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Exploration of Cytotoxicity and Anti-tumor Activity of *Manilkara hexandra* Leaves on Ehrlich Ascites Carcinoma Model

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Abstract

Manilkara hexandra (Sapotaceae) is an evergreen, medium-sized tree that grows in tropical and temperate regions. The different parts of this plant are traditionally used as an expectorant, antileprotic, febrifuge, purgative, aphrodisiac, and anthelmintic. At dosages of 50, 100, 200, 400, and 800 µg/mL, the anti-cancer efficacy of the ethanolic extract of *Manilkara hexandra* (EELMH) against Ehrlich Ascites Carcinoma (EAC) cells was evaluated using MTT and trypan blue assays. It exhibited a cytotoxic effect with IC50 values of 612 µg/mL and 624.97 µg/mL in MTT and trypan blue assays, respectively, after 48 hours. DAPI staining after treating the EAC cells at an IC50 value of 612 µg/ml showed morphological changes in the nucleus. The further anti-tumor activity of EELMH was evaluated in vivo using EAC-bearing Swiss albino mice at 200 and 400 mg/kg body weight. The effect of EELMH on tumor growth, cell viability, lifespan, hematological, and biochemical parameters of EAC-bearing mice was assessed. In addition to extending the lifespan of EAC-bearing mice, EELMH demonstrated a significant reduction in tumor volume, packed cell volume, and viable cell count. The hematological and serum biochemical parameters returned to nearly their original levels in mice treated with EELMH, specifically at a dose of 400 mg/kg.

Keywords: Anti-tumor; Cytotoxic; EAC cells; *Manilkara hexandra*; *Sapotaceae*; *IC50.*

1. Introduction

Cancer is characterized by uncontrolled changes in the natural process of cell division and has become a major health issue that kills many people every year all over the globe [1, 2]. Based on the given statistics, >19.3 million (19,300,000) new instances of cancer were diagnosed and reported lately; this is anticipated to result in around 10 million fatalities in 2020 [3]. Effective medicines for the treatment of various carcinomas are in high demand due to the ongoing global increase in cancer cases, which claim millions of lives each year [4- 6].

Ehrlich ascites carcinoma (EAC) is a common experimental tumor used for modeling purposes. Before being used as an experimental tumor by Ehrlich and Apolant (1905) by subcutaneous tumor tissue transplantation from mice to mice, it first manifested as spontaneous breast cancer in a female mouse. Due to the presence of ascites liquid in addition to cancer cells,

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Loewenthal and Jahn (1932) identified the liquid form in the mouse peritoneum and called it "Ehrlich ascites carcinoma" [7, 8]. Lettre *et al.* (1972) contributed not only to the detection of tumors but also to the transformation of a test system suitable for both qualitative and quantitative cancer research, as a result of their World War II investigations [9].

Currently, available cancer treatments include radiation therapy, hormone therapy, chemotherapy, and surgery; despite having better results, they often have negative adverse effects [10]. The WHO reports that nearly 80% of individuals worldwide opt to treat their ailments using herbal and traditional remedies [11]. In India, the use of alternative and complementary therapies has increased dramatically during the past two decades. An alternate strategy to mitigate the negative effects of synthetic medications is to use complementary and alternative treatments [12]. Chemotherapy is a relatively new method that offers an appealing alternative for managing cancer. The goal of experimental cancer chemotherapy studies is to find agents that can do one or more of the following: (i) prolong cancer latency periods; (ii) stop or delay tumor development; (iii) lower cancer metastasis as well as mortality; (iv) stop secondary tumors from growing again; and (v) stop tumors from growing. The discovery, characterization, and development of novel and safe cancer chemopreventive drugs have been the primary focus of modern chemotherapy research [13].

Natural phytochemicals derived from therapeutic plants are gaining recognition for their potential to treat various clinical disorders in humans, including cancer. Extracts from plants have considerable potential as preventive measures to halt the carcinogenic process. Many plants have been utilized in the conventional medical system to treat cancer [14, 15]. Notably, 60% of the anti-cancer drugs currently in use are derived from natural sources, including plants, microbes, and marine organisms. Plants have long been a substantial source of potent anti-cancer medicines [16, 17]. Chemotherapy, a cancer treatment, has undoubtedly benefited from plant-based medicine, and a significant amount of research has been conducted on the interactions between various phytochemicals and cancer cells. The pharmacological analysis of many plants employed in the traditional Indian medical system is of great interest. Natural compounds derived from plants, such as flavonoids, steroids, and terpenoids, have garnered considerable interest recently due to their diverse pharmacological properties, including anti-cancer and antioxidant actions. One of the most abundant and diverse groups of naturally occurring substances, flavonoids are widely distributed in various plant components, including leaves, roots, bark, seeds, flowers, and fruits [18]. Plant flavonoids are of enormous scientific significance due to their potential health advantages and medical qualities. These features include anti-cancer, antimicrobial, anti-inflammatory, and antioxidant effects [19, 20].

The *Manilkara hexandra*, which is a member of the Sapotaceae family, bears small fruits with one or two hard seeds within. This plant is widely distributed in the tropical forests of central and western India, as well as in other wet regions. Several states, such as Maharashtra, Gujarat, Rajasthan, and Madhya Pradesh, refer to the tree by the common names "Rayan," "Khirni," and "Raina" [21]. This native plant of South Asia is widely used in traditional medicine as a febrifuge, astringent, and tonic for the treatment of a range of severe gastrointestinal disorders [22]. Its seeds, bark, and fresh fruits are all used in medicine. There has been no scientific research conducted on anti-tumor activity. Therefore, we have attempted to evaluate the efficacy of this plant against the EAC cell line. Ehrlich ascites carcinoma (EAC) is an artificial tumor that grows quickly, behaves aggressively, and mimics real tumors [23]. For this reason, EAC cells were employed to assess the in vitro anti-cancer and in vivo anti-tumor activity of an ethanolic extract of *M. hexandra* leaves.

2. Materials And Methods

2.1. *Collection of plant*

Manilkara hexandra leaves were collected from Panakudi, Tirunelveli District, Tamil Nadu. It is authenticated by Dr. S. Mutheeswaran (Ph.D.), a scientist at St. Xavier's College, Xavier Research Foundation, Palayamkottai, Tamil Nadu, India. A voucher specimen was deposited in the Pharmacognosy department, PES College of Pharmacy, Bangalore.

2.2. *Extraction*

After being dried in the shade, the leaves were ground into coarse powder. The solvent used in the Soxhlet extraction process was 95% (v/v) ethanol. It was

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observed that the extraction yield was 11.09%. The resultant extract was concentrated using a vacuum rotary evaporator at low temperatures. To identify the plant-based constituents in the extract, a preliminary phytochemical analysis was conducted using standard screening tests [24].

2.3. *Chemicals*

Minimum Essential Media, Streptomycin sulphate, Penicillin G sodium salt, Trypsin-EDTA solution, Foetal Bovine Serum, Trypan blue, DAPI dihydrochloride, MTT Reagent, Paraformaldehyde solution (4%), Triton X-100, D-PBS, DMSO, and 5-Fluorouracil were obtained from Hi-Media Laboratories, Mumbai

2.4. *Animals*

The Swiss albino mice, weighing an average of 25 ± 4 g, were obtained ethically from Vertebrates, a registered facility located on Magadi Road, Bangalore-02, with registration number 2138/po/RcBiBt/s/21/CPCSEA. The experiment was conducted with sufficient attention to the animals' welfare. They had free access to standardized pelleted feed and clean drinking water, and they were kept in a temperature-controlled setting. The experimental protocols were approved by the Institutional Animal Ethics Committee (PESCP/IAEC/139/2022) and closely followed the criteria established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

2.5. *EAC Cells*

Ehrlich's ascites carcinoma (EAC) cells were obtained from the National Centre for Cell Sciences in Pune, India. For the in vitro study, cells were subcultured in minimal essential medium at 37°C in 5% CO_2, supplemented with 15% penicillin/streptomycin, and Fetal Bovine serum (10%). For the in vivo study, EAC cells were maintained through the intraperitoneal inoculation of 2×10^{6} cells/mouse.

2.6. *In vitro cytotoxic activity*

2.6.1. *Trypan Blue*

Seeded 200µl cell suspension in a 96-well plate at the required cell density (20,000 cells per well), without the test agent, and allowed the cells to grow overnight. Appropriate concentrations of the test agents (50, 100, 200, 400, and 800 µg/ml) in 0.1% DMSO were added to respective wells. Incubated the plate for 48 hours at 37°C in a 5% CO_2 atmosphere. After the incubation period, the cells in each well were trypsinized with 50 µl of 0.25% trypsin-EDTA solution and collected into specifically labeled 1.5 ml Eppendorf tubes. 10 µl of the cell suspension was mixed with 10 µl of 0.4% trypan blue stain. 10 µl of the mixture was loaded into a cell counting slide. The viable cells and non-viable cells were calculated using the LUNA-II Automated Cell Counter (#L40002, South Korea) [25].

Cell viability (%) = (Total viable cells) / (Total cells) ×100

2.6.2. *MTT assay*

Seeded 200µl cell suspension in a 96-well plate (triplicate) at the required cell density (10,000 cells per well), without the test agent, and allowed the cells to grow overnight.

Appropriate concentrations of the test agents (50, 100, 200, 400, and 800 µg/ml) in 0.1% DMSO were added to the respective wells, and the plate was incubated for 48 hours at 37°C in a 5% CO2 atmosphere. After the incubation period, the spent media was removed from the wells, and MTT reagent was added to each well at a final concentration of 0.5 mg/mL. The total volume was then incubated for 3 hours. Removed the MTT reagent, and then 100µl of solubilization solution (DMSO) was added to each well. Read the absorbance on an ELISA reader (ELX-800, BioTek, USA) at 570nm. The IC50 value was determined using a logarithmic equation, i.e., $Y = M\ln(x) + C$. Here, Y = 50, M, and C values were derived from the viability graph [26].

2.6.3. *DAPI staining*

Without the test agent, a 200µl cell solution was seeded in a 96-well plate at the necessary cell density of 10,000 cells/well. The cells were incubated for 24 hours. After incubation, cells were treated with EELMH, with an IC50 value of (~) 612 µg/ml in 0.1% DMSO. The plate was incubated at 37°C with 5% carbon dioxide in air for a total of 48 hours. Following the incubation time, the spent medium was removed from the wells, cleaned twice with PBS, and then treated with 2% paraformaldehyde for 30 minutes at room temperature. Fixed cells were permeabilized for 10 minutes using 0.1% Triton X-100 and washed with PBS. After five

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minutes of DAPI (1µg/ml) incubation, the cells were examined under an Inverted fluorescence microscope (Zeiss Axio Observer 7, Germany) [27].

2.7. *In vivo Anti-Tumor Activity*

EAC Carcinoma Model

The mice were divided into five groups (n = 12), with one group serving as the control. After 24 hours, all other groups received tumor inoculation of $2 \times 10^{\wedge 6}$ EAC cells. The II group, which served as the disease control, received $2 \times 10^{\wedge 6}$ EAC cells. The treatment groups III and IV received EELMH extract at 200 and 400 mg/kg, p.o. respectively, and the V group served as the standard, receiving 5-Fluorouracil (20 mg/kg) once daily for 9 days. To estimate hematological and serum biochemical parameters, blood was extracted from six animals in each group via direct heart puncture on the last day, after the last dose, and after an 18-hour fast. Later, mice were killed by cervical dislocation to get ascitic fluid, which was used to determine the packed cell volume (PCV), tumor volume, and tumor weight. The remaining six animals were kept alive to determine the mean survival time and the percentage increase in lifetime [28].

2.7.1. Hematological parameters

2.7.1.1. Measurement of hemoglobin (Hb)

The acid haematin technique was used to measure the amount of hemoglobin. A Sahli's hemoglobinometer tube was filled with 20 µL of EDTA-anticoagulated blood, up to 2 g%, and mixed. Gradually add distilled water and stir until the color matches the comparator. The tube reading was used to determine the final reading.

2.7.1.2. *Total count of WBC*

Dilute EDTA anticoagulant blood with WBC diluting fluid 1:20 in a WBC pipette. After proper mixing, it was used to count WBC with the help of a hemocytometer (Neubauer`s counting chamber).

WBC = (dilution factor X number of WBC counted)/ (area X Fluid depth)

2.7.1.3. *Total count of RBC*

Twenty microliters of EDTA-anticoagulated blood were added to the tube containing RBC diluting fluid. Neubauer`s chamber was used to count RBCs at a lower magnification. N X 10,000 (N = Total RBCs 5 square) equals the RBC count/ mm3

2.7.2. *Tumor growth response*

EELMH`s anti-tumor efficacy was assessed by using the following metrics:

Tumor Volume, (PCV) Packed cell volume, and Tumor Weight

The ascitic fluid was taken from the peritoneal cavity. A graduated centrifuge tube was used to measure the volume, and then centrifugation at 1000 rpm for 5 minutes was performed to determine the packed cell volume. The mice's weight before and after the ascitic fluid was extracted from the peritoneal cavity was used to calculate the tumor weight.

2.7.3. Viable and Non-viable Tumor Cell Count

The ascitic fluid was diluted 20 times with PBS in a WBC pipette. A drop of diluted solution was added to Neubauer's chamber and stained with 0.4% Trypan blue dye. The cells that did not absorb the dye were viable, while those that accepted the stain were non-viable.

The total number of viable and non-viable cells was counted using the following formula:

Cell count = (number of cells X dilution factor) / (area X thickness of liquid film)

2.7.4. Percentage Increased Life Span (%ILS)

The experimental mice's mortality was recorded to find the impact of EELMH on tumor progression. The %ILS was obtained using the formula: MST of the treated group / MST of the control group)-1 X 100

Where MST = (Day of first death + Day of final death)/2

2.7.5. *Biochemical parameters*

After allowing blood samples to clot at 4^0 °C for 45 minutes, the serum was separated using centrifugation for 10 minutes at 5000 rpm. It was then used to estimate serum glutamate pyruvic transaminase (SGPT) and serum glutamic oxaloacetate transaminase (SGOT) by commercially available kits made by Transia Biomedical Ltd., Himachal Pradesh, India, using a semi-auto bioanalyzer from Remi India.

2.7.6. *Statistical Analysis*

The data were given as Mean ± SEM. The study data were subjected to one-way ANOVA followed by Dunnett's Test using GraphPad Prism 10 software.

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3. Results and Discussion

Preliminary phytochemical screening of ethanol extracts of *Manilkara hexandra* revealed the presence of alkaloids, flavonoids, phenolic compounds, terpenoids, saponins, steroids, and sugar.

3.1. *In vitro* cytotoxicity

3.1.1. *Trypan blue*

In this experiment, dye was added to the cell solution, and the cells' ability to absorb or reject the dye was assessed visually. The cytoplasm of living cells was transparent, whereas that of dead cells was blue. **Table 1** lists the results of the trypan blue assay at various concentrations. The extract demonstrated a dose-dependent reduction in cell viability. At higher doses, specifically 800 mg/kg, a notable increase in the percentage of dead cells or a decrease in cell viability is observed (**Figure 1a**). This implies that the extract causes cytotoxicity in EAC cells, possibly through mechanisms such as apoptosis induction, membrane disturbance, or metabolic interference. The IC50 value of EELMH at 48 hours is 624.97 µg/mL.

Table 1. Effect of EELMH on cell viability in Trypan blue assay

Concentrations (µg/ml)	**Cell viability%**
Untreated	100±0
50	86.24±0.354****
100	76.21±0.349****
200	65.93±0.231****
400	56.02±0.547****
800	46.83±0.678****

Values are represented as mean ± SEM (n = 3). Values are ****P < 0.0001 significant compared to the untreated group

3.1.2. *MTT Assay*

The purpose of this investigation was to assess the sample's potential for cytotoxicity against the EAC cell line. The EELMH exhibited a statistically significant dose-dependent reduction in the viability of EAC cells (**Table 2**). With higher concentrations, a significant lowering of the values of % viability of EAC cells was noticed (**Figure 1b**), signifying diminishing cell viability, which can be due to the bioactive compounds in the extracts that could have induced apoptosis by influencing major signaling pathways like mitochondrial apoptotic pathway, caspase activation, or induction of oxidative stress. Phytochemicals, such as flavonoids and polyphenolic compounds, have been reported to have these effects [29]. The IC50 value of EELMH at 48 hours was found to be (~) 612 µg/ml.

Table 2. Effect of EELMH on cell viability in MTT assay

Concentrations (µg/ml)	Cell viability%
Untreated	100±0
50	86.85±0.018****
100	76.75±0.008****
200	65.52±0.027****
400	59.57±0.008****
800	44.07±0.024****

Values are represented as mean ± SEM (n = 3). Values are ****P < 0.0001 significant compared to the untreated group.

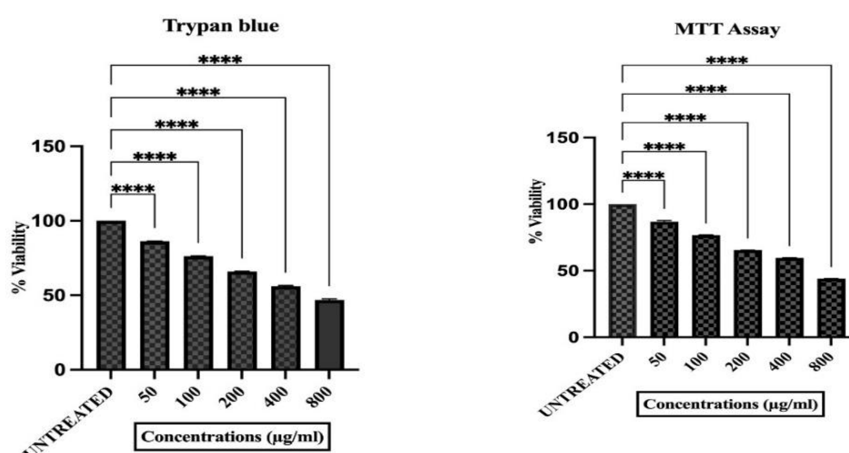

Figure 1. a Effect of EELMH on EAC cells in Trypan blue assay and **b** MTT assay

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3.1.3. *DAPI staining analysis*

The analysis of nuclear morphology is critical in evaluating the apoptotic and cytotoxic activity of potential anti-cancer drugs. The DAPI staining technique is commonly used to visualize nuclear alterations characteristic of cell death. In the present investigation, the EELMH at its IC50 concentration on EAC cells exhibited pronounced nuclear changes compared to the untreated control, as shown in **Figure 2**. From previous studies, it can be concluded that EELMH-induced morphological changes are possibly due to the bioactive compounds in the extract, which may have disrupted the assembly of mitochondria, increasing cytotoxicity by inducing apoptosis in the EAC cell line [30].

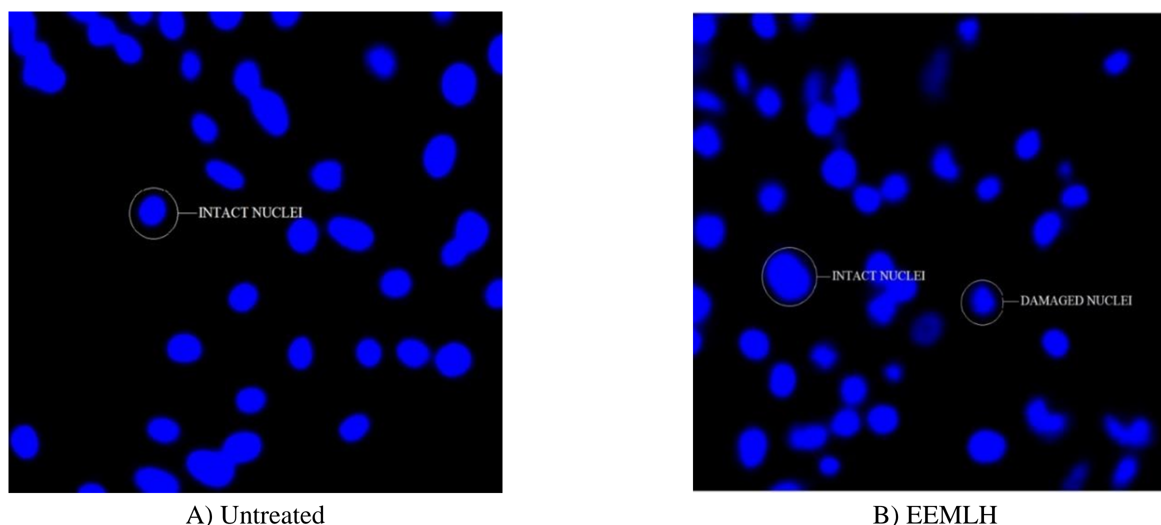
The use of herbal remedies for cancer management has drawn more attention in recent years due to their diverse Phyto-metabolic contents and range of biological actions. Based on the in vitro findings, it was determined that the EELMH has strong cytotoxic properties. The lowest percentage of viable cells was observed at a concentration of 800 µg/mL, with IC50 values ranging between approximately 612 µg/mL and 624.96 µg/mL.

3.2. *In vivo anti-tumor activity by EAC carcinoma model*

3.2.1. *Hematological parameters*

In the disease control (DC) group, a marked increase in total white blood cell (WBC) count was observed, accompanied by a reduction in hemoglobin (Hb) and total red blood cell (RBC) counts. However, after 14 days of treatment with the extract, a significant improvement in hematological parameters was noted at the 400 mg/kg dose. Specifically, WBC levels significantly decreased from $14.26 \pm 0.23 \times 10^3$ cells/mm^3 in the DC group to $8.53 \pm 0.08 \times 10^3$ cells/mm^3 in the 400 mg/kg treated group (****$P < 0.0001$). Conversely, RBC counts significantly increased from $3.39 \pm 0.15 \times 10^6$ cells/mm^3 to $4.89 \pm 0.04 \times 10^6$ cells/mm^3 (****$P < 0.0001$), and Hb levels rose from 6.17±0.16 gm% to 10.58 ± 0.25 gm% (****$P < 0.0001$). These improvements were comparable to those observed with the standard drug, 5-Fluorouracil. Significant major side effects of cancer chemotherapy include myelosuppression and anemia [31]. These altered hematological parameters are restored to nearly normal levels, indicating the extract's effect on the hematopoietic and immune systems (**Figure 3**).

The resultant rise in RBC and HB count indicates that the EELMH can have hematoprotective effects, which could be achieved by inducing erythropoiesis or by extracts having antioxidant potential, thereby reversing EAC-induced oxidative stress and inflammatory mediators known to inhibit erythropoiesis [32, 33]. A reduction in the WBC count could suggest that the bioactive compound present in EELMH may suppress inflammatory reactions and prevent leukocytosis, which is usually seen in EAC-bearing mice [34].



A) Untreated B) EEMLH

Figure 2. Morphological changes in nuclei of EELMH-treated EAC cells.

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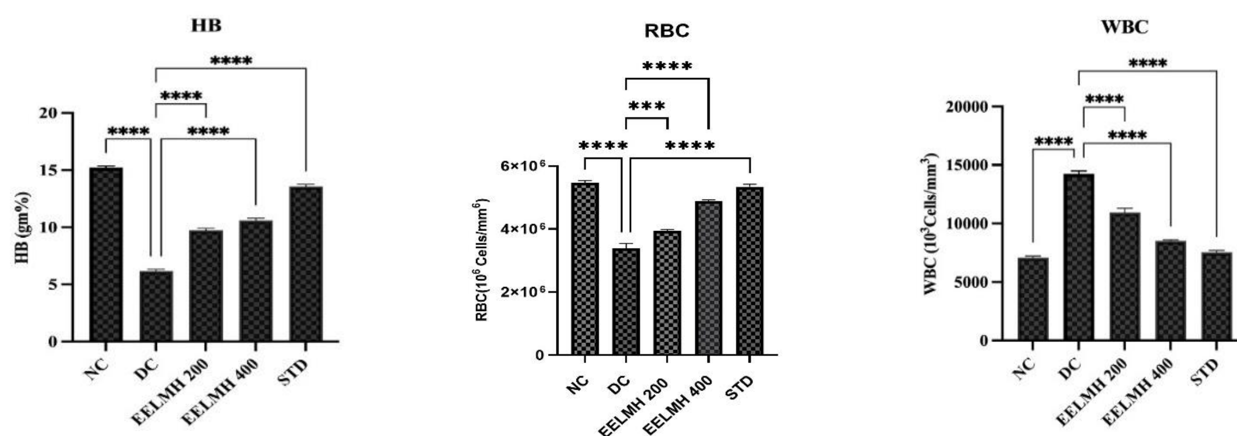

Figure 3. Effect of EEMLH on WBC, RBC, and HB Counts.

3.2.2. Tumor growth response and tumor cell viability

Treatment with EELMH at a dose of 400 mg/kg demonstrated a significant anti-tumor effect, as evidenced by a decrease in tumor growth parameters and a reduction in viable cell count. There was a significant decrease in tumor weight from 5.5±0.34gm to 3.5±0.42gm (**P < 0.01), tumor volume from 4.2±0.17 mL to 03±0.20 mL (****P < 0.0001), and packed cell volume from 1.6 ± 0.05 mL to 0.73 ± 0.07 mL (****P < 0.0001) at 400 mg/kg. Additionally, the viable tumor cell count was significantly reduced from 7.91 ± 0.06 × 10^7 cells/mL to 4.15 ± 0.14 × 10^7 cells/mL (****P < 0.0001), accompanied by a significant increase in non-viable cell count from 0.39±0.04 ×10^7 cells/mL to 1.29±0.09×10^7 cells/mL (****P < 0.0001). In contrast, treatment with EELMH at 200 mg/kg did not produce statistically significant changes in tumor volume, tumor weight, or non-viable cell count compared to the disease control group (Figures 4 and 5). The observed decrease in viable cells and increase in non-viable cells in the 400 mg/kg group may suggest the induction of apoptosis, which was further supported by the nuclear fragmentation observed in DAPI staining.

Tumor-bearing mice tend to have elevated body weights due to the accumulation of ascitic fluid and tumor burden. The decrease in tumor volume, tumor weight, and packed cell volume at higher dose (400mg/kg) indicates that the extract is cytotoxic or antiproliferative. This may be mediated by preventing angiogenesis and direct cytotoxicity against EAC cells [35].

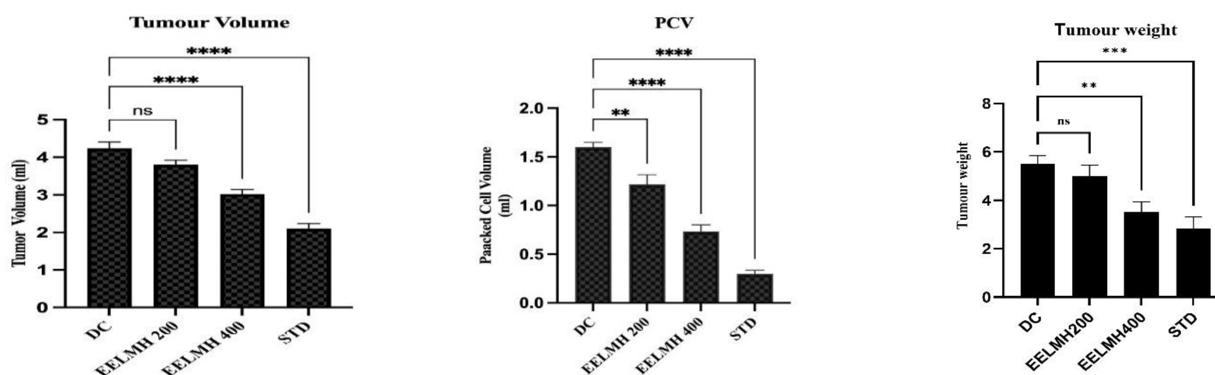

Figure 4. Effect of EELMH on Tumor volume, Packed cell volume, and Tumor weight.

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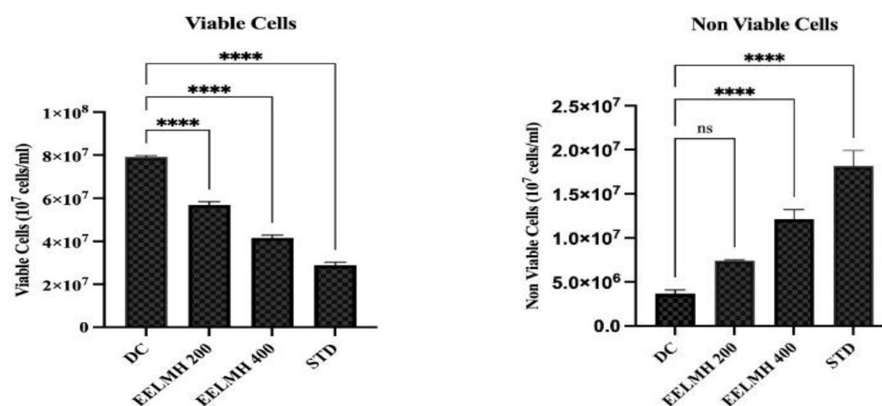

Figure 5. ffects of EELMH on tumor cell viability.

3.2.3. *Survival analysis*

In the disease control group, the mean survival time (MST) was recorded as 20 days. Treatment with EELMH resulted in a dose-dependent increase in MST, with 28 days observed at 200 mg/kg and 36 days at 400 mg/kg. In comparison, the standard drug 5-Fluorouracil (20 mg/kg) exhibited an MST of 41 days (**Table 3**). An increase in the lifespan of tumor-bearing mice is a well-established indicator of anti-cancer efficacy. The observed prolongation of survival in the EELMH-treated groups thus highlights the extract's potential anti-cancer activity [36].

3.3. *Biochemical parameters*

The effect of EELMH on SGOT and SGPT is evident in **Figure 6**. The disease control group exhibited significantly elevated levels of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT), recorded as 216.58 ± 3.9 IU/L and 93.41± 2.6 IU/L, respectively, indicating potential hepatocellular damage. Treatment with ethanolic extract of *Manilkara hexandra* leaves (EELMH) at both 200 mg/kg and 400 mg/kg resulted in a significant reduction in these enzyme levels. At 200 mg/kg, SGPT and SGOT levels decreased to 109.32 ± 3.96 IU/L and 82.21 ± 2.40 IU/L, respectively. A more pronounced effect was observed at 400 mg/kg, with SGPT levels reduced to 72.72 ± 5.44 IU/L (****P < 0.0001) and SGOT to 76.47 ± 2.95 IU/L (*P < 0.1). These findings suggest that EELMH exhibits hepatoprotective activity by reversing biochemical markers of liver injury.

The decrease in hepatic enzyme levels can be attributed to the antioxidant and anti-inflammatory activities of bioactive compounds, such as flavonoids, polyphenols, terpenoids, and saponins, present in EELMH, which can counteract liver injury caused by tumor growth [37].

Table 3. Impact of EELMH treatment on the survival time.

Groups	Normal	DC	EELMH (200 mg/kg)	EELMH (400mg/kg)	5-Fluorouracil (20mg/kg)
MST(Days)	0	20±1.19	28±1.08***	36±0.5****	41±1.5****
%ILS	--	--	40	80	105

Values are expressed as the mean of 6 animals ± SEM. ****P < 0.000, ***P < 0.001 significant compared to the EAC Control group.

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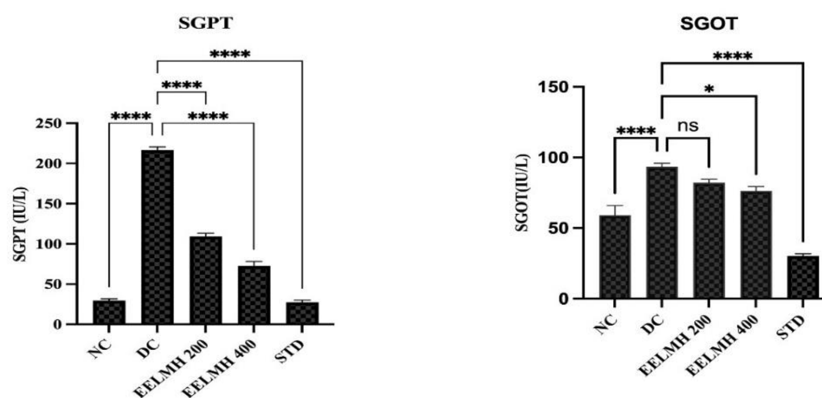

Figure 6. Effect of EELMH on SGPT and SGOT.

Cancer occurs due to a series of molecular changes that severely disrupt normal cell functioning. Traditional cancer therapies like chemotherapy usually come up with very severe side effects, such as neutropenia and anemia. Additionally, chemotherapy drugs may damage healthy cells that are proliferating rapidly, further weakening the patient. Lastly, chemotherapy drugs tend to be expensive, creating financial burdens for patients. Plant-derived natural compounds offer a promising alternative, with several plant-based treatments already showing valid potential as anti-cancer agents.

An effort was undertaken in the current work to create a new anti-cancer medication using a common plant source. We found in the literature that the anti-cancer potential of plants in the Sapotaceae family is well established. Therefore, a plant that is widely accessible and belongs to the Sapotaceae family was chosen for the current study. *Manilkara hexandra* was well recognized for its therapeutic properties. The presence of secondary metabolites, such as terpenoids, saponins, flavonoids, and phenolic compounds, in extracts has been proven to be an antioxidant and anti-cancer agent [38-40]. This might also be responsible for the anti-cancer activity of EEMLH.

4. Conclusion

The rising incidence of cancer globally emphasizes the need for safer and more efficient anti-cancer drugs. Although traditional treatments like chemotherapy and radiotherapy are extensively used, they tend to involve major side effects and decreased patient quality of life. For these reasons, there is heightened interest in anti-cancer drugs derived from plants because they have the potential to be effective, exhibit minimal toxicity, and are readily available. Results against the EAC cell line demonstrated that EELMH could inhibit the proliferation of tumor cells in a dose-dependent manner, which is comparable to that of the widely used drug 5-fluorouracil. This may be due to the presence of bioactive compounds in the extract. Further studies are required to elucidate the exact molecular mechanism and identify the lead compound present in *M. hexandra* leaves associated with its anti-cancer properties. Further investigation to isolate and characterize the compounds is in progress.

List of Abbreviations

EELMH: Ethanolic extract of leaves of *Manilkara hexandra*
EAC: Ehrlich's ascites carcinoma
DC: Disease control
WHO: World Health Organisation
ILS: Increase in life span
MTT: [3- (4,5) -dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MST: Mean survival time
SGOT: Serum glutamate oxaloacetate transaminase
SGOP: Serum glutamate pyruvate transaminase
PBS: Phosphate buffered saline
DAPI: 4',6- diamidino-2 phenylindole
STD: Standard

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Using artificial intelligence chatbots

There was no use of artificial intelligence in the making of this article.

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