Research Article

Inhibitory Effect of the Fruit Pods of *Carum Copticum* B. against Ehrlich Ascites Carcinoma (EAC) Bearing Mice.

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ABSTRACT

This study was aimed to evaluate the inhibitory effect of the fruit pods of *Carum copticum* B. against Ehrlich Ascites Carcinoma (EAC) bearing mice. Animals were divided into four groups of four mice each. Group I: Normal untreated mice. Group II: Ehrlich Ascites Carcinoma cell line (1X10^{6}cells). Group III: Ehrlich Ascites Carcinoma cell line (1X10^{6}cells) + Treated with AECF at a dosage of 150 mg/kg b.w/day for 14 days. Group IV: Ehrlich Ascites Carcinoma cell line (1X10^{6}cells) + Treated with standard drug 5- Fluorouracil at a dosage of 20mg/kg.bw/day for 14 days. After completing the experiment, the liver markers and antioxidants were analysed. Phytochemical screening of aqueous extract of *Carum copticum* leaves indicates the presence of flavonoids, tannins, glycosides, phenol, steroids, alkaloids, quinone, saponin and coumarin. Supplementation of *Carum copticum* significantly restored the liver markers and antioxidant markers on Ehrlich Ascites Carcinoma (EAC) bearing mice. The results of our study showed that the aqueous extract of *C. copticum* B. fruit pods has significant anti-tumour activity against EAC cell line, probably due to its phytochemicals.

Keywords: *Carum copticum,* Phytochemicals, Ehrlich Ascites Carcinoma cell line, Antioxidant.

INTRODUCTION

Cancer is one of the most dreaded diseases of centuries, characterized by uncontrolled cellular proliferation, tissue invasion and metastases.[1] Overproduction of peroxides and free radicals (reactive oxygen species, ROS) due to aberrant metabolism involving redox enzymes and/or exposure to a plethora of exogeneous chemicals/factors can cause damage to cellular macromolecules such as nucleic acids, proteins or lipids leading to many degenerative diseases including cancer, alzheimer's disease, arthritis and ischemic reperfusion[2-5] More and more recent evidences suggest that this potentially cancer inducing oxidative damage or 'oxidative stress' can be prevented or limited in part by natural antioxidants which may mediate their effects by directly quenching the ROS or chelating the catalytic metal ions of redox enzymes.[6] Natural products including fruits and vegetables have drawn considerable interest as chemotherapeutics because of their widespread antioxidant and anticancer principles. Among the natural antioxidants polyphenolic compounds such as flavonoids, flavonols and terpenoids etc. from plant origin have appeared as favoured choice. By virtue of being electron rich these molecules can donate electrons to ROS and neutralize these chemical species[7-9].

Carum copticum (Family: Apiaceae) is an Egyptian aborigine plant. This plant grows in arid and semiarid fields in different regions of central Europe, Asia, India (most crops are in the states of Rajasthan, Gujarat, and West Bengal), Iran (especially eastern regions of Baluchistan), Iraq, Afghanistan, and Pakistan.[10,11] *C.copticum* Seeds also has anti-bacterial, germicide, antifungal and anesthetic properties. They are rich in various vitamins, minerals, fibers and antioxidants. These seeds can cure spasmodic pains due to indigestion, flatulence and various infections.[12] Therapeutic uses of *C. copticum* seeds also include carminative, antiseptic, amoebiasis expectorant, antimicrobial, anti-parasitic, antiplatelet aggregatory, and antilithiasis as well as treating common cold and acute pharyngitis.[12] Abortifacient, galactogogic, and diuretic activities have been observed for this plant.[13,14] There is also anti-carcinogenic potential evidence for *C. copticum*. It has been shown that this plant has also foetotoxicity, abortion potential, and galactogogue properties.[15] The aim of the present study was to evaluate the inhibitory effect of the fruit pods of *Carum copticum* B. against Ehrlich Ascites Carcinoma (EAC) bearing mice.

MATERIALS AND METHODS

Collection of Plant Material

The fruit pods of *Carum copticum* B. were purchased from local market, Kumbakonam, Thanjavur district, Tamil Nadu, India. The plant was authenticated by Department of Botany, Government Arts College for Men's, Kumbakonam. The fruit pods were shade dried for 5 days. The dried fruit pods were powdered with an electrical blender and stored in air tight container at room temperature for our study.

Preparation of AECF (Aqueous Extract of *Carum copticum* Fruit pods)

100g of coarse powder of the fruit pods was taken in 600 ml of water and boiled. The boiling was continued till the contents were reduced to one third and the filtrate obtained was then evaporated to dryness. Paste form of the extract obtained was stored in an airtight container at

4°C. This aqueous extract was then subjected to pre-clinical screening.

Preliminary Phytochemical Screening of *Carum copticum*

Preliminary phytochemical screening of *Carum copticum* B. was carried out as per the standard procedures.[16]

Anti Tumor Screening

Experimental Animals

Healthy adult Swiss Albino mice, weighing 25-35g were obtained from Kovai Medical Research Centre and Hospital, Kovai. The animals were allowed to acclimatize under laboratory conditions for a period of 5 days prior to the experiment. Animals were housed in standard polypropylene cages. Six animals were housed per cage, so as to provide them with sufficient space, and to avoid unnecessary morbidity and mortality. Animals were maintained under standard condition of 12: 12- hour's light/ dark cycle and at an ambient temperature at 23 ± 2°C, with 65 ± 5 % humidity. Animals were fed with standard rat chow pellet and water *ad libitum*. All the studies were conducted according to the ethical guidelines of CPCSEA after obtaining necessary clearance from the committee. (Approval NO: SAC/IAEC/BC/2015/M.Sc-004).

Ehrlich Ascites Carcinoma

Since the first description of the Ehrlich ascites tumour in 1932 by Loewenthal and Jahn the ascites tumour has been used comparatively and frequently by investigators for chemotherapeutic studies. The Ehrlich ascites tumour is a useful tool for testing the activity of chemicals and besides the ascites tumour provides an easy challenge to chemotherapeutic agents. Ascites tumour was obtained from the Ehrlich mouse carcinoma, which originated as a tumour of the mammary gland. Intraperitoneal injection of the tumour emulsion produces ascites. Solid tumours were obtained by subcutaneous injection of fresh ascetic fluid containing cancer cells, and ascites tumours were induced by intraperitoneal injection.

Maintenance of cells

Ehrlich Ascites Carcinoma cells were obtained through the courtesy of Amla Cancer Research Centre, Thrissur and were maintained by weekly intraperitoneal inoculation of $1X10^6$ cells/mouse.

Experimental Design

Animals were divided into four groups of four mice each. The experimental design given below has been followed for the aqueous extract of *Carum copticum* B. selected for the study.

Group I : Normal untreated mice

Group II : Ehrlich Ascites Carcinoma cell line ($1X10^6$cells)

Group III: Ehrlich Ascites Carcinoma cell line ($1X10^6$cells) + Treated with AECF at a dosage of 150 mg/kg b.w/day for 14 days.

Group IV: Ehrlich Ascites Carcinoma cell line ($1X10^6$cells) + Treated with standard drug 5- Fluorouracil at a dosage of 20mg/kg.bw/day for 14 days.

Treatment with the plant extract started after 24hrs of EAC inoculation. The plant extract administered orally for 14 days at the dose of 150mg/kg bw. At the end of the experimental period, the Ascites fluid was collected and the animals were sacrificed by cervical decapitation. Blood was collected and used for haematological and biochemical estimations. Liver tissues were homogenized, in 0.1 M phosphate buffer, pH 7.4 and used for analysing various antioxidant and biochemical parameters.

Biochemical Parameters

Determination of Haemoglobin, Red and White Blood cells count Wintrobe, Armour,[17] respectively. Determination of Hexosamine and Sialic Acid by Warren, Wanger respectively. Determination of Lipid Peroxides And Glutathione Peroxidase And Reduced Glutathione by the method of Ohkawa,[18] Rotruck,[19] and Moron[20] respectively. Determination of Superoxide Dismutase and Catalase by the method of Misra[21] and Maehly[22] respectively.

In-Vitro Studies

Tryphan Blue Dye Exclusion Method[23]

Short-term *in-vitro* cytotoxicity was assessed using Ehrlich Ascites Carcinoma cell lines cells by incubating different concentrations of the ethanolic extracts of the selected plant drugs at 37°C for 3 hours. The tumour cells were aspirated from peritoneal cavity of tumour bearing mice using an insulin syringe and transferred to a test tube containing isotonic saline. The cells were then washed in normal saline and cell number was determined using a Haemocytometer and adjusted at $10x10^6$cells/ml. For the cytotoxicity assay, different concentrations of the extracts (25-500 µg/ml) were added to each tubes and the final volume was adjusted to one ml with normal saline. Control tubes were kept with the saline, tumour cells and without the drugs. All the tubes were incubated at 370°C for 3 hours. After incubation 0.1ml of 0.4% tryphan blue dye in isotonic saline was added to each tube and the number of viable (unstained) and dead (stained) cells were counted using haemocytometer.

$$\%\ of\ Dead\ cells = \frac{Total\ cells\ counted - Total\ viable\ cells}{Total\ cells\ counted} \times 100$$

Statistical Analysis

All values were expressed as mean ± S.E.M (Standard error of mean). Statistical analysis was performed with one way analysis of variance (ANOVA) and P values <0.05 were considered significant.

RESULTS AND DISCUSSION

Cancer is one of the leading causes of human death.[24] In modern medicine, chemotherapy, radiotherapy and surgery are the major treatments available for cancer.[25] Intervention with chemo preventive agents in the early

stage of carcinogenesis is theoretically more rational than attempting to eradicate fully developed tumours with chemotherapeutic drugs.[26] These agents have a narrow margin of safety and the therapy may fail due to drug resistance and dose-limiting toxicities, which may severely affect the host normal cells.[24] Hence the use of natural products is an attractive alternative in the control and eradication of cancer.[27] Medicinal plants used as folk medicine have strong anti-tumour activity against the Ehrlich ascites carcinoma (EAC) cell line.[28] In this present study one of the plant drugs *Carum copticum* B. was scientifically validated for its anti-tumour potential against Ehrlich Ascites Carcinoma induced cancer in experimental animals.

The retention times of various phenolics (including phenolic standards) identified in this study were approximately in the following ranges: 8.0–31.4 min for phenolic acids (except for rosmarinic acid: 46.8 min); 5.7–40.0 min for tannins; 11.0–30.2 min for flavanols (flavan-3-ols); 21.5–51.0 min for glycoside forms of flavones, isoflavones, flavonols, and flavanones, and 24.8–75.0 min for their aglycone forms; 44.0–75.6 min for chalcones; 23.5–41.5 min for anthocyanins; 20.8–48.6 min for coumarins; 79.0–82.4 min for curcuminoids; 47.5–86.6 min for quinones; 46.0–88.7 min for lignans; 65.0–81.5 min for phenolic diterpenes; and 62.0–90.0 min for some volatile oils (e.g., aromatic compounds: 62.4 min for eugenol, 75.1 min for carvacrol).[29]

A report from Cairo indicated the presence of c-terpinene (24.0%), p-cymene (24.0%), thymol (42.0%) and carvacrol (4.7%).[30] An earlier report shows the major components of *C. copticum* fruits essential oil as thymol, c-terpinene and p-cymene with a minor amount of carvacrol. In two other reports major components of the oil were reported as thymol (35.4% and 49.0%), c-terpinene (28.6% and 30.8%) and p-cymene (29.2% and 15.7%) with no carvacrol.[31,32] However, in other reports from India the major compounds were reported as p-cymene (41.98%) carvacrol (45.20%) and thymol (0.48%).[33] The major components of essential oil of *C. copticum* fruits in that study were thymol (54.50%), c-terpinene (22.96%), p-cymene (19.38%) and carvacrol (0.46%) in the oil. Due to significant difference between amount of thymol and carvacrol in different reports and previous reports about thymol and carvacrol chemotypes,[34] we suggest that the chemical variations for *C. copticum* from different regions should be regarded as chemodemes and they speculate two different chemotypes as thymol and carvacrol chemotypes for *C. copticum*. According to different activities of thymol and carvacrol,[35] this difference between constituents of the essential oil will be important in nutritional and medicinal uses.

The constituents of the seed of *C. copticum* included carbohydrates (38.6%), fat (18.1%), protein (15.4%), fiber (11.9%), tannins, glycosides, moisture (8.9%), saponins, flavone, and mineral matter (7.1%) containing calcium, phosphorous, iron, cobalt, copper, iodine, manganese, thiamine, riboflavin, and nicotinic acid.[12] It was also reported that thymol (45.9%), γ-terpinene (20.6%), and o-cymene (19%) are the major components of the oil of *C. copticum* but ethylene methacrylate (6.9%), β-pinene (1.9%), and hexadecane (1.1%) were the other constituents of the plant.[36] Thymol (72.3%), terpinolene (13.12%), and o-cymene (11.97%) were also identified as constituents of *C. copticum*.[37] Chemical composition of *C. copticum* in two areas in Iran was assessed and results showed that the plant in Kamfiruz contains γ-terpinene (48.07%), p-cymene (33.73%), and thymol (17.41%) compared to the composition of plant in Eghlid area which included γ-terpinene (50.22%), p-cymene (31.90%), and nerolidol (4.26%) as main components.[38]

The essential oil of *Carum copticum* procured from Shiraz (Iran) contained mainly thymol (54.5%), γ-terpenine (22.9%) and p-cymene (19.3%); it was devoid of aromatic, aliphatic and sesquiterpene constituents.[39] A report from Cairo (Egypt) indicated that the presence γ-terpinene (24.0%) and p-cymene (24.0%), thymol (42%) and carvacrol (4.7%).[40] An earlier report showed that the major components of *C.copticum* fruit essential oil as thymol, γ-terpinene and p-cymene. In two other reports major components of the oil were identified as thymol (35.4% and 49.0%) and γ-terpinene (28.6% and 30.8%) with no carvacrol.[31,32] The essential oil of *C.copticum* possessed mainly p-cymene (33.1%), γ-terpinene (28.6%), and thymol (24.1%). However, in other report the major compounds were characterized as p-cymene (41.9%), carvacrol (45.2%) and thymol (0.48%).[33] The significant variation of the chemical constituents of the *C.copticum* oil from different regions may be regarded as chemotypes as thymol, p-cymene, carvacrol, and γ-terpenine as chemotypes. These chemical constituents possess different bioactivities.

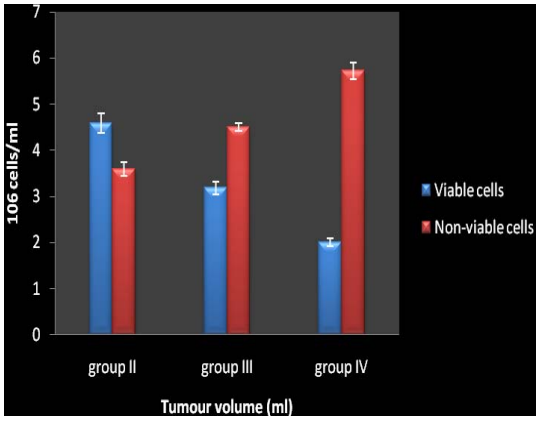

Figure 1: Effect of AECF treatment on tumor growth, Viable & Non-Viable cell count.

Values are expressed as mean ± SEM (n=6)

*p<0.05 statistically significant when compared with Group I

**p<0.05 statistically significant when compared with Group II

Group I: Normal control : Group II: EAC control : Group III: EAC+150mg/kg bw. of AECF : Group IV: EAC+ 20 mg/kg bw. of 5-fluoro uracil.

Fig: 1. predicted the tumour growth response. The EAC inoculation to the experimental animal resulted in the increased production of Ascites fluid (Tumour volume) on treatment with test drug test drug increased dose level decreased the tumour volume. Further the test drug increased the Non-Viable cell counts and decreased the Viable cell count in a plant dose treated (Group III).

The Ehrlich tumour is a rapidly growing carcinoma with very aggressive behaviour[41] and able to grow in almost all strains of mice.[42] The Ehrlich ascitic tumour implantation induces *perse* a local inflammatory reaction with increasing vascular permeability, which results in an intense edema formation, cellular migration and a progressive ascetic fluid formation.[43] The ascitic fluid is essential for tumour growth, since it constitutes a direct nutritional source for tumour cells.[44]

A reliable criterion for assessing the potential of any anticancer agent is the prolongation of life span of the animals.[45] Decrease in tumour volume and viable tumour cell count observed in that present experiment can be considered as an important indication of the reduction of tumour burden and enhancement of life span of EAC-bearing mice.

Treatment with Pterostilbene (PTER) and its conjugate inhibited tumour volume and also increased the lifespan of tumour bearing mice. Further, to understand the underlying mechanism for this regression in tumour volume they checked the expression level of apoptotic marker proteins like caspase-3 and Bax in tumour samples. In that data showed that the expressions of apoptotic marker proteins were up-regulated after treatment with conjugate. These results clearly support the anti-tumour action of the conjugate molecule. *In-vivo* experimental studies have shown that tumour growth and metastasis are dependent on angiogenesis.[46] Angiogenesis is a property of most solid tumour sand is necessary for their continues growth. Thus inhibiting tumour angiogenesis may halt the tumour growth and decrease metastatic potential of tumours. Further, it has been earlier reported that ascetic tumour growth including EAC cells are angiogenesis dependent.[47s] and Vascuslar Endothelial Growth Factor (VEGF) being a permeability fact or plays a fundamental role in the fluid accumulation and tumour growth in ascetic tumour. By secreting VEGF, the permeability of pre-existing microves sellining of peritoneal cavity is enhanced which stimulates ascites formation thereby tumour progression. Inhibition of fluid accumulation, tumour growth, and micro vessel density by neutralization of VEGF has been demonstrated underlining the importance of VEGF in malignant ascites formation.[48,49]

Ascitic fluid has severally been shown to be the direct source of nutrition for tumour cells. Since ascetic fluid has an inhibitor factor which suppresses cell proliferation, Ehrlich Ascites Carcinoma cells decrease with increasing concentration of ascetic fluid.[50] These results of this study showed that pre-carcinoma administration of Rho-Kinase inhibitor Y-27632 reduced the ascitic burden as depicted by the body weights of mice.

Table 2 predicted the hemoglobin (6.58±0.27 (%)), RBC count (2.73±0.18 cells/mm^3) and WBC count (1050±15.81 cells/mm^3) of tumour bearing mice. On treated with the concentration of 150 mg/kg bw. of the plant drug AECF increased the hemoglobin (7.65±0.21 (%)), RBC count (3.33±0.11 cells/mm^3) and WBC count(6700±182.57 cells/mm^3).

The anaemia encountered in tumour bearing mice is mainly due to a reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions.[45]

Recovery of the haemoglobin content, RBC and WBC cell count in the experimental mice indicates the protective action of Ethyl acetate extract from the flower of *Calotropis gigantea* on the haemopoietic system.

In this experimental tumour model, host defense cells with prevalence of neutrophils were observed infiltrating the solid tumour or adjacent to disseminated tumour cells.

The number of peritoneal leukocytes has been reported to be increased as a consequence of tumour growth.[43]

The activation process of leukocytes is accompanied by the intensive production of reactive oxygen species.

There is evidence that treatment with Y-27632 result in a significant reduction in the accumulation of neutrophils. Polymorphonuclear leukocytes aggregation can also be decreased by Y-27632.

In cancer chemotherapy the major problems are myelosuppression and anaemia.

The anaemia encountered in tumour bearing mice is mainly due to a reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions.[45]

The development of hypoglycaemia and hyperlipidaemia in experimental animals with carcinoma has been previously reported.

The total white blood cell (WBC) count, proteins and packed cell volume (PCV) were found to be increased with a reduction in the haemoglobin content of red blood cell (RBC).

The differential count of WBC showed that the percentage of neutrophils increased (P<0.001) while that of lymphocytes decreased (P<0.001). At the same time interval, complexes (100 mg/kg/day, p.o) treatment could change these altered parameters to near normal.

The reliable criteria for judging the value of any anti-cancer drug are prolongation of lifespan and decrease of WBC from blood.[51,52]

Myelosuppression and anaemia have been frequently observed in ascites carcinoma[53] and similar findings were also observed in that study. In EAC control mice, elevated WBC count and reduced hemoglobin and RBC count was observed. Anemia (reduced hemoglobin) encountered in ascites carcinoma mainly occurs due to iron deficiency, either by haemolytic or myelopathic conditions, which finally lead to reduced RBC number. In that study showed that alcoholic extract of PH (ALC) treatment prevented the fall in hemoglobin content and maintained normal values of RBC and WBC. Treatment with ALC maintains the normal values of whole blood count, which supports its hematopoietic protecting activity. Mechanism behind the hematopoietic protection is beyond the scope of the present study. Induction of myelotoxicity in EAC mice is considered to be of immunological significance to meet the adverse situation developed by the introduction of foreign bodies in the blood.

Table 1: Preliminary phytochemical screening of *Carum copticum*

S. No	Test	Reagents Required	Reaction	Observation
1.	Saponin	Water shakes	Leather formation	(-) ve
2.	Tannin	Lead acetate solution.	White precipitate	(-) ve
3.	Sterol	Acetic anhydride + H_2SO_4.	Bluish green color	(-) ve
4.	Terpenoids	Warmed with Tin & Thionyl chloride.	Pink color	(-) ve
5.	Flavonoid	Magnesium bits + Conc. HCl.	Magenta color	(+) ve
6.	Coumarin	10% Sodium hydroxide.	Yellow color	(+) ve
7.	Quinines	Conc. H_2SO_4.	Red color	(+) ve
8.	Alkaloids	Warmed with Dragendroff's reagent.	Orange color	(+) ve
9.	Glycosides	Anthrone and Conc. H_2SO_4.	Dark green color	(+) ve
10.	Sugar	Warmed with Fehling's A & B.	Red or Green color	(+) ve
11.	Phenols	Alcohol + Ferric chloride.	Red color	(-) ve

(+) ve indicates present; (—) ve indicates absent.

Table 2: Effect of AECF on Serum Hemoglobin, RBC & WBC in Experimental Animals

Particulars	Hemoglobin (g %)	RBC (10^6/mm^3)	WBC (cells/mm^3)
Group I	11.00 ± 0.32	4.80 ± 0.13	6325.00 ± 131.50
Group II	6.58 ± 0.24*	2.73 ± 0.18*	1050 ± 15.81*
Group III	7.65 ± 0.21**	3.33 ± 0.11**	6700.00 ± 182.57**
Group IV	9.75 ± 0.19	4.08 ± 0.06	7050.00 ± 0.18

Values are expressed as mean ± SEM (n=6); *p<0.05 statistically significant when compared with Group I; **p<0.05 statistically significant when compared with Group II

Table 3: Effect of AECF on levels of enzymic and Non-enzymic antioxidants in Experimental Animals

Particulars	LPO (nM of MDA Formed/g of tissue)	Glutathione peroxidase (µg of GSH hydrolyzed/g tissue/minute)	Reduced glutathione (µg/g tissue)	SOD (mg of epinephrine oxidized/g tissue)	Catalase (µ moles of H_2O_2 hydrolyzed/g tissue/minute)
Group I	171.17±8.54	0.40±0.01	6.33±0.14	12.18±0.67	39.22±0.23
Group II	620.67±13.87*	0.14±0.01*	2.74±0.11*	24.06±0.35*	27.68±0.40*
Group III	425.83±4.48**	0.26±0.01**	4.43±0.21**	16.95±0.48**	32.86±0.16**
Group IV	290.33±3.05	0.35±0.01	5.52±0.08	16.51±0.52	30.83±0.13

Values are expressed as mean ± SEM (n=6); *p<0.05 statistically significant when compared with Group I; **p<0.05 statistically significant when compared with Group II

Table 4: Effect of AECF on levels of Hexosamine in experimental animals

Groups	Hexosamine (µg/g tissue)	Sialic acid (µg/g tissue)
I	581.25±83.15	4725.00±339.74
II	2325.00 ±78.06*	16470.00±810.00*
III	1218.75 ±121.03**	6210.00±307.41**
IV	806.25 ±39.03	8325±86.17

Values are expressed as mean ± SEM (n=6); *p<0.05 statistically significant when compared with Group I;

**p<0.05 statistically significant when compared with Group II

Table 3 predicted the enzymatic and non-enzymatic status of experimental animals Lipid Peroxides (LPO), Glutathione peroxidase, Reduced glutathione, SOD and Catalase level of tumour bearing mice treated with the concentration 150 mg/kg b.w. of the plant drug AECF increased the glutathione peroxidase, reduced glutathione and catalase level than the EAC control level (group II). The level of LPO and SOD decreased than the group II level.

Lipid peroxidation was analysed by the method of.[18] After treatment (groups I–XV), a reaction mixture was prepared in a final volume of 2.0 ml cell lysate, 10µl of 10% SDS, 600µl of 20% glacial acetic acid, 600µl of 0.8% ThioBarbituric Acid (TBA), and water. The mixture was placed in a boiling water bath for 90 min and immediately shifted to crushed ice bath for 10 min. The mixture was centrifuged at 2000×g for 10 min. The amount of thiobarbituric acid reactive substances (TBARS) formed was measured based on the optical density of the supernatant at 535 nm. The activity was expressed as nmoles of TBARS/mg of protein. GPx concentration was determined according to the manufacturer's protocol (Sigma–Aldrich, MO). The activity was expressed as µmol/min/mg protein.

A number of studies have indicated that tumour growth can cause antioxidant disturbances and acceleration in lipid peroxidation in vital organs of the tumour host.[54-57] Increased Lipid Peroxidation (LPx) would cause degeneration of tissues and lipid peroxide formed in the primary site would be transferred through circulation and provoke damage by propagating the process of LPx.[58] Malondialdehide (MDA), the end product of LPx was reported to be higher in carcinomatous tissue than in non-diseased organs.[59] and their levels were correlated with advanced clinical stages and the impairment is related to tumour progression.[60] Our results for MDA levels in plasma of (Solid Ehrlich Carcinoma) SEC-bearing mice are in agreement with earlier reports that showed increased levels of LPx in tumour and liver tissues of mice-bearing human breast cancer[61] and in the sera of patients with breast cancer,[62] laryngeal carcinoma,[6,64] gastric cancer and lung carcinoma.[65] It has been suggested that active oxygen species such as superoxide anion, hydrogen peroxide and hydroxyl radical generated in inflamed tissues can damage the target cells, resulting in DNA damage and contributing to tumour development.[66] Moreover, it has been claimed that MDA acts as a tumour promoter and co-carcinogenic agent because of its high cytotoxicity and inhibitory action on protective enzymes.[67]

The present data revealed marked depletion in glutathione (GSH) content as well as the activity and gene expression of the antioxidant scavenger enzymes, GPx, GST, SOD and CAT in the blood and liver tissues of tumour-bearing mice. The relationship among cancer growth, GSH content, and the antioxidant system has been also studied. GSH, a potent inhibitor of the neoplastic process, plays an important role as an endogenous antioxidant system that is found in particularly high concentration in the liver and is known to have a key function in the protective process.[58]

Reduced activities of the glutathione (GSH) and GSH related enzymes such as glutathione peroxidase (GPx) in cancer patients were also reported by others.[68-70] It was reported that during cancer growth, glutathione redox (GSH/GSSG) decreases in the blood of Ehrlich ascites tumour–bearing mice, mainly due to an increase in blood GSSG levels as a result of oxidative stress. This increase may be caused by an increase in peroxide production by tumour cells that can lead to GSH oxidation within the red blood cells and increased GSSG release from different tissues into the blood.[71] Early studies hypothesized that the enzyme inactivating action of reactive oxygen species (ROS) or lipid peroxides can overcome enzyme synthesis capacity. GSH Px plays an important role in metabolizing lipid peroxides in the liver and this enzyme decrease is potentially ascribable to inactivation by the increase in ROS or lipid peroxide formations.[72]

In the current study, we also observed that the detoxifying enzyme glutathione-S-transferase (GST) activity significantly dropped in the liver of the SEC-bearing mice. Our results are in accordance with others who detected low liver GST activity in SEC-bearing mice and in lung cancer-bearing animals.[72]

Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) are involved in the clearance of superoxide and hydrogen peroxide. SOD catalyses the diminution of superoxide into H_2O_2, which has to be eliminated by GPx and/or CAT. The decline in SOD activity was observed in different tissues of SEC-bearing mice (Solid Ehrlich Carcinoma).[55,73] The loss of Mn SOD activity was attributed to the loss of mitochondria membrane potential, which leads to a decrease in total SOD activity in different tissues of the tumour host.[74] It is worth mentioning that SOD activity plays an important role in the anti-tumour effects of active oxygen-forming anticancer agents. However, when the oxidative damage is extreme as a result of tumour growth, ROS scavenging enzymes such as SOD and catalase are degraded. The inhibition of CAT activity in different tissues of mice-bearing Ehrlich tumour as a result of tumour growth was also reported by several investigators.[75] This was also expressed in the present study by the decrease in CAT mRNA expression.

Blask and Wilson[76] claimed that the anti-proliferating effect of melatonin on MCF-7 human breast cancer cells might involve intracellular GSH levels. On the other hand, Chen[77] reported that melatonin's inhibitory effect on growth of ME-180 human cervical cancer cells is not related to intracellular glutathione concentrations. The present results confirmed the findings of Chen[77] and showed that melatonin reduced cell viability, and volume of Ehrlich Ascites Carcinoma (EAC) cells implanted in mice and increased the survival time of the tumourized animals

paralleled with insignificant changes in GSH levels in EAC cells. This is also in harmony with recent findings that intracellular GSH levels did not play any role in the sensitivity of breast cancer cells to melatonin.[78]

The increased activity of GST has been demonstrated in various head and neck squamous carcinoma cell lines.[79] It is suggested that elevated levels of GST may be due to amplification of the GST gene on 11q 13 in EAC cells obtained from melatonin treated mice. This is supported by the report of Reiter[80] that melatonin may bind to DNA, chromatin or heterochromatin. Thus, it is reasoned that the enzyme GST might be influenced by melatonin.

Table 4 showed that the membrane associated carbohydrate such as Hexosamine was found to be higher in hepatic tissue of tumour bearing mice. Oral administration of the dose level of AECF for 14 days, to the experimental animals (Group III) effectively decreased the membrane bound carbohydrates. In the IV animals treated with the standard drug 5-Fluoro uracil also showed marked decrease in the membrane bound carbohydrate.

Glucose enters the glycolytic pathway, where the majority is metabolized to pyruvate; however, a small quantity of fructose 6-phosphate combines with the amino acid glutamine to form glucosamine 6-phosphate. The rate-limiting enzyme controlling this step, and hence flux through the whole pathway, is glutamine: fructose-6-phosphate aminotransferase (GFAT; EC 2.6.1.16). Glucosamine 6-phosphate is further metabolized to UDP-N-acetylglucosamine, which serves as an essential precursor for the synthesis of glycoproteins, glycolipids and proteoglycans.[81]

The effects reported in the present study were obtained with a pre-incubation of just 2 h in high glucose or glucosamine. It is likely that additional effects will be obtained with longer pre-incubation times. Indeed, in hyperglycaemic streptozotocin diabetic rats, a decrease in the intrinsic activity of glucose transporters was an early event but, with time, hyperglycaemia induced a defect in transporter translocation in response to insulin.[82] Furthermore, a defect in the insulin-mediated translocation of GLUT4 has recently been reported in rat muscle following a 4 h infusion of glucosamine.[83]

Table 4: predicted the levels of sialic acid in tumour bearing mice treated with the 150mg/kg b.w. of the plant drug showed the increased level of sialic acid than the EAC control group. The standard drug 5-fluorouracil also showed the significantly restore values.

Sialic acids will have a great future in medical sciences and one of the most recent examples are encephalopathies caused by prions, the glycosylation residues of which are sialylated.[84] Sialyltransferase inhibitors are becoming more important, since "over-sialylation" is a widely studied phenomenon of various tumour cells.[85,86] and selectins recognizing Sia (Sialic acid) have been shown to be involved in metastasis.[87]

Carbohydrates also play an eminent role in immunology.[88] The effect of Sia on cells being "self " and the role of Siglecs, for instance enabling the communication between B and T lymphocytes has been mentioned. Here, the problem of xenotransplantation of pig tissues is addressed, since these animals express, apart from the immogenic epitope α-galactose, relatively large amounts of N-glycolylneuraminic acid (Neu5Gc).[85,89] The antigenicity of this Sia species for man may create a further problem in the transplantation of tissues from pig.

Polysialic acids represent a further large and fast developing area of sialobiology, which has not been discussed in this review.[90] Their great impact in the regulation of cell adhesion and growth, especially in developmental, neurological and tumour biology.[91,92] and also in insect larvae.[93,94] render research in this area rewarding.

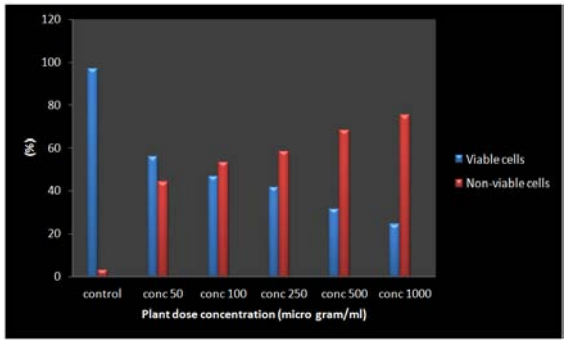

Figure 2: Cytotoxic effect of AECF on EAC cells (trypan blue method)

- Non-Viable cells= Stained with trypan blue dye.
- Viable cells= Not stained with trypan blue dye.

Fig: 2. showed that the aqueous extract of *Carum copticum* B. fruit pods showed pronounced activity against the EAC cell line. Several bacterial pore-forming toxins can cause host cell damage by perforating the host cell membranes.[95-97] It is conceivable that the pores formed by the toxin may allow trypan blue to enter the host cell, which would consequently be considered to be dead. Indeed, the trypan blue is a relatively small molecule (960 Da) and may therefore be able to penetrate into cells through pores formed by toxins. However, the issue of whether pore-forming toxins may cause membrane leakage without inducing immediate cell death has not been extensively considered. In this case, trypan blue staining would reveal pore formation in the host cell membrane but not necessarily host cell death.

The effect of HlyII on host cell membrane integrity, murine J774 macrophages were incubated with various doses of purified GST-tagged HlyII (0–0.5 μg/mL).[98] After 2 h of incubation, trypan blue dye was added to the preparation and cells were visualized under the microscope. The percentage of total cells that was blue was calculated by counting, for each condition, 100 cells from three independent experiments. HlyII at a

concentration of 0.2 µg/mL resulted in 50% of the cells to be stained blue, and at 0.5 µg/mL nearly 100% of the cells were stained blue. HlyII has been described as a pore-forming toxin.[99-101] The previous study showed that HlyII induces macrophage apoptosis after 24 h incubation[98] and this seemed in apparent contradiction with a trypan blue staining as indicator of cell death after 2 h of incubation. Indeed, if the trypan blue dye staining reflects cell death as usually described, the cells should be already dead after 2 h of incubation with the toxin, and therefore be unable to undergo apoptosis subsequently. Therefore, we hypothesized that the trypan blue dye, which is a small molecule, may enter the cells through the pores formed by HlyII. To investigate this issue, we used various techniques to test whether the trypan blue staining detected within 2 h of incubation with HlyII was a consequence of pore formation rather than immediate cell necrosis.

The results revealed that the aqueous extract of *C.copticum* B. fruit pods showed pronounced activity against the EAC cell line. Thus the results revealed that the aqueous extract of *C.copticum* B. fruit pods has significant anti-tumour activity against EAC cell line. *In-vitro* cytotoxic assay (Trypan blue) also used for this study. These parameters were assessed after treatment with AECF in the dose of (150mg/kg b.w. for 14 days) and compared them with EAC control group. Anti-tumour activity of AECF due to the phytochemicals such as Flavonoids, Coumarins, quinones, Alkaloids and Glycosides were present.

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