RESEARCH ARTICLE

In-vitro and *In-vivo* Anticancer Activity of *Indigofera cordifolia* against Ehrlich Ascites Carcinoma

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ABSTRACT

The research study investigated the anticancer properties of *Indigofera cordifolia* in relation to ehrlich ascites carcinoma (EAC) in mice. The substance was administered intraperitoneally at 10 mg per kilogram of body-weight. The extract was supplied nine successive days and mice were euthanized after 24 hours since the previous dosage and after fasting for 18 hours. The antitumor impact was evaluated by measuring tumor dimensions, counting viable & non-viable cancer cells, measuring the weight of tumor, & analyzing hematological considerations of the host harboring EAC. A histopathological examination of the liver sections was conducted. The study found a statistically considerable ($p < 0.001$) improvement in endurance times for tumor-bearing mice treated with ethanolic extraction of *I. cordifolia*. The extract also reduced the weight of subjects with EAC tumors. Hematological analyses indicate a reduction in hemoglobin (Hb) levels in mice treated with EAC, while extract-treated animals showed a restoration of Hb levels close to normal. Noted with a statistically significant decrease ($p < 0.001$) in red blood cell count up & boost in white blood cell count in mice supplied with the extract/fraction compared to those treated with EAC. The study stated that extract had noteworthy anticancer activity, which was equivalent to that of doxorubicin. The histological sections of the liver tissue showed enhanced hepatic architecture following treatment with a 400 mg/kg dosage of the test extract.

Keywords: *Indigofera cordifolia,* Hematological parameters, Ehrlich ascites carcinoma, Cell line, doxorubicin.

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INTRODUCTION

Melanoma is a significant and perilous health concern that poses a serious threat to life in both industrialized and developing countries.[1] Leukaemia and cancer reacting to lung, breast, prostate, cervical, bone, & ascites are considered the prevalent types of cancer worldwide, & all of such diseases have the potential to be fatal.[2] It refers to a collection of disorders that occur due to the lack of control over the cell cycle, resulting in aberrant and unregulated development of cells.[3] Development of malignancy is linked to the adjustment of oncogenes, tumor-suppressing genes, & DNA mend genes.[4] Tobacco, radiation, chemicals, & pathogenic organisms, & endogenous factors, like hereditary mutations and immunological states, are considered responsible for the development of cancer or as risk factors.[5]

It has a significant load on public health, & its treatment remains systematically complex.[6] Traditional methods for treating cancer include chemotherapy, radiation, & surgery. Each of such traditional therapy modules is associated with significant adverse consequences.[7] The researchers were driven to explore for novel & much effective treatments with fewer side effects.[8] Scientists are always searching for natural chemicals that can heal cancer due to the existing constraints.[9]

A wide range of natural chemicals, including phenolic acids, terpenoids, tannins, lignans, quinones, flavonoids, alkaloids, and coumarins, have been identified in plants. These compounds have strong antioxidant properties and are known to be valuable in the treatment of cancer.[10] Multiple investigations have demonstrated that antioxidant molecules exhibit antitumor, anti-inflammatory, anti-mutagenic, & anti-carcinogenic properties.[11] They were found in nature that possess antioxidant properties have the ability to directly impede the growth of cells and enhance the functioning of the immune system.[12] Recently, the pharmaceutical industry has heavily relied on natural substances as a primary supply of medication. According to the data, more than 60% of the lately utilized anticancer medications have a connection to plant sources.[13-15] These are widely recognized globally as a kind of complementary & substitute therapy for many disorders, including cancer. They provide us with reasonably secure, efficient, and cost-effective therapeutic alternatives, especially in the case of cancer, when therapy is prolonged and costs are too high.[16]

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The Indian subcontinent is rich in plant species having therapeutic characteristics due to its excellent environmental conditions. Ayurveda is an ancient medicinal practice that originated in India and is based on a fundamental premise.[17] This treatment technique is derived from plant materials and has been operating seamlessly since ancient times up to the present day. Ayurveda has a rich history and is widely recognized for its significant contributions to the field of medicine. Although contemporary medicine has made significant advancements in the fields of physical, chemical, and natural sciences, Ayurvedic medicine has also made valuable contributions to alleviating human suffering.[18] The present objective is to uncover numerous bioactive chemicals that can treat cancer with anticancer properties. *Indigofera cordifolia*, sometimes known as the heart-leaf indigo, belongs to the Fabaceae family & is a flowering plant species. This work aimed to examine anticancer effects of an ethanolic extract of *I. cordifolia* (EEIC) on the ehrlich ascites carcinoma (EAC) cell line, both *in-vitro* & *in-vivo*.

MATERIALS AND METHODS

Plant

I. cordifolia is the scientific name of a plant species. Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Tirupati, authenticated the specimen. The specimens labeled Pt 0219 and Pt 0831 were conserved in the herbarium.

Extraction

A total of 5 kilograms plant material was dehydrated in the shade (25–35ºC) for seven days. The desiccated botanical matter was pulverized using a grinding apparatus. The desiccated botanical powder underwent soxhlet extraction utilizing petroleum ether and ethanol to derive the corresponding extract. Subsequently, each extract underwent filtration by cotton plugs and a Whatman filter. Filtrates were further intensified, desiccated at low pressure using a rotary evaporator & finally freeze-dried for powdered form.

Initial Phytochemical Analysis

Primary and secondary metabolites of *I. cordifolia* were analyzed in all of its extract/fractions to assure the presence of many 1^o metabolites, including amino acids, carbohydrates, proteins, & lipids, as well as 2^o metabolites, such as tannins, alkaloids, saponins, phenols, steroids, flavonoids, glycosides, & resins, using established ways.

Acute Oral Toxicity Studies

The animal model used in this study was wistar rats, in accordance with the standards provided by the Organisation for Economic Co-operation and Development (OECD) 423. The experiment involved administering a limit test dosage of 2000 mg/kg body weight. Prior to commencing each experimental trial, all animals underwent overnight fasting while being allowed free access to water. The animal subjects were segregated into five groups (n = 6). The initial grouping acted as a control with no expected effects, whereas the subsequent groups received different amounts of plant extracts, namely 5, 50, 300, and 2000 mg/kg of body weight, by oral route. Prior to administering test substances, a body weight of each animal was recorded to ensure precise dosage. Toxicological effects were initially observed after four hours after administering the extract. The continued observation was made for a period of three days, during which various physiological and behavioral factors were closely analyzed. These factors included body weight, urine production, food and water intake, breathing rate, occurrence of seizures or tremors, movement of the digestive system (constipation), and change in eye & skin coloration.

Animals

A group of Swiss albino mice weighing between 25 and 30 gm were separated into four groups: normal control, illness control, positive control, and test categories. Each category/group consisted of 6 animals. These were housed in a climate-controlled (22 ± 1°C, 55 ± 1%). The room had a 12-hour brightness and dark rotation. The subjects were provided with a regular industrial mice pellet meal and unrestricted water access. The animals were given seven days to acclimatize to the surroundings before the experimental session. Animal experiments protocol received approval from the Institutional Animal Ethics Committee under reference number 1447/PO/Re/S/11/CPCSEA-76/A.

In-vitro Cytotoxicity

Cytotoxicity of extracts of *I. cordifolia* was assessed *in-vitro* using a semiautomated technique with sulforhodamine-B (SRB).[19-21] The cancer cell lines were cultivated in culture flasks at 37°C, in a setting containing 5% carbon dioxide & 90% RH, using a complete growth media. Flasks containing cells at the sub-confluent point of development were chosen, & the cells was collected by treating them with trypsin-EDTA. A hemocytometer determined the concentration of cells in each millilitre of suspension. Cell density was standardized to 10,000 cells per 100 µL in the cell suspension or modified accordingly for each specific cell line. Cell suspension (100 µL) was dispensed in to separate 96-well plates using a handy-step device. The plates were placed in an incubator (37°C; 5% CO_2; 90% RH; 24 hours). Subsequently, a volume of 100 µL of the running solution of the test substance was introduced into wells of 96-well plates. Extracts were made as stock solutions by a 20 mg/mL concentration in DMSO. A growth medium then diluted this solution to obtain working solutions with 10, 30, and 100 µg/mL concentrations. The concluding DMSO concentration ranged from 0.5 to 0.001%. These working solutions were kept into the 96 wells. These were filled with cells that were seeded in suitable amounts. For example, breast (MCF-7), lung (A549), prostate (DU 145), and colon (HT29) cells were seeded at 8000 cells per 100 µL, while other cells were planted at 10000 cells per 100 µL. All medium controls had the same amount of DMSO.

Plates was placed in an incubator and kept at 37°C for 48 hours. The incubator was set for 5% carbon dioxide & 90% RH. Subsequently, 50 µL of refrigerated trichloroacetic

acid (50%; TCA) was carefully introduced into the plates, resulting in a concluding concentration of 10%. Plates were placed in a cold environment at 4°C for 1-hour to immobilize the cells that were adhered to the bottom of wells. Plates were thereafter rinsed 5 to 6 times by distilled water & air-dried. SRB dye (100 µL; 0.4% w/v in 1% acetic acid) was applied and allowed to sit for 30 minutes at room ambiance. Subsequently, plates were cleansed using a solution of 1% acetic acid. Post-drying, these were treated with 100 µL of Tris buffer (10 mM; pH 10.5). The plates were agitated for a duration of 10–15 minutes using a mechanical shaker. The blank wells were filled with culture; however did not include any cells. On the other hand, the control wells had cells but did not have any test samples. The microplate reader estimated the plate wells' optical density (OD) at 540 nm and recorded data.

Transplantation of Cancer Cells

The EAC cells was acquired from the National Centre for Cell Sciences (NCCS), Pune, India. EAC cells was sustained *in-vivo* in Swiss albino mice by intra-peritoneal transplantation of 2×106 cells suspended in PBS/animal all 10 days. Ascitic fluid was collected from mice with EAC cell tumors during the logarithmic phase of tumor formation on days 7 to 8. Each test animal was injected with 0.1 mL of cancer cell solution with 2×106 cells directly into the peritoneal cavity (i.p.). The given text is a list containing the numbers 22 and 23.[22,23]

Treatment Schedule

Each group was administered EAC cells (2×106 cells/mouse, i. p.), excluding for group I, which received normal saline as control (5 mL/kg body weight, i. p.). 2nd group functioned as control group for the sickness. After 24 hours of EAC administration, the 3rd group was administered doxorubicin at 0.3 mg/kg body weight by intraperitoneal route (positive control). The test groups received the same treatment for nine consecutive days. After a 24-hour period since the previous treatment, the animals had an 18-hour fasting period. Following this, six Mice from each group were slaughtered by heart puncture in order to measure hematological and biochemical markers. The surviving mice were given unlimited admittance to food & water & be monitored to assess any potential alterations in their longevity.

Tumor & Packed Cell Volume

Subjects was dissected & ascitic fluid was obtained through peritoneal void. A graduated conical centrifuge tube detected volume. Subsequently, packed cell volume (PCV) was estimated by centrifuging the fluid with a force of 1,000 times acceleration due to gravity (1,000 × g) at 4°C for 5 minutes.[24]

Viable and Non-Viable Cancer Cell Count

Ascetic fluid was extracted using a pipette specifically designed for WBC & then diluted by a factor of 100 using phosphate-buffered saline (PBS). Afterward, a small amount of the diluted mixture was applied onto the Neubauer counting compartment, & cells were dyed using trypan blue (0.4% solution in normal saline). Cells that do not absorb dye were classified as viable, while that did was classified as non-viable.[25]

The following formula is used to calculate cell count:

$$\text{Cell count} = (\text{number of cells} \times \text{dilution factor}) / (\text{area} \times \text{thickness of liquid film})$$

Median Survival Time & Increase in Lifespan

Mortality was monitored by recording the percentage increase in lifespan (%ILS) and the median survival time (MST), according to the following formula:[26]

$$\text{ILS\%} = [(\text{Mean survival time of treated group} / \text{Mean survival time of control group}) - 1] \times 100$$

Were,

$$\text{Mean survival time} = (\text{day of first mortality} + \text{day of last mortality})/2$$

Hematological Parameters

The collected blood was utilized to determine hemoglobin, RBC, and WBC levels. Leishman-stained blood smears were used to produce differential counts of WBC.[27-29]

Biochemical Parameters

Blood was allowed to clot & serum was collected (2500 rpm; 15 minutes) for serum glutamate pyruvic transaminase (SGPT), alkaline phosphatase (ALP), total proteins, serum glutamic oxaloacetate transaminase (SWAS), and bilirubin content by standard protocols analysis.

Histopathological Studies

The mice were euthanized and their livers were removed, cleaned with normal saline, trimmed, and prepared for histological analysis. The liver samples were entrenched in paraffin, sliced into sections with a width of 4 to 5 µm, stained by hematoxylin & eosin, and later analyzed by light microscope.

RESULTS

The current study examined the extraction of *I. cordifolia* using petroleum ether and ethanol. The analysis revealed the presence of several phytoconstituents like flavonoids, triterpenoids, phenolic compounds, saponins, tannins, proteins, amino acids, & carbohydrates. After conducting a phytochemical study & determining the %yield, it was decided to proceed with ethanolic extraction for both plants in order to conduct GC-MS analysis. The tests conducted for proteins, amino acids, carbohydrates, alkaloids, triterpenoids, cardiac glycosides, flavonoids, saponins, tannins, phenolic compounds, and steroids were positive, while gums showed negative results.

The EEIC extract exhibited inhibitory efficacy on cancer cell growth compared to the standard doxorubicin. The EEIC extract demonstrated greater effectiveness (IC_{50} = 62.422.37) in the breast (MCF-7) cell line. The EEIC extract demonstrated an IC_{50} value of 52.191.75 in the A549 lung cell line. The EEIC extract did not achieve the 50% growth inhibition concentration within the studied range of concentrations in the prostate (DU 145) & human umbilical vein endothelial cell line. Nevertheless, both extracts exhibited inhibitory effects in

the colon (HT29) cell line, indicating a greater efficacy level than the EEIC extract (IC_{50} = 32.85 ± 2.35; Table 1).

Acute Oral Toxicity Studies

This was conducted following Organisation for Economic Co-operation and Development (OECD) guideline 423. The study employed wistar rats and supplied with a unit dosage of 2000 mg/kg body weight as a limit test. Prior to each trial, all animals were subjected to an overnight fasting period, during which they had unrestricted access to water. The creatures were categorized into five categories, each comprising six species. The first category was used as a negative control, while the second, third, fourth, and fifth groups were nominated as experimental categories/groups. Experimental groups received extracts at doses of 5, 50, 300, and 2000 mg/kg body weight orally. Prior to administering the dose, the body weight of the subject was measured, & dose was calculated based on body weight (Table 2). The subject was monitored for toxicological impact during the initial 4-hour period following treatment. Additional animals were examined over a period of three days to assess several parameters like body weight, food intake, urine, respiration, water intake, tremor, convulsion, constipation, temperature, skin color, eye color, etc.

Both extracts showed no toxicity symptoms at 2,000 mg/kg body weight. About 400 mg/kg body weight is selected as the therapeutic dose for future *in-vivo* studies.

Findings from the experiment using mice with EAC-bearing tumors illustrate the effectiveness of various therapies in suppressing tumor development and prolonging lifetime (Table 3). The normal control group consists of mice that did not undergo any form of therapy. The subjects had a stable body weight of 38.08 units, showed no signs of tumor growth or cancer cell presence, and had a median survival period of 0 days due to their healthy condition. The disease control group consists of mice with untreated EAC tumors. They saw a reduction in body weight by 32.05 units and observed tumor development of 5.57 mL (Figure 1). Additionally, they found a substantial quantity of viable cancer cells at 3.42×106 per millilitre and non-viable cancer cells at 9.86×106 per millilitre. The median survival time was 0.57 days, showing rapid illness development. According to standard protocol, the control group was administered doxorubicin, a chemotherapeutic medication. Their body weight remained generally constant at 37.39, and there was a notable decrease in tumor volume to 0.40 mL (Figure 2). They had no viable cancer cells and a small number of non-viable cells (0.55 × 106/mL). The median survival time rose to 1.46 days, indicating a 107.19% increase in longevity relative to the illness control category. Test group was treated with EEIC. The mice were administered varying dosages of the EEIC, namely 200 and 400 mg/kg. Both extracts shown favorable outcomes in diminishing tumor volume, viable & non-viable cell count up, and enhancing median survival time. The increased dose of both extracts showed superior effectiveness (Figure 3). The administration of EEIC extract at a dosage of 400 mg per kilogram of body weight significantly improved 69.49% in a lifetime.

Based on the aforementioned findings, it can be concluded that the disease control group had a significant increase in tumor development, a loss in body weight, and a shorter median survival time, which emphasizes the severity of the EAC tumor model. The administration of doxorubicin, a commonly used chemotherapeutic agent, resulted in a substantial decrease in tumor size, eradication of viable cancer cells, and an extension of the median survival period. The analysis of the EEIC extract demonstrated favorable outcomes, as greater dosages resulted in a drop in tumor size, reduced counts of both viable & non-viable cells, and an improvement in the median duration of survival.

The control group, which did not receive any medication, showed normal levels of hematological markers, such as Hb content, RBC count, and WBC count (Table 4, Figure 4). The proportions of monocytes, lymphocytes, and neutrophils were within the expected limits. The disease control group, which consisted of mice with untreated EAC, exhibited a notable reduction in hemoglobin content (7.05 g/dL) and red blood cell count (3.06 million/µL). The WBC count was considerably

Table 1: IC_{50} of extracts on cancer cell lines in MTT assay

Cell lines	*EEIC*	*Doxorubicin*
Breast (MCF-7)	62.42 ± 2.37	1.52 ± 1.28
Lung (A549)	52.19 ± 1.75	1.06 ± 1.34
prostate (DU 145)	>100	1.82 ± 1.55
Colon (HT29)	32.85 ± 2.35	1.95 ± 0.97
Human umbilical vein endothelial cells (Healthy cells)	>100	42.36 ± 1.22

Table 2: Impact of the extracts on the body weight

Treatment	*EEIC*		
	Day 0	*Day 7*	*Day 14*
Control	33.5 ± 1.71	36.8 ± 2.56	38.1 ± 1.91
5 mg/kg	33.8 ± 1.43	35.5 ± 9.60	36.5 ± 2.01
50 mg/kg	35 ± 2.89	36 ± 1.70	35.66 ± 3.7
300 mg/kg	36.5 ± 2.05	37.6 ± 1.16	36.66 ± 4.93
2000 mg/kg	36.7 ± 2.15	37.45 ± 1.43	37.87 ± 4.41

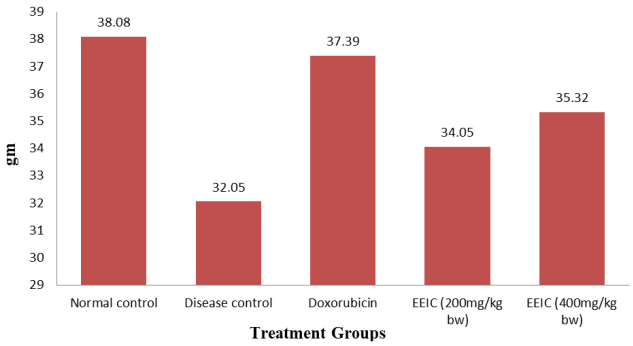

Figure 1: Impact of EEIC on EAC-bearing mice on body weight

Table 3: Effect on EAC-bearing mice

Treatment	*Body weight (gm)*	*Tumor volume (mL)*	*Packed cell volume (mL)*	*Viable cell count (cells×10^6 /mL)*	*Non-viable cell count (cells×10^6 /mL)*	*Median survival time (days)*	*Percentage increase of lifespan (ILS%)*
Normal control	38.08 ± 2.64	0.00	0.00	0.00	0.00	0.00	0.00
Disease control	32.05 ± 1.8	5.57 ± 0.32	3.42 ± 0.3	9.86 ± 0.26	0.57 ± 0.22	18.57 ± 0.27	0.00
Doxorubicin	37.39 ± 2.53***	0.40 ± 0.25***	0.00***	0.55 ± 0.26***	1.46 ± 0.23***	38.47 ± 1.05***	107.19***
EEIC (200 mg/kg bw)	34.05 ± 1.84**	3.22 ± 0.59**	1.26 ± 0.16**	3.75 ± 0.49**	1.02 ± 0.22**	30.68 ± 0.81**	65.24***
EEIC (400 mg/kg bw)	35.32 ± 2.32***	1.19 ± 0.42***	0.51 ± 0.21***	1.22 ± 0.16***	1.07 ± 0.25***	31.47 ± 1.1***	69.49***

Results are expressed in mean ± SEM (n = 6); statistical significance at p < 0.05*, 0.01** and 0.001***, in comparison to control group

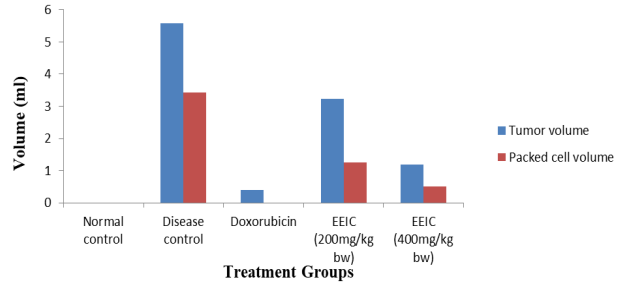

Figure 2: Impact of EEIC on EAC-bearing mice on tumor volume and packed cell volume

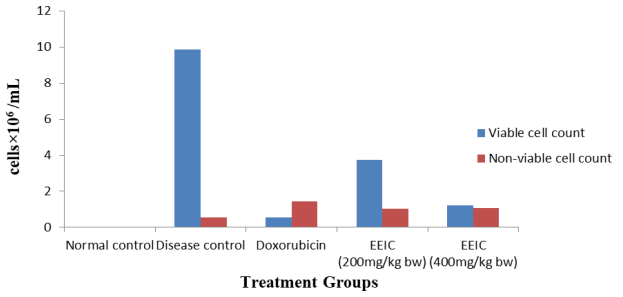

Figure 3: Effect of EEIC on EAC-bearing mice on viable cell count & non-viable cell count

increased (9.47 thousand/µL), suggesting an immunological response to the illness. The lymphocyte percentage reduced to 35.08%, but the neutrophil percentage considerably increased to 92.34%, indicating an inflammatory response. The mice receiving doxorubicin showed enhanced hematological

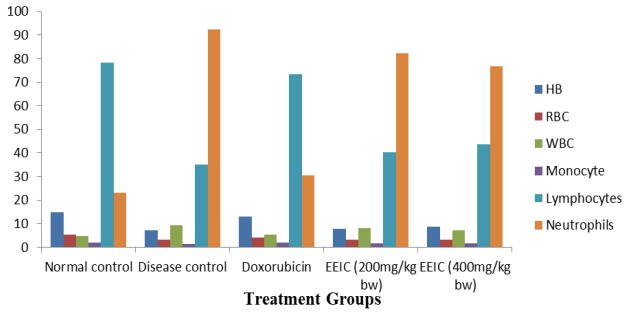

Figure 4: Effect on hematological parameters

parameters compared to the group that did not get any treatment for the condition. The Hb concentration climbed to 13.09 g/dL, and RBC count grew to 4.04 µL. However, the WBC count declined to 5.23 thousand per µL. The lymphocyte percentage (73.35%) and neutrophil percentage (30.43%) have reached levels that are close to normal, suggesting a partial recovery of immunological function. The mice who received the EEIC treatment showed varied impacts on their hematological markers. The Hb, RBC count up, & WBC count up were consistently lower than those of normal control group, but exhibited a little improvement compared to the illness group. The lymphocyte percentages exhibited variation, with certain groups seeing a rise in percentages while others displayed a drop. The percentages of neutrophils exhibited variability, with certain groups nearing values that are considered normal. The findings suggest that the disease control group displayed notable deviations in hematological indices, like reduced Hb content, RBC count, & modified

Table 4: Effect on hematological parameters

Treatment	*Hb content*	*RBC*	*WBC*	*Monocyte*	*Lymphocytes*	*Neutrophils*
Normal control	14.71 ± 1.02	5.26 ± 0.28	4.79 ± 0.25	2.07 ± 0.29	78.32 ± 3.68	23.13 ± 1.09
Disease control	7.05 ± 0.4	3.06 ± 0.18	9.47 ± 0.5	1.49 ± 0.21	35.08 ± 2.0	92.34 ± 5.27
Doxorubicin	13.09 ± 0.89***	4.04 ± 0.47***	5.23 ± 0.12***	1.86 ± 0.26***	73.35 ± 3.97***	30.43 ± 3.59***
EEIC (200 mg/kg b. w)	7.95 ± 0.58**	3.11 ± 0.18**	8.05 ± 0.43**	1.62 ± 0.21**	40.34 ± 2.3**	82.33 ± 0.97**
EEIC (400 mg/kg b. w)	8.83 ± 0.37***	3.27 ± 0.21***	7.17 ± 0.34***	1.64 ± 0.23***	43.57 ± 2.49***	76.69 ± 3.86***

Results are expressed in mean ± SEM (n = 6); statistical significance at *p* < 0.05*, 0.01** and 0.001***, in comparison to control group

Table 5: Effect on biochemical parameters

Treatment	*SWAS (IU/L)*	*SGPT (IU/L)*	*SALP (IU/L)*	*Total protein (mg/dL)*	*Bilirubin (mg/dL)*
Normal control	33.75 ± 1.6	28.86 ± 0.82	79.20 ± 1.33	9.28 ± 0.49	1.14 ± 0.1
Disease control	82.24 ± 3.87	78.78 ± 1.44	124.61 ± 2.34	4.82 ± 0.26	4.03 ± 0.23
Doxorubicin	37.78 ± 1.87***	33.66 ± 0.96***	82.66 ± 1.83***	7.98 ± 0.42***	1.73 ± 0.15***
EEIC (200 mg/kg)	82.44 ± 4.39**	73.64 ± 2.15**	116.89 ± 2.09**	4.88 ± 0.22**	3.55 ± 0.07**
EEIC (400 mg/kg)	75.86 ± 2.13***	64.80 ± 1.89***	113.78 ± 2.99***	5.81 ± 0.14***	3.26 ± 0.19***

Results are expressed in mean ± SEM (n = 6); statistical significance at $p < 0.05$*, 0.01** and 0.001***, in comparison to control group

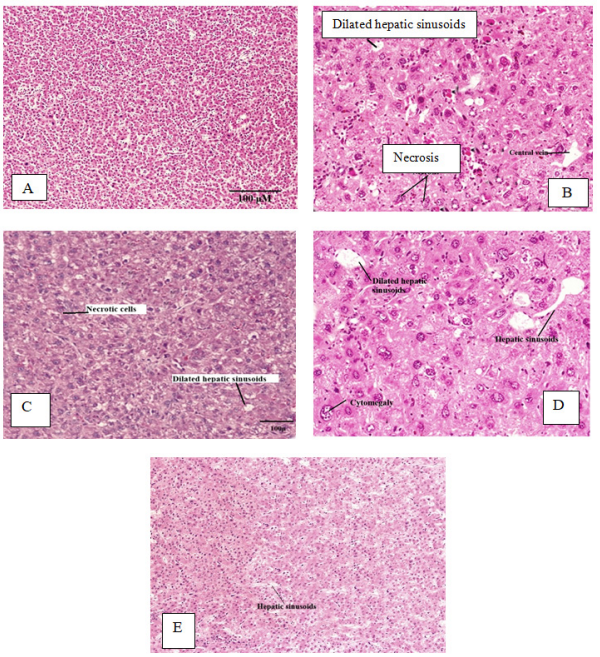

Figure 5: Effect of EEIC on anatomical features of HE-stained mice liver A) Normal control; B) Disease control – Enlargement of hepatic sinusoids, cellular necrosis, resembled Ehrlich ascites carcinoma; C) Doxorubicin Improved the abnormal architecture of the hepatic tissue; D) EEIC treatment 200 mg/kg depicted a notable improvement in inflammation & necrosis of the liver cells; E) EEIC treatment 400 mg/kg demonstrated a remarkable reversal of abnormal anatomical features in the hepatocytes.

populations of immune cells. Administration of doxorubicin somewhat enhanced these metrics. The EEIC demonstrated favorable outcomes, with varied levels of enhancement found in several hematological markers. Nevertheless, the impact of these effects was typically constrained in assessment to the normal control group.

The control group, which did not receive any medication, showed normal levels of the assessed biochemical indicators, suggesting healthy liver function and protein levels. The disease control group, which consisted of mice with untreated EAC, had markedly increased levels of SWAS (82.24 IU/L), SGPT (78.78 IU/L), SALP (124.61 IU/L), and bilirubin (4.03 mg/dL), indicating decreased liver function (Table 5). The total protein levels exhibited a decrease (4.82 mg/dL) compared to standard range, indicating disruptions in protein metabolism. The mice who received doxorubicin exhibited SWAS levels of 37.78 IU/L, SGPT levels of 33.66 IU/L, SALP levels of 82.66 IU/L, and bilirubin levels of 1.73 mg/dL, which were pretty normal. This suggests that their liver function improved compared to disease control group. Additionally, total protein levels were partially restored to 7.98 mg/dL. Nevertheless, there was variation in the total protein levels across the groups, as the 200 mg/kg b. w group exhibited a total protein level of 4.88 mg/dL, while the 400 mg/kg bw group had a slightly enhanced 5.81 mg/dL level.

The data reveal that the disease control group exhibited notable liver function and protein metabolism abnormalities. This is evident from the higher levels of SWAS, SGPT, SALP, and bilirubin, as well as the decreased total protein levels. Administration of doxorubicin partially enhanced hepatic function and protein concentrations. The ME and IC extracts had variable effects on the examined biochemical markers, with certain groups displaying indications of possible liver impairment.

DISCUSSION

India possesses a vast reservoir of medicinal plants, many of which play a crucial role in the formulations used in many medical systems like Ayurveda, Unani, and Siddha. These plants are also extensively utilized in traditional and folk remedies practiced in rural areas. However, only a limited number of them have undergone scientific investigation using globally recognized scientific protocols and tests. This study aims to confirm the anti-tumoral effectiveness of *I. Cordifolia*.[30] The anticancer impacts were especially evaluated at the advanced phases (10 days after tumor injection) to gather significant translational data for potential future human trials. Doxorubicin, a synthetic medication often used in clinical settings, was employed as a positive control.

All animals saw a fast growth in ascites tumor volume when the EAC was injected into the peritoneal cavity. This resulted in a rise in body weight; by day 10 there was an estimated 15 to 24% growth. Results are consistent with previous findings with EAC.[31] The administration of doxorubicin resulted in a significant reduction in the rate of weight gain in addition to an improvement in the survival rate of the mice with tumors. The antineoplastic activity of the alcoholic extract, administered at a dosage of 6.5 mg/kg body weight (equivalent to one-tenth of LD0/30), was ineffective compared to doxorubicin. Nevertheless, it is crucial to take into account that the amount of doxorubicin used (3.5 mg/kg body weight) is about one-fifth of the LD50/30 dosage. It induces genotoxicity & systemic

toxicity in recipient animals. Nevertheless, *I. cordifolia* did not exhibit any adverse effects, indicating its safety.

Upon comparing the extracts, it was noted that alcoholic extract exhibited higher potency than aqueous. This strongly indicates that specific kind & concentrations of phytochemicals in attendance in the extract are accountable for the observed impact. The initial analysis of the plant *I. cordifolia* revealed that it has alkaloids and flavonoids as its primary components. Alkaloids are shown to have higher solubility in organic solvents like ethanol compared to water, but most flavonoids exhibit the opposite trend. Typically, alkaloids are found to be more poisonous to cells, which might explain the strong toxic impact of ethanol extract. *In-vitro* experiments conducted using EAC cells have demonstrated that all treatments resulted in an elevated proportion of deceased cells, as indicated by tryphan blue-positive staining. This suggests that the treatments exhibited cytotoxic impacts.[32] When extract was administered to tumors developing in the peritoneal cavity of mice, a comparable process may be taking place, which would slow down tumor weight development and lead to an increase in survival.

The histological tests demonstrated the impact of the ethanolic extract of *I. Cordifolia* on the morphology of the liver in mice (Figure 5). The normal mice had a healthy liver with a well-structured architecture of the hepatic tissue, displaying a normal arrangement of cells and no signs of inflammation or necrosis. The hepatic sinusoids in the diseased control animals exhibit an enlarged or dilated appearance. This may be due to changes in blood flow and microcirculation in the liver. Regions of cellular demise or necrosis were seen in the hepatic tissue. The aggressive nature of Ehrlich ascites carcinoma may result in the destruction of liver cells, which might be a potential outcome. The normal organization of hepatocytes into functional cords was disturbed. This suggests an atypical arrangement of liver tissue, which might potentially affect liver function. The administration of doxorubicin resulted in a notable enhancement of the atypical structure of the liver tissue. However, a small number of expanded blood vessels in the liver and some dead cells were also seen. The administration of EEIC at a dosage of 200 mg/kg body weight resulted in a noteworthy amelioration of liver cell inflammation and necrosis. Nevertheless, some histological findings, such as infrequent dilated hepatic sinusoids and cytomegaly, persisted in the liver tissue. The intracellular (IC) therapy, administered at a dosage of 400 mg/kg, exhibited a notable reversal of the aberrant morphological characteristics observed in the hepatocytes. Instances of necrotic cells were hardly detected. In addition, there was no evidence of inflammation in the liver tissue, and the hepatic sinusoids looked to be normal and healthy.

CONCLUSION

The current discovery that the ethanolic extract of *I. Cordifolia* exhibited inhibitory effects on tumor growth in advanced stages of cancer is highly promising and likely attributable to its complex composition. Some lesser chemicals can increase the effectiveness of the main compounds, leading to an additive or synergistic impact. This can also lessen harmful effects of therapy, overturn changes in blood parameters of tumors, & provide significant advantages. Further research is being organised to study the efficacy of I's ethanol extract. *Cordifolia* in more relevant tumor models is used to assess its potential for clinical use. Ongoing research is being conducted to determine the specific active ingredients responsible for the anticancer benefits and how they work.

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