Original Article

Anticancer activity of *Ipomoea carnea* on Ehrlich Ascites Carcinoma Bearing Mice

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ABSTRACT

The current study was aimed to study the anticancer potential of *Ipomoea carnea* towards Ehrlich ascites carcinoma (EAC) bearing mice. Ethanolic extract of *Ipomoea carnea* (leaves) was assessed for *in vivo* anticancer potential. The albino mice of either sex separated into five groups of six animals each. Group I served as normal control, group II served as tumor control, group III and IV animals received different concentrations of plant extract of tumor inoculation (150 mg/kg.bw, 300mg/kg.bw) after 24 h for 14 days. Standard drug 5 fluorouracil (20mg/kg.bw) was given to group V animals for 14 days. Before the treatment, all the group's mice were inoculated with EAC (1×10^6cells/mice) except the group I animals. After an experimental time, the tumor volume, tumor cell count, viable and non-viable cell count were analyzed using the ascites fluid. The animals were slaughtered by cervical decapitation, the blood was stored and used to hematological and biochemical studies. The liver homogenate was used for antioxidant and glycoprotein profiles. The ethanolic extract of *Ipomoea carnea* (EEIC) administration at different dose increased the life span and reduced the viable cell counts and ascites fluid volume. Administration of plant extract restored the altered levels of antioxidant parameters like SOD, reduced glutathione, LPO and catalase activity. It also decreased the nucleic acid content and glycoprotein level of a tumour bearing animals. Thus the study revealed that *Ipomoea carnea* has significant anticancer activity against EAC bearing mice.

Key words: *Ipomoea carnea,* Ehrlich ascites carcinoma, Albino mice, Ascites fluid, Anticancer activity.

INTRODUCTION

Cancer is a group of disease characterized by abnormal cell growth that grows and divides in an uncontrolled manner. These 'malignant' cells invading normal tissues and organs and eventually spreading (metastasize) throughout the body. *Cancer* incidence and *mortality* rates have been increasing in both developed and developing countries, whereas in 2018 around 11, 57, 294 new cases were diagnosed and 7, 84, 821 deaths occur around the world.[1]

Ipomoea carnea, the morning glory belongs to the family of Convolvulacea. It has heart-shaped leaves that are 6-9 inches long with green rich color. *Ipomoea carnea* grows to a height of 6m on terrestrial land, but acquires a shorter height in the aquatic habitats. It has thick stem and several branches from base. The stem is erect, woody, hairy and cylindrical shape. Normally it attains 1.25 – 2.75 long and 0.5-0.8 in diameter. The plant has simple and petiolate, attains 4.5-7cm in length, 2.5-3mm in diameter. The upper surface of leaf is pale green and lower surface is paler.[1a]

The plant has many medicinal values and it was used in the ancient system of medicine in many countries. It contains a component alike marsilin, a sedative and anticonvulsant. *I. carnea* that contain saponin, a secondary metabolite which has proven anticarcinogenic and cytotoxic properties.

Plant derived secondary metabolites such as alkaloids, flavonoids, steroids, terpenoids

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and etc., which has antioxidant and antitumor activity. Antioxidants have the ability to inhibit and scavenge the free radicals, thus protect the humans against infections and degenerative diseases. Though the plant *I. carnea* have been extensively used in folklore medicine, information from organized searches for its anticarcinogenic activity.[2]

MATERIALS AND METHODS

Collection of plant material

The *Ipomoea carnea* leaves were collected from the herbal garden, Thanjavur and identified at the Centre for Advanced Research in Indian System of Medicine (CARISM), SASTRA University, Thanjavur and the herbarium was submitted in CARISM. The plant was confirmed Rapinat Herbarium (RHT-77), St. Joseph's College, Trichy, Tamil nadu, India.[3]

The leaves were allowed to shade dried for 10 days. The dried leaves were powdered with an electric blender and stored at room temperature in an air tight container.

Preparation of plant extract

250g of dried plant sample was soaked for 72 h with ethanol at room temperature. Further addition of solvent showed no green colour in the sample. Filtered the solution and the filtrate was vaporized to dryness. The dried residue was dissolved and used for further study.

Animals

Swiss albino mice were purchased from Biogen Laboratory, Bangalore. The animals of either sex were taken and fed with standard pellet diet. The mice were acclimatized in laboratory condition for 10 days before commencement of experiment. Temperature condition maintained is -25°C ± 2°C. The animals got approval from ethical committee approval from SAC/IAEC/BS/2016/MSc003. Each group consists of six animals, total thirty six. They were maintained in poly propylene cages with standard pellet diet (Sai Durga Feeds, Bangalore and water *ad libitum*).

EAC Cell line

Ehrlich-Lettre ascites carcinoma (EAC) is also known as Ehrlich cell. It was originally established as an ascites tumor in mice. EAC is referred to as an undifferentiated carcinoma and is originally hyperdiploid, has the high transplantable capability, no- regression, rapid proliferation, shorter life span, 100% malignancy and also does not have tumor specific transplantation antigen (TSTA).

Experimental design

Six albino *mice* of *both sexes were* randomly *divided into five groups.*

Group I - Normal Control

Group II - Ehrlich Ascites Carcinoma cell line ($1x10^6$cells/mouse) (Disease Control)

Group III - EAC cell line ($1x10^6$cells/mouse) treated with *Ipomoea carnea* at a dosage of 150 mg/kg for 14 days.

Group IV - EAC cell line ($1x10^6$cells/mouse) treated with *Ipomoea carnea*a dosage of 300mg/kg for 14 days.

Group V - EAC cell line ($1x10^6$cells/mouse) treated with 20mg/kg of Standard drug 5- Flurouracil for 14 days. (This compound is a well established model to study anticancer properties both *in vivo* and *in vitro*)

Survival time

The Swiss albino mice were divided into five groups of six animals each, animals were inoculated with $1x10^6$cells/mouse on day 0 and treatment with EEIC started after 24 h of tumor inoculation at a dose of 150 and 300 mg/kg.bw. 0 to group III, IV and V respectively for 14 days. Group II animals served as a tumour control and received normal saline.

The percentage increases in life span were calculated as follows,

$$ILS(\%) = \left\{ \frac{\text{mean survival time of treated group}}{\text{mean survival time of control group}} - 1 \right\} \times 100$$

$$MST = \frac{(\text{Day of first death} + \text{day of last death})}{2}$$

Tumor growth response

Tumor volume

The ascitic fluid was taken from the peritoneal cavity of the mice. Using graduated centrifuge tube the packed cell volume was measured by centrifugation for 10 min at 1000 rpm.

Tumor cell count

The ascitic fluid was collected with a help of WBC pipette and diluted 100 times. A drop of diluted cell suspension was fixed on the Neubauer counting chamber

by using 64 small squares, the number of cells was counted.

Viable/non-viable tumor cell count

The ascitic fluid was collected with a help of WBC pipette and diluted 100 times. The tryphan blue (0.4% in normal saline) dye was used to stain the cells. If the cells absorbed the dye, it is considered as viable otherwise it is non-viable.

Thus the viable and non-viable cells were counted.

$$\text{Cell count} = \frac{\text{No. of cells} \times \text{Dilution}}{\text{Area} \times \text{Thickness of liquid film}}$$

Hematological studies

Red Blood Cells (RBC), White Blood Cells (WBC) and Hemoglobin was collected from each mice by intra cardialpunture and anticoagulant (EDTA) was added.

Determination of other parameters

By using liver homogenate anti-oxidant level and hepatic glycoprotein levels were determined. Hepatic marker enzymes were determined by using the serum.

RESULTS AND DISCUSSION

Gastric cancer is a vigorous disease that remains the fourth most common type of cancer and is the second leading cause of cancer death among worldwide. Gastric cancer is frequently identified at an advanced stage that demands continued attention and research with regard to prevention, early detection and novel therapeutic options.[4] The keystone of treatment is surgical resection with adjuvant chemotherapy or chemo radiation that has led to improved survival.[5,6]

Medicinal plants were used from many centuries as a medicine and have strong anti-tumor activity and anti-oxidant activity.[7] In the present study one of the plant *Ipomoea carnea* was selected to asses scientifically for its anti-tumor potential against EAC induced cancer in experimental animals.

Qualitative phytochemical screening of ethanolic extract of *Ipomoea carnea* is represented in Table 1. Ethanol extracts of *Ipomoea carnea* leaves showed the presence of phenol, tannins, alkaloids, flavonoids, glycosides, steroid and terpenoids. Our observation is in line with Vanlalhruaii *et al.* 2014.[8] The presence of secondary metabolites in *Ipomoea carnea* extract play an important role in bioactivity of medicinal plants and exert antimicrobial activity through a different mechanism.

Flavonoids are fifteen carbon compounds usually dispersed through the plant kingdom. They also play a major role in disease resistance.[9] Flavonoids are free radical scavengers and strong water soluble antioxidants that avoids the oxidative cell damage and also have strong anticancer property.[10] As antioxidant flavonoids from plant provide anti-inflammatory activity.

Table 1: Qualitative phytochemical analysis of an ethanolic extract of *Ipomoea carnea* leaves.

S. No.	Phytochemical	Observation
1	Phenol	Present
2	Flavonoids	Present
3	Glycosides	Present
4	Saponins	Absent
5	Alkaloids	Present
6	Steroids	Present
7	Tannins	Present
8	Terpenoids	Present
9	Anthraquinone	Absent
10	Carbohydrates	Present

Alkaloids are a group of highly diverse natural products that constitute most of the valuable drugs. They produce a wide range of physiological effects in animals.[11] Solaodine, an alkaloid have been specified as an initial material in the production of steroidal drugs.[12] The isolated alkaloids and their derivatives are used as basic medicinal agents due to their antibacterial and analgesic effects.

Glycosides are the unique group of secondary molecules and play an important role in living organisms. Many plants store important chemicals in the form of inactive glycosides. If these chemicals are needed, the sugar part is broken down to become active glycosides. Many such plant glycosides are used in medications. In animals, poisons are often bound to sugar molecules in order to remove them from the body.[11]

Tannins are the part of a phytochemical constituent that incubate the cell protein synthesis by forming irreversible complexes with proline rich protein. Previous studies reported that the tannin is known to react with protein to provide the typical tanning effect that plays a vital role in the treatment of ulcerated tissue.[13] Tannin present in the herbs are used to treat intestinal disorders like dysentery and diarrhea. Li and Wang reviewed the biological activities of tannins. These observations support the use of *Ipomoea carnea* in herbal remedies and suggesting that it has potential anticancer activity hence used in cancer treatment and prevention.[14]

Table 2 depicts that the effect of EEIC on tumor growth in EAC bearing mice. Results showed increased tumor volume in group II animal, whereas on treatment with plant extract decreased the tumor volume. Further,

Table 2: Effect of EEIC treatment on tumour growth, viable and non-viable cell count.

Group	Tumour volume (ml)	Viable cell (10^6 cells/ml)	Non-viable cells (10^6 cells/ml)
Group I	-	-	-
Group II	5.4±0.16	7.3±0.16	1.8±0.08
Group III	4.06±0.12	5.73±0.12	3.66±0.20
Group IV	2.9±0.08	3.1±0.16	5.23±0.24
Group V	1.56±0.24	2.5±0.16	6.1±0.16

Values are expressed as mean ± S.E, *n*= 5.

Table 3: Effect of EEIC on Hb, RBC and WBC count in EAC bearing mice.

Group	Hb (g %)	RBC (10^6 cells/mm^3)	WBC (10^6 cells/mm^3)
Group I	12.3±0.16	5.19±0.04	7.53±0.24
Group II	6.23±0.09	3.19± 0.05	15.23±0.24
Group III	8.22±0.06	3.9±0.08	11.63±0.20
Group IV	11.06±0.12	4.42±0.07	9.10±0.12
Group V	11.7±0.08	4.9±0.08	8.30±0.16

Values are expressed as mean ± S.E, *n*= 5
Hb: Haemoglobin; RBC: Red Blood Cells; WBC: White Blood cells.

group III and IV animals showed an increase in non-viable cells with a concomitant decrease in viable cells. Group V treated with the standard drug 5-fluorouracil showed a significant reduction in tumor growth as compared with group II mice.

The Ehrlich tumor was a spontaneous murine mammary adenocarcinoma. It is a rapidly growing carcinoma with very aggressive behavior and is able to grow in all strains of mice. The Ehrlich ascetic tumor implantation induces a local inflammatory reaction with increasing vascular permeability, which results in an intense edema formation, cellular migration and a progressive ascitic fluid formation. The ascitic fluid is essential for tumor growth, since it constitutes a direct nutritional source for tumor cells.[15]

Table 3 represents RBC, WBC count and level of hemoglobin in experimental animals. An increase in WBC was observed with decrease in RBC level and hemoglobin count in group II, on contrary an increase in RBC count, hemoglobin level and a decrease in WBC count were observed in plant extract treated groups. Nearly normal values of the hematological parameter were observed in the group treated with the standard drug.

Reduction of RBC or hemoglobin occurs due to myelopathy condition or iron deficiency leads to anemia in tumor bearing mice.[16]

The anemia encountered in tumor bearing mice is due to the reduction of RBC or hemoglobin and this may occur either due to iron deficiency or myelopathy conditions.[16] Ethanolic extract of *Ipomoea carnea*re covers the RBC and hemoglobin content in experimental mice that indicates the protective action of the leaves on the haemopoietic system.

Table 4 shows the activity of enzymatic (SOD and catalase) and the level of non-enzymatic status (LPO and reduced GSH) in experimental animals. SOD are a family of metallo enzymes that catalyze the spontaneous dismutation of superoxide to give hydrogen peroxide and hence diminish toxic effects occurs due to the free radicals derived from the secondary reaction.

Superoxide anion plays an important role in plant tissues and also involved in the formation of other cell-damaging free radicals. The methanol extract of *Dimocarpus longan L our* has potent antioxidant capability have been detected by the scavenging potential of the superoxide anion. *Ricinus communis L* has high phenolic content. It is known that the hydroxyl group of the phenolic content contributes the superoxide anion scavenging activity which confirmed that the plant has antioxidant property. Naturally medicinal plants have the antioxidant potential. The superoxide anion scavenging potential of the plant increased with an increase in the plant extract

Table 4: Effect of EEIC on the activity of enzymatic and the level of non-enzymatic anti-oxidants in experimental animals.

Group	LPO (ng/g)	Reduced GSH (µmole/g)	SOD (µg of adrenochrome /g)	Catalase (mg/min/g tissue)
Group I	15.44±0.269	27.38±0.098	37.12±0.065	339.10±11.694
Group II	28.26±0.130	17.73±0.289	12.39±0.136	123.03±4.548
Group III	24.93±0.36	21.23±0.200	17.60±0.408	208.53±7.880
Group IV	21.43±0.579	26.08±0.129	19.13±0.136	266.86±4.922
Group V	18.2±0.285	26.60±0.153	35.41±0.205	277.40±3.321

Values are expressed as mean ± S.E, *n*= 5
LPO: Lipid Hydroperoxide; GSH: Glutathione; SOD: Superoxide Dismutase.

Table 5: Effect of EEIC on the activity of serum hepatic marker enzymes and level of protein in experimental animals.

Groups	ALT (U/L)	AST (U/L)	LDH (U/L)	Protein (gm/dl)
Group I	62.43±1.510	88.03±3.065	673.5±6.307	5.10±0.135
Group II	306.3±0.216	394.7±2.993	2623.5±22.441	3.28±0.083
Group III	261.7±0.524	288.8±2.242	1724.4±11.937	3.93±0.057
Group IV	160.8±0.249	156.0±3.399	915.10±25.79	4.62±0.082
Group V	109.4±0.368	108.3±1.228	765.9±14.079	4.73±0.106

Values are expressed as mean ± S.E, *n*= 5
ALT: Alanine Aminotransaminase; AST: Aspartate Transaminase; LDH: Lactate Dehydrogenase.

concentration. The antioxidant potential of *Ricinuscommunis* L to scavenge superoxide anion may be due to the presence of secondary metabolites such as alkaloids and flavonoids.[17]

LPO, is a free radical-related process in biologic systems that may occur under enzymatic control. Malondialdehyde (MDA) was the end product of LPO, reported to be higher in cancer tissues than in non-diseased organs. It was also reported that the presence of tumors in the human body or an experimental animal, even not interfere directly with organ function, it affects many functions of vital organs especially the liver.[18] Hence the study reported that *T. catappa* significantly reduced the elevated levels of lipid LPO and GSH content in EAC-treated mice.

SOD, is a powerful natural antioxidant enzyme. It has been reported that, a decrease in SOD activity in EAC cells that decrease the total SOD activity in the liver of EAC bearing mice. Treatment with *T. catappa* brought back the levels of scavenges and reduced the level of LPO. The results were compared with the standard drug 5-fluorouracil. Thus the reports revealed that the *T. catappa* possesses significant antitumor and antioxidant potential against EAC bearing mice.

Phenols are secondary molecules that are present in plants. Studies state that phenols that play a vital role in the antioxidant function. Phenolic compounds have antioxidant properties because of their ability to scavenge the free radicals.[19] The phenolic compound of plant origin showed their antioxidant effect by various mechanisms like scavenging the free radicals, active various antioxidant enzymes and inhibition oxidizes.[20]

On treatment with EEIC, there was an increase in the reduced glutathione and the activity of SOD and catalase than the EAC control animals (group II). The level of LPO, an index of lipid peroxidation, was found to be decreased when compared with group II animal.

Table 5 represents the activity of serum hepatic marker enzymes such as ALT, AST and LDH in experimental animals. The marker enzymes activity was found to be increased in group II when compared to group I.

The changes in the marker enzyme levels will reflect in hepatic structural integrity. The rise in the AST is usually accompanied by elevation of ALT which play a vital role in the conversion of ketoacids from aminoacids. Results state that, this plant might have potent anticancer activity. The elevated serum enzyme levels like AST and ALT are indicative of cellular leakage and functional integrity of the cell membrane in the liver.[20] Treatment with *Ipomoea carnea* decreases the serum level of AST and ALT towards their respective normal value that is an indication of stabilization of plasma membrane as well as repair of hepatic tissue damage which is caused by EAC.

On treatment with plant extract the enzyme activity restored to near normal. Similar results were observed in serum protein level, group II animal showed decreased protein level as compared with the control group. Animals treated with plant extract group III and IV and animals treated with 5 flurouracil showed near normal level of protein. This may be due to the presence of flavonoids and other constituents occur in the extract that reduces the free radicals and thus decreased the damage of liver and release of hepatic markers.

Table 6 shows the level of hepatic glycoproteins such as hexose, hexosamine and fucose. A significant increase in glycoproteins was observed in group II animals. On administration of plant extract in group III, IV animals, a significant change was observed in hepatic hexose, hexosamine and fucose level. The increased glycoproteins could be due to the damage of hepatocytes and is a characteristic feature of a cancer cell.

Table 7 represents the survival time of the tumor bearing mice. In the EAC control group, the mean survival time was 15.33±1.24 days. An increase in MST was observed in group III and IV for 23±0.81 and 27.33±1.24days respectively. The standard drug 5-fluorouracil treated group had a mean survival time of 32.3±2.0 days. The percentage increase in life span of a tumour bearing

Table 6: Effect of EEIC on hepatic glycoprotein level in experimental animals.

Groups	Hexose (mg/g)	Hexosamine (mg/g)	Fucose (mg/g)
Group I	1.86±0.306	2.45±0.285	3.54±0.250
Group II	4.85±0.302	5.89±0.216	11.53±0.384
Group III	3.73±0.302	5.24±0.142	5.81±0.106
Group IV	3.10±0.179	3.79±0.216	4.27±0.025
Group V	2.73±0.174	2.97±0.142	3.64±0.038

Values are expressed as mean ± S.E, *n*= 5

Table 7: Effect of EEIC on survival time of the tumour bearing mice.

Group	MST (days)	ILS (%)
Group I	15.33±1.24	_
Group II	23±0.81	_
Group III	27.33±1.24	50.03
Group IV	32.3±2.0	78.27
Group V	15.33±1.24	86.70

Values are expressed as mean ± S.E, *n*= 5
MST: Mean Survival Time; ILS: Increased Life Span.

mice was found to be 78.27% in plant treated group and 86.70 % (ILS) in the standard drug (5 fluorouracil) treated group.

CONCLUSION

The anti-proliferative effect of *Ipomea carnea* on EAC bearing mice was investigated. EAC bearing mice showed a marked reduction in Hb, RBC and WBC, whereas the EEIC treated group restored the above heamatological parameters to near normal, which is almost similar to 5 flourouracil treated group. The non enzymic antioxidant (GSH), level of LPO and enzymic antioxidant activities were found to improve in EEIC treated groups. On treatment with EEIC the membrane bound carbohydrates such as hexose, hexosamine and fucose were found to be decreased as compared to EAC control group which is due to the antiproliferative effect of the plant. This study confirmed that the leaves of *Ipomoea carnea* possess various phytoconstituents which may contribute its antioxidants and anti-tumor activity. This plant can be subjected to isolate novel pharmacologically active compound investigate its anticancer property could be useful for treating infectious disease and metabolic disorders.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

EAC: Ehrlich Ascites Carcinoma; **EEIC:** Ethanolic Extract of *Ipomoea carnea*; **SOD:** Superoxide Dismutase; **LPO:** Lipid Peroxidation; **CAT:** Catalase; **RBC:** Red Blood Cells; **WBC:** White Blood Cells; **EDTA:** Ethylene Diamine Tetraacetic Acid.

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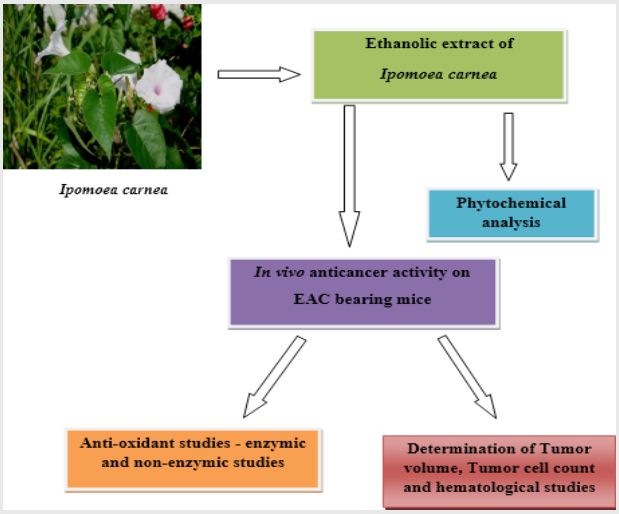
PICTORIAL ABSTRACT



SUMMARY

Our findings explore the anti-proliferative effect of *Ipomoea carnea*. The information should aid to use this plant extract for developing new therapies against variety of cancer.

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Prof. Renuka Saravanan is working as an Assistant Professor in Department of Chemistry and Biosciences at SASTRA Deemed to be University, Srinivasa Ramanujan Centre, Kumbakonam. She has more than 10 years of teaching experience and 8 years of research experience. Her areas of interest are Cancer biology, Clinical Biochemistry and Herbal Medicine.

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