

ANTICANCER STUDY OF HEMATOLOGICAL AND SURVIVAL PERIOD IN BRASSICA RAPA CHINENSIS LINN. USING IN VIVO MODEL IN MICE

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ABSTRACT

Aim: Dalton's Lymphoma Ascites (DLA) cells are a cancer of the lymphocytes, a type of cell that forms part of the immune system. The present study aims to evaluate the anticancer study of hematological and survival period in brassica rapa chinensis linn. using in vivo model in mice. **Methods:** Hematological study- The experimental mice were divided to 5 groups. The methanolic extract (800 mg/kg) and nanoparticles of methanolic extract (1mg/kg) of *Brassica rapa* Chinensis leaves, was administered to mice of group III and IV respectively. The mice were induced with DLA. Group I mice served as normal control and group II as DLA control. Cyclophosphamide at 3mg/kg b.wt was administered to group V. Survival period- The animals were divided into eight groups, containing six animals in each group. The survival period and change in body weight of these animals, during and after the development were monitored up to 58 days. **Results:** Treatment with methanolic extract and nanoparticles of methanolic extract of *Brassica rapa* Chinensis leaves (800, 1mg/kg, orally) against Dalton's Lymphoma Ascites (DLA) in mice by the activities of hematological parameters as Hemoglobin (Hb), White blood cell count (WBC), Red blood cell count (RBC) & PCV levels in blood and body weight & life span. The extract also enhanced in a dose dependent manner with 3mg dose revealing more defending effect in line with the standard drug, Cyclophosphamide. **Conclusion:** The observed results indicate that the *Brassica rapa* Chinensis leaves extract at both the doses were effective in curbing the toxic insult of DLA.

KEY WORDS - Dalton's Lymphoma Ascites, *Brassica rapa* Chinensis, hematology, survival period.

INTRODUCTION

Cancer is a group of diseases where cell growth is aggressive, abnormal, invasive and metastatic many times leading to [1]. The "war on cancer" is now in its fourth decade since the National Cancer Act was passed in 1971. Cancer cells usually invade and destroy normal cells. These cells are born due to imbalance between the free radicals and antioxidants in the body and by correcting this imbalance, the cancer may be treated [2].

Lymphoma is a type of cancer that begins in immune system cells called lymphocytes. Like other cancers, lymphoma occurs when lymphocytes are in a state of uncontrolled cell growth and multiplication. Lymphoma occur when lymphocyte B or T cells transform and begin growing and multiplying uncontrollably [3].

Free radicals and oxidants play a dual role as both toxic and beneficial compounds to the body. They are produced either from normal cell metabolisms in situ or from external sources like pollution, cigarette smoke, radiation and medication [4]. The accumulation of free radicals in the body generates a phenomenon called oxidative stress. This process plays a major part in the development of chronic and degenerative illnesses such as cancer, autoimmune disorders, ageing, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases [5].

The pharmacological industries has produced considerable number of commercial antibiotics time to time but resistance in pathogens towards these drugs too has increased at high rate and multi drug resistant microorganisms have exacerbated the situation [6]. Natural products, especially those from plants, have been a valuable source of new cancer drugs for many decades. Medicinal plants are the most exclusive source of life saving drugs for the majority of the world's population [7].

In the present scenario, there is an urgent and continuous need of exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines. Traditional medicine is an important source of potentially useful new compounds for the development of chemotherapeutic agents [8].

The phytonutrients found in bok choy are powerful antioxidants that are capable of strengthening the immune system. Intake of this vegetable could reduce the risk of osteoporosis [9].

The present study was undertaken to evaluate the anticancer study of hematological and survival period in *Brassica rapa chinensis* linn. using in vivo model in mice.

MATERIALS AND METHODS

Plant sample collection

Bok choy, *Brassica rapa* Chinensis was obtained from local department store, Coimbatore, Tamilnadu, India. The plant sample, *Brassica rapa* Chinensis was authenticated by Dr. V.S. Ramachandran (Taxonomist), Professor, Bharathiar University, Coimbatore, Tamilnadu, India.

Plant extract preparation

The Fresh leaves were cleaned to remove adhering dust particles, washed under running tap water, and rinsed with distilled water. The leaves were shade dried and powdered. 15g of dried powder was extracted in 150ml of methanol for 6 hours using a series of soxhlet extractor. The extract was filtered through Whatmann No.1 filter paper. The filtered sample was concentrated and dried under room temperature, which is denoted as methanolic extract of *Brassica rapa* Chinensis. The extract yielded a green residue solid weighing 2.5g and was preserved in a refrigerator at 4°C until further experiments.

Silver nanoparticles preparation

Fresh leaves were collected and cleaned to remove adhering dust particles, washed under running tap water, gently blotted dry between folds of tissue paper and 10g of the leaf sample was weighed, cut into small pieces and added to 100ml of methanol. The flask was stored in the dark with mild shaking for 24 hours. The mixture was then filtered through whatman no.1 filter paper. The extract was stored at 4°C until further experiments. The leaf extract (10ml) was added to 90ml of 3mM silver nitrate (AgNO_3) Solution. The experimented for the synthesis of the nanoparticles as elaborated below. The extent of nanoparticle synthesis was monitored by measuring the absorption at 400-600nm. To separate the synthesized silver nanoparticles, samples were centrifuged at 13,000 rpm for 20 minutes under refrigeration and washed three times with deionized water. A dried powder of the silver nanoparticles was obtained by freeze-drying.

Drugs and Chemicals

Dalton ascites lymphoma cells (DLA) were obtained from KMCH, Coimbatore, India for the induction of DLA in mice and Cyclophosphamide was purchased from Alagu Pharmacy, Coimbatore, India. All other chemicals used in this study were obtained commercially and were of analytical grade.

Experimental animal

Female Swiss albino mice with an average weight of 20-25g were obtained from Small Animals Breeding Center of KMCH, Coimbatore. The animals were housed in large spacious cages, maintained at controlled condition of 12 hours light/darkness, humidity and temperature. They were fed with standard pellet diet and water. Lymphoma cancer was induced with (i.p) DLA (1×10^6 cells). The institutional animal ethics committee no. 659/02/a/CPCSEA approved the experimental design.

Experimental design and Biochemical Analysis

The animals were divided into 5 groups of five animals each. In group I as a control, In group II as a DLA control, In group III, animals were treated with methanolic extract of *Brassica rapa* Chinensis leaves (MEBRC), In group IV animals were treated with nanoparticles of methanolic extract of *Brassica rapa* Chinensis leaves (NMBRC) and group V animals were treated with the standard drug of Cyclophosphamide, simultaneously from the next day of the induction of tumor, for 20 days orally, because the tumor bearing animals were found to be started dying from the 20th day onwards which was observed from survival period experiments.

On 21st day, the experimental animals were sacrificed after an overnight fast by decapitation for analysis of biochemical parameters and histological studies. Blood was collected in conventional way and used for the estimation of Hemoglobin (Hb) by the method of Drabkin and Austin, 1932 [10], White blood cell count (WBC) and Red blood cell count (RBC) was determined by Chesbrough and Me Arthur, 1972 [11] and PCV were analysed.

The animals were divided into eight groups, containing six animals in each group. The survival period and change in body weight of these animals were monitored up to 58 days. Mean survival time (MST) was found.

EXPERIMENTAL DESIGN	
Group I	Control
Group II	DLA control
Group III	DLA induced+ treatment with methanolic extract of <i>Brassica rapa</i> Chinensis leaves 800mg/kg body weight from 1 st day induction to till death.
Group IV	DLA induced+ treatment with methanolic extract of <i>Brassica rapa</i> Chinensis leaves 800mg/kg body weight from 11 th day induction to till death.
Group V	DLA induced+ treatment with nanoparticles of methanolic extract of <i>Brassica rapa</i> Chinensis leaves 1mg/kg body weight from 1 st day induction to till death.
Group VI	DLA induced+ treatment with nanoparticles of methanolic extract of <i>Brassica rapa</i> Chinensis leaves 1mg/kg body weight from 11 th day induction to till death.
Group VII	DLA induced+ treatment with drug cyclophosphamide 3mg/kg body weight from 1 st day induction to till death.
Group VIII	DLA induced+ treatment with drug cyclophosphamide 3mg/kg body weight from 11 th day induction to till death.

All these animals were weighed on the day of tumor transplantation and at 5 days intervals throughout the treatment period. Survival period of treated groups (T) were compared with control group (C) and percent increase in life (T/C %) was calculated by the formula.

$$\% \text{ Increase in Life span} = [T/C \times 100] - 100$$

or

$$\% \text{ Increase in Life span} = \left(\frac{\text{MST of treated group}}{\text{MST of DLA control group}} \right) \times 100 - 100$$

Histopathological Examination

The spleen tissue of each animal were dissected out and then fixed in buffered formalin for 12 hours and processed for histopathological examination. Four μm -thick paraffin sections were stained with hematoxylin and eosin for light microscope examination using conventional protocol.

Statistical Analysis

The data are expressed as mean \pm S.D. Statistical comparison was done at significance level, $p < 0.05$ using SPSS package version 10.0. One way ANOVA followed by post hoc analysis of LSD was performed.

RESULTS

It includes the analysis of hematological parameters such as hemoglobin, RBC, WBC and PCV in blood. The methanolic extract of *Brassica rapa* Chinensis leaves on hematological parameters are summarized in the table 1.

Table 1 and Figure 1 represent the effect of methanolic extract of *Brassica rapa* Chinensis leaves on hematological parameters in control and experimental animals. There was a significant decrease ($P < 0.05$) in Hb and RBC levels but significant increase ($P < 0.05$) in the levels of WBC and PCV in lymphoma bearing mice (Group II) when compared to normal control group (Group I). Methanolic extract of *Brassica rapa* Chinensis leaves treated groups (Group III) were able to reverse the changes in the hematological parameter when compared with Group II animals. Nanoparticles of methanolic extract of *Brassica rapa* Chinensis leaves treated mice (Group IV) showed a ($P < 0.05$) significant decreased when compared with Group II animals. Similar trend was observed on treatment with the standard drug cyclophosphamide (Group V) when compared to Group II animals. Nanoparticles of methanolic extract of *Brassica rapa* Chinensis treated mice (Group IV) increased ($P < 0.05$) significantly when compared with Group III animals. Cyclophosphamide treated (Group V) animals also showed a significant decrease ($P < 0.05$) when compared with Group III animals.

Table 1. Effect of methanolic extract of *Brassica rapa* Chinensis leaves on hematological parameters in serum of control and experimental animals

Groups	Haemoglobin (g/dl)	Red Blood Cells Counts (Mill/Cumm)	Total White Blood Cells/ml×10 ³	PCV (%)
Group-I (Control)	13.46±0.37	10.16±0.47	7.08±0.47	15.26±0.20
Group-II (DLA Induced)	8.16±0.49 ^a	5.36±0.41 ^a	15.7±0.85 ^a	17.33±0.37 ^a
Group-III (DLA+MEBRC leaves 800mg/kg b.wt)	11.85±0.78 ^b	7.22±0.38 ^b	10.96±0.54 ^b	16.88±0.17 ^b
Group-IV (DLA+NMBRC leaves of 1mg/kg b.wt)	12.39±0.61 ^{cc}	8.77±0.40 ^{cc}	9.99±0.35 ^{cc}	15.96±0.66 ^{cc}
Group-V (DLA+ Cyclophosphamide 3mg/kg b.wt)	11.00±0.91 ^{df}	5.96±0.63 ^{df}	12.01±0.21 ^{df}	17.26±0.35 ^{df}

Values are expressed as mean ± SD (n=5)

Group comparison significant at 5% (p<0.05)

a) G-II vs G-I b) G-III vs G-II c) G-IV vs G-II d) G-V vs G-II e) G-IV vs G-III f) G-V vs G-III

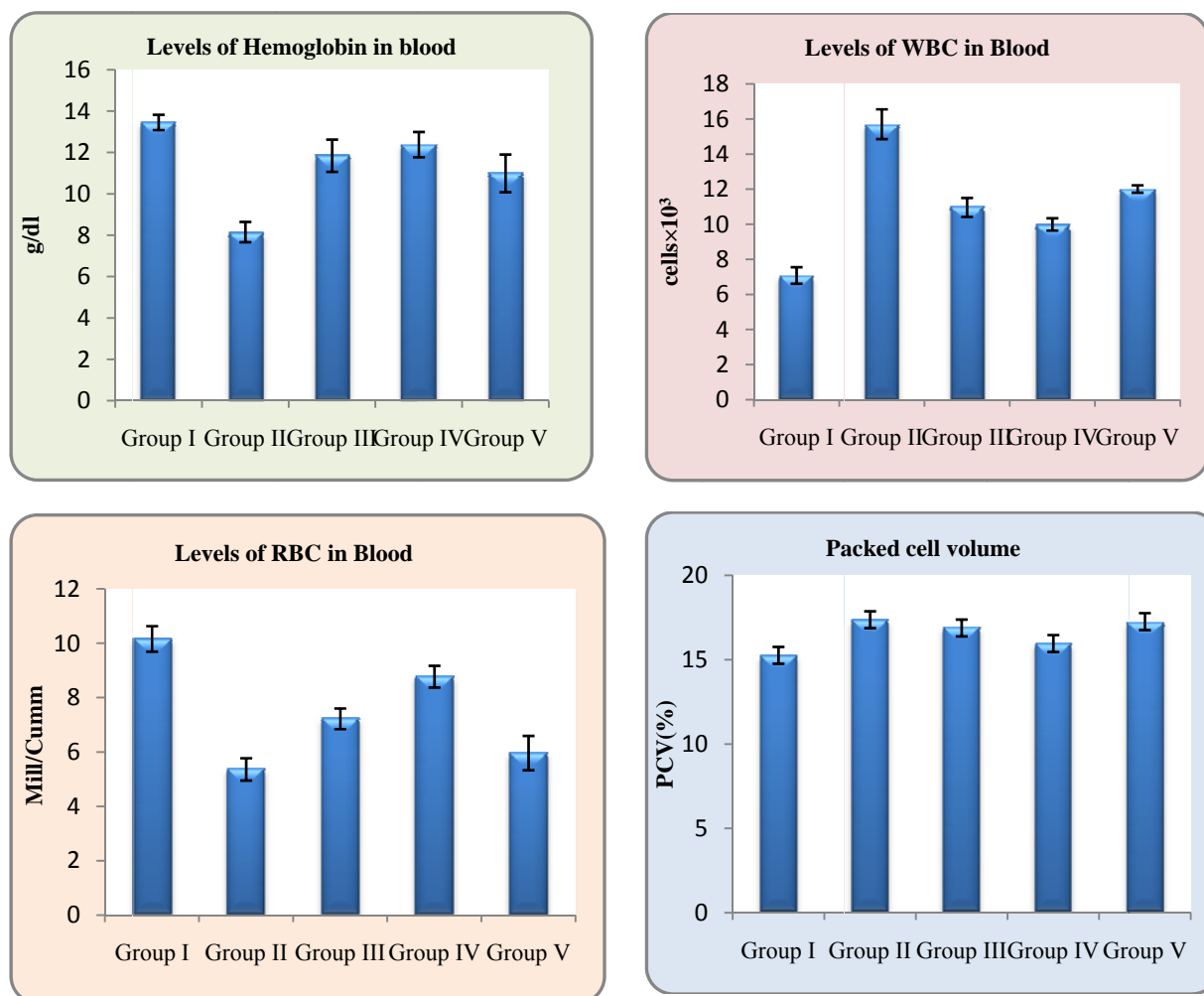


Figure 1. Effect of methanolic extract of *Brassica rapa* Chinensis leaves on Hematological parameters in serum of control and experimental animals

Histological Examination

The results of the histopathological analysis of the lymphoma spleen tissue of experimental animals are presented in fig 2-6. Figure 2 - The spleen Sections shows normal splenic tissue. Figure 3- The DLA tumor bearing mice showed the spleen has the presence of more number of megakaryocytes indicating moderate to severe extra medullary heamatopoiesis. Figure 4- The DLA tumor bearing mice treated with methanolic extract of *Brassica rapa* Chinensis leaves showed the sections show splenic tissue. There is infiltration by atypical cells which are medium sizes to large with scanty to moderate amount of cytoplasm and lobulated nuclei. Few show conspicuous nuclei. Congested blood vessels are seen. Figure 5- The DLA tumor bearing mice treated with nanoparticles of methanolic extract of *Brassica rapa* Chinensis leaves showed almost near to normal histological appearance of hepatocytes. Figure 6- The DLA tumor mice treated with standard drug cyclophosphamide showed the presence of more number of megakaryocytes indicating moderate to serve extra medullary heamatopoiesis.

Histopathological investigation of control and experimental animals- spleen

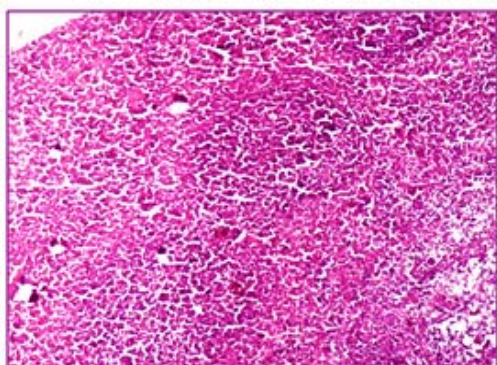


Figure 2: Group I (Control)

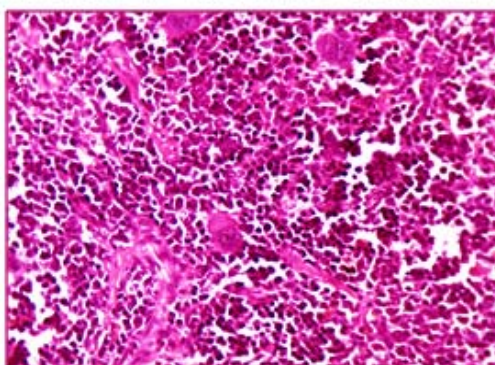


Figure 3: Group II (DLA induced)

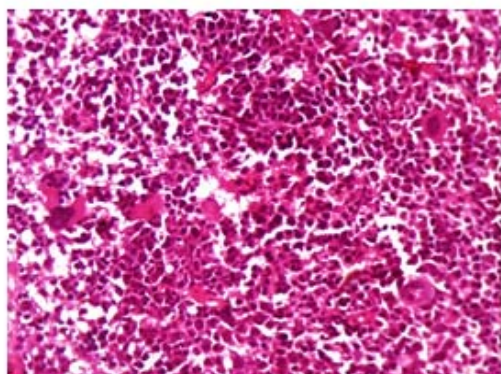


Figure 4: Group III
(DLA+ MEBRC leaves 800mg/kg b.wt)

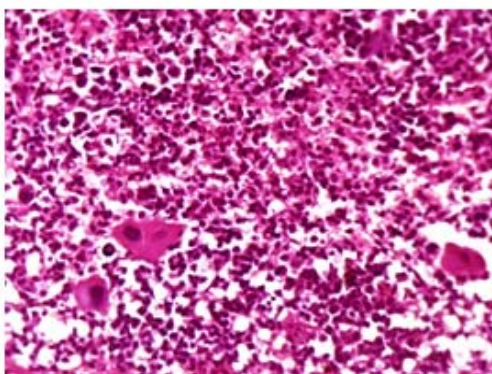


Figure 5: Group IV
(DLA+ NMBRC leaves 1mg/kg b.wt)

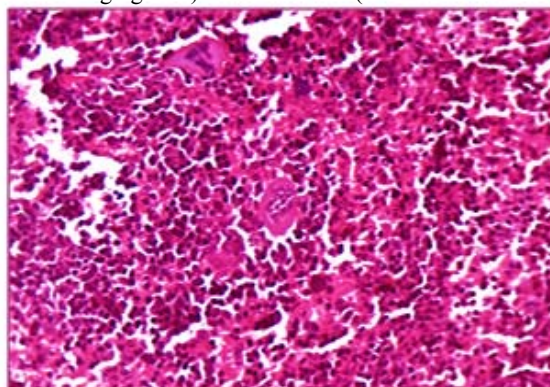


Figure 6: Group V
(DLA+ Cyclophosphamide 3mg/kg b.wt)

The tumor transplantation, the increase in body weight with sluggish movement of the animals was noted from 5th day onwards. The changes in the body weight were observed on every 5 days interval from the period of tumor transplantation and the results obtained are presented in figure 7.

The increase in body weight of the DLA tumor bearing mice (Group-II) was significantly increased when compared with normal control (Group-I). The methanolic extract and nanoparticles of methanolic extract of *Brassica rapa* Chinensis leaves treated from 1st & 11th day of induction (Group-III, IV, V and VI) showed a decrease in body weight when compared to induced group (Group-II). Treatment with cyclophosphamide from 1st & 11th day of induction showed a decrease in body weight when compared to Group-II.

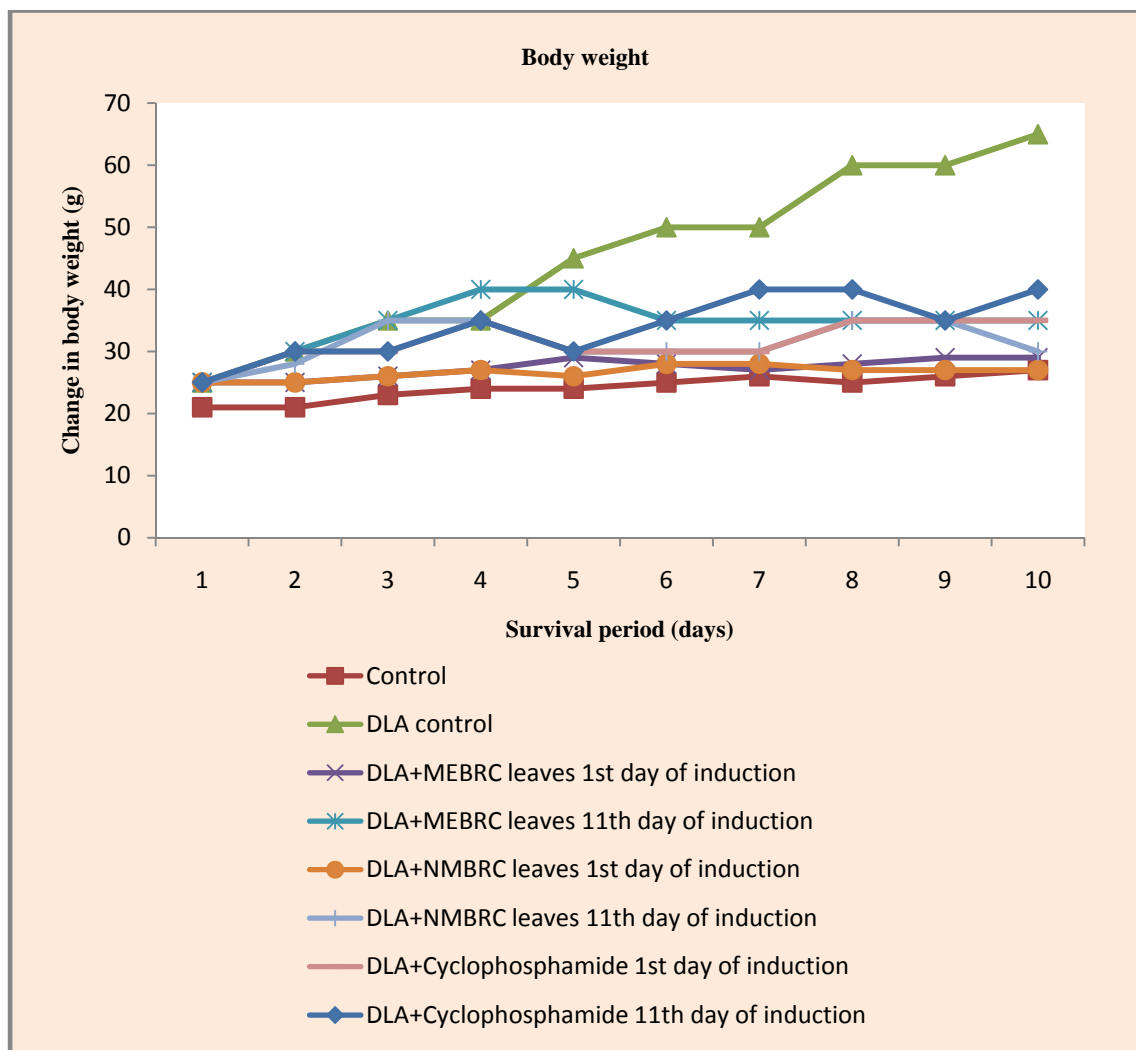


Figure 7: Effect of methanolic extract of *Brassica rapa* Chinensis leaves on body weight of DLA tumor bearing mice

The effect of methanolic extract of *Brassica rapa* Chinensis leaves on the survival period of DLA tumor bearing mice given in **Table 2**. The survival period of DLA tumor bearing mice (Group-II) significantly decreased when compared with control (group-I). Treatment with methanolic extract of *Brassica rapa* Chinensis leaves (group-III and group-IV), nanoparticles of methanolic extract of *Brassica rapa* Chinensis leaves (group-V and group-VI) and cyclophosphamide (group-VII and group-VIII) at various development stages of tumor showed the significant increase in life span when compared to the DLA control (Group-II).

Table 2: Effect of *Brassica rapa* Chinensis leaves on survival period of DLA tumor bearing mice

Groups	Survival in days	Life span (%)	% increases in life span
Group-I Control	>58	-	-
Group-II(DLA induced)	20.33±1.52 ^a	100	-
Group-III (DLA+ MEBRC leaves treatment 1 st day of induction)	36.66±1.366 ^{bc}	180.32	80.32
Group-IV (DLA+MEBRC leaves treatment 11 th day of induction)	32.00±0.89	157.40	57.40
Group-V (DLA+ NMBRC leaves treatment 1 st day of induction)	40.33±0.51 ^{cf}	198.376	98.376
Group-VI (DLA+NMBRC leaves treatment 11 th day of induction)	39.00±0.89	191.834	91.834
Group-VII (DLA+ Cyclophosphamide treatment from 1 st day of induction)	28.33±1.03 ^d	139.350	39.350
Group-VIII (DLA+ Cyclophosphamide treatment from 11 th day of induction)	25.66±0.51	126.217	26.217

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

Group comparison significant at (p<0.05): a) G-II vs G-I b) G-III vs G-II & IV c) G-V vs G-II & VI d) G-VII vs G-II & VIII e) G-III vs G-V f) G-V vs G-VI

DISCUSSION

The anemia is common complication in cancer and the situation aggravates further during chemotherapy since a majority of antineoplastic agents exert suppressive effects on erythropoiesis and thereby limiting the use of these drugs. The improvement in the hematological profile of the tumor bearing mice following the treatment with the extract could be due to the action of the different phytoconstituents present in the extracts [12]. The hemoglobin content and RBC count were reduced in DLA-bearing mice as compared to normal animals.

The haemoglobin content, RBC, platelets and differential count of WBC had been reversed by the treatment. Elevation of WBC levels may be due to its adverse effect on the haemopoietic system [13]. The important criteria for assessing the value of any antitumor drug are prolongation of life span and decrease of WBC count from blood [14]. PCV may be considered as comparable to that volume of solid tumors. The major problems that are being encountered are myelo suppression and anemia [15].

Our observation correlates with previous report Santhosh kumar *et al.*, 2008 [16] showed that there was significantly increase in the level WBC and neutrophils and significant reduction in the levels of RBC and lymphocytes. The levels were normalized after supplemented with methanolic extract of *Hypericum hookerianum*.

Similar results have been obtained by Priya *et al.* 2011 [17] where the treatment of DLA bearing mice with the root extracts of *L.inermis* almost brought the levels of RBC, total WBC count and platelets closer to the normal level. Raju *et al.* 2011 [18] reported that the extract of *Indigofera cassioides* showed protective effect on haemopoietic system on DLA and EAC (Erhlich's Ascites Carcinoma) bearing mice.

Treatment with methanolic extract of *Brassica rapa* Chinensis leaves caused an increase in hemoglobin content & RBC, and reduction the WBC count. Thus, it clearly indicated that the methanolic extract of *Brassica rapa* Chinensis leaves possess protective action on the hemopoietic system of DLA tumor bearing mice and this may occur either due to iron deficiency and due to hemolytic or myelopathic conditions [19].

In DLA- tumor bearing mice, a regular rapid increase in ascetic tumor volume was observed [20]. The increase in life span and change in the body weight of the animals suggest that the tumor growth inhibiting activity of the methanolic extract of *Brassica rapa* Chinensis leaves is due to the presence of secondary metabolites like flavonoids, phenols and other metabolites present in the methanolic extract of *Brassica rapa* Chinensis leaves.

Malaya Gupta *et al.*, 2004 [21] reported that the significant decrease in body weight was observed in cancer bearing animals when treated with *Caesalpinia bonducella* leaves. The results are in agreement with the report of Sivakumar *et al.*, 2008 [22] who showed that the body weight of DLA bearing mice was decreased when treated with *Triumboidea* leaves.

There are several *in vitro* studies and rodent *in vivo* studies suggesting that certain herbs and spices may have a chemopreventive effect in the early (initiating) stages of cancer. Herbs may act through several mechanisms to provide protection against cancer [23]. Induction of apoptosis in tumor cells is considered very useful in the management and therapy as well as the prevention of cancer.

Apart from physiological stimuli there are exogenous factors which can contribute to induction of apoptosis. Certain phytochemicals from herbs or herb extracts have been shown to inhibit one or more of the stages of the cancer process (i.e., initiation, promotion, growth and metastasis) [24].

Life span of DLA tumor bearing mice was found to be extended by simultaneous treatment with methanolic extract and nanoparticles of methanolic extract of *Brassica rapa* Chinensis leaves and from the time of tumor induction and when compared to untreated tumor bearing mice.

In another experiment, treatment was given to the animals after the development of tumor induction. i.e. from the 11th day of tumor induction. Survival period of this group of animals were found to be increased when compared to the untreated Group II.

In both the experimental conditions the effect of the standard drug cyclophosphamide was found to be having better effect. However, the anticancer effect exhibited by methanolic extract and nanoparticles of methanolic extract of *Brassica rapa* Chinensis leaves should be considered which might be due to the presence of secondary metabolites such as phenols, flavonoids and other phytochemicals in the methanolic extract and nanoparticles of methanolic extract of *Brassica rapa* Chinensis leaves.

Gorelik *et al.*, 2008 [25] reported the life span of DLA tumor bearing mice was found to be 15-25 days with the average life span of 20 days. Antioxidant and Antitumorigenic efficacy of methanolic extract of *Gloriosa superba* and Silver Nanoparticles of methanolic extract of *Gloriosa superba* to DLA tumor cells Treatment with MGsSTL and AgMGsSTL reduced the intraperitoneal tumor burden, by detoxifying the tumor cells and increased the life span of the tumor induced mice. The steadfast criteria for judging the potency of any anticancer drug is the prolongation of life span of tumor induced animals.

Our results coincide with the results of Balasubramanian *et al.*, 2007 [26] who reported that the administration of *Phyllanthus polyphyllus* increased the mean survival time in EAC bearing mice. Our results are in accordance with the earlier report of Malaya Gupta *et al.*, 2004 [21] who showed that the survival period of cancer bearing mice was prolonged when treated *Bauhinia racemosa* stem bark.

Conclusion

In conclusion, treatment with methanolic extract of *Brassica rapa* Chinensis leaves caused an increase in hemoglobin content & RBC, and reduction the WBC count. Thus, it clearly indicated that the methanolic extract of *Brassica rapa* Chinensis leaves possess protective action on the hemopoietic system of DLA tumor bearing mice and this may occur either due to iron deficiency and due to hemolytic or myelopathic conditions.

The histological examination of the spleen of DLA tumor bearing mice showed abnormal histological changes whereas treatment with *Brassica rapa* Chinensis leaves and cyclophosphamide caused a remarkable decrease in the abnormal changes in the spleen. The above effects might be due to the synergistic activity of the various components and secondary metabolites present in the *Brassica rapa* Chinensis leaves.

In the present study, it was found that MEBRC and NMBRC leaves have anticancer activity which in turn raised the life span of DLA induced mice and confirmed their anticancer effect. It may be concluded that MEBRC and NMBRC leaves increased the life span of DLA bearing mice and proved their anticancer effect by arresting the tumor growth.

All our findings suggested that the MEBRC and NMBRC leaves exhibited antitumor activity as revealed by the significant increase in the % of MST, % ILS and change in body weight. Active compounds present in the methanolic leaf extract of MEBRC and NMBRC leaves might be responsible for the remarkable antitumor activity.

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