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ANTITUMOR ACTIVITY OF METHANOLIC EXTRACT OF ROOT OF *DECALEPIS HAMILTONII* IN DALTON'S LYMPHOMA ASCITES (DLA) BEARING MICE

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ABSTRACT: The antitumor activity of methanolic extract of *Decalepis hamiltonii* was evaluated using Dalton's Lymphoma Ascites (DLA) tumor model in Swiss albino mice. Methanolic extract of *Decalepis hamiltonii* was administered at a dose of 200mg and 400mg/kg b.wt once daily for 14 days after 24h of tumor inoculation. Cyclophosphamide was used as a standard drug at a dose of 25mg/kg b.wt. The result showed that methanolic extract of *Decalepis hamiltonii* root increased the mean survival time and percentage increase in life span and also decreased the body weight of the DLA tumor bearing mice. Hematological studies reveal that increase in hemoglobin content and RBC count and decrease in WBC count was observed in methanolic extract of root of *Decalepis hamiltonii* and cyclophosphamide treated mice. Administration of methanolic extract of root of *Decalepis hamiltonii* and standard control significantly decrease the serum marker enzymes (aspartate transaminase, alanine transaminase) and protein level to nearly normal levels in DLA tumor bearing mice. The methanolic extract of root of *Decalepis hamiltonii* at a dose of 400mg shows potent antitumor activity.

INTRODUCTION: Cancer is an abnormal type of tissue growth in which the cells exhibit an uncontrolled division, relatively in an autonomous fashion, leading to a progressive increase in the number of dividing cells ¹.

Cancer is one of the most dreaded diseases of the 20th century and spreading further continuously and increasing incidence in 21st century.

Cancer is caused by internal factors (tobacco, chemicals, radiation and infectious organisms) and external factors (mutation, hormones and immune conditions) ² and can be treated with surgery, radiation, chemotherapy, hormone therapy and biological therapy. Cancer treatment is usually accompanied by diverse side effects to different body organs.

Cancer presents a serious clinical problem and poses significant social and economic impacts on the health care system. Despite improved imaging and molecular diagnostic techniques, the disease still impacts millions of patient's worldwide ³. Cancer is the leading cause of mortality worldwide and the failure of conventional chemotherapy to effect major reduction in the mortality indicates that new approaches are critically needed.



An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents to block the development of cancer in humans⁴.

A variety of bioactive compounds and their derivatives have been shown to inhibit carcinogenesis in a number of experimental systems involving initiation, promotion and progression⁵. Plants, vegetables and herbs used as folk and traditional medicine have been accepted currently as one of the main sources of cancer chemoprevention drug discovery and development⁶.

Living organisms, including plants, microbes and marine organisms provide rich sources of chemically diverse bioactive compounds. More than 40% of the chemicals, thus far identified as natural products, have not been chemically synthesized. Natural products and their derivatives including Vinblastine, Paclitaxel and Etoposide already play critical roles in cancer chemotherapy^{7,8}.

In addition to substances that pose cancer risk, the human diet also contains vegetables, fruits and beverages, which not only provide essential vitamins and minerals, but include important chemopreventive agents capable of protecting against some forms of human cancer⁹. Such chemopreventive agents are known as anticarcinogens, and ideally they should be non-toxic.

The area of anticancer remedial gained massive attentions from scientists all over the world¹³. With better indulgent of pathophysiology of the disease. Since cell homeostasis depends on the balance between proliferation and apoptosis, effective compounds inducing apoptosis appear to be a relevant strategy to suppress tumor growth¹⁰. Many chemotherapeutic drugs eliminate cancer cells by inducing a genetically programmed form of cell death¹¹.

Medicinal plants have been used for years in daily life to treat diseases all over the World. Drugs derived from unmodified natural products or drugs semi-synthetically obtained from natural sources corresponded to 78% of the new drugs approved by the FDA between 1983 and 1994. This evidence contributes to support and quantify the importance of screening of natural products¹². Plants have a long history of use in the treatment of cancer. Drug discovery from plants is a multi-disciplinary

approach which combines various botanical, ethnobotanicals, phytochemical and biological and chemical separation techniques¹³.

Plant and plant products both as extracts and derived compounds are known to be effective and versatile chemopreventive drugs against various types of cancer¹⁴. Identification of phytochemicals is found to be effective chemopreventive agents in the field of chemoprevention research. Herbal remedies have been used for various treatments from the day of careman. Traditional medicine has been widely used to heal cancer in about 75-80% of world population¹⁵. Increased demand for herbal products is the 'back-to-nature' global trend and the belief of the general public is that natural products are much safer without deleterious side effects.

Antitumor activities have been reported in several plant species^{16, 17} however, up to now, few researches have been done to investigate this traditionally used plant in the recognition of their mechanism, guaranteeing in the future its scientific and therapeutic use. Selection of plants based on ethno medical knowledge and testing the selected plants efficacy as well as safety is one of the best approaches for the isolation of anticancer lead molecules from the medicinal plants¹⁸.

Plant derived natural products such as flavonoids, terpenoids and steroids etc have received considerable attention in recent years due to their diverse pharmacological properties including antioxidant and antitumor activity¹⁹.

The present was designed to evaluate the antitumor activity of methanolic extract of root of *Decalepis hamiltonii* in Dalton's Lymphoma Ascites bearing mice.

MATERIALS AND METHODS

Plant material: The root of *Decalepis hamiltonii* used for the investigation was purchased from a plant supplier in Chennai, Tamil Nadu, India. The plant was authenticated taxonomically at Plant Anatomy and Research Center, Chennai, Tamil Nadu, India.

Preparation of extract: The collected root of *Decalepis hamiltonii* were dried in shade, crushed to coarse powder. The powder was defatted with petroleum ether (60 – 80°C) and then extracted with 90% methanol using soxhlet extractor.

The solvent was evaporated under reduced pressure and dried in vacuum and the filtrate obtained was used for further studies.

Animals: Swiss male Albino mice weighing 20 – 25g were obtained from animal house, JSS college of Pharmacy, Ooty and sanctioned by animal ethical committee and proposal number is JSSCP/IAEC/M.Pharm/PH.COG/06/2009-10. The animals were kept in microloan boxes in a controlled environment (temperature $25\pm 2^{\circ}\text{C}$ and 12h dark and light cycle) with standard pellet diet and water ad libitum.

Tumor cells: The Daltons cells were obtained from JSS College of Pharmacy, Ooty.

Maintenance of DLA cell lines: The DLA cells were propagated in the peritoneal cavity of the mice by injecting 10^6 cells. The cells were aspirated aseptically from developed tumor mice, during the log phase on the 15th day of tumor transplantation using 18 gauge needles by withdrawing the fluid from peritoneal cavity.

The ascitic fluid was washed three times in PBS (phosphate buffer saline) and the cell pellet was re-suspended in PBS. The tumor cell count was done using trypan blue dye exclusion method in a hemocytometer. The cell suspension was diluted to get $2 \times 10^6/0.1$ ml.

In vitro Cytotoxicity activity by MTT assay method ²⁰: The methanolic extract of *Decalepis hamiltonii* root was subjected for *in vitro* cytotoxicity activity by MTT assay method using two types of cell lines i.e. Vero cell line and A₅₄₉ cell line (cancerous cell line).

The ability of cell to survive a toxic insult has been the basis of most cytotoxicity assays. This assay is based on the assumption that dead cells or their products do not reduce tetrazolium. The assay depends both on the number of cells present and on the mitochondrial activity per cell. The cleavage of MTT to a blue formazan derivative by living cells is clearly a very effective principle on which the assay is based.

The principle involved is the cleavage of tetrazolium salt 3- (4, 5 dimethyl thiazole- 2yl)- 2, 5-diphenyltetrazolium bromide (MTT) into a blue colored products (formazan) by mitochondrial enzyme succinate dehydrogenase.

The number of cells was found to be proportional to the extent of formazan production by the cells used.

Requirements:

- 1) Confluent mono layer cell cultures
- 2) F-12 Conn's medium with antibiotics
- 3) New born calf serum
- 4) Micro titer plate (96 wells)
- 5) MTT (prepared in HBBS without phenol red, 2mg/ml)
- 6) Propanol

Procedure:

- 1) The monolayer cell culture was trypsinized using TPVG and cell count was adjusted to 1.0×10^5 cells/ml using medium containing 10 % new born calf serum.
- 2) To each well of the 96 well micro titer plates, 0.1 ml of the diluted cell suspension (approximately 10,000 cells) was added.
- 3) After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100 μl of methanolic extract of (1000 to 15.6 $\mu\text{g/ml}$) *Decalepis hamiltonii* was added to the cells in micro titer plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours.
- 4) After 72 hours, the drug solutions in the wells were discarded and 50 μl of MTT (MTT: prepared in Hank's Balanced Salt Solution without phenol red) [(HBSS-PR), 2 mg/ml, Sigma Chemicals] was added to each well.
- 5) The plates were gently shaken and incubated for 3 hours at 37°C in CO_2 atmosphere.
- 6) The supernatant was removed and the 50 μl of propanol was added and the plates were gently shaken to solubilize the formed formazan.
- 7) The absorbance was measured using a micro titer reader (ELISA Reader, Biorad) at a wavelength of 540 nm.

The percentage growth inhibition was calculated using the formula below:

$$\% \text{ Growth inhibition} = 100 - \left[\frac{\text{Control OD} - \text{Sample OD}}{\text{Total cells}} \right] \times 100$$

In vivo anticancer activity: Male Swiss albino mice were divided into 5 groups of 6 mice each. All the groups were inoculated with $2 \times 10^6/0.1\text{ml}$ DLA cells intraperitoneally except normal control group.

1. Group 1: Normal control group
2. Group 2: Cancer control
3. Group 3: Cancer mice received Cyclophosphamide (25mg/kg ip)
4. Group 4: Cancer mice received 200mg/kg of methanolic extract of *Decalepis hamiltonii*
5. Group 5: Cancer mice received 400mg/kg of methanolic extract of *Decalepis hamiltonii*.

Treatment was started after 24 hours of inoculation of DLA cells, once daily for 14 days. After 14 days, mice were sacrificed, blood samples were collected from all groups to evaluate hematological and biochemical parameters.

The anti-tumor activity measurements: The anti-tumor activity was measured in DLA bearing mice with respect to the following parameters.

- 1) Body weight analysis
 - 2) Mean survival time (MST)
 - 3) Percentage increase in life span (% ILS)
 - 4) Hematological parameters
 - 5) Biochemical estimation
1. **Body weight analysis:** Body weight were recorded at the beginning of the experiment (day 0) and sequentially on every 7th day (weekly interval) during the treatment period. Average body weight and percentage decrease in body weight was calculated with the formula;

% Decrease in body weight =

$$\frac{\text{Gain in body weight of control} - \text{Gain in body weight of treated group} \times 100}{\text{Gain in body weight of control}}$$

❖ Determination of MST and %ILS

At the end of the experiment, the effect of methanolic extract of *Decalepis hamiltonii* root on tumor growth was monitored by recording the mortality daily for 5 weeks (MST) and percentage increase in life span (% ILS) was calculated ²¹.

$$\text{Mean survival time} = \frac{\text{Day of 1}^{\text{st}} \text{ death} + \text{Day of last death}}{2} \quad (\text{MST})$$

$$\% \text{ ILS} = \frac{\text{MST of treated group} - \text{MST of control group}}{\text{MST of the control group}} \times 100$$

2. **Hematological parameters:** Effect of methanolic extract of *Decalepis hamiltonii* and standard drug on hematological parameters on DLA tumor model was determined. The estimation of hemoglobin, Red blood cells ²² and White blood cells count ²³ was done by standard procedures.
3. **Biochemical estimation:** The effect of methanolic extract of root of *Decalepis hamiltonii* and standard drug on various biochemical parameters like aspartate transaminase, alanine transaminase by Reitman and Frankel method ²⁴ and total protein by Lowry method ²⁵ was determined.

Statistical Analysis: Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by student's 't' test. The values are mean \pm SD for six rats in each group. Statistical significance was considered at $p < 0.05$.

RESULTS:

In-vitro cytotoxicity by MTT assay method: The cytotoxic effect of methanolic extract of *Decalepis hamiltonii* root was tested on Vero cell line and A-549 cell lines by MTT method. The CTC₅₀ value of methanolic extract of *Decalepis hamiltonii* root for Vero cell line and A-549 cell lines were 60 $\mu\text{g/ml}$ and 215 $\mu\text{g/ml}$ respectively. The methanolic extract of *Decalepis hamiltonii* showed good cytotoxicity against cell lines (Table 1).

TABLE 1: CTC₅₀ VALUE OF METHANOLIC EXTRACT OF *DECALEPIS HAMILTONII*

Cell type	Cell line	Methanolic extract (CTC ₅₀)
Normal	Vero	60 µg/ml
Cancer	A-549	215 µg/ml

In-vivo Anticancer Activity:

Mean survival time and percentage ILS: In DLA control group, mean survival time and percentage increase in life span was decreased (**table 2**). On administration of 200mg and 400mg of methanolic extract of root of *Decalepis hamiltonii* increase the mean survival time and percentage increase in life span in DLA control group. Treatment with 400mg of methanolic extract of *Decalepis hamiltonii* showed significant increase in mean survival time compared to 200mg of methanolic extract of *Decalepis hamiltonii*.

TABLE 2: EFFECT OF METHANOLIC EXTRACT OF ROOT OF *DECALEPIS HAMILTONII* ON MEAN SURVIVAL TIME AND % ILS OF DLA BEARING MICE

Groups	Mean Survival Time	% ILS
Cancer control	21.4 ± 0.58 ^a	-
Std. Control	32.4 ± 0.40 ^b	41.94
200 mg MEDH	26 ± 0.63 ^c	17.11
400mg MEDH	31 ± 0.48 ^b	30.20

Values are expressed as mean ± SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at P ≤ 0.05.

Table 4: Anticancer effect of methanolic extract of *Decalepis hamiltonii* on weekly body weight analysis of DLA bearing mice

Groups	Weekly body weight					
	0 day	7 day	14 day	21 day	28 day	32 day
Cancer Control	27.8 ± 1.28	30.6 ± 1.20	35.4 ± 0.81	40.5 ± 0.64 ^a	All animals dead	All animals dead
Standard Control	28.4 ± 0.67	28.2 ± 0.80	32.2 ± 0.73	33.2 ± 0.80 ^b	33.0 ± 0.12	31.67 ± 0.33
200 mg MEDH	27.8 ± 0.58	29.6 ± 1.16	34.0 ± 0.94	38.2 ± 0.96 ^c	All animals dead	All animals dead
400 mg MEDH	27.4 ± 2.15	28.6 ± 1.86	33.0 ± 1.64	32.8 ± 1.06 ^b	31.6 ± 0.86	All animals dead

Values are expressed as mean ± SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at P ≤ 0.05.

Hematological parameters: As shown in **table 5**, the hemoglobin content and RBC count in DLA bearing mice were significantly decreased when compared to normal mice. Treatment with methanolic extract of *Decalepis hamiltonii* at the dose of 200mg and 400mg/kg increased the hemoglobin content, RBC count to more or less normal levels. The total WBC counts were significantly higher in the DLA bearing mice when

Body weight analysis: **Table 3** shows the effect of methanolic extract of *Decalepis hamiltonii* on average body weight in DLA bearing mice. The average body weight was found to have a significant increase in cancer control as compared to normal control. On administration of 200mg and 400mg of methanolic extract of *Decalepis hamiltonii* shows the reduction in the body weight in DLA bearing mice. Hence the extract treatment at 200mg and 400mg was found to be more potent than the standard drug.

TABLE 3: ANTICANCER EFFECT OF METHANOLIC EXTRACT OF *DECALEPIS HAMILTONII* ON BODY WEIGHT OF DLA BEARING MICE

Groups	Average body weight in gms
Normal	30.0 ± 0.90 ^a
Cancer control	40.4 ± 0.74 ^b
Std. Control	30.2 ± 1.24 ^a
200 mg MEDH	38.5 ± 1.07 ^c
400 mg MEDH	32.8 ± 1.02 ^a

Values are expressed as mean ± SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at P ≤ 0.05.

Since the cancer control animals were survived up to 21.4 days, percentage decrease in body weight was calculated on 20th day. Antitumor effect of methanolic extract of *Decalepis hamiltonii* on weekly body weight analysis on DLA bearing mice was shown in **table 4**.

compared with normal. On administration of methanolic extract of *Decalepis hamiltonii* at the dose of 200mg and 400mg/kg reduced the WBC count as compared to DLA control. Treatment with 200mg/kg of methanolic extract of *Decalepis hamiltonii* also recovered these altered depleted parameters towards normal though treatment with 400mg/kg was found to be more effective.

TABLE 5: EFFECT OF METHANOLIC EXTRACT OF *DECALEPIS HAMILTONII* ON HEMATOLOGICAL PARAMETERS OF DLA BEARING MICE

Groups	RBC (X10 ⁹ /ml)	WBC (X10 ⁶ /ml)	Hb (g %)
Normal	12.03 ± 0.30 ^a	7.64 ± 0.56 ^a	14.46 ± 0.43 ^a
Cancer control	7.33 ± 0.4 ^b	16.48 ± 0.53 ^b	8.7 ± 0.74 ^b
Std Control	10.59 ± 0.28 ^c	8.32 ± 0.39 ^a	13.9 ± 0.44 ^a
200 mg MEDH	9.69 ± 0.66 ^d	10.96 ± 0.27 ^d	10.16 ± 0.20 ^d
400 mg MEDH	10.25 ± 0.48 ^c	9.44 ± 0.29 ^c	12.94 ± 0.41 ^c

Values are expressed as mean ± SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at P ≤ 0.05.

Biochemical parameters: Biochemical estimations indicate that significantly elevated the levels of serum aspartate transaminase, alanine transaminase and total protein in DLA bearing mice with respect to normal control. However on administration of methanolic extract of *Decalepis hamiltonii* at the dose of 200mg and 400mg/kg and standard drug

significantly reduced the levels serum aspartate transaminase, alanine transaminase and total protein as compared to cancer control group indicating the protection of the tumor cell induced hepatotoxicity by methanolic extract of *Decalepis hamiltonii* (table 6).

TABLE 6: ANTICANCER EFFECT METHANOLIC EXTRACT OF *DECALEPIS HAMILTONII* ON BIOCHEMICAL PARAMETERS OF DLA BEARING MICE

Groups	ALT (U/L)	AST (U/L)	Total Protein(g/dl)
Normal	32.2 ± 0.58 ^a	85.6 ± 0.81 ^a	3.4 ± 0.24 ^a
Cancer control	50.8 ± 0.86 ^b	103.6 ± 1.20 ^b	7.4 ± 0.24 ^b
Std. control	33.6 ± 0.58 ^a	88.8 ± 0.73 ^c	4.4 ± 0.24 ^a
200mg MEDH	40 ± 1.04 ^c	87 ± 0.70 ^c	5.4 ± 0.24 ^c
400mg MEDH	35.6 ± 1.20 ^a	82.6 ± 1.24 ^d	4.6 ± 0.24 ^a

Values are expressed as mean ± SD. ANOVA followed by Duncan's multiple range tests. Values not sharing a common superscript differ significantly at P ≤ 0.05.

DISCUSSION: The present investigation was carried out to evaluate the antitumor activity of methanolic extract of *Decalepis hamiltonii* in DLA tumor bearing mice. Many natural products have served as anticancer agents and also as lead compounds for further research. Many plants are used to treat tumors in the Indian traditional system of medicine, but most of the plants have not been scientifically evaluated²⁶. Hence enormous scope exists for identifying potent anticancer plants. The use of chemotherapeutic drugs in cancer involves the risk of life threatening host toxicity. Several studies were conducted to minimize the side effects after treatment with the chemotherapeutic agents using different modalities²⁷.

A successful anticancer agent should kill or incapacitate cancer cells without causing excessive damage to normal cells. Certain products from plants are known to induce apoptosis in cancer cells but not in normal cells. Thus, it is important to screen apoptotic inducers from plants, either in the form of crude extracts or as active isolated components.

However plant phytochemicals serve to continue as a viable source of drugs for the world population and several plant-based drugs are in extensive clinical use²⁸.

The present study revealed that methanolic extract of *Decalepis hamiltonii* at the dose of 400mg/kg significantly increased the life span of the mice when compared to the DLA control. The reliable criteria for judging the potency of any anticancer drug are prolongation of life span²⁹, inhibition of gain in average body weight and decrease of WBC from blood³⁰.

The methanolic extract delayed the cell division, thereby suggesting the reduction in DLA volume and increased survival time in mice which suggested the antiproliferative effect of the extract³¹. Methanolic extract of *Decalepis hamiltonii* at the dose of 400mg/kg significantly improved the mean survival time in tumor bearing mice. The cytotoxicity of chemotherapeutic agents is effective on malignant as well as all the rapidly dividing cells.

Hair loss, mucosal ulceration and suppression of hemopoiesis occur due to same reason. Usually, in cancer chemotherapy the major problems that are being encountered are of myelosuppression and anemia (reduced hemoglobin)³². In DLA control mice, elevated WBC count; reduced Hemoglobin and RBC count were observed. In this study, it was observed that the oral administration of methanolic extract of *Decalepis hamiltonii* extracts restored hemoglobin content and maintained the normal values of RBC and WBC, thus supporting its hematopoietic protecting activity without induced myelotoxicity, the most common side effects of cancer chemotherapy. The improvement in hematological profile of the tumor bearing mice following the treatment with extract could be due to the action of the different phyto constituents present in the extract.

Anemia encountered in ascites carcinoma mainly due to iron deficiency, either by hemolytic or myelopathic conditions which finally lead to reduced RBC number³³. Decrease in hemoglobin is common in cancer patients and given its known adverse impact on physical functioning and quality of life variable including fatigue and cognitive function. Associations have been found between low hemoglobin levels and decreased survival in cancer patients as well as solid tumors³⁴. Present results were coinciding with the fact that cancer has been well described for many years as a cause of microangiopathic hemolytic anemia and thrombocytopenia³⁵.

Recently, compelling evidence is that immune responses are impaired in patients with oral squamous cell carcinoma (SCC)³⁶. Some studies have shown that a growing tumor burden correlates with aggravating changes in immunity. The leucocytes of the innate immune system, including neutrophils, macrophages and NK cells, infiltrate the tumor site for a multipronged killing response³⁷. The significant increase in WBCs and neutrophils in tumor bearing mice due to the fact that these cells are the first one to arrive at sites of infection, where they can release chemokines and proteases that can in turn recruit both non-specific and specific immune effector cells³⁸. They can also release toxic granules against neighboring cells, suggesting potential anti-tumor activity³⁹.

Recent physiological, pharmacological and biochemical studies appear to support the wisdom of the traditional dietary practices. They proved that phytochemicals from fruits and vegetables have shown to exert varied beneficial biological function. Preliminary phytochemical screening revealed the presence of carbohydrates, phytosterols, fixed oils and phenolic compounds, saponins, proteins, tannins and flavanoids. Flavanoids have been shown to possess antimutagenic and antimalignant effect⁴⁰. Moreover, flavanoids have a chemo preventive role in cancer through their effects on signal transduction in cell proliferation and angiogenesis⁴¹. The cytotoxicity and anticancer activity of methanolic extract is probably due to the presence of flavanoids.

Phytosterols present are able to get incorporated in the cell membrane, alters membrane fluidity and the activity of membrane-bound enzymes. They also alter signal transduction in pathways leading to tumor growth and stimulate apoptosis in tumor cell lines. They also have been shown to enhance in-vitro human peripheral blood lymphocyte and T-cell proliferation in vitro which suggests a possible stimulation of the immune system function⁴².

Significant elevation in the levels of aspartate transaminase, alanine transaminase and protein reflects the hepatocellular damages caused by a number of agents. Biochemical measurements of these parameters showed that to some extent hepatotoxicity was associated after inoculation with DLA. It was reported that the presence of tumor in humans or experimental animals is known to affect many functions of the vital organs especially the liver, even when the site of the tumor does not interfere directly with organ functions⁴³.

Treatment with methanolic extract of *Decalepis hamiltonii* restored the elevated biochemical parameters more or less to normal range, indicating the protection of the tumor cell induced hepatotoxicity by methanolic extract of *Decalepis hamiltonii*.

However, further investigation to explore the potential of the methanolic extract of *Decalepis hamiltonii* in tumor treatment may prove to be worthwhile. Further studies to characterize the active principles and elucidate the mechanism of the action of *Decalepis hamiltonii* are in progress.

CONCLUSION: In the present study, the antitumor effect of methanolic extract of *Decalepis hamiltonii* was assessed by elevating tumor weight and hematological parameters of DLA tumor bearing mice. The antitumor activity of methanolic extract of *Decalepis hamiltonii* is probably due to its flavanoid content. However further research is ongoing to isolate the pure bioactive compounds.

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