

Full Length Research Paper

Antitumor and antimicrobial activities and inhibition of *in-vitro* lipid peroxidation by *Dendrobium nobile*

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The aim of the study was to determine the antitumor and antimicrobial activities as well as the inhibition of *in vitro* lipid peroxidation evoked by *Dendrobium nobile*. Antimicrobial activity of different solvent extracts of flowers and stem of *D. nobile* against *Escherichia coli*, *Bacillus subtilis*, *Proteus*, *Salmonella typhi* and *Staphylococcus aureus* were analyzed separately. The swiss albino mice were induced with Dalton's Lymphoma Ascites (DLA) cell lines and the antitumor effect of *D. nobile* was studied by administering orally the aqueous extracts of the flowers of *D. nobile*. The antitumor activity was measured in DLA cell line induced mice. Inhibition of *in vitro* lipid peroxidation activity of the *D. nobile* in both liver homogenate and RBC ghosts was also carried out. The aqueous extracts of stem and flower of *D. nobile* showed better zone of bacterial inhibition than that of ethanol and chloroform extracts. The plant extracts treated DLA cell induced mice showed better tumor regression, and the flowers of the plant inhibited the *in vitro* lipid peroxidation, significantly. The present work indicates that the *D. nobile* flower extracts possessed antimicrobial and antitumor properties in addition to antiperoxidative activity.

Key words: Antimicrobial activity, antitumor activity, *Dendrobium nobile*, DLA cell line, lipid peroxidation inhibition.

INTRODUCTION

Today more and more people take plant medicine as an alternate therapy. The basic similarity of all life chemistry has inspired the use of plants as biopharmaceuticals (Taylor, 2001). Medicinal plant exhibits antibacterial activity since they contain innumerable biologically active chemical constituents. The use of plant preparation as foodstuff, insecticides, CNS active, cardioactive, antitumor and antimicrobial agents are some examples of immense chemical diversity in plants, which are as old as mankind (Al-Sereiti et al., 1999).

Orchids are known all over the world as plants of ornamentation and referred to as gems in the field of horticulture. The variation in color and form of flower is indeed fantastic and the flower mimic bee, wasps, butterflies, moths, swans, doves, frogs, lizards and even human (Khoshoo, 2000). They are flowering plants and are estimated to have 25,000 to 35,000 species (Saito

et al., 1994). Orchids have long been used for medicinal purposes. In ancient China during 2800 B.C. there are records of some species of *Dendrobium* used for medicinal purposes (Hedge and Ingal halli, 1988). In ancient India, orchids used for medicinal purposes were described as medicinal and ornamental plant. Indigenous people of Eastern Himalayas believe that *Dendrobium nobile* flowers can cure eye ailments (Mandal and Datta, 2003).

Cancer, characterized by uncontrolled growth and spread of abnormal cells, is caused by both external and internal factors. Environmental factors and chemical carcinogens play a predominant role in the induction of DNA lesions and other genomic abnormalities (Madhumitha et al., 2004). Developments of anticancer drug is always a fascinating challenge and are classified in various ways according to their nature, sources, mechanisms of action, etc, which may be of synthetic chemicals, microbial products, plant or animal extracts (Sanyal et al., 2002).

D. nobile has compounds called gigantel and moscatilin which have antimutagenic activity (Miyazawa et al.,

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1997). The plant has two phenanthrenes which also have anticancer activity (Jin-Ming et al., 2003). Lipid peroxidation (LPO) has been identified as one of the basic reactions involved in free radical induced cellular damages in cells (Hallwell and Gutteridge., 1992). Antioxidants are a broad group of compounds that destroy reactive oxygen species and protect the body against oxidative damage to cells (Express Pharma Plus, 1999). The main objective of the present study is to: 1) test the antimicrobial activity of *D. nobile* on different pathogenic microorganism, 2) evaluate the antitumour activity of the flower extract of *D. nobile* in DLA (Dalton's Lymphoma Ascites) induced mice and 3) determine the *in vitro* inhibition of lipid peroxidation in liver homogenate and erythrocyte ghost models by stem and flower extract of *D. nobile*.

MATERIAL AND METHODS

Collection of plant materials

Fresh plant materials of *D. nobile* were collected from Flowers Blooms Bouquets Shop in Coimbatore, Tamilnadu, India.

Preparation of plant extract

Approximately 10 g of samples were weighed and homogenate using mortar and pestle. Homogenate is transferred into 3 conical flasks. Then ethanol, chloroform and distilled water were added respectively and kept in a shaker for 48 h. After filtering the contents using Whatmann No 1 filter paper, the supernatant was kept in an electric water bath at 100°C to evaporate fully. The remaining powder was then taken for assay by dissolving each extracts with 1 ml of respective solvents.

Collection of microbial strain

The strains were collected from the Postgraduate Department of Microbiology and Biotechnology of Dr. N. G. P. Arts and Science College, Coimbatore, Tamilnadu, India. The bacterial strains used in this study were *Escherichia coli*, *Bacillus subtilis*, *Proteus*, *Salmonella typhi* and *Staphylococcus aureus*.

Preparation of bacterial culture

The stock culture of bacteria was maintained on nutrient agar medium at 37°C for 24 h that was used in the antimicrobial screening. The antimicrobial activity of the above mentioned different solvent extracts of flower and stem were assayed separately using the strip plate method employing overnight cultures of test bacterial strain and the resultant zone of inhibition was measured. The standard antibiotic ampicillin was used for comparison.

Animals

Swiss Albino mice (male) were housed in well ventilated cages and fed with standard mouse chow (Lipton India Ltd) and water ad libitum. Animal experiments conducted during the present study were approved by Institutional Animal Ethics Committee (IAEC).

The Wistar strains of male albino rats weighing between 100-150

gm were purchased from Perundurai Medical College, Erode, Tamilnadu, India. The animals were housed in larger spacious cages and they were fed with commercial pelleted rat chow marketed by Hindustan Lever Ltd. Bangalore, India under the trade name "Gold Mohur Rat Feed" and had free access to water ad libitum. The animals were well acclimatized to standard environmental conditions of temperature (22°C ± 5°C) and humidity (55 ± 5 %) for 12 h light-dark cycles throughout the experimental period. The animals used in the present study were approved by the institutional Ethical Committee constituted as per the directions of the committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) under Ministry of Animal Welfare Division, Government of India, New Delhi, India.

In the experiment, a total of 24 mice were used. The experimental animals were divided into four groups:

- A): Normal untreated (Containing 8 mice)
- B): Negative control - DLA cell line alone (Containing 8 mice)
- C): Curative control - DLA cell line with Stem extract (Containing 8 mice)
- D): Curative control - DLA cell line with Flower extract (Containing 8 mice).

Cell line

Dalton's Lymphoma Ascites (DLA) cells were obtained from Amala Cancer Institute Kerala, India. 1×10^6 cells in 0.1 ml of phosphate buffered saline (PBS) were injected in the peritoneal cavity of the mice.

Propagation of tumor cell line

Dalton's Lymphoma Ascites (DLA) cells were propagated as transplantable ascites tumors in Swiss albino mice (male). Four peritoneal cavities of mice were washed thrice with saline and one million cells were inoculated to fresh animals (Groups B and C mice) to propagate the cell lines.

Preparation of plant extract

Flower was extracted with distilled water. The extract was concentrated and evaporated to dryness. The yield of the extract was 10%. The dried extract suspended in distilled water and is used for further studies. The flower extract (50 µg) was given orally to Group C mice every day for 15 days, and then the peritoneal cavity layer was subjected to histopathological examination.

Induction of *in vitro* lipid peroxidation

An *in vitro* model of goat liver homogenate and RBC ghosts was used for induction of lipid peroxidation, mediated by FeSO₄ as prooxidant. Application of the relevant extract of *Dendrobium* flower and stem in the medium was tried with an objective of assessing the extent of inhibition of *in vitro* lipid peroxidation by the measurement of thiobarbituric acid reactive substances (TBARS) in the experimental mixtures. TBARS were measured spectrophotometrically at 535 nm. The extent of inhibition of lipid peroxidation in the presence and absence of the extract was determined in liver as proposed by Okhawa et al. (1979) and in RBC membrane as proposed by Dodge et al. (1963).

RESULTS AND DISCUSSION

The extracts of (flower and stem) of *D. nobile* were tested

Table 1. Antimicrobial activity of stem and flower extracts of *Dendrobium nobile* (strip plate method).

Name of the organism	Zone of inhibition of stem extract (diameter in cm)					
	Ethanol (50 µg)		Chloroform (50 µg)		Aqueous (50 µg)	
	Stem	Flower	Stem	Flower	Stem	Flower
<i>Bacillus subtilis</i>	0.86±0.11	0.8±0.2	0.7±0.14	0.6±0.11	1.2±0.20	0.86±0.2
<i>Escherichia coli</i>	0.6±0.2	0.73±0.11	0.26±0.11	0.3±0.11	0.46±0.12	0.53±0.11
<i>Proteus</i>	0.36±0.05	0.6±0.2	0.46±0.11	0.6±0.11	1.13±0.13	1.16±0.15
<i>Staphylococcus Aureus</i>	1.03±0.15	1.0±0.2	1.0±0.2	1.0±0.15	1.2±0.10	1.2±0.2
<i>Salmonella typhi</i>	1.2±0.2	0.86±0.20	0.73±0.11	0.88±0.11	0.46±0.12	0.7±0.1

Values are mean ± SD triplicates.

Table 2. Comparison of antimicrobial activity of aqueous extract of stem and flower of *Dendrobium nobile* with standard antibiotic (ampicillin).

Organism	Control (ampicillin) (diameter in cm)	Aqueous extract (diameter in cm)	
		Stem	Flower
<i>Bacillus subtilis</i>	2.2	1.2±0.20	0.86±0.20
<i>Escherichia coli</i>	1.5	0.46± 0.11	0.53± 0.11
<i>Proteus</i>	2.0	1.13± 0.11	1.16± 0.15
<i>Staphylococcus aureus</i>	2.2	1.2 ± 0.10	1.2 ± 0.20
<i>Salmonella typhi</i>	1.0	0.46± 0.11	0.7± 0.10

Table 3. Histopathology study in normal and *Dendrobium nobile* extract treated mice.

Pathological features	Animal groups			
	A	B	C	D
Microscopic features regarding presence of cancer cells in peritoneum	-	+++++	++	Occasional

- = Negative (no cancer cells); + + + + + = plenty of cancer cells are present; + + = few cancer cells are present; occasional = cancer cells are present sparsely.

for their antimicrobial activity against *E. coli*, *B. subtilis*, *Proteus*, *S. typhi* and *S. aureus*. Among the four different extract of flower and stem used, namely petroleum ether, ethanol, chloroform and aqueous, the aqueous extract of flower and stem of *D. nobile* showed better zone of bacterial inhibition when compared with other three extracts (Table 1). The outcome of the study was compared with the standard antibiotic ampicillin and indicated in Table 2.

In comparison, the pathogenic microorganism was more sensitive towards aqueous extract of flower than the aqueous extract of the stem. Enthused with the above result, a protective role of only the aqueous extract of flower *D. nobile* against tumor cell lines in mice was carried out. The peritoneal cavity layer of Groups A, B and C mice were subjected to histopathological study. The outcome of the histopathological study is represented in Figure 1. The aqueous flower extracts of *D. nobile* were used for the study of *in vitro* cytotoxicity. The anti-oxidative activity of *D. nobile* in both liver homogenate

and RBC ghost is represented in Figure 2. Group A (normal mice) had normal fibrous peritoneal tissue. Group B (mice with only DLA induced cancer) showed abundant malignant epithelial growth in peritoneum. Group C (cancer induced mice treated with *D. Nobile* stem extract) showed very much regressed malignant epithelial growth with peritoneal tissue. While Group D (cancer induced mice treated with *D. nobile* flower extract) also showed very much regressed malignant epithelial growth with peritoneal tissue (Table 3). Remission of cancer cells is very much significant in the flower extracts of *D. nobile*.

A dedicated balance between the availability of the substrate for lipid peroxidation (namely PUFA) in membranes and cellular antioxidant system, are essential to protect body against oxidative stress (Narayana, 2000). The percent inhibition of LPO by the aqueous extracts of stem and flowers of *D. nobile* against goat liver homogenate and RBC ghosts are given in Figure 2. The *in vitro* LPO was inhibited to a good extent by the

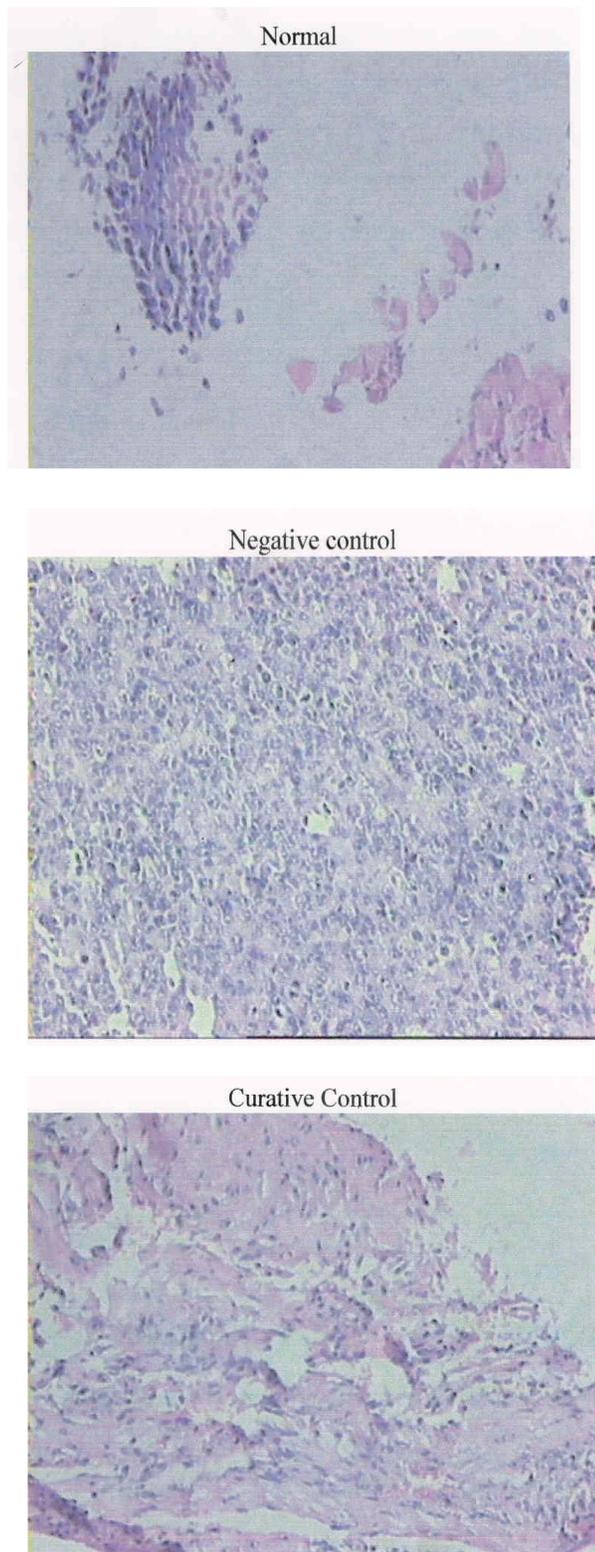


Figure 1. Results of the histopathological study.

stem and flowers of *D. nobile*, the extent of inhibition by flowers and stem being higher in RBC membrane model than that of liver homogenate. This difference could be

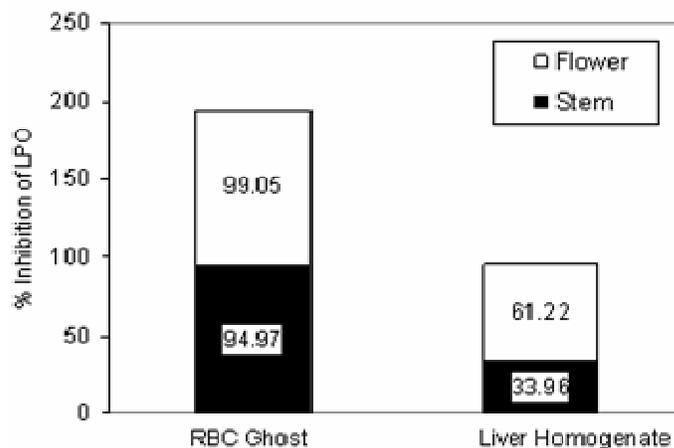


Figure 2. Extent of inhibition of *in vitro* lipid peroxidation in RBC ghost and goat liver homogenate by the *Dendrobium nobile*

due to the nature of the RBC ghosts, which contain plasma membranes alone, and liver homogenate that may contain other lipid constituents like intracellular membrane having different lipid composition. Khajuria (1996) has reported that the mammalian cells have evolved myriad interrelated antioxidant defense mechanisms, which minimize the injurious events that result from toxic chemicals and normal oxidative products of cellular metabolism. But the extent of inhibition differed with the type of extract used, indicating that the antioxidants present in the flowers and stem react differently towards different lipid groups.

In vitro cytotoxicity assay indicated that the percentage of cytotoxicity is maximized against DLA cell lines at the dose of 50 μ g of the flower extract of the plant.

Conclusion

The present findings demonstrate that the aqueous extracts of stem and flowers of *D. nobile* showed better zone of bacterial inhibition than ethanol and chloroform extracts. However, the flower extract has more antimicrobial and antitumor activity. Moreover its antiperoxidative effect could represent a protective role of the plant extract against the free radical mediated tissue damage. Hence the present study supports the possibility of including stem and flowers of *D. nobile* as a source of medicinal preparations to combat the plethora of disorders linked to oxidative damage. Progression of studies in this medicinal plant will help its use to control diseases and infections.

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