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Antitumor Activity and Antioxidant Role of *Brassica oleracea Italica* against ehrlich ascites Carcinoma In Swiss Albino Mice

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ABSTRACT

To study the antitumor effect and antioxidant role of *Brassica Oleracea Italica*. Antitumor activity and antioxidant status of ethanolic extracts of 200,300,400 mg/kg of *Brassica Oleracea Italica* flower was evaluated against Ehrlich ascites carcinoma (EAC) tumor in mice. Acute and short-term toxicity studies were performed initially in order to ascertain the safety of ethanolic extracts of *Brassica Oleracea Italica*. After 24 h of tumor inoculation, the extract was administered daily for 14 d. After administration of the last dose followed by 18 h fasting, mice were then sacrificed for observation of antitumor activity. The effect of ethanolic extracts of *Brassica Oleracea Italica* on the growth of transplantable murine tumor, life span of EAC bearing hosts and simultaneous alterations in the haematological profile and liver biochemical parameters (lipid peroxidation, antioxidant enzymes) were estimated. The ethanolic extracts of *Brassica Oleracea Italica* showed decrease in tumor volume, packed cell volume and viable cell count, and increased the nonviable cell count and mean survival time thereby increasing life span of EAC tumor bearing mice. Haematological profile reverted to more or less normal levels in extracts treated mice. Treatment with ethanolic extracts of *Brassica Oleracea Italica* decreased the levels of lipid peroxidation and increased the levels of glutathione, superoxide dismutase and catalase. The ethanolic extracts of *Brassica Oleracea Italica* flower exhibited antitumor effect by modulating lipid peroxidation and augmenting antioxidant defense system in EAC bearing mice.

Keywords *Brassica Oleracea Italica*; Ehrlich's ascites carcinoma; lipid peroxidation; antioxidants.

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INTRODUCTION

Cancer is a cellular tumour that, unlike benign tumour cells, can metastasize and invade the surrounding and distant tissues [1]. Cancer is the abnormal growth of cells in our bodies that can lead to death. These cells are born due to imbalance in the body and by correcting this imbalance the cancer may be treated. The major causes of cancer are smoking, dietary imbalances, hormones and chronic infections leading to chronic inflammation. The important preventive methods for most of the cancers include dietary changes, stopping the use of tobacco products, treating inflammatory diseases effectively, and taking nutritional supplements that aid immune functions [15]. Broccoli is a cruciferous vegetable, a member of the cabbage family *Brassicaceae* (formerly *Cruciferae*), classified as the Italica Cultivar Group of the species *Brassica oleracea*. It is densely packed with vitamins, namely vitamins A and C and minerals that promote health. It has broad antibiotic properties – including nematocidal, antimicrobial, antiprotozoal and insecticidal activities. The woody stalk (base of the plant) and also the flowering portion of the plant (broccoli) contain antibacterial compounds. It may be helpful in treatment against bacterial infection with *Helicobacter pylori* associated with a marked increase in the risk of gastric cancer. Cruciferous vegetables like broccoli are known for containing isothiocyanates and indoles namely indole-3-carbinol (I3C) are phytochemical that are well-known protectors against the development of cancer suggesting that greater intakes of these vegetables may lower the risk of several types of cancer including Bladder cancer, Prostrate cancer, Breast cancer, Non-Hodgkin's lymphoma. It also contains lutein, another phytochemical with health benefits [22]. Broccoli contains the compound glucoraphanin, leading to an anticancer compound sulforaphane. Broccoli is believed to play a role in protecting against cancer. It is rich in several potential anticancer substances such as indolesglucosinolates, beta-carotene [29]. The ability of antioxidants to scavenge free radicals in the human body and thereby decrease the amount of free radical damage to biological molecules like lipids and DNA may be one of their protective mechanisms [16]. However, clinical trials using 'nutritional' antioxidants in food such as vitamins C and E have given equivocal results [4, 5]. From this viewpoint the present study was carried out to evaluate the antitumor activity, lipid peroxidation and antioxidant status of ethanolic extracts of *Brassica Oleracea Italica* against Ehrlich's ascites carcinoma (EAC) in Swiss albino mice.

MATERIALS AND METHODS

Collection and Preparation of Ethanolic Extract

Flower of *Brassica Oleracea Italica* (Broccoli) were collected from the daily market in Coimbatore. The flowers were washed under running tap water, dried and cut into small pieces. The flowers were shade dried for 30 days. Then homogenized to get a coarse powder.

The dried powder material of the flower of *Brassica Oleracea Italica* was extracted with ethanol (yield 9.25 %), in a soxhlet apparatus. The ethanolic extracts were then distilled,

evaporated, and dried in vacuum in order to remove the solvent completely. This powder was stored in an air tight container and used for further successive extraction.

Chemicals:

All the chemicals were of analytical grade. 1-Chloro-2,4-dinitrobenzene (CDNB), bovine serum albumin (Sigma chemicals), Thiobarbituric acid, Nitrobluetetrazolium chloride (NBT) (Loba Chemie, Bombay India), 5,5'-dithio *bis*-2-nitrobenzoic acid (DTNB) (SICCO Research Laboratory, Bombay). EAC cells were obtained from Amala Cancer Institute, Kerala; India. The EAC cells were maintained by intraperitoneal inoculation of 2×10^6 cells/mouse. Studies were carried out using male Swiss albino mice weighing 18 ± 25 g were obtained from KMCH College of Pharmacy, Coimbatore, Tamil nadu, India.

Antitumor Activity

Experimental Design:

Male Swiss albino mice were divided into 5 groups ($n=12$). All the groups were injected with EAC cells (0.2 ml of 2×10^6 cells/mouse) intraperitoneally except the normal group. This was taken as day zero, from the first day normal saline 5 ml/kg/mouse/day and propylene glycol 5 ml/kg /mouse/day was administered to normal and EAC control groups respectively for 14 days intraperitoneally. Similarly ethanolic extracts *Brassica Oleracea Italica* at 200, 300 mg/kg/day in group 3, 4 and standard drug 5-flurouracil (20 mg/kg/day) were administered in groups 5, respectively, after the administration of last dose followed by 18 hrs. Fasting 6 mice form each group was sacrificed for the study of antitumor activity, haematological and liver biochemical parameters. The remaining animals in each of the groups were kept to check the mean survival lime (MST) and percent increase in life span of the tumor bearing hosts [10,3,21] Various parameters like Body weight of animals, Life span of animals, Cytological studies on cell lines, Haematological parameter, RBC, WBC, Haemoglobin, differential count, Biochemical parameters evaluated in the present study. Anticancer effect of *Brassica Oleracea Italica* was assayed by observation of change with respect of body weight, ascitic tumor volume, packed cell volume, viable and non viable tumor cell count, mean survived time (MST) and percentage increase in life span (%ILS) [14].

Experimental Animals:

Male Swiss albino mice weighing between 18-25 gm were used for present study. They were maintained under standard environmental conditions and were fed with standard pellet diet of water. The mice were acclimatized and laboratory condition for 10 days before commencement of experiment. All procedure described were reviewed and approved by the Institutional Animal Ethical Committee of Kovai Medical Centre Hospital and Research (KMCH) College of Pharmacy, Coimbatore.

Cancer Cell line

EAC cells were obtained from Amala Cancer Research Center, Thrissur, and Kerala, India. They were maintained by weekly intraperitoneal inoculation of 10^6 cells / mouse.

Tumor Transplantation

Ehrlich's Ascites Carcinoma was maintained by serial transplantation from tumor bearing Swiss Albino mice. Ascetic fluid was drawn out from tumor bearing mice at the log phase (day 78 of tumor bearing) of the tumor cells. The tumor cell number was adjusted to 2×10^6 tumor cells/ml. Sample showing more than 90 % viability was used for transplantation. Each animal received 0.2 ml of tumor cell suspension containing 2×10^6 cells / ml intraperitoneally [14].

Tumor Cell Volume and Packed Cell Volume

The mice were dissected to collect ascitic fluid from peritoneal cavity and centrifuged to determine packed cell volume at 1000 rpm for 5 min [25]. The transplantable murine tumor was carefully collected to measure the tumor volume.

Viable and non viable cell count

Viable and non viable cell counting of the ascetic cell was done by staining with trypan blue (0.4 % in normal saline), dye exclusion test and count was determined in a Neubauer counting chamber. The cells that did not take up the dye were viable and those that took the stain were not viable [14].

Mean survival time and percent increased in life span

The effect of *Brassica Oleracea Italica* on tumor growth was observed by MST and % ILS. MST of each group continuing 6 mice were monitored by recording the mortality daily for 6 weeks and % ILS was calculated by using following equation [14,25].

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

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

Methods

Toxicity Study

An acute toxicity study relating to the determination of LD_{50} was performed. A short-term toxicity study was also carried out for a period of 14 d which is period of the study of

antitumor activity. Healthy Swiss albino mice were divided into 5 groups of 12 animals in each group received ethanolic extracts *Brassica Oleracea Italica* at the doses 200, 300 and 400 mg/kg intraperitoneally once daily for 14 d. After 24 h of the last dose including 18 h of fasting the mice were sacrificed. Blood collected and haematological parameters were determined as described in haematological studies. Liver and other important internal organs were removed, weighed, and observed for pathological changes. Serum glutamate pyruvate transaminase (ALT) [8] and Serum glutamate oxaloacetate transaminases [8] were determined using a portion of the blood collected. Urea by enzymatic method [27] was estimated from serum. Further, liver biochemical parameters were estimated by methods described in estimation of biochemical parameters.

Haematological Studies

Haemoglobin content [18], red blood cell (RBC) and white blood cell (WBC) [18], counts were measured from freely flowing tail vein blood. Differential WBC leukocyte count was carried out from Leishaman stained blood smears of normal, EAC control, and *Brassica Oleracea Italica* treated groups respectively.

Estimation of Biochemical Parameters

After the collection of blood samples the mice were sacrificed and their liver were excised, rinsed in ice-cold normal saline followed by cold 0.15 mol/L Tris-HCl buffer (pH 7.4), blotted dry, and weighed. A 10 % w/v homogenate was prepared in 0.15 mol/L Tris-HCl buffer and a portion utilized for the estimation of lipid peroxidation another portion of the same after precipitating proteins with TCA was used for the estimation of glutathione [19]. The remaining homogenate was centrifuged at 1500 rpm for 15 min at 4 °C. The supernatant thus obtained was used for the estimation of superoxide dismutase [2], catalase [24], Lipid peroxidation [6] and protein content [11].

Statistical Analysis

Values were represented as mean \pm SEM. Data were analysed by one-way analysis of variance (ANOVA) followed by Dunnett's test using statistical package for social sciences (SPSS) version 10.0. $P < 0.05$ was considered significant. The toxic control group was compared with the normal control group and all other treatment groups were compared with the toxic control group.

RESULTS

Short Term Toxicity Studies

When the mice were observed for the behavioral changes after intraperitoneally administration of a single dose of the extract, none of the mice exhibited any abnormal

behavioral responses at doses of 200, 300 mg/kg but mice which received 400 mg/kg or above showed slight toxic symptoms. These include inactiveness, loss of appetite, slow movement, dizziness, erection of hairs, and hypothermia. Administration of repeated daily doses of 200, 300, and 400 mg/kg for 14 d did not influence the body weight of the mice. The weights of liver, kidney, brain, and spleen were also not altered by the treatment. Haematological parameters like haemoglobin and RBC count remained unaltered at the doses of 200, 300 mg/kg. But there was a marginal increase in WBC count. The haematological parameters, urea, and transaminase activities increased at the dose of 400 mg/kg.

Effect of Ethanolic Extracts of *Brassica Oleracea Italica* on Mean Survival Time and Tumor Growth

In the EAC control group the mean survival time was 18.0 ± 0.11 days, while it increased to 30.31 ± 0.19 (200 mg/kg) 37.6 ± 0.15 (300 mg/kg) days, respectively in ethanolic extracts of *Brassica Oleracea Italica* -treated groups. The group treated with the standard drug 5-fluoruracil (20 mg/kg) showed 40.6 ± 0.22 days for the same. Treatment with ethanolic extracts of *Brassica Oleracea Italica* at the doses of 200, 300 mg/kg reduced the body weight, tumor volume, packed cell volume, and viable tumor cell count in a dose-dependent manner as compared to that of EAC control group. Further, nonviable tumor cell counts at the different doses of ethanolic extracts of *Brassica Oleracea Italica* were increased when compared with the EAC control (Tab 1).

Effect of Ethanolic extracts *Brassica Oleracea Italica* on Haematological Parameters

Treatment with Ethanolic extracts *Brassica Oleracea Italica* at the doses of 200; 300 mg/kg increased the haemoglobin content and RBC count to more or less normal levels. The total WBC counts and protein were found to be increased in EAC control group when compared with normal group. Administration of Ethanolic extracts of *Brassica Oleracea Italica* at the different doses of 200, 300 mg/kg in EAC bearing mice reduced both WBC count and protein as compared with EAC control. In the differential count of WBC, increase of neutrophils and the lymphocyte count decreased in EAC control group. Treatment with Ethanolic extracts of *Brassica Oleracea Italica* at different doses changed these altered parameters more or less normal (Tab 2).

Effect of Ethanolic Extracts *Brassica Oleracea Italica* on lipid peroxidation and glutathione

Table. 3 showed that the levels of lipid peroxidation in liver tissue were increased by 2.92 ± 0.01 in EAC control group as compared to the normal group ($P < 0.05$). After administration of Ethanolic extracts of *Brassica Oleracea Italica* at doses (200, 300 mg/kg) to EAC bearing mice the level of lipid peroxidation were reduced by 1.85 ± 0.36 , 1.98 ± 0.06 respectively in comparison to EAC control group ($P < 0.05$). Inoculation of EAC drastically decreased the GSH content to 2.32 ± 0.01 in EAC control group when compared with normal group. The administration of Ethanolic extracts of *Brassica Oleracea Italica* at the doses of 200,

300 mg/kg to the EAC bearing mice increased GSH levels by 3.89 ± 0.52 , 3.12 ± 0.29 respectively as compared with EAC control group ($P < 0.05$).

Effect of Ethanolic Extracts of *Brassica Oleracea Italica* on antioxidant enzymes

The effect of Ethanolic extracts of *Brassica Oleracea Italica* on the antioxidant enzymes was given in Tab 3. The levels of superoxide dismutase (SOD) in the liver of EAC bearing mice decreased by 3.82 ± 0.23 ($P < 0.05$) in comparison with normal group. After administration of Ethanolic extracts of *Brassica Oleracea Italica* at the doses of 200, 300 mg/ kg increased levels of SOD by 4.01 ± 0.13 , 4.58 ± 0.35 respectively as compared to that of EAC control group ($P < 0.05$). The catalase (CAT) level in EAC. Control group decreased by 10.8 ± 0.07 compared with normal group. Treatment with Ethanolic extracts *Brassica Oleracea Italica* at the doses of 200, 300 mg/kg increased CAT levels by 9.58 ± 1.76 , 16.82 ± 0.05 , respectively when compared to that of EAC control ($P < 0.05$).

DISCUSSION

The present study was carried out to evaluate the toxicity, antitumor activity, lipid peroxidation and antioxidant status of *Brassica Oleracea Italica* on EAC bearing mice. In short-term toxicity study the *Brassica Oleracea Italica* at the high dose level (400 mg/kg) increased the urea and transaminase activity indicating its hepatorenal dysfunction and metabolism. The *Brassica Oleracea Italica* treated animals at the different doses of 200; 300 mg/kg inhibited the body weight, tumor volume, packed cell volume, tumor cell count and also brought back the haematological parameters to more or less normal levels. The extracts also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as antioxidant enzymes such as SOD and CAT in tumor bearing mice to near normal levels. Short-term toxicity studies indicate that at the different doses of 200, 300 mg/kg for 14 d *Brassica Oleracea Italica* did not exhibit any adverse effect. Reliable criteria for judging the value of any anticancer agents is the prolongation of life span of animals [7]. A decrease in tumor volume and viable tumor cell count as mentioned above finally reduced the tumor burden and enhanced the life span of EAC bearing mice. In cancer chemotherapy the major problem are of myelo suppression and anaemia [17, 13]. The anaemia encountered in tumor bearing mice is mainly due to reduction in RBC or haemoglobin percentage and this may occur either due to iron deficiency or due to haemolytic or myelopathic conditions. Treatment with *Brassica Oleracea Italica* brought back the haemoglobin content, RBC and WBC cell count near to normal values. This indicates that *Brassica Oleracea Italica* posse's protective action on the haematopoietic system. Excessive production of free radicals resulted in oxidative stress, which leads to damage of macromolecules such as lipids can induce lipid peroxidation *in vivo* [28]. Increased lipid peroxidation would cause degeneration of tissues. Lipid peroxide formed in the primary site

Table. 1 Effect of Ethanolic extracts of *Brassica Oleracea Italica* On Body Weight, Mean Survival Time, % ILS, Tumor Volume, Packed Cell Volume, Viable And Non-Viable Tumor Cell Count Of EAC Bearing Mice. n=6.

Parameters	Normal Group Animals Group-I	EAC Control (2x10 ⁶ cells/mouse) Group- II	Brassica Oleracea Italica (200mg/kg) + EAC Group- III	Brassica Oleracea Italica (300mg/kg) + EAC Group- IV	Standard 5- fluorouracil (20mg/kg) + EAC Group-V
Body weight/g	28.1 ± 0.14	27.02 ± 1.92	21.16±0.25	20.4 ± 0.17a*	19.87 ± 0.16b*c ^{ns}
Mean survival time/d	18.32 ± 0.17	18.0 ± 0.11	30.31±0.19	37.6 ± 0.15a*	40.6 ± 0.22b*c*
Increase life Span %	-	-	65.15±0.33	99.73 ± 0.68	116.53 ± 0.72
Tumor volume / ml	-	4.92 ± 0.09	0.62±0.48	0.82 ± 0.11a*	-
Packed cell volume/ ml	-	2.52 ± 1.0	0.26±0.15	0.19 ± 0.15b*	-
Viable tumor cell Count/10 ⁶ cells L ⁻⁴	-	13.5 ± 0.37	2.13±0.11	2.82 ± 0.11a*	4.02 ± 0.66 b*c ^{ns}
Non viable tumor cell count / 10 ⁶ cells L ⁻⁴	-	0.76 ± 0.11	0.48±0.06	0.4 ± 0.51a*	-

Values are mean ± SD of Six samples

Group comparison : a- Group II vs. Group IV; b- Group II vs. Group V; c – Group IV vs. Group V

Statistical significance: *- significant (p<0.05) ns – not significant

Table. 2 Effect of Ethanolic Extracts of *Brassica Oleracea Italica* on Haematological Parameters Of EAC Bearing Mice. n=6.

Parameters	Normal Group Animals Group-I	EAC Control (2x10 ⁶ cells/mouse) Group-II	Brassica Oleracea Italica (200mg/kg) + EAC Group-III	Brassica Oleracea Italica (300mg/kg) + EAC Group-IV	Standard 5- fluorouracil (20mg/kg) + EAC Group-V
Haemoglobin /g %	16.02 ± 0.92a*	10.01 ± 1.12b*	12.3±0.52	13.06 ± 0.73c*	14.02 ± 1.37
RBC/ 10 ¹⁵ -L ⁻⁴	8.62 ± 0.03a*	3.52 ± 0.09 b*	4.32±0.86	5.92 ± 0.03c*	8.67 ± 0.03
WBC/ 10 ¹² -L ⁻⁴	5.57 ± 0.03a*	19.03 ± 0.92b*	7.2±0.11	5.02 ± 0.06c ^{ns}	4.92 ± 0.09
Monocyte / %	1.82 ± 0.01a*	1.0 ± 0.02ab	1.81±0.03	1.82 ± 0.01c ^{ns}	1.86 ± 0.01
Neutrophil / %	18.81 ± 0.15a*	72.48 ± 0.17b*	98.02±0.15	19.12 ± 0.12c ^{ns}	20.14 ± 0.14
Lymphocyte / %	89.42 ± 0.23a*	50.05 ± 0.42 b*	67.11±0.68	79.29 ± 0.46c*	84.01 ± 0.26
Eosinophil / %	0.66 ± 0.019a*	1.89 ± 0.042b*	0.79±0.36	0.79 ± 0.038c*	0.89 ± 0.052

Values are mean ± SD of six samples

Group comparison : a - Group I vs. Group II; b – Group II vs. Group V; c – Group IV vs. Group V

Statistical significance: *- significant (p<0.05) ns – not significant

Table 3. Effect of Ethanolic Extracts of *Brassica Oleracea Italica* on lipid peroxidation glutathione content and antioxidant enzymes in the liver of EAC bearing mice. n=6.

Parameters	Normal Group Animals Group-I	EAC Control (2x10 ⁶ cells/mouse) Group-II	Brassica Oleracea Italica (200mg/kg) + EAC Group-III	Brassica Oleracea Italica (300mg/kg) + EAC GroupIV	Standard 5- fluorouracil (20mg/kg) + EAC Group-V
Lipid peroxidation / nmol MDA mg ⁻¹ (protein)	1.96 ± 0.02	2.92 ± 0.01a*	1.83±0.36	1.98 ± 0.06b*c ^{ns}	1.96 ± 0.09b*c ^{ns}
Glutathione content / mg ⁻¹ (wet tissue)	3.42 ± 0.09	2.32 ± 0.01a*	3.89±0.52	3.12 ± 0.29b*c ^{ns}	3.32 ±0.32b*c ^{ns}
Superoxide dismutase / U-mg ⁻¹ (protein)	5.32 ±0.37	3.82 ± 0.23a*	4.01±0.13	4.58 ± 0.35b*c*	4.97 ± 0.29b*c ^{ns}
Catalase / U-mg ⁻¹ (protein)	28.46 ± 0.09	10.8 ± 0.07a*	9.58±1.76	16.82 ± 0.05b*c*	18.19 ± 0.07b*c*

Values are mean ± SD of Six samples

Group comparison : a - Group I vs Group II; b- Group II vs Group IV & V; c- Group I vs Group IV & V

Statistical significance: *- significant (p<0.05) ns – not significant

would be transferred through the circulation and provoke damage by propagating the process of lipid peroxidation [23], the end product of lipid peroxidation was reported to be higher in carcinomatous tissue than in non diseased organ [28]. Glutathione, a potent inhibitor of neoplastic process plays an important role as an endogenous antioxidant system that is found particularly in high concentration in liver and is known to have key function in the protective process [23]. *Brassica Oleracea Italica* reduced the elevated levels of lipid peroxidation and increased the glutathione content in EAC bearing mice. On the other hand the free radical scavenging system, SOD and catalase are present in all oxygen metabolizing cells and their function is to provide a defense against the potentially damaging reactivity's of superoxide and hydrogen peroxide [26], reported a decrease in SOD activity in EAC bearing mice which might be due to loss of Mn SOD activity in EAC cells and the loss of mitochondria, leading to a decrease in total SOD activity in the liver. The inhibition of SOD and CAT activities as a result of tumor growth was also reported [12]. Similar findings were observed in the present investigation with EAC bearing mice. The administration of *Brassica Oleracea Italica* at different doses increased the SOD and CAT levels in a dose dependent manner, which may indicate the antioxidant and free radical scavenging property of plant derived extracts containing antioxidant principles showed cytotoxicity towards tumor cells [9] and antitumor activity in experimental animals [20]. The lowering of lipid peroxidation and increase in levels of GSH, SOD and catalase in *Brassica Oleracea Italica* -treated group indicates its potential as an inhibitor of EAC induced intracellular oxidative stress. We propose that the additive and synergistic antioxidant activity of phytochemical such as flavonoids, phenols, alkaloids, triterpenoids, steroids, etc, present in *Brassica Oleracea Italica* are responsible for the its potent antitumor activity which can be inferred from the increased the life span of EAC tumor bearing mice.

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