

IN VITRO AND IN VIVO ANTI-CANCER ACTIVITY OF LEAF EXTRACT OF BARLERIA GRANDIFLORA

NISHANT MANGLANI^{1*}, SHILPA VAISHNAVA¹, DHAMODARAN P¹, HEMANT SAWARKAR²

¹JSS College of Pharmacy Ooty, Tamilnadu India- 643001, ²Shri Rawatpura Sarkar Institute of Pharmacy Durg Chhattisgarh- 490042, ^{1*}Research and Development Centre Department of The Himalaya Drug Company Bangalore 562 162 India. Email: bittu6488@gmail.com

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ABSTRACT

Objective: To evaluate anticancer activity of the *Barleria grandiflora* (Acanthaceae) leaf on the different cancerous and normal cell lines such as A-549 (human lung cancer) cells, Dalton's lymphoma Ascites (DLA tumour cells) and Vero (African green monkey kidney) normal cells.

Methods: *In vitro* cytotoxicity activity was carried out to screen cytotoxicity potency of *Barleria grandiflora* alcoholic extract (MeBG) and aqueous extract (WtBG) against above mentioned cell lines which concluded the effectiveness of MeBG against DLA tumour cells and hence the particular MeBG extract was taken for further *In Vivo* anti tumour activity by assessing tumor volume, viable and nonviable tumor cell count, tumor weight, hematological parameters and biochemical estimations.

Results: In *in vitro* cytotoxicity study, MeBG extract showed direct cytotoxic effect on the A-549 and DLA cell line in a concentration dependent manner and the IC₅₀ value was found to be 143.4 µg/mL (MeBG) and 210.8 µg/mL (WtBG) 137.2 µg/mL (MeBG) and 217.8 µg/mL (WtBG) respectively while both the extract were less toxic to Vero cell line and IC₅₀ value was found to be 148.7 µg/mL (MeBG) and 152.6 µg/mL (WtBG) (Fig.1) *In vitro* cytotoxicity study shows that MeBG is more cytotoxic for DLA tumour cells hence MeBG was taken for further *in vivo* anti tumour study. At doses of 200mg/kg and 400mg/kg MeBG exhibited significant (P<0.01) decrease in the tumor volume, viable cell count, tumor weight, and elevated the span of DLA tumor bearing mice. The hematological parameters were reverted to normal level in MeBG treated mice.

Conclusion: The present study states that the alcoholic leaf extract of *Barleria grandiflora* showed a significant *in vitro* and *in vivo* anti tumour activity against DLA cells and also less toxic for human cells.

Keywords: Anti cancer, A-549 cell line, Vero cell line, Cytotoxicity activity, Anti tumour, *Barleria grandiflora*.

INTRODUCTION

Cancer is a large group of diseases, all of which have one thing in common i.e. cells growing out of control or fundamentally a disease of tissue growth regulation failure. In order for a normal cell to transform into a cancer cell, the genes which regulate cell growth and differentiation must be altered [1]. Though many diseases (such as heart failure) may have a worst prognosis than most cases of cancer, cancer is the subject of widespread fear and taboos, there are 200 different types of cancer that afflict humans [2]. The causes of cancer are diverse, complex, and only partially understood. Many things are known to increase the risk of cancer, including tobacco use, dietary factors, certain infections, exposure to radiation, lack of physical activity, obesity, and environmental pollutants [3]. Cancers are classified by the type of cell that the tumor cells resemble and are therefore presumed to be the origin of the tumor. Although, several classes of anti cancer are currently being used, due to clinical limitations and adverse effects there is critical interest in development of efficient and safe drugs for treatment of cancer.

Herbal medicines constitute a major substitute for cancer prevention and treatment in anomalous countries around the globe. The effect of plant extracts as anti cancer was widely studied due to their low toxicity and side effects. Due to the aforementioned concerns, such studies investigating medicinal herbs have been steadily held with interests. Currently, the number of plants reported to possess anti cancer properties are more than 3000 [4, 5]. *Barleria grandiflora* (Family: Acanthaceae) commonly known as Dev koranti in Marathi which is frequently distributed in Central India especially in Vidarbha area of Maharashtra. Ethnobotanical survey reveals the leaf juice is used for treating mouth ulcer [6]. The principle constituents of leaf and stem circumscribe the presence of glycosides i.e. barlerin, shanzhiside, methyl ester [7, 8]. Present work aims to evaluate anti-cancer potency of *Barleria grandiflora* leaves using various cancer and normal cell lines *in vitro* and *in vivo*.

MATERIALS AND METHODS

Collection of leafs and extraction

The leaf of *Barleria grandiflora* Dalz (Family: Acanthaceae) was collected from Amravati region (Maharashtra) in the month of July and was authenticated by Prof. Dr. B.K. Auti, Professor and Head Department of Botany, Radhabai K.M. Ahmednagar (Maharashtra). A specimen was submitted in JSS College of Pharmacy Ooty. (Specimen no: JSSCP/ M.Pharm/P'cognosy /29/2011-12). Leaf material was shade dried in room temperature for 3 days and then coarsely powdered. Powdered plant material (500g) was defatted using hexane and defatted material was extracted with (2000 ml×3) of methanol which was kept overnight (cold maceration) and filtered, filtrate was completely evaporated under reduced pressure using Rotavapor (MeBG) yield 13.6%w/w. The spent material after methanol extraction was extracted with distilled water (2000 ml×3) filtered and filtrate was completely dried under reduced pressure using Rotavapor (WtBG) yield 25.2% w/w [10]. The preliminary phytochemical study of defatted methanol extract indicated the presence of Anthraquinone glycosides, flavanoids, phenols, carbohydrates [10].

Animals

Swiss albino mice of about eight weeks old were used for the experiment. The mice were grouped and housed in poly acrylic cages (38cm×23cm×10cm) with not more than six animals per cage. The animals were maintained under standard laboratory conditions (temperature 25±2°C and 55±5% relative humidity with dark/light cycle 14/10h) and were allowed free access to standard dry pellet diet and water *ad libitum*. The mice were acclimatized to laboratory condition for seven days before the commencement of the experiments. All the described procedures were reviewed and approved by J.S.S. University animal ethics committee approved by (JSSCP/IAEC/M.Pharm 02/2011-12).

Transplantation of tumor cells

DLA (Dalton's lymphoma Ascites) cells were procured from Amla Cancer Institute Amla nagar, Trissur, Kerala India. The cells were maintained *in vivo* in Swiss albino mice *i.p.* The ascitic fluid was drawn out from DLA tumor bearing mouse at log phase (days 7-8 of tumor bearing) of tumor cells. The cells withdrawn were used for both *in vitro* and *in vivo* study [11].

Assay for *in vitro* cytotoxicity study

In vitro cytotoxicity assay of MeBG and WtBG was performed by using Vero (African green Monkey Kidney), A-549 (Human lung) and Dalton's Lymphoma Ascites (Tumour cells) cell line. Briefly, 1×10^6 cells were suspended in 0.1 ml of phosphate buffered saline (PBS, 0.2 M, pH 7.4) and mixed with 100 μ l of various concentration (25,50,100,150,200 and 300 μ g/ml) of MeBG and WtBG and standard drug 5-fluorouracil.

The final volume was adjusted 1 ml with PBS and was incubated at 37°C for 3 h after the incubation was over, the viability of the cells was determined using trypan blue (0.4% in normal saline) method and the percentage of cytotoxicity was determined by calculating percentage inhibition and IC_{50} value [12, 13].

Acute toxicity

The acute oral toxicity of MeBG in Swiss albino mice was performed as per OECD guidelines [14]. The extract was safe up to the dose of 2g/kg b.w. P.O. for mice.

Treatment schedule for assessment of *in vivo* antitumor potential

The Swiss albino mice (20-25 g) were divided into five groups (n=12). Except, Group-I all the animals in DLA group were being injected with DLA cells (2×10^6 cells/mouse, *i.p.*). This was marked as day "0". Group-I was served as normal saline control (5 mL /kg, *i.p.*) and group-II was served as DLA control. After 24h, DLA transplanted group-III and IV were

being injected MeBG (200 and 400mg/kg b.w. *i.p.*) once daily for 11 consecutive days. Group V received standard drug 5-Fluorouracil (20 mg/kg *i.p.*) for 11 consecutive days [15]. After administrations of last dose 6 mice from each group were kept fasting for 18h and blood was collected by direct cardiac puncture for the estimation of haematological determination.

Rest of animals in each groups were kept alive with food and water *ad libitum* to check the percentage increase in life span of the tumor host and also to determine the mean survival time (MST). Antitumor activity of MeBG extract was assessed by observation of changes with respect to the following parameters [11].

Tumor volume and weight

The mice were dissected and the ascitic fluid was collected from the peritoneal cavity. Volume of the fluid was measured by taking it in graduated centrifuge tube and expressed in millilitre (ml). Tumor weight was measured by taking the weight of the mice before and after the collection of the ascitic fluid from peritoneal cavity and expressed in gram (g).

Percentage increase life span (ILS)

The effect of MeBG on tumor growth was monitored by recording the mortality of the experimental mice. The percentage increase in life span (ILS) was calculated by the following formula:

Mean survival time (MST) in days = (day of first death + day of last death)/2

ILS (%) = [(MST of the treated group/MST of the control group)-1] \times 100

Tumor cell (Viable/nonviable) count

The ascitic fluid was taken in a pipette and diluted upto 20 times with PBS solution. Then a drop of the diluted cell suspension was

placed on Neubauer's counting chamber and the number of cells in the 64 small squares were counted.

The viability and non-viability of the cell were determined by trypan blue assay. The cells were stained with trypan blue (0.4% in normal saline) dye. The cells that did not take up the dye were viable and those that took the dye were nonviable. These viable and non viable cells were counted using the under-mentioned formula:

Cell count = (number of cells \times dilution factor)/ (area \times thickness of liquid film).

Haematological parameters

The collected blood was used for the estimation of hemoglobin (Hb), red blood cell (RBC) and white (WBC) count by standard procedures [16].

Statistic analysis

All the experimental data are expressed as the mean SEM. The data was statistically analyzed by using one way Analysis of Variance (ANOVA) followed by Dunnett's post-hoc test by Instat using Graph Pad Prism 5.0.

RESULTS AND DISCUSSION

In *in vitro* cytotoxicity study of MeBG extract and WtBG extract, MeBG extract showed direct cytotoxic effect on the A-549 and DLA cell line in a concentration dependent manner and the IC_{50} value was found to be 143.4 μ g/ml (MeBG) and 210.8 μ g/ml (WtBG) 137.2 μ g/ml (MeBG) and 217.8 μ g/ml (WtBG) respectively while both the extract were less toxic to Vero cell line and IC_{50} value was found to be 148.7 μ g/ml (MeBG) and 152.6 μ g/ml (WtBG) Figure 1. *In vitro* cytotoxicity study shows that MeBG is more cytotoxic for DLA tumour cells and less cytotoxic for normal cell line i.e. A-549 cell line hence MeBG was taken for further *in vivo* anti tumour study.

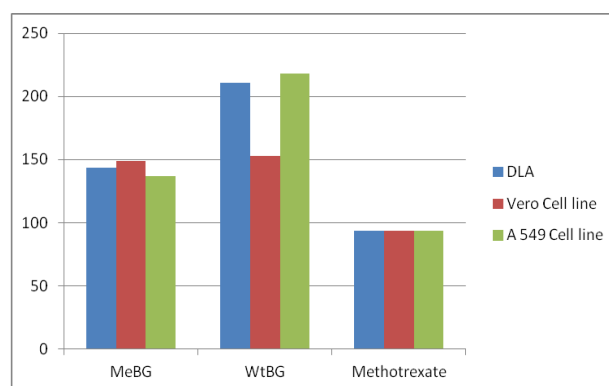


Fig. 1: CTC50 value by Trypan blue dye exclusion technique MeBG showed potent cytotoxicity activity against DLA and A-549 cell line.

Anti tumor activity of MeBG against DLA tumor bearing mice was assessed by the parameters such as tumor volume, tumor weight, cell count (viable and nonviable), mean survival time and percentage increase in life span. The tumor volume, tumor weight and viable cell count were found to be significantly ($P < 0.01$) increased and non viable cell count was significantly ($P < 0.01$) declined in DLA control animals, when compared with normal control animals (Table 1).

Administration of MeBG at the doses of 200 and 400mg/kg significantly ($P < 0.01$) decreased the tumor volume and viable cell count. Non viable cell count was significantly ($P < 0.01$) higher in MeBG treated animals comparing to DLA control animals. These results could connote either a direct cytotoxic effect of MeBG on tumor cells or an indirect local effect, which may involve macro- phage activation and vascular permeability inhibition.

Table 1: Effect of MeBG on tumor volume (ml), tumor weight (g), viable (cells × 10⁶ cell/ml) and nonviable cell count (cells × 10⁶ cell/ml), median survival time (MST), percentage increase life-span (% ILS) and haematological parameters like RBC (cells × 10⁶ μl⁻¹), WBC (cells × 10³ μl⁻¹) and HB content (g/dl) in DLA bearing mice.

Parameters	Normal control (5ml/kg)	DLA Control(2x10 ⁶ cell/ml)	DLA+MEBG (200mg/kg)	DLA+MEBG (400mg/kg)	DLA+5-FU(20mg/kg)
Tumor Volume	-	3.01 ± 0.18	1.81 ± 0.17 ^{b,*}	1.17 ± 0.15 ^{b,*}	0.61 ± 0.05 ^{b,*}
Tumor weight	-	2.92 ± 0.21	1.33 ± 0.14 ^{b,*}	0.98 ± 0.07 ^{b,*}	0.59 ± 0.08 ^{b,*}
Viable cell	-	8.55 ± 0.28	3.67 ± 0.18 ^{b,*}	1.40 ± 0.16 ^{b,*}	0.78 ± 0.04 ^{b,*}
Non Viable cell	-	0.35 ± 0.07	1.29 ± 0.15 ^{b,*}	2.57 ± 0.18 ^{b,*}	3.01 ± 0.13 ^{b,*}
MST (days)	-	21.6 ± 0.47	30.7 ± 0.90	37 ± 0.39	41.7 ± 0.65
%ILS	-	00	42.87	74.41	96.54
RBC	6.08 ± 0.06	3.14 ± 0.38 ^{a*}	4.13 ± 0.24 ^{b,*}	5.19 ± 0.18 ^{b,*}	5.75 ± 0.28 ^{b,*}
WBC	5.18 ± 0.06	12.14 ± 0.76 ^{a*}	8.39 ± 0.49 ^{b,*}	6.39 ± 1.12 ^{b,*}	5.78 ± 0.32 ^{b,*}
Hemoglobin	13.18 ± 0.06	7.21 ± 0.41 ^{a*}	9.18 ± 0.96 ^{b,*}	10.93 ± 0.60 ^{b,*}	11.83 ± 0.98 ^{b,*}

Values are represented as mean ± SEM, where n=6, ^a P<0.01 as compare to normal control, ^b P<0.01 as compare to DLA control

The prolongation of the animal life span was being considered as a reliable criterion for the depiction of efficacy of an anticancer agent [11]. Furthermore, the median survival time was increased to 30.7 ± 0.90 (%ILS = 42.87) and 37±0.39 (%ILS = 74.41) on administration of MeBG in a dose dependant manner. The rapid increase in ascitic fluid volume was observed in DLA bearing mice, ascetic fluid is the direct nutritional source for tumors growth it meets the nutritional requirements of tumor cells [17]. The increase of life span of tumor bearing mice indicates reduction of nutritional fluid volume and seization of the tumor growth is a positive result and further determines the antitumor effect of MeBG. The major problems encountered in cancer chemotherapy are myelosuppression and anemia. The anemia exhibited in tumor bearing mice is mainly due to reduction of RBC or hemoglobin percentage and etiology is either due to iron deficiency or hemolytic/myelopathic condition [10]. There was significantly (P<0.01) elevated level of WBC and significantly (P<0.01) reduced level of RBC and hemoglobin (Hb) in DLA control group as compared to normal control group (Table 1). But, treatment with MeBG at the doses of 200 and 400 mg/kg in DLA bearing mice significantly (P<0.01) increased both the RBC count and Hb content while WBC count was reduced significantly (P<0.01) when compared with the DLA control group.

CONCLUSION

The present study stating that the alcoholic leaf extract of *Barleria grandiflora* showed a significant *in vitro* and *in vivo* anti tumour activity against DLA cells. These important and significant preliminary finding can be taken as the basis upon which further studies should be carried out to delineate the detailed profile of these anti cancer actions of *Barleria grandiflora*.

REFERENCES

- Priya K, Krishankumari S, Vijaykumar M *Cyathula prostrata*: A potent source of anti cancer agent against Dalton's ascites in Swiss albino mice. Asian Pacific J Trop Med 2013; 6: 776-779.
- Rajkumar V, Guha G, Kumar A Antioxidant and anti neoplastic activities of *Picrorhiza kurroa* extract. Food Chem Toxicol 2011; 49: 368-369.
- Malcolm RA. Cancer: ENCYCLOPEDIA OF LIFE SCIENCES / Nature Publishing Group: 1-8, (2001).
- Mulla SK, Swamy P Anti cancer activity of ethanol and polyphenol extracts of *Portulaca quadrifida* Linn on human colon cancer cell lines. Intl Journal of Pharma and Bio Sci 2012; 3(3): 488 – 498.
- Jain R, Jain SK Screening of *in vitro* cytotoxic activity of some medicinal plants used traditionally to treat cancer in Chhattisgarh states, India. Asian Pacific J Trop Biomed 2011; 1: 147-150.
- Newman DJ, Cragg GM, Snader KM Natural products as sources of new drugs over the period 1981-2002. J Nat Prod 2003; 66: 1022-1037.
- Sawarkar HA, Khadabadi SS, Wandhare MD The Antioxidant Activity of the Leaves of *Barleria grandiflora* Dalz. (Acanthaceae). J of Herbal Med Toxicol 2009; 2: 63-66.
- Kirtikar KR, Basu BD. *Indian Medicinal Plant*, International Book Distributors, Dehradun, 2: 1876-1882 (1987).
- Yoganarasimhan SN. *Medicinal Plant of India Tamil Nadu*. Regional Research institute Bangalore India, 2: 69-71(2000).
- Evans WC. *Text book of Pharmacognosy* 14th ed Bailleire Tindwall W.B. Saunders Company Ltd London; 1996.
- Haldar PK, Bhattacharya S, Kar B, Bala A, Mazumder UK Chemopreventive role of Indigofera as palathoides in 20-methylcholanthrene induced carcinogenesis in mouse. Toxicol and Enviro Chem 2010; 92: 1749–1763.
- Manojkumar P, Kochupappy RT, Subbucchettiaar G Synthesis of coumarin heterocyclic derivatives with antioxidants activity and *in vitro* cytotoxic activity against tumor cells. Acta Pharmaceutica 2009; 5: 159-170.
- Patel S, Gheewala N, Suthar A, Shah A *In vitro* cytotoxicity activity of *Solanum nigrum* extract against Hela cell line and vero cell line. Int J of Pharm and Pceutical Sci, 2009; 1: 38-46.
- OECD, 2008 guidelines for testing of chemicals/ Section 4: Health Effects Test No. 425: Acute oral Toxicity: Up and Down procedure. Organization for Economic Co-operation and development Publishing Paris.
- Bala A, Kar B, Haldar PK, Mazumder UK, Bera S Evaluation of anticancer activity of *Cleome gynandra* on Ehrlich's ascites carcinoma treated mice. J Ethno pharmacol 2010; 129:131–134 (2010).
- D'Armour FE, Blood FR, Belden DA The Manual for *Laboratory Work in Mammalian Physiology* 3rd ed. The University of Chicago: Chicago 1965.
- Kumar AS, Juvvadi RK, Kumar VK, Reddy M, Reddy V Effect of methanolic extract of *Adenathera palvonina linn* on Dalton's Ascitic Lymphoma. Indian J Res in Pharm and Biotech 2011; 1: 138-141.
- Kumar A, Kaurand R, Arora S Free radical scavenging potential of some Indian medicinal plants. J of Med Plants Res, 2010; 4 (19) : 2034-2042.