

## Evaluation of Antitumor Activity of Bark Extract of *Magnolia grandiflora* Linn. *in vivo* and *in vitro*

L. S. Singh<sup>+</sup>, Montessori Sagolsem and G. J. Sharma

Centre of Advanced Study in Life Sciences, Manipur University, Imphal- 795003, India

**Abstract.** Antitumor activity of 50% ethanol bark extract of *Magnolia grandiflora* Linn. was evaluated against tumors induced in mice using dimethyl benzanthracene (DMBA) and 3-methyl cholanthrene (MC). The activity was assessed using the ability of the plant extract in reducing tumor weight, tumor volume and lung weight. The plant extract treated groups showed significant reduction in tumor weight, tumor volume and lung weight. The cytotoxic/cytostatic activity of the plant extract on human cancer cell line Bu25Tk<sup>-</sup> cells was also evaluated using 3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. Cells at proliferative phases were treated with the plant extracts and incubated for 48 hrs at 37°C, 5% carbon dioxide (CO<sub>2</sub>) and 100% relative humidity. After 48 hrs, MTT working solution was added to the culture, incubate for 4 hrs and centrifuged at 1700 rpm for 10 min. The supernatant was removed and dimethyl sulfoxide (DMSO) was added, thoroughly mixed and the absorbance was measured at 546 nm. The results of the present experiment indicate the ability of the plant extract in reducing the tumor weight and volume as well as lung weight in mice and growth of human cervical cancer cell Bu25Tk<sup>-</sup> on a dose dependent manner.

**Keywords:** Carcinogen, Cervical carcinoma, 3-(3,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), IC<sub>50</sub>, Histopathology

### 1. Introduction

Plants have been used in traditional medicine for the treatment of cancer and 60% of the currently used anti cancer agents are derived from natural sources including plants [1]. *Magnolia grandiflora* Linn. (Family: Magnoliaceae) is traditionally used in the treatment of various diseases including inhibition of skin tumor promotion [2]. It also has antioxidant and antineoplastic properties [3,4,5,6]. The present study was carried out to evaluate the anti-tumor properties of 50% ethanol extract of the bark of *M. grandiflora* Linn. against the tumors induced in Swiss albino mice and human cervical cancer cell Bu25Tk<sup>-</sup>.

### 2. Materials and Methods

#### 2.1. Plant Material and Extraction

The bark of *Magnolia grandiflora* Linn. was collected from the campus of Manipur University. The plant was identified at the Botanical Survey of India (BSI), Shillong. The herbarium of the plant was deposited to the BSI, Shillong (Accession Number: 18972). The barks were washed in tap water and shade-dried at room temperature. The dried parts were kept in the oven at 55-60°C for a week and ground to coarse powder. The powdered parts were again extracted using Soxhlet apparatus with 50% ethanol as solvent, concentrated using rotary vacuum evaporator, lyophilized and kept at 4°C.

#### 2.2. Chemicals

Fetal bovine serum (FBS) was purchased from Invitrogen. Dulbecco's modified eagle medium (DMEM), dimethyl sulfoxide (DMSO), trypsin phosphate versene glucose (TPVG), 3-(3,5-dimethylthiazol-2-yl)-2,5-

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<sup>+</sup> Corresponding author.  
E-mail address: gjs1951@yahoo.co.in

diphenyl tetrazolium bromide (MTT), dimethyl benzanthracene (DMBA), 3-methyl cholanthrene (MC) were procured from Sigma Aldrich, St. Louis, USA. All other chemicals and reagents were of analytical grade and obtained from Merck, India.

### 2.3. In Vivo Tumor Growth Inhibition

Tumors were induced in the Swiss albino mice (*Mus musculus*) having an average weight of 20±2 gm and 10-12 weeks old. The animals were housed in animal cages (Tarsons 29X22X14 cm<sup>3</sup>) bedded with rice husk in a hot and cold controlled air-conditioned room (temperature 22±1 °C, humidity 85% and 12 hrs dark/light cycle) with standard laboratory diet and water *ad libitum*. The study was conducted after obtaining Institutional Ethics Committee approval and guidelines of the Committee for the Purpose of Control and Supervision of the Experiments on Animals (CPCSEA) were followed. 1mg of DMBA dissolved in 0.5 ml of olive oil was orally administered to mice once a week for six consecutive weeks. Three months after the administration of DMBA, the mice were again administered a single dose of 1mg MC dissolved in 0.5 ml of olive oil. The animals were separated into 2 groups of 15 animals each— first group received 0.5 ml PBS daily and second group was given 833 µg of plant extract per kg body weight dissolved in 0.5 ml of PBS. The animals receiving neither DMBA nor MC nor plant extracts were used as control group. At the first and last day of experiment, the body weight of each mouse was measured. The changes in dietary habit of the animals during the experiment were observed. At the end of the experiment, mice were sacrificed and necropsies were performed. Weights of the vital organs as well as tumor were measured. The tumor volume was estimated on the basis of two dimensional tumor measurements and resorting to the formula suggested by Kato *et al.* 1994 [7]:

Tumor volume =  $0.5AB^2$  where A is the longest diameter and B, the shortest diameter of the tumor.

The tumor inhibitory rate was calculated using the formula:

Tumor inhibitory rate (%) =  $100 \times (\text{mean tumor weight in control mice} - \text{mean tumor weight in treated mice}) / \text{mean tumor weight in control mice}$ .

### 2.4. Histopathological Examination

Tumors developed in the lungs were taken and fixed at 10% paraformaldehyde (pH 7.5) and processed for paraffin embedding according to the standard histological procedures. 5µm thick sections were prepared and stained with hematoxyline-eosine (HE) stain. Histopathological examinations were completed using Leica light microscopy.

### 2.5. MTT Assay

The effect of the plant extract on the growth of human cervical cancer cell line Bu25Tk<sup>-</sup> was evaluated *in vitro* using MTT assay with slight modification [8]. Human cervical cancer cell Bu25Tk<sup>-</sup> was procured from National Centre for Cell Science, Pune. Exponentially growing cells were maintained in a humidified atmosphere of 5% CO<sub>2</sub> at 37°C. Medium was changed at 48 hrs intervals. Cells at the logarithmic growth phase were used for the assay. Single cell suspensions were obtained by trypsinization of the monolayer cells of the adhesive cultures. Counting of the cells was done with haemocytometer. 100 µl of cell culture containing 400–1000 cells were dispensed in 96-well culture plates. Following 24 hrs incubation at 37°C, 5% CO<sub>2</sub>, 100% RH (Shellab 2123 CO<sub>2</sub> incubator), 100 µl of plant extract at different concentrations or culture medium or drug carrier (PBS) were dispensed and incubated for 48 hours. After 48 hrs, 50µl MTT working solution (1mg/ ml PBS) were added to each well, incubated for 4 hrs and centrifuged at 1700 rpm for 10 minutes. Supernatant was removed from the wells using 18 gauge needle leaving around 20–30 µl in each well. 150 µl of DMSO (Sigma) were added to each well. The plate was thoroughly mixed using a plate shaker and measured the absorbance at 546 nm with Huma Reader HS human microwell plate reader. All results represented the average of 9 wells. Percent inhibition of growth of the cells was calculated by using the formula:

Inhibition of growth % =  $\{1 - (\text{Test absorbance at 546 nm} / \text{control absorbance})\} \times 100$ .

### 2.6. Statistical Analysis

All values were expressed as mean±SEM. The data were statistically analyzed by one-way ANOVA, followed by Duncan multiple comparison test. P values < 0.05 were considered significant.

### 3. Results and Discussion

#### 3.1. In Vivo Tumor Growth Inhibition

DMBA is one of the most potent tumor initiating agents known. Besides, initiation and cause of tumors in skin, mammary tissue and kidney, it also causes tumors in lungs [9]. Application of MC also causes tumors in lungs [10]. When a single carcinogen is applied to the animals at a time, the rate of development of tumor is slow and the number of tumor developed is small. It was found that if both the carcinogens were applied at the same time, then the rate of tumor development was fast as well as number of tumors developed were large. All the animals exposed to carcinogens developed tumors in the lungs while no tumor was developed in the control animals. When the animals were exposed to carcinogens (1mg DMBA dissolved in 0.5 ml olive oil, given orally once a week for six consecutive weeks followed by a single dose of 1 mg MC dissolved in 0.5 ml olive oil) in combination, good number of tumors were induced in the lungs (Fig. 1a). The development of tumors was confirmed by histopathological examination (Fig. 1b).

The weight as well as the volume of the tumors developed in the carcinogen exposed *M. grandiflora* bark extract untreated animals were larger than the tumors developed in the carcinogen exposed plant extract treated animals. The present study showed the anti-tumor activity of the 50% ethanol bark extract of *M. grandiflora*. The treatment of the animals with *M. grandiflora* bark extract reduced the volume of the tumors induced by carcinogens. In the control group, the average tumor volume was  $0.555 \pm 0.072 \text{ cm}^3$  but it was reduced to  $0.375 \pm 0.051 \text{ cm}^3$  in the animals treated with the plant extract showing significant reductions can be effected in the volume of the tumors in animals by 32.43% ( $p < 0.001$ ). The weight of the tumor was also reduced in the plant extract treated groups by 22.38% ( $p < 0.05$ ) compared to the control group (Table 1). The above results indicate that the 50% ethanol bark extract of *M. grandiflora* is active in the reduction of the tumor volume as well as tumor weight induced by carcinogens DMBA and MC, and the results obtained corroborates the results obtained by Park *et al.* 2008 [11], Lee *et al.* 1991 [12] and Roomi *et al.* 2009 [13].

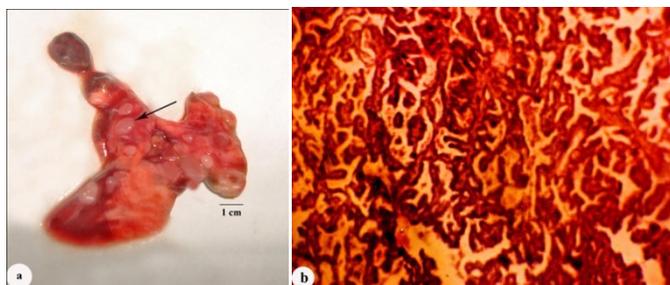


Fig. 1: Tumors (a) in the lungs of mice and (b) section of tumor, 10X magnification

Table 1: Effect of plant extract (PE) on tumor volume, tumor weight and lungs weight of mice

Organ/Tumor	Effects
Tumor volume	32.43% reduced, $p < 0.001$
Tumor weight	22.38% reduced, $p < 0.05$
Lung weight	30.78% reduced, $p < 0.001$

The two carcinogens did induce tumors in the lungs of the animals. The average weight of the lungs was greater in the carcinogen-treated control animals than the untreated control ones. The average weight of the lungs in the plant extract treated animals was reduced significantly from the carcinogen-treated animals but higher than that of the carcinogen untreated control ones. In the plant extract treated animals, there was a reduction in the average weight of the lungs by 30.78% ( $p < 0.001$ ) than the carcinogen-treated control group (Table 1). There was no significant change in the average body weight of the carcinogen treated control groups and plant extract treated groups in the animals. Since there was no significant difference in mean body weights of the mice, these findings indicate that the observed differences in the average lung weight among the groups of mice was due to difference in the tumor growth.

The mean number of tumors, on the other hand, did not show any significant changes. The mean number of tumors in the carcinogen (DMBA/MC) treated control group was  $15.33 \pm 2.10$  and that of the plant extract treated groups was  $15.75 \pm 1.97$ . Similarly, there was no significant change in the morphology as well as the weights of other vital organs between the carcinogen exposed plant extract treated group and carcinogen unexposed control group (Table 2).

Table 2: Effect of plant extract on weights of body, vital organs and tumors on male mice

Body/organ	Body/Organ weight (gm)	
	Control (mean $\pm$ SE)	PE (mean $\pm$ SE)
Whole body	16.19 $\pm$ 0.91	16.33 $\pm$ 0.70
Liver	2.44 $\pm$ 0.10	2.28 $\pm$ 0.11
Lung	1.27 $\pm$ 0.10	0.85 $\pm$ 0.05
Kidney	0.77 $\pm$ 0.05	0.70 $\pm$ 0.03
Heart	0.24 $\pm$ 0.04	0.24 $\pm$ 0.19
Spleen	0.11 $\pm$ 0.012	0.11 $\pm$ 0.11
Tumor number	15.33 $\pm$ 2.10	15.75 $\pm$ 1.97
Tumor weight*	218.54 $\pm$ 10.97	169.63 $\pm$ 10.57
Tumor volume**	0.55 $\pm$ 0.07	0.37 $\pm$ 0.05

\*Tumor weight expressed in mg

\*\* Tumor volume expressed in  $\text{cm}^3$

All the organs have been examined and found to be free of tumors, except in the lungs. At the given dose of the *M. grandiflora* bark extract which was administered orally, the animals did not show any sign of toxicity. Neither the life span nor the body weight of the plant extract given animals decreased compared to the carcinogen exposed control group.

To determine whether there was a relationship among the lungs weight and tumor volume, lungs weight and tumor weight and tumor volume and tumor weight, *in vivo* study data were pooled and analyzed. The analysis showed that there was a significant positive correlation between lungs weight and tumor volume (correlation coefficient  $r = 0.5660$ ,  $p < 0.0001$ ), lungs weight and tumor weight (correlation coefficient  $r = 0.7685$ ,  $p < 0.0001$ ) and tumor volume and tumor weight (correlation coefficient  $r = 0.6614$ ,  $p < 0.0001$ ).

### 3.2. Histopathological Examination

Histopathological observation showed the loss of characteristic features of the normal lung cells in the tumors obtained from the lungs of carcinogen exposed animals. Neoplastic and hyperplastic cells were present and the cells become elongated and spindle-shaped.

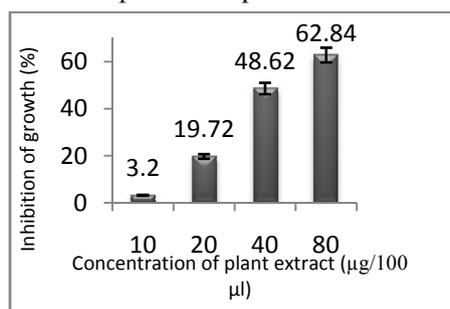


Fig. 2: Effect of plant extract on the growth of human cervical cancer cell Bu25Tk<sup>-</sup>.

### 3.3. MTT Assay

The effect of the plant extract on the growth of human cervical cancer cell line Bu25Tk<sup>-</sup> was evaluated by MTT assay. When the cells were incubated with the 50% ethanol bark extract of *Magnolia grandiflora* for 48 hrs with various concentration of the plant extract (10-80  $\mu\text{g}/100 \mu\text{l}$ ), relative growth of the cells reduced progressively in a dose dependent manner. The  $\text{IC}_{50}$  of the plant extract was found to be  $55.11 \mu\text{g}/100 \mu\text{l}$ . The *in vitro* studies of Mohamed *et al.* 2009 [14] showed that the bio-active compounds magnoflorine and lanuginosine present in this plant had cytotoxic activities in cancer cells HEPG2 (human hepatocellular carcinoma cell) and U251 (human brain tumor cell). The result of the present work also

indicates the inhibitory effect of this plant on the growth of human cervical cancer cell Bu25Tk-. These perhaps suggest that the 50% ethanol extract of the *M. grandiflora* bark has the cytotoxic activity against tumor cells *in vitro*.

#### 4. Acknowledgement

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