500) micromolar in 5 ml of seawater for 24 h. A drop of the suspension was added to each vial. Vials were maintained under illumination. After 24 h, the number of surviving Nauplli was counted using >3 magnifying glass, and the percentage cytotoxicity and IC50 values were determined [12].

In vivo antitumor activity of ALCM and AQCM against EAC inoculated ascites tumor model
EAC cells were procured from the peritoneal cavity of an EAC bearing mouse, after 15 days of tumor transplantation. The ascitic fluid was withdrawn and suitably diluted in phosphate buffer saline to obtain a stock cell concentration of 107 cells/ml. For ascitic tumor 2.5×106; EAC cells (0.25 ml of stock suspension) were injected intraperitoneally to each mouse [13]. Treatment was started after 24 h of tumor inoculation and was continued for 15 days, and the various parameters was evaluated [14]:

% Increase in body weight as compared to “0” day weight
The animals were weighed on the day of inoculation and once in 3 days after that in the post-inoculation period. The % increase in body weight was calculated as follows [15]:

% increase in weight = \( \frac{(\text{Animal weight on a respected day}) - 1 \times 100}{\text{Weight of animal on day 0}} \)

Mean survival time (MST) and percentage increase in life span (%ILS)
A total number of days an animal survived from the day of tumor inoculation were counted. Subsequently, the MST was calculated. The %ILS was calculated as:

\[ \frac{([\text{Mean survival time test} - \text{Mean survival time concentration}] - 1) \times 100}{\text{Mean survival time concentration}} \]

Hematological parameters
To assess the hematological status of treated animals, blood was withdrawn from retro-orbital plexus of the animals. The collected blood was transferred into sodium citrate treated microcentrifuge tubes, and the following parameters were monitored [16]:

Determination of total white blood cell (WBC) count
Blood was drawn up to 0.5 marks of WBC pipette, and excess of blood from the tip was wiped off. The collected blood was diluted with WBC diluting fluid (1% acetic acid, 1 ml glacial acetic acid was added to 99 ml distilled water) up to mark 11 and was mixed thoroughly for 1 min. The fluid was charged on the counting chamber of hemocytometer. The total number of cells in all the four corner squares of WBC counting area was counted using ×10 objective. Then, the total WBC count was calculated from the number of cells/mm using the formula:

Total WBC count = Total number of cells in the WBC counting area × 50

Determination of total red blood cell (RBC) count
Blood was drawn up to 0.5 marks of RBC pipette, and the excess of blood from the tip was wiped off. The collected blood was diluted with RBC diluting fluid and mixed thoroughly for 1 min. The fluid was charged on the counting chamber of hemocytometer. The total number of cells in the RBC counting area was counted using ×45 objective. The average number of cells/mm3 was calculated using the formula:

Total RBC count = Total number of cells in the RBC counting area × 1000

Where 1000 is the volume of RBC counting area.

Estimation of serum biomarkers

Estimation of serum glutamic oxaloacetic transaminase (SGOT)
Four volumes of reagent-1 (TRIS, L-Aspartate, malate dehydrogenase, and lactate dehydrogenase [LDH]) were mixed with one volume of
Tumor volume

Vernier caliper was used to measure the radii of developing tumor at 5 days interval for 1 month, and tumor volume was calculated using the formula:

\[ V = \frac{4}{3} \pi dc^2 \]

Where c and d represent the major and minor radii.

Tumor weight

Animals were sacrificed at the end of the 4th week under anesthesia using diethyl ether; the tumor was extirpated and weighed. The percentage inhibition was calculated by the formula:

\[ \% \text{Inhibition} = \left( \frac{1-D}{C} \right) \times 100 \]

Where C is the average tumor weight of control group, D is that of the treated group.

In vivo immunomodulatory activity

Carbon clearance test

Swiss albino mice of 25–30 g of body weight were used in the experiment. Animals were randomized into the following six groups comprising six animals each. Animals were given the test extracts for 2 days. The mice were administered with carbon ink suspension through tail vein after 48 h of intraperitoneal injection. Blood samples were collected from the retro-orbital vein using glass capillaries at 5 min and 15 min [21]. Collected blood samples were mixed with 0.1% sodium carbonate solution for the lysis of erythrocytes. Phagocytic activity was measured using the spectrophotometer at 675 nm. The phagocytic activity was expressed by the phagocytic index (K) [22].

\[ K = \frac{(\ln OD_1 - \ln OD_2)}{\left( t_1 - t_2 \right)} \]

Where,

\( OD_1 \) and \( OD_2 \) are optical densities at time \( t_1 \) and \( t_2 \).

The clearance rate is expressed as the half-life period of carbon ink in the blood.

\[ t_{1/2} = 0.693/K \]

Delayed type hypersensitivity

Daily treatment of test extracts was given to the mice 5 days before the challenge. Control received the vehicle on each day. On day 0, all animals were immunized with 20 µl of sheep RBC (SRBC) solution (5×10^6 SRBC/ml) injected subcutaneously into their right hind footpad. After 5 days of treatment, the thickness of each animal’s footpad was measured just before the challenge using a digital plethysmometer. The animals were then challenged by injecting the 20µl of SRBC solution into the left hind footpad (deemed time 0). Foot thickness was remeasured after 24 h [23]. The difference between the thickness of left foot just before and after challenge (in mm) was taken as the measure of DTH.

\[ \text{DTH response} = \frac{\text{SRBC treatment paw} - \text{saline treated paw}}{\text{SRBC treated paw} \times 100} \]

Statistical analysis

All experiments were done in triplicate, and values were reported as mean±standard error of the mean. Statistical analysis was by one-way ANOVA followed post hoc Tukey’s multiple comparison analysis. The level of significance was set up at p<0.05.

RESULTS

DPFF-free radical scavenging assay

Both extracts of ALCM and AQCM exhibited dose-dependent free radical scavenging activity. The IC_{50} of AQCM and ALCM was found to
Fig. 1: 3-Ethylbenzothiazoline-6-sulfonic acid radical scavenging activity of alcoholic extracts of *Crateva magna* (CM) and aqueous extracts of CM with respect to the standard. All values are represented as mean±standard error of the mean, n=3

![Fig. 1: 3-Ethylbenzothiazoline-6-sulfonic acid radical scavenging activity of alcoholic extracts of *Crateva magna* (CM) and aqueous extracts of CM with respect to the standard. All values are represented as mean±standard error of the mean, n=3](image1)

Fig. 2: 3-ethylbenzothiazoline-6-sulfonic acid radical scavenging IC$_{50}$ of alcoholic extracts of *Crateva magna* (CM) and aqueous extracts of CM with respect to the standard. All values are represented as mean±standard error of the mean, n=3

![Fig. 2: 3-ethylbenzothiazoline-6-sulfonic acid radical scavenging IC$_{50}$ of alcoholic extracts of *Crateva magna* (CM) and aqueous extracts of CM with respect to the standard. All values are represented as mean±standard error of the mean, n=3](image2)

Fig. 3: Reducing power activity of alcoholic extracts of *Crateva magna* (CM) and aqueous extracts of CM with respect to the standard. All the values are represented mean±standard error of the mean, n=3

![Fig. 3: Reducing power activity of alcoholic extracts of *Crateva magna* (CM) and aqueous extracts of CM with respect to the standard. All the values are represented mean±standard error of the mean, n=3](image3)

be 89.75±0.001 µg/ml and 55.97±0.002 µg/ml, respectively, when compared to ascorbic acid with an IC$_{50}$ of 3.6±0.001 µg/ml which are shown in Fig. 1.

ABTS-decolorizing assay
Both the extracts ALCM and AQCM exhibited dose-dependent free radical scavenging activity. The IC$_{50}$ of AQCM and ALCM was found
to be 268.70±0.5 µg/ml and 270.07±0.38 µg/ml, respectively, when compared to ascorbic acid with an IC_{50} of 22.16±0.4 µg/ml, as shown in Fig. 2.

**Reducing power assay**

Reducing power assay of ALCM and AQCM is shown in Fig. 3:

Figure 4: Percentage cytotoxicity of alcoholic extracts of *Crateva magna* (CM) and aqueous extracts of CM on Ehrlich Ascites Carcinoma cells by Trypan blue exclusion assay. All values are expressed as mean±standard error of the mean, n=3

**Trypan blue exclusion assay**

*In vitro* cytotoxicity of extracts, ALCM and AQCM on EAC cells were determined by Trypan blue exclusion assay. Both the extracts showed dose-dependent cytotoxicity with an IC_{50} of 298±0.50 and 356±0.62 µg/ml, respectively. The percentage cytotoxicity of ALCM and AQCM on EAC cells by Trypan blue exclusion assay is shown in Fig. 4.

Figure 5: Percentage lethality of alcoholic extracts of *Crateva magna* (CM) and aqueous extracts of CM by brine shrimp lethality assay. All values are expressed as mean±standard error of the mean, n=3

Figure 6: Effect of alcoholic extracts of *Crateva magna* (CM) and aqueous extracts of CM on body weight changes in Ehrlich Ascites Carcinoma inoculated mice. All values represent mean±standard error of the mean, n=6. Where *p<0.05* when compared to normal, *p<0.05* when compared to control. Data were analyzed by one-way ANOVA followed by post hoc Tukey’s multiple comparisons. Where, Control (CM 0.25%), Standard (cycle 10 mg/kg), AQCM (100 mg/kg), AQCM (200 mg/kg), ALCM (100 mg/kg), ALCM (200 mg/kg)
A significant increase in serum SGPT level was seen in EAC inoculated control animals with a maximum growth of 75.0±4.5% on day 15 compared to day 0. The cyclophosphamide treatment significantly reduced body weight (18.67±3.6%) compared to control. ALCM treatment at 200 mg/kg significantly decreased the tumor-induced percentage increase in the body weight (37.19±1.1%), and the efficacy was compared with standard. The effect of ALCM and AQCM on body weight changes in EAC inoculated animals is shown in Fig. 5.

Effect of ALCM and ALQCM on body weight changes
Substantial increase in body weight was seen in EAC inoculated control animals with a maximum growth of 75.0±4.5% on day 15 compared to day 0. The cyclophosphamide treatment significantly reduced body weight (18.67±3.6%) compared to control. ALCM treatment at 200 mg/kg significantly decreased the tumor-induced percentage increase in the body weight (37.19±1.1%), and the efficacy was compared with standard. The effect of ALCM and ALQCM on body weight changes in EAC inoculated animals is shown in Fig. 6.

Effect of ALCM and ALQCM on MST and % increase in the life span of EAC inoculated mice
MST of EAC inoculated mice was 15.16±0.7 days. Standard cyclophosphamide treatment at 10 mg/kg significantly improved the MST to 22.83±2.22 days when compared to control. ALCM at 200 mg/kg increased the MST to 2.00±0.816 days, respectively, when compared to control. The %ILS of mice treated with ALCM at 200 mg/kg was 31.87%.

Effect of ALCM and ALQCM on hematological parameters in EAC inoculated mice
To assess the influence of ALCM and ALQCM treatment on hematological parameters, the total RBCs, WBCs, and hemoglobin content of all the treatment groups were checked on the 15th day of tumor inoculation.

Effect on total WBC
A significant rise in total WBC count was seen in EAC inoculated control animal (20.63±0.91 cells/mm³) when compared to the normal animal (7.01±0.53 cells/mm³). Standard cyclophosphamide treatment at a dose of 10 mg/kg resulted in a reduction in the WBC count to (6.05±0.86 cells/mm³) when compared with control. ALCM at dose 100 mg/kg and 200 mg/kg significantly reversed the elevated WBC to 11.67±0.69 cells/mm³ and 13.72±0.63 cells/mm³ when compared to control.

Total RBC
A significant decrease in total RBC count was seen in EAC inoculated control animal (2.84±0.05 cells/mm³) when compared with the normal mice (5.53±0.34 cells/mm³). Treatment with cyclophosphamide 10 mg/kg significantly reversed this reduction to 5.16±0.09 cells/mm³ as compared to control; and ALCM at 100 mg/kg doses increased the total RBC count to 3.51±0.81 and 4.08±0.19 cells/mm³ when compared to control animal.

Effect of ALCM and ALQCM on serum enzyme levels
To assess the influence of ALCM and ALQCM treatment on serum enzyme levels, the ALP, SGOT, and SGPT of all the treatment groups were checked on the 15th day of tumor inoculation.

Effect on serum glutamic oxaloacetic transaminase (SGOT)
A significant increase in serum SGOT level was seen in EAC inoculated control animal (74.96±2.5 mg/kg) when compared to the normal animal (39.98±3.305 mg/kg). Cyclophosphamide at 10 mg/kg reversed tumor-induced elevation in SGOT level (53.6±2.565 mg/kg) when compared with control. ALCM and ALQCM at dose of 200 mg/kg decreased the elevated SGOT level to 6.08±0.2735 mg/kg and 61.86±7.3635 mg/kg compared to control.

Effect on SGPT
A significant increase in serum SGPT level was seen in EAC inoculated control animal (62.30±3.195 mg/kg) when compared to the normal animal (19.82±1.275 mg/kg). Cyclophosphamide at 10 mg/kg reversed tumor-induced elevation in SGPT level (35.75±3.605 mg/kg) when compared with control. ALCM and ALQCM at both doses significantly decreased the elevated SGPT level to 41.70±3.44 5 mg/kg and 44.467±1.765 mg/kg compared to control.

Effect on ALP
A significant increase in serum ALP level was observed in the EAC inoculated control animal (69.85±1.45 mg/kg) when compared to the normal animal (22.69±1.605 mg/kg). Standard cyclophosphamide treatment at 10 mg/kg reversed tumor-induced elevation in the ALP level (30.10±0.845 mg/kg) when compared with control. ALCM and ALQCM at the dose of 200 mg/kg significantly decreased the elevated ALP level to 39.10±0.845 mg/kg, and the reduction in ALP level was comparable to standard.

Effect on serum LDH
A significant increase in serum LDH level was seen in EAC inoculated control animal (238.00±12.35 mg/kg) when compared with control. ALCM at 200 mg/kg dose reversed tumor-induced elevation of LDH to 140.32±2.35 mg/kg.

Evaluation of liver endogenous antioxidant enzymes
To assess the influence of ALCM and ALQCM treatment on endogenous liver antioxidant levels, all the treatment groups were checked on the 15th day of tumor inoculation.

Effect of GSH
A significant decrease of GSH concentration was observed in liver homogenate of ECA inoculated control mice 0.85±0.15 mg/kg when compared to normal animal 2.95±0.35 mg/kg. Cyclophosphamide at a dose of 10 mg/kg significantly reversed the tumor-induced decrease in GSH concentration 1.78±0.15 mg/kg when compared with control. ALCM at 200 mg/kg significantly reversed GSH concentration to 1.57±0.2305 mg/kg, and the efficacy was compared with the standard group.

Effect of SOD
A significant decrease in SOD concentration was observed in liver homogenate of ECA inoculated control mice 2.28±0.25 mg/kg when compared to normal animal 4.54±0.25 mg/kg. Standard cyclophosphamide treatment at 10 mg/kg dose significantly reversed the tumor-induced decrease in SOD concentration 3.92±0.155 mg/kg when compared with control. ALCM at 200 mg/kg reversed GSH concentration to 3.09±0.25 mg/kg significantly in treated animals, the efficacy was compared with the standard group.

Influence of extracts on DNA fragmentation assay
The effect of extracts on DNA fragmentation assay is shown in Fig. 7:

In vivo anticancer activity of ALCM and ALQCM on DLA inoculated solid tumor model
The weight of solid tumor in the control animal was found to be 6.85±0.226 g by the end of the 4th week. Cyclophosphamide at 10 mg/kg dose showed a significant reduction in the solid tumor weight by 85.76±1.02 g when compared to control. ALCM and ALQCM at the dose of 200 mg/kg caused a significant reduction in the solid tumor weight when compared with control. ALCM at a dose of 200 mg/kg was more effective in reducing the tumor weight by 54.02±6.3%. The extent of tumor growth inhibition was not comparable to standard.

Carbon clearance test
There was an increased clearance of carbon particles from blood after administration of ALCM at 100 mg/kg and 200 mg/kg p.o. It is specified by a significant rise in the phagocytic index when compared to control. The effect of ALCM and ALQCM on the phagocytic index is shown in Fig. 8.
The effect of ALCM and AQCM on the paw volume in mice when compared with the control group. ALCM 100 mg/kg and 200 mg/kg p.o. showed a dose-dependent increase in volume. The data were analyzed by one-way ANOVA followed by post hoc Tukey's multiple comparison test.

**CONCLUSION**

The present study indicated that ALCM exhibit more antioxidant potential by well-established methods. Both the extracts of CM were assessed for their in vivo antioxidant activity in both EAC and DLA transplanted tumor-bearing animals. Both the extracts reflected their cytotoxic potential as evident in vitro cytotoxicity assays such as brine shrimp lethality assay and Trypan blue cytotoxicity assay. ALCM exhibited more potent cytotoxicity as compared to AQCM. In the EAC model, ALCM showed a reduction in increased body weight, as well as an increment in the survival time, which indicates inhibition in the tumor progression. The alcoholic extract at the dose of 200 mg/kg body weight reversed the hematological parameters and restored the endogenous antioxidant and serum enzymes, which signified with that of control. Treatment by ALCM and AQCM at 200 mg/kg dose reversed the enhanced WBC count in the treated group, which was more significant to that of control. Standard cyclophosphamide at 10 mg/kg dose decreased the cell count than the normal reflecting myelosuppression. There was a decrease in RBC count in the control group, which was not reflected in the treated groups. ALCM treatment at a dose of 200 mg/kg enhanced the survival time up to 27.47%. %ILS by 25% or more when compared to control is considered as an effective anticancer response. The therapy with ALCM and AQCM at 200 mg/kg reversed the cell count than the normal indicating its ability to enhance the immunomodulatory activity. Both the extracts showed the enhancement in lifespan, more than that of control. The influence of tumor growth rate was evaluated by the reduction of solid tumor weight and the delay in tumor progression. The alcoholic extract of CM at the dose of 200 mg/kg substantially decreased the body weight to 37.1 g and 40.6 g. It is evident that ALCM demonstrated significantly more activity when compared to AQCM. MST enhanced by ALCM treatment at a dose of 200 mg/kg. The life span was increased up to 31.87% by ALCM at a dose of 200 mg/kg. AQCM 200 mg/kg also enhanced the survival time up to 27.47%. %ILS by 25% or more when compared to control is considered as an effective anticancer response. Both the extracts showed the enhancement in lifespan, more than that of control. ALCM showed a significant increase in the survival time up to 27.47%. %ILS by 25% or more when compared to control is considered as an effective anticancer response. The therapy with ALCM and AQCM at 200 mg/kg reversed the tumor-induced alternation in hematological parameters.
ACKNOWLEDGMENT
The authors are thankful to Principal, JSS College of Pharmacy, Mysore, Academy of Higher Education and Research, Mysore, for providing the necessary infrastructure in completing the project.

AUTHOR’S CONTRIBUTIONS
Ms. Ankitha Deep performed the in vivo and in vitro studies at JSS College of Pharmacy. Ms. Sindhu R prepared the manuscript. Santhepete N Manjula and Kenagangora Mruthunjaya designed and guided to carry out the experiments and evaluated the result part of in vitro and in vivo studies and reviewed the manuscript.

CONFLICTS OF INTEREST
The authors declare that they have no conflicts of interest.

REFERENCES