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In-vivo Anticancer Activity of Ethanol Extract of *Brassica Oleracea var. Italica* (Broccoli) Against Dalton's Lymphoma Ascites Induced Cancer Using Female Swiss Albino Mice

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Abstract

The study aimed at evaluating the effect of Ethanol Extract of *Brassica Oleracea var. Italica* (EEBO) against Dalton's Ascitic Lymphoma (DAL) in Swiss Albino mice. The mice were injected with DAL cells (106 cells/mouse) intraperitoneally, followed by treatment with EEBO for 14 days at a dosage of 200mg and 500mg/kg. A significant decrease in the cancer cell number, tumour weight, tumour volume and increased life span of mice were observed. These results suggested the protective effect of EEBO against DAL.

Keywords: Anticancer Activity, *Brassica Oleracea var. Italica,* Dalton's Ascitic Lymphoma Swiss Albino Mice

1. Introduction

Cancer is the second leading cause of death in the world after cardiovascular diseases. Cancer may be regarded as a group of diseases characterized by an (i) abnormal growth of cells (ii) ability to invade adjacent tissues and even distant organs, and (iii) the eventual death of the affected patient if the tumor has progressed beyond that stage when it can be successfully removed[1].

Cancer is the uncontrolled growth and spread of cells. It can affect almost any part of the body. The growths often invade surrounding tissue and can metastasize to distant sites. Many cancers can be prevented by avoiding exposure to common risk factors, such as tobacco, smoke. In addition, a significant proportion of cancers can be cured by surgery, radiotherapy or chemotherapy, especially if they are detected early[2].

According to American Cancer Society (2017), some cancers cause fluid to build up in the abdomen. This can make belly swollen and feel uncomfortable. The fluid can also push on your lungs and make it hard to breathe[3].

NCI Dictionary of Cancer Terms defined ascites is an Abnormal build up of fluid in the abdomen that may cause swelling. In late-stage cancer, tumor cells may be found in the fluid in the abdomen. Ascites also occurs in patients with liver disease[4].

As broccoli (*Brassica oleracea var. italica*) has high content of bioactive phytochemicals such as glucosinolates, phenolic compounds, vitamin C, and mineral nutrients it is marketed as a health-promoting

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food. A diet rich in broccoli helps in the preventing chronic diseases, such as cardiovascular and carcinogenic pathologies, and breast and prostate cancers. Further broccoli possesses antioxidant activity which prevents oxidative stress related to many diseases (Hwang et *al.*, 2015). The vegetable has antioxidant, antimicrobial, anti-inflammatory and anticancer activities. It actively kills cancer stem cells and slows tumor growth. The broccoli extract also shows the bioactive and Phytochemical compounds which possess many nutraceutical benefits[5].

Thus, the main objective of the study is to determine the nutrient content of Brassica *oleracea var. italica* (Broccoli) and to study the anti-cancer activity of ethanol extract of *Brassica oleracea var. italica* in experimental Swiss albino mice.

2. Methods and Materials

2.1 Selection of Sample

Fresh flowers of *Brassica oleracea var. italica* (Broccoli) were collected in the month of October to November from Preethi Agriculture farm, Ooty of Tamil Nadu. The flowers of broccoli were dried, powdered and stored in airtight container for the study.

2.2 Processing and Preparation of Broccoli Extract

The collected fresh flowers were cleaned by washing in running tap water for two to three times until they are rendered free from dirt and dust particles. The cleaned fresh flowers were shade dried for six to seven days. Then the dried flowers were made into powder by using electric mixer. The air-dried powdered material was extracted successively by using ethanol. The extract of *Brassica oleracea var. italica* was concentrated, evaporated to dryness until solid or semisolid mass was obtained.

2.3 Phytochemical Analysis

Preliminary Phytochemical analysis was carried out for the extract as per standard procedures.

2.4 Selection of Rats

Swiss albino mice weighing 25 to 50 gm were obtained from the Mass Biotech animal house, Chengalpet, Chennai, Tamil Nadu. The animals are caged and maintained under standard laboratory conditions of temperature (22-24^0 C), humidity (30-60%) in Erode College of Pharmacy. The experimental protocol was approved by the Institutional Animals Ethical Committee. Certificate of Animal Ethical Committee Ref. No. 2025/GO/Re/S/18/CPCSEA Dated 3rd August 2018 and Proposal No. ECP/IAEC/003/2019-20.

2.5 Induction of Cancer

DAL cells were originally obtained through the courtesy of Amala Cancer Research Center, Thrissur, Kerala, India. They were maintained by weekly intraperitoneal inoculation of 10^6cells/mouse. After inoculation of DAL cells in the animals they were maintained under standard environmental conditions and fed with standard food pellets and water.

2.6 Experimental Design for Animal Study and Supplementation

5-6 Weeks of healthy young Swiss albino mice weighing about 25-50 gm, were selected and divided into 4 groups, each consisting of six animals as Normal Control (G1), Cancer Control (G2), Test Control 1 (G3), Test Control 2 (G4).

2.7 Physical examination of Rats

The rats in each group were weighed daily for 14 days and their weights were recorded.

2.8 Biochemical Examination of the Rats

2.8.1 Hematological Parameters of Selected Experimental Animals

Erythrocyte (RBC), Haemoglobin (Hb) and Leukocyte (WBC) were measured in animal house of Erode College of Pharmacy, Erode.

2.8.2 DAL Viable and Non-Viable Cell Count in Experimental Animals

The Trypan Blue dye exclusion test is used to determine the number of viable cells present in a cell suspension.

DAL viable and non-viable cell count were assessed in all experimental groups on the 14th day of the study.

2.8.3 Tumor Weight of DAL Bearing Mice

In this study, Tumor weight was assessed in all DAL bearing mice in G2, G3 and G4 on the 14th day of the study.

2.8.4 Volume of DAL Bearing Mice

Tumor volume: The size of a cancer can be measured by the amount of space taken up by the tumor. Tumor volume was assessed in all DAL bearing mice in G2, G3 and G4 on the 14th day of the study.

2.8.5 Survival Time and Life Span of Dal Bearing Mice

The survival was monitored and assessed in all DAL bearing mice in G2, G3 and G4 by recording median survival time (MST) and percentage increase in life span (%ILS).

3. Results and Discussion

3.1 Phytochemical Content of *Brassica Oleracea*

Florets and stem of *Brassica oleracea* contain phenols, phenolic acids, polyphenols, sophoroside-glucosides, flavonoids, alkaloids, steroids, tannins, saponins, glutathione, glucosinolates (glucoraphanin, glucobrassicin, neoglucobrassicin), terpenoids, coumarins, xanthoproteins, glycosides, carotenoids (zeaxanthin, lutein, β-carotene), tocopherols, phytosterols, chlorophyll, free sugars and vitamin C.

3.2 Weight Changes in Experimental Animals

From Table 1 it is evident that body weight gradually increased during the study period. The normal control (G1) animals showed maximum increase in body weight compared to the other experimental groups. Due to regular intake of ethanol extract of *Brassica*

Table 1. Body weight of experimental animals

Group	Treatment	Initial body weight (g)	Final body weight (g)	Percentage increase in body weight (%)
Group I	Normal control 0.5% CMC (1 ml/kg)	23.88±0.38	26.68±1.54	10.49%
Group II	DAL control 0.5% CMC (1 ml/kg)	24.05±0.62	35.30±2.60^{***a}	46.77%
Group III	DAL + Extract 200 mg/kg	23.68±0.47	30.23±2.76^{**b}	27.66%
Group IV	DAL + Extract 400 mg/kg	23.46±0.50	29.86±1.44^{**b}	27.28%

(All values are expressed as mean ± S.E.M, n=6 in each group. One-way ANOVA followed by Dunnett's test was used to compare experimental groups.

[a]Values are significantly different from control group; ns-non significant;

[b]Values are significantly different from DAL control group; ns-non significant; *P < 0.05; **P < 0.01; ***P <0.001)

Table 2. Haemoglobin content in selected experimental animals

Group	Treatment	Haemoglobin (g/dl)
Group I	Normal control 0.5% CMC (1 ml/kg)	16.80±0.08
Group II	DAL control 0.5% CMC (1 ml/kg)	9.83±0.54^{***a}
Group III	DAL + Extract 200 mg/kg	11.90±0.08^{*b}
Group IV	DAL + Extract 400 mg/kg	13.87±0.71^{***b}

(All values are expressed as mean ± S.E.M, n=6 in each group. One-way ANOVA followed by Dunnett's test was used to compare experimental groups.

^{a}Values are significantly different from control group; ns-non significant;

^{b}Values are significantly different from DAL control group; ns-non significant; *P < 0.05; **P < 0.01; ***P < 0.001)

Table 3. Erythrocytes (red blood cells) level in selected experimental animals

Group	Treatment	RBC (million cells/ cu.mm)
Group I	Normal control 0.5% CMC (1 ml/kg)	33.12±0.58
Group II	DAL control 0.5% CMC (1 ml/kg)	3.68±0.54^{***a}
Group III	DAL + Extract 200 mg/kg	13.77±1.53***^{b}
Group IV	DAL + Extract 400 mg/kg	27.77±2.16^{***b}

(All values are expressed as mean ± S.E.M, n=6 in each group. One-way ANOVA followed by Dunnett's test was used to compare experimental groups.

^{a}Values are significantly different from control group; ns-non significant;

^{b}Values are significantly different from DAL control group; ns-non significant; *P < 0.05; **P < 0.01; ***P < 0.001)

oleracea var. Italica in G3 and G4 showed normal increase in body weight. Further it was noted that the increase in body weight was more in G2 animals than G3 and G4.

3.3 Hematological Parameters of Selected Experimental Animals

From Table 2 it is evident that G1 showed normal level in haemoglobin and the cancer control group (G2) showed low in haemoglobin than test control groups (G3 and G4).

From the above Table 3 it is evident that G1 showed slightly higher level in red blood cells and the cancer control group (G2) showed very low in red blood cells than other experimental groups. Due to regular intake of ethanol extract of *Brassica oleracea* var. Italica in G3 and G4 showed normal increase in red blood cells. High

Table 4. Leukocytes (white blood cells) level in selected experimental animals

Group	Treatment	WBC (cells/cu.mm)
Group I	Normal control 0.5% CMC (1 ml/kg)	4687±55.06
Group II	DAL control 0.5% CMC (1 ml/kg)	11885±319.4^{*a}
Group III	DAL + Extract 200 mg/kg	21838±3128^{**b}
Group IV	DAL + Extract 400 mg/kg	20167±2464^{*b}

(All values are expressed as mean ± S.E.M, n=6 in each group. One-way ANOVA followed by Dunnett's test was used to compare experimental groups.

^{a}Values are significantly different from control group; ns-non significant;

^{b}Values are significantly different from DAL control group; ns-non significant; *P < 0.05; **P < 0.01; ***P < 0.001)

Table 5. DAL viable cell count in experimental animals

Group	Treatment	Viable cells (x10^7 cells)
Group I	Normal control 0.5% CMC (1 ml/kg)	-
Group II	DAL control 0.5% CMC (1 ml/kg)	64.45±0. 95
Group III	DAL + Extract 200 mg/kg	46.90±2.03***
Group IV	DAL + Extract 400 mg/kg	44.78±2. 73***

(All the values are expressed as mean ± S.E.M, n=6 in each group. One-way ANOVA followed by Dunnett's test was used to compare experimental groups.

^{a}Values are significantly different from control group; ns-non significant;

^{b}Values are significantly different from DAL control group; ns-non significant; *P < 0.05; **P < 0.01; ***P < 0.001)

dosage of broccoli extract showed high results in red blood cell count.

From Table 4 it is evident that G1 showed normal level in white blood cells and the cancer control group (G2) showed slightly higher in white blood cells than normal control group. Due to regular intake of ethanol extract of *Brassica oleracea var. Italica* in G3 and G4 showed highly increased in white blood cells. High dosage of broccoli extract showed high results in white blood cell count and it is significantly different from DAL control group.

From Table 5 it is evident that G1 showed high level of live cancer cells and the test control group (G2) showed low amount of live cancer cells than cancer control group. Due to regular intake of ethanol extract of *Brassica oleracea var. Italica* in G3 and G4 showed slow progression of cancer cells than cancer control group. High dosage of broccoli extract showed low level of cancer

Table 6. DAL viable non-viable cell count in experimental animals

Group	Treatment	Non-Viable cells ($x10^6$ cells)
Group I	Normal control 0.5% CMC (1 ml/kg)	-
Group II	DAL control 0.5% CMC (1 ml/kg)	4.45±1.53
Group III	DAL + Extract 200 mg/kg	13.83±1.72**
Group IV	DAL + Extract 400 mg/kg	14.83±2.28**

(All the values are expressed as mean ± S.E.M, n=6 in each group. One-way ANOVA followed by Dunnett's test was used to compare experimental groups.
[a]Values are significantly different from control group; ns-non significant;
[b]Values are significantly different from DAL control group; ns-non significant; *P < 0.05; **P < 0.01; ***P < 0.001)

Table 7. Tumour weight of DAL bearing mice

Group	Treatment	Tumor weight (g)
Group I	Normal control 0.5% CMC (1 ml/kg)	-
Group II	DAL control 0.5% CMC (1 ml/kg)	15.99±0.16
Group III	DAL + Extract 200 mg/kg	11.61±0.81**
Group IV	DAL + Extract 400 mg/kg	9.09±1.81**

(All the values are expressed as mean ± S.E.M, n=6 in each group. One-way ANOVA followed by Dunnett's test was used to compare experimental groups.
[a]Values are significantly different from control group; ns-non significant;
[b]Values are significantly different from DAL control group; ns-non significant; *P < 0.05; **P < 0.01; ***P < 0.001)

cell proliferation and it is significantly different from DAL control group.

From Table 6 it is evident that G1 showed very low amount of dead cancer cells and the test control group(G2) showed high level dead cancer cells than cancer control group. Due to regular intake of ethanol extract of *Brassica oleracea* var. Italica in G3 and G4 showed greater impact in cancer cells. High dosage of broccoli extract showed high amount of cancer dead cells and it is significantly different from DAL control group.

From Table 7 it is evident that G2 showed high level of tumour weight and the test control group (G3) showed lower weight in tumour than cancer control group. G4 showed low level of tumour weight comparing than G3 and G2. Due to regular intake of ethanol extract of *Brassica oleracea var.* Italica in G3 and G4 showed low

Table 8. Tumour volume of DAL bearing mice

Group	Treatment	Tumor volume (ml)
Group I	Normal control 0.5% CMC (1 ml/kg)	-
Group II	DAL control 0.5% CMC (1 ml/kg)	16.68±0.17
Group III	DAL + Extract 200 mg/kg	12.90±0.99*
Group IV	DAL + Extract 400 mg/kg	9.70±1.08***

(All values are expressed as mean ± S.E.M, n=6 in each group. One-way ANOVA followed by Dunnett's test was used to compare experimental groups.
[a]Values are significantly different from control group; ns-non significant;
[b]Values are significantly different from DAL control group; ns-non significant;
*P < 0.05; **P < 0.01; ***P < 0.001)

Table 9. Survival time and life span of dal bearing mice

Group	Treatment	Mean survival time (days)	Percentage increase in life span (%)
Group I	Normal control 0.5% CMC (1 ml/kg)	-	-
Group II	DAL control 0.5% CMC (1 ml/kg)	19.5	-
Group III	DAL + Extract 200 mg/kg	23	17.94 %
Group IV	DAL + Extract 400 mg/kg	23.5	20.51%

Mean survival time (MST) = First death + last death/2
Percentage Increase in life span = (T – C)/C×100

amount of tumour weight. High dosage of broccoli extract showed high results in tumour weight and it is significantly different from DAL control group.

From Table 8 it is evident that G2 showed high amount of tumour volume and the test control group (G3) showed slightly lower than cancer control group. G4 showed very low amount of tumour volume comparing than G3 and G2. Due to regular intake of ethanol extract of *Brassica oleracea* var. Italica in G3 and G4 showed low amount of tumour volume. High dosage of broccoli extract showed high results in tumour volume and it is significantly different from DAL control group.

From Table 9 it is evident that G2 showed low survival period and the test control group (G3) showed slightly increased in life span than cancer control group. G4 showed highly increased in survival period comparing than G3 and G2. Due to regular intake of ethanol extract of *Brassica oleracea* var. Italica in G3 and G4 showed increase in their lifespan. High dosage of broccoli extract showed high results in increasing life span than cancer

control group and it is significantly different from DAL control group.

4. Conclusion

From the results of the current study, it is seen that High dose (400 mg/kg of Body Weight) of ethanol extract of *Brassica oleracea* var. Italica showed good results on Body weight and the extract had immediate and high impact in Hematological parameters (Hemoglobin level, RBC and WBC Count) and better impact on Viable and Non-viable count of cells, Tumour weight, Tumour volume, Survival time and Life Span of the animals when compared with Normal control and Cancer Control group animals. Thus, it is proven that adequate dosage of ethanol extract of *Brassica oleracea* var. Italica shows the anticancer activity on Dalton′s ascites lymphoma induced cancer in Swiss albino mice.

5. References

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