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A STUDY TO EVALUATE THE ANTICANCER ACTIVITY OF BARLERIA GRANDIFLORA DALZ (BG): AN *IN VIVO* STUDY

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ABSTRACT

One of the best approaches in searching novel anticancer agents from plant resources is selection of plants based on ethnomedical practices and testing their efficacy and safety in light of modern science. Various parts of Barleria grandiflora dalz (BG) have been traditionally used as ethnomedicine for a number of disorders. The aim of the present study is Evaluation of Anticancer Activity of *Barleria grandiflora dalz* (BG) in Experimental Animals: An *In-vivo* Study. The Ethanol extract of leaves of Barleria grandiflora dalz was prepared by Soxhlet extraction. The *in-vivo* anticancer activity of BG extract was investigated in EAC induced Ascites tumor model. The toxicity of drug before starting the in vivo was checked by acute toxicity test, where the MTD was seen more than 5000mg/kg body weight in Swiss Albino rats. The *In-vivo* Anticancer activity was assessed by administering 250mg and 500mg/kg of BG extract orally for 9 days and Cisplatin (3.5mg/kg, i.p., Single dose). The *in-vivo* activity of BG 500mg/kg was shown more significant than the BG 250mg/kg. Hence 500mg/kg was taken for combination study with standard drug Cisplatin. The different parameters like Mean Survival Time, Percentage Increase in Life Span were restored towards normal and significant reduction in tumor weight was observed in 500mg and combination group as compared to control. The Biochemical parameters and Hematological parameters were also restored to normal.

KEYWORDS: Barleria grandiflora, anticancer, Cisplatin, EAC induced Ascites tumor model.

INTRODUCTION

Cancer is a state of illness characterized by cells failing to control cell growth resulting in the development of tumours made up by a mass of unregulated cells. Unlike normal tissues comprised by cells in which cell growth is tightly regulated, cancer cells grow abnormally since they lack the signal transduction network that regulates cell growth and therefore they proliferate uncontrollably. [1] The inactivation of these pathways is due to different mutations depending on the cell type, ethnicity and genetic background of the patients, epigenetic factors etc. In general, tumours can be classified into 2 categories: benign and malignant. [2]

Benign tumours refer to tumours that are non-metastatic and grow and stay within the tissue of origin. [2] These tumours can develop in most tissues and will increase in size but will not invade surrounding normal tissues. The non-invasiveness nature of benign tumours results in the development of a tumour surrounded by a capsule of connective tissue which allows benign tumours to be easily removed surgically.[1] On the other hand, malignant tumours possess cellular abnormalities and are highly invasive towards surrounding tissues.[1] Malignant tumours invade blood and lymphatic vessels enabling the translocation of tumour cells to other sites in the body resulting in cancer metastasis and development of secondary tumours.[1] The ability to escape suicide (cell death) is a hallmark of most cancer cells. The etiology for this is still in debate and is related to multiple factors including exposure to carcinogens, environmental conditions (anoxia, oxidative stress), oncogenic viruses, genetic predisposition, sex, age and race which contribute to the development of cancer.

Genetic instability is reflected in changes in the numbers of genes in cancer cells. This can be the result of small duplications or deletions, translocations of material from one chromosome to another or even changes that affect entire chromosomes. Plants have a long history of use in the treatment of cancer. [3] The search for anti-cancer agents from plant sources started in the late 1950's, with the discovery and development of the vinca alkaloids, (vinblastine and vincristine) and isolation of cytotoxic podophyllotoxins. As a result, the United States National Cancer Institute (NCI) initiated an extensive plant collection program in 1960. This led to the discovery of many other compounds such as taxanes, camptothecins [4] and combrestatins.[4] Paclitaxel (Taxol), vinorelbine (Navelbine), teniposide (Vumon) and various water-soluble analogs of camptothecin (e.g., Hycamtin) which are being used in cancer treatment with varied degrees of success. More over plant based drugs are cheap, locally available, and free from severe side effects. Over 60% of currently used anti-cancer agents

are derived in one-way or another from natural sources, including plants, marine organisms and microorganisms. [5] Barleria is a perennial, ornamental plant which is found abundantly in India and Myanmar. *Barleria* is a carefree shrub that blooms in both spring and fall. An extract of Barleria leaves has been used in traditional medicines for the treatment of anemia, toothache, and inflammation. Scientific studies have confirmed that it has strong anti-inflammatory properties. The Hypoglycemic agent in the plant is found to be useful in the treatment of diabetes. The leaves of the plant have germicidal properties. They are used in beauty preparations and shampoos. The plant is used for relieving fever and joint pain. The leaves and roots are used for cough and inflammations. Seeds are used as antidote for snake bites. The herb Yellow Barleria is considered *Vajradanti* and is described in Ancient Ayurvedic literature. [6]

MATERIALS AND METHODOLOGY

Collection and preparation of plant extract

The plant material (Leaves of *Barleria grandiflora* Dalz) was collected from Mysore district, Karnataka, India and was authenticated by Dr. M.N. Naganandini, Assistant Professor, Dept of Pharmacognosy, JSSCP, and Mysore. The Leaves of plant were cleaned to remove impurities and shade dried. The coarsely powdered leaves were weighed and stored in air tight containers. The coarsely powderd shade dried leaves of the plant Barleria grandiflora Dalz (200g) was extracted with ethanol by soxhlet extraction method for 25h. After completion of extraction the extract was filterd, concentrated using flash rotator evaporator and dried under vaccum.

Cell lines

EAC (Ehrlich Ascites Carcinoma) cells, obtained from JSS College of Pharmacy, Mysore, Karnataka, India. The cell lines were maintained and propagated intraperitonially by serial transplantation in adult Swiss albino mice.

Animals

The experiments were carried out on 8-10 weeks old Swiss albino mice of either sex weighing 25 ± 5 gm and female Wistar albino rats weighing around 175±25gm. Animals used in the study were procured from a registered breeder. The animal care and handling was carried out in accordance to guidelines issued by the Institutional Animal Ethics Committee, JSS Medical College, Mysore, and Karnataka. Animals were acclimatized to the experimental room for one week prior to the experiment. Animals were maintained under controlled

conditions of temperature (23 ± 3^0C) and humidity (50 ± 5 %) and were caged in sterile polypropylene cages containing sterile paddy husk as bedding material with maximum of four animals in each cage. The mice were fed on standard food pellets and water *ad libitum*. The studies conducted were approved by the Institutional Ethical Committee, JSS Medical College, Mysore, and Karnataka.

***In vivo* anticancer activity of alcoholic extract of BG against EAC cell lines by liquid tumor model Ehrlich ascites carcinoma (EAC) induced tumor model. [7]**

Induction of Liquid tumor

EAC cells were aspirated from the peritoneal cavity of EAC bearing mice, after 15 days of tumor transplantation. The ascitic fluid was drawn using an 18-gauge needle into a sterile syringe and a small amount was tested for microbial contamination. Total number of viable cells/ml was counted by Trypan blue [8] and the ascitic fluid was suitably diluted in PBS to obtain a stock cell concentration of 10^7 cells per ml. To induce ascitic tumor 2×10^6 EAC cells (0.25 ml of stock suspension) was injected intraperitonially to each mice. Treatment was started after 24 h tumor inoculation and continued for 9 days.

Treatment groups: (n=12)

Group I	**Normal**	**No treatment**
Group II	Control	EAC cells + $D.H_2O$ p.o
Group III	Standard	EAC cells + Cisplatin(3.5mg/kg) i.p
Group IV	BG dose I	EAC cells + Alcoholic extract(250mg/kg) p.o
Group Vl	BG dose II	EAC cells + Alcoholic extract(500mg/kg) p.o
Group VI	Cisplatin + Selected dose of BG	EAC cells + Cisplatin (1.75mg/kg) i.p + Alcoholic extract (500mg/kg) p.o

Parameters Monitored

1. % Increase in body weight as compared to day "0"weight

Upon weighing the animals on the day of inoculation and after once in 3 days in the post inoculation period the % increase in body weight was calculated as follows:

% increase in wt = (animal wt on respective day/animal wt on day 0)-1 x 100

2. Mean survival time (MST) and Increase in life span [%ILS]

Total number of days an animal survived from the day of tumor inoculation was counted. Subsequently the mean survival time was calculated. The %ILS was calculated as follows:

$$\%\ ILS = \left(\frac{MST\ of\ treated\ group - MST\ of\ control\ group}{MST\ of\ Control\ Group}\right) \times 100$$

An enhancement of life span by 25% or more over that of control was considered as effective antitumor response.

Hematological parameters

In order to assess the influence of treatment on the hematological status of EAC bearing mice, blood was collected intracardially from the animals into heparinised and EDTA treated micro centrifuge tubes on 10th day and following parameters were monitored.

1. White blood cell total count
2. Red blood cell total count
3. Hemoglobin contents.

Biochemical estimation [9]

SGPT, SGOT, ALP, Blood Urea [10], Serum Creatinine [10], Total protein. [11]

RESULTS AND OBSERVATIONS

Effect of BG extract on change in the body weight in EAC inoculated mice

Substantial increase in body weight was observed in EAC inoculated control mice with a maximum gain of (67.19±6.178 %) on day 15 compared to day 0. The development of tumor was observed on day 6th and continued till end of study. The Standard Cisplatin (3.5mg/kg) treatment significantly reduced body weight (-29.02±2.22%) compared to control. BG at a dose of 250mg/kg treatment significantly reduced the tumor induced % increase in the body weight (14.8±4.75%) and BG at a dose of 500mg/kg with (9.48±1.988%) when compared to control. The combination (Cisplatin 1.75mg/kg + BG 500mg/kg) reduced significantly with reduced tumor induced % (-2.91±1.408%) when compare to control and efficacy was comparable to standard. On 12th, 15th day all treated groups including the standard Cisplatin group significantly inhibiting the percentage rise in body weight as compared to control.

Table: 1: Effect of Bg Extract on Body Weight Changes In Eac Inoculated Mice.

	MEAN % OF BODY WEIGHT COMPARE TO DAY '0'				
Days	**(3) day MEAN ±SEM**	**(6) day MEAN ±SEM**	**(9) day MEAN ±SEM**	**(12) day MEAN ±SEM**	**(15) day MEAN ±SEM**
Control	7.08±1.352	25.38±1.587	33.70±2.363	45.33±2.717	67.9±6.178
Cisplatin(3.5mg/kg)	5.7±2.452	1.150±2.767^{a}	1.33±3.74^{a}	-11.74±2.66^{a}	-29.02±2.22^{a}
BG 250mg	6.7±2.168	22.35±2.376^{b}	29.74±2.017^{b}	20.47±2.306^{ab}	14.80±4.075^{ab}
BG 500mg	3.59±1.304	10.04±1.677^{a}	23.75±2.14^{b}	17.18±1.993^{ab}	9.48±1.988^{ab}
Cisplatin(1.75mg/kg+ BG 500mg)	4.668±1.547	7.02±2.687^{a}	11.10±2.768^{a}	-1.13±2.262^{ab}	-2.91±1.408^{ab}

*All the values are MEAN ±SEM of six mice, [a]p < 0.05 compared to control, [b]p < 0.05 compared to standard. All data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

Effect of extract BG on mean survival time and % increase in life span of EAC inoculated mice.

Mean survival time of EAC inoculated mice was 21.17 ± 0.749 days. Standard cisplatin treatment at 3.5mg/kg also significantly enhanced the mean survival time to 54.50 ± 5.937 days when compared to control. The BG plant extract of 250mg/kg and 500mg/kg significantly increase the MST to 30.00 ± 0.57774 and 41.67 ± 1.430. Respectively when compared to control the combination (cisplatin 1.75mg/kg + BG 500mg) significantly increased the MST to 48.83 ± 2.52 and efficacy was comparable to standard.

The percent increase in lifespan (% ILS) of animal treated with BG 250mg/kg and BG 500mg/kg was 42.18% and 96.88%, the percentage increase in lifespan of combination (cisplatin 1.75mg/kg + BG 500mg) treated was 130%. The efficacy of combination treated in enhancing lifespan of tumor bearing animal was comparable to that of standard (cisplatin 3.5mg/kg) which was 158%.

Table - 2: Effect of Extract Bg on Mean Survival Time And % Increase In Life Span In Eac Inoculated Mice.

Treatment	MST	%ILS
Control	21.17±0.749	-
Cisplatin(3.5mg/kg)	54.50±5.9372[a]	158%
BG 250mg/kg	30.00±0.5774[b]	42.18%
BG 500mg/kg	41.67±1.430[a,b]	96.88%
Cisplatin(1.75mg/kg+ BG 500mg/kg)	48.83±2.52[a]	130%

*All the values are Mean ± SEM of six mice, where [a] p < 0.05 compared to control, [b] p < 0.05 compared to standard. All data were analyzed by one way ANOVA followed by post hoc Tukey's multiple comparison tests.

Effect of BG extract on hematological parameters in EAC inoculated mice

To assess the influence of BG treatment on hematological parameters, the total RBC, WBC and hemoglobin content of all the treatment groups were checked on 10th day of tumor inoculation.

Effect on total RBC

A significant reduction in total RBC count was observed in EAC inoculated control mice (2.58±0.14) when compared with the normal mice (4.91±0.06). Treatment with Cisplatin 3.5mg/kg significantly reversed this reduction to (4.06±0.09) as compared to Control. BG at both doses increased the total RBC count to near normal & the efficacy was comparable with standard Cisplatin. Combination (Cisplatin 1.75mg/kg + BG 500mg/kg) treated significantly reversed the RBC count (4.2±0.14) when compared to control and was not significant to standard. (table 3).

Effect on total WBC

A significant increase in total WBC count was observed in EAC inoculated control mice (24.77 ± 0.729) when compared to normal animal (9.06 ± 0.12). Standard Cisplatin treatment at a dose of 3.5 mg/kg significantly reversed the tumor induced elevation in WBC count to (11.23±0.26) when compared with control. Both doses of BG and combination treatment significantly reversed the elevated WBC, when compared to control. (table 3).

Effect on hemoglobin content

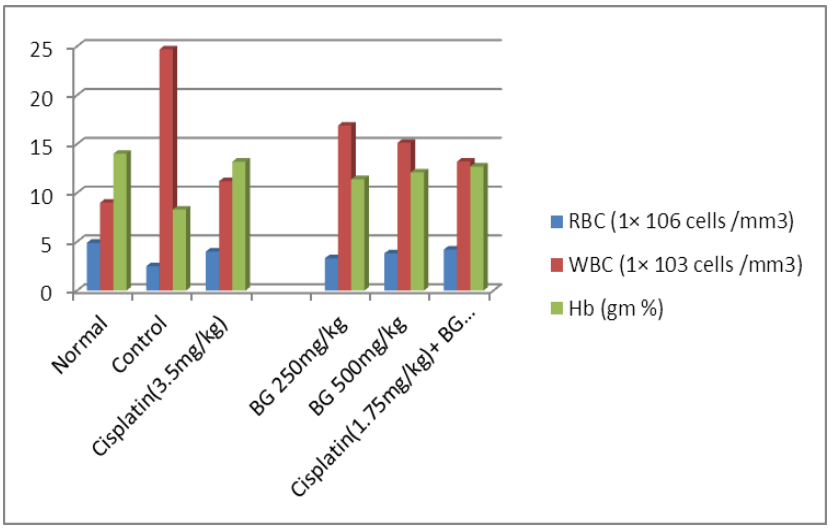
A significant reduction in hemoglobin level was observed in EAC inoculated control (8.30± 0.15) mice as compared to normal (14.02 ± 0.14). Standard Cisplatin treatment at a dose of 3.5mg/kg significantly reversed the tumor induced reduction in hemoglobin level to (13.28±0.15) when compared to control .The combination treated significantly reversed the decrease in hemoglobin content when compared to control. BG treatment of both the doses also caused an significant increase in hemoglobin content compared to control (table 3).

Table - 3: Effect of extract BG on total RBC, WBC and Hb% in eac inoculated mice.

Treatment	RBC (1× 106 cells /mm3) MEAN ± SEM	WBC(1× 103 cells /mm3) MEAN ± SEM	Hb(gm %) MEAN ± SEM
Normal	4.9±0.0654	9.0±0.1229	14.0±0.142
Control	2.5±0.1493[a]	24.7±0.7279[ac]	8.3±0.1528[ac]
Cisplatin(3.5mg/kg)	4.0±0.0900[ab]	11.2±0.2629[ab]	13.2±0.1537[ab]
BG 250mg/kg	3.3±0.1585[abc]	16.9±0.2704[abc]	11.4±0.2033[abc]
BG 500mg/kg	3.8±0.1249[ab]	15.13±0.256[abc]	12.1±0.1759[ab]
Cisplatin(1.75mg/kg)+ BG (500mg/kg)	4.2±0.1406[ab]	13.21±0.1579[abc]	12.7±0.163[ab]

*All the values are Mean ± SEM of six mice, [a] $p < 0.05$ compared to normal, [b] $p<0.05$ compared to control, [c] $p < 0.05$ when compared to standard. The data were analyzed by one way ANOVA followed by post hoc Turkey's multiple comparison tests.

Fig 1: Showing effect of extract BG on total RBC, WBC and Hb% in eac inoculated mice.



Effect of BG extract on Biochemical parameters in EAC inoculated mice

To assess the influence of BG treatment on Biochemical parameters, SGOT, SGPT, ALP, S. Creatinine, S. Urea, and Total Protein content of all the treatment groups were checked on 10^{th} day of tumor inoculation.

Effect on serum glutamate oxaloacetic transaminase (SGOT)

A significant increase in serum SGOT level was observed in EAC inoculated control mice (82.00±3.055) when compared to normal animal (42.00±1.36). Cisplatin at a dose of 3.5 mg/kg significantly reversed the tumor induced elevation in SGOT level (52.67±1.606) when compared with control. BG at both doses significantly decreased the elevated SGOT level compared to control, but the combination showed significant reduction in SGOT level when compared to control. (Table 4)

Effect on Serum glutamate pyruvate transaminase (SGPT)

A significant increase in serum SGPT level was observed in EAC inoculated control mice (95.17±2.040) when compared to normal animal (64.33±2.076). Cisplatin at a dose of 3.5mg/kg significantly reversed the tumor induced elevation in SGPT level (70.33±1.25) when compared with control. Both the doses of BG significantly decreased the elevated SGPT level significantly compared to control, the combination significantly decrease the SGPT level compared to control and decrease SGPT level was comparable with standard. (Table 4)

Effect on Alkaline phosphatase (ALP)

A significant increase in serum ALP level was observed in EAC inoculated control mice (189.3±1.282) when compared to normal animal (126.2±2.197). Standard Cisplatin treatment at a dose of 3.5mg/kg significantly reversed the tumor induced elevation in ALP level (129.7±2.34) when compared with control. BG at both doses significantly decreased the elevated ALP level when compared to control, the combination treated significantly increased in ALP level when compared to normal. (Table 4).

Effect on Serum creatinine

Standard Cisplatin 3.5mg/kg induced toxicity was manifested by significant increase in serum creatinine (1.52±0.09) when compared to normal (0.47±0.018). BG dose at 250mg/kg significantly increase (1.177±0.0896) when compared to normal.BG dose at 500mg/kg significantly decreased (0.915±0.0639) when compared to standard cisplatin. Combination showed induced toxicity (1.418±0.141) when compared to normal. (Table 4)

Effect on Serum urea

Standard Cisplatin 3.5mg/kg induced toxicity was manifested by significant increase in serum urea (57.67±1.9) when compared to normal (22.83±0.018). BG dose at 250mg/kg (37.33±2.61) and combination (49.00±1.653) showed significantly increase when compared to normal.BG dose at 500mg/kg(32.50±1.204) significantly decreased when compared to standard cisplatin. (Table 4).

Effect on Total protein

Standard Cisplatin showed significant decreased (7.25±0.19) when compared to control (9.25±0.18) .BG at both the doses and combination showed significant decreased when compare to control. (Table 4)

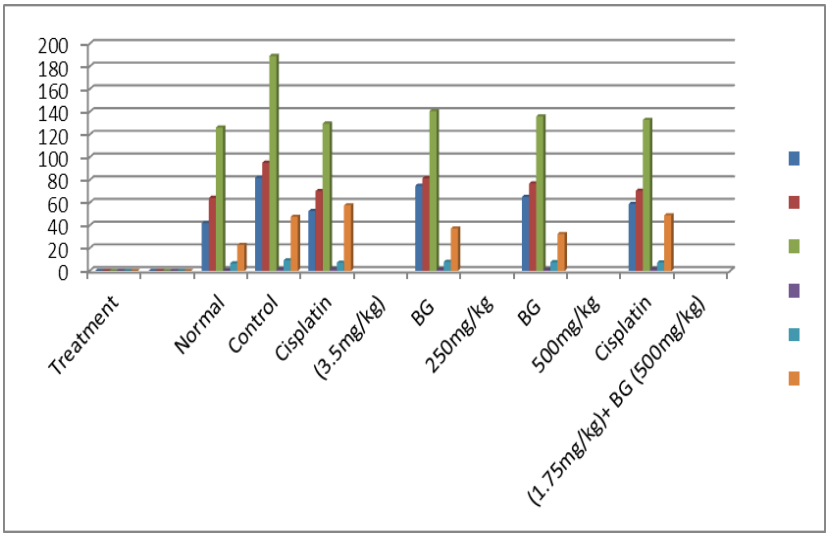
Table - 4: Effect of extract BG on SGOT, SGPT, ALP, S.creatinine, S.urea, and Total protein in EAC inoculated mice.

Treatment	SGOT MEAN±SEM	SGPT MEAN±SEM	ALP MEAN±SEM	S.Creatinine MEAN±SEM	T.Protein MEAN±SEM	S.Urea MEAN±SEM
Normal	42.00±1.36	64.33±2.07	126.2±2.19	0.475±0.01	6.667±0.21	22.83±1.07
Control	82.00±3.05ac	95.17±2.04ac	189.3±1.28ac	1.283±0.11a	9.250±0.18ac	47.67±2.34ac
Cisplatin (3.5mg/kg)	52.67±1.60ab	70.33±1.25b	129.7±2.34b	1.523±0.09a	7.250±0.19b	57.67±1.99ab
BG 250mg/kg	75.00±1.84ac	81.50±1.94abc	140.7±1.38abc	1.177±0.08a	8.050±0.23ab	37.33±2.61abc

BG 500mg/kg	65.17±2.08abc	77.00±1.34ab	136.0±2.36b	0.915±0.06c	7.700±0.21ab	32.50±1.20abc
Cisplatin (1.75mg/kg)+ BG (500mg/kg)	59.00±1.06ab	70.67±0.88b	133.0±2.04b	1.418±0.01a	7.433±0.16b	49.00±1.65a

*All the values are mean ± SEM of six mice, [a] $p < 0.05$ compared to normal, [b] $p<0.05$ compared to control, [c] $p < 0.05$ when compared to standard. The data was analyzed by one way ANOVA followed by post hoc Turkey's multiple comparison tests

FIG 2: showing effect of extract BG ON SGOT, SGPT, ALP, S.creatinine, S.urea, AND Total protein in EAC inoculated mice.



DISCUSSION

In spite of the spectacular advances made by medical sciences during the present century, cancer still remains as major threat against human race. Extensive progress has been made to fight against cancer. The search for a selective and less toxic molecule for cancer treatment is an ongoing process. Plants have played an important role as a source of effective anticancer agents and about 60% of the currently available anticancer drugs are derived from plant sources.[12] The global trend is to also towards natural bioactive substances due to their low toxicity and cost. The exploration of medicinal plants for their therapeutic efficacy still holds the hope for the treatment and prevention of cancer The present study was designed to explore the possible in *vivo* anticancer activity of plant alcoholic extract of BG. Generally, anticancer screening involves use of expensive and sophisticated techniques viz. using human cell lines and grafted or implanted tumor mice models. The "appropriate" transplantable mouse tumors models still have their place in the drug development programs and are used to

investigate the antineoplasic effects of several chemical compounds and plant extracts. Hence in the present study the in vivo anticancer efficacy of plant was assessed in transplantable tumor model Ehrlich's Ascites Carcinoma in mice. In this Ascites tumor model, a substantial increase in body weight of the animals was observed in EAC bearing control mice owing to the rapid and progressive accumulation of Ascites tumor cells. Treatment with BG combination dose caused marked reduction in the body weight of the animal as compared to the lower doses indicating the inhibition tumor cell progression. BG combination treatment enhances the MST of tumor bearing mice in a dose dependant manner and enhancement was observed at 500mg / kg body weight and BG combination. The percent increase in life span of tumor bearing mice, following treatment with BG 500 mg / kg was 96.88% and combination was 130%. But lower dose of BG was less effective in increasing the life span of tumor bearing mice. Prolongation of life span is a reliable criteria for judging the anticancer efficacy of any compound.[13] An enhancement of life span by 25% or more over that of control was considered as effective antitumor response. In the present study BG extract meets this criteria. Myelosuppression and anemia have been frequently observed in ascites carcinoma. In EAC control mice elevated WBC count, and reduced hemoglobin and RBC count was observed. Anemia (reduced hemoglobin) encountered in ascites carcinoma mainly due to iron deficiency, either by hemolytic or myelopathic conditions which finally lead to reduced RBC number. The major problems of cancer chemotherapy with the conventional drugs are myelosuppression and anemia. The combination group and BG 500mg/kg reversed the EAC induced alteration in hematological parameters such as elevation of hemoglobin content and total RBC count and reduction of elevated total WBC count. It also restored the serum enzyme levels SGPT, SGOT, ALP, Serum Creatinine, Total protein and Serum Urea to near normal which indicates the less toxicity.

Many tumor cells have pro-oxidant status and promote oxidative stress. This increases the survival potential of cancer cells by inducing mutations, activating redox signaling and stimulating pro-survival factors such as NF αB and AP-1.[14] Previous studies reported the potent antioxidant activity of BG in invitro antioxidant model. BG extract significantly shows lower absorbance in both FTC and TBA methods, which indicates that, the hydro alcoholic extract has high antioxidant activity.[15] Thus it could be suggested that BG alter the intracellular redox state, thereby contributes to enhance antitumor activity. It was reported that plant derived extracts containing antioxidant principles showed cytotoxicity towards cells and antitumor activity in experimental animals.[16] The cytotoxic and antitumor activity of

plant derived product is either through induction of apoptosis or inhibition of neovascularization.[17] Plants with high phenol content are reported to possess effective antioxidant and antitumor properties.[17] Since the alcoholic extract of BG showed similar anticancer activity as that of standard, the mechanism of action may be because of the potent antioxidant activity of the extracts. However, further studies are required to support the assumption.

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