Anticarcinogenic activity of the methanolic extracts of *Pandanus odoratissimus*

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**ABSTRACT**

The methanolic extract of *Pandanus odoratissimus* was evaluated for its anticarcinogenic activity against 7, 12-dimethylbenzanthracene (DMBA) in Swiss albino mice. Methanol extract of *P. odoratissimus* on skin papilloma, at low dose of 50 mg/kg body weight was treated to papilloma induced mice. It is observed that the average number of tumour per tumour per tumour (tumour burden) was reduced from 6.46 ± 2.61 to 3.88 ± 2.69, whereas at high dose 100 mg/kg body weight skin papilloma treated group have showed the tumour per tumour (tumour burden) 3.12 ± 2.4 when compared to control 6.46 ± 2.61, after methanolic extract treatment.

**KEY WORDS:** Pandanus odoratissimus, Methanolic extract, Anticarcinogenic, 7, 12-dimethylbenzanthracene (DMBA), mice.

**INTRODUCTION:**
Cancer is the leading cause of mortality across the world and the failure of conventional chemotherapy to effect major reduction in the mortality indicates that new approaches are essentially needed (Kapadia GJ et al., 2000). An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents to block the development of cancer in humans (Gupta M et al., 2004). A variety of bioactive compounds and their derivatives have been shown to inhibit carcinogenesis in a number of experimental system involving initiation, promotion and progression (Ho CT et al., 1994). Plants, vegetables and herbs are used as folk drug and traditional medicines have been accepted currently as one of the main sources of cancer chemoprevention drug (Abdullaev et al., 1998).

Development of cancer is the result of a continuous process that occurs several years during which time there is a damage of numerous regulatory genes eventually may result in premalignant and then metastatic stages (Belinsky N and Jaiswal AK., 1993). Cancer is a heterogeneous disease composed of complex genetic changes driving uncontrolled growth and metastatic spread (Chemoprevention working group, 1999). Reactive oxygen species and reactive metabolic intermediates generated from various chemical carcinogens are known to play an important role in cell damage and in the initiation and progression of carcinogenesis (Hakama M., 1998).

Pandan is said to be a restorative, deodorant, indolent and phylactic, promoting a feeling of wellbeing and acting as a counter to tropical lassitude. It may be chewed as a breath sweetener or used as a preservative on foods. It is also said to have healthful properties, including antiviral, anti-allergy, antiplatelet, anti-inflammatory, antioxidant and antitumor activity. Ayurvedic science finds the medicinal action of the essential oil yielded by the screw pine’s highly scented flowers to be useful in headaches, earaches, and as a liniment for rheumatic pains. The distilled water extract of the flowers of *Pandanus odoratissimus* are used for inducing perspiration. It is also prescribed as a stimulant and an antispasmodic. The flowers themselves are powdered and included in medicines, which are either sniffed like snuff or smoked for asthma and other bronchial infections (Keerthikar and Basu., 2000). Hence, the present investigation is aimed to study anticarcinogenic property of *Pandanus odoratissimus*.

**MATERIAL AND METHODS:**

**Collection of sample:**
Leaves of the plants *Pandanus odoratissimus* (PO) were collected from Gurmitkal, near Gulbarga district. The samples were authenticated in Botany Department, Gulbarga University, Gulbarga. Plant materials were shade dried and stored in container and were powdered to mesh as and required. The drug was prepared by taking amount of volume/weight.

**Preparation of Extract:**
The dried material of *Pandanus odoratissimus* was taken for extraction of bioactive compounds with methanol using Soxhlet apparatus. The solvent was removed under reduced pressure and a semi-solid mass was obtained. The extract at different doses of 50 and 100mg/Kg body weight was suspended in Tween 80 solution was used for the treatment.

**Animals**
Swiss albino mice of 8-9 weeks old, weighing 25 ±2g from inbred colony were used for the study. They were maintained in animal house at Department of Biotechnology, Gulbarga university, Gulbarga, with a 12–h light/12-h dark cycle, and fed with standard food pellets as described by Central Food and Technological Research Institute (CFTRI, Mysore) formula and provided tap water ad libitum.

7, 12-dimethylbenzanthracene (DMBA) obtained from sigma chemical Co., DMBA was dissolved in acetone at a concentration of 0.05 mg in 0.05 ml acetone. *P. odoratissimus* suspension was prepared in a mixture of acetone and double distilled water (ratio 1:1 ml) and was administered topically to mice at two different dose level viz., 50 and 100mg/kg body weight/day.

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The DMBA induced skin papillomagenesis was studied in Swiss albino mice as described (Yasukawa et al., 1995). The hairs on the dorsum (2 cm diameter) of the mice were clipped off three days before the application of the chemicals or modulator and animals in the resting phase of hair growth cycle were selected for the experiment. The animals were assorted into following groups. Each group consist Minimum six animals.
Group-I
This group animals are treated with the mixture of acetone + double distilled water (ratio 1:1ml) topically for 21 days which was used as vehicle for suspending the modulator. On day 14, a single dose of DMBA (0.05mg/0.05ml acetone) was applied on the shaved area of skin. Two weeks after the carcinogen application, 0.05 ml of 1% croton oil in acetone was applied twice a week continued until termination of the experiment and this group is served as positive control.

Group-II
All animals of this group are treated topically with 0.1ml of methanolic extract of *P. odoratissimus* leaf suspended in the control vehicle at dose of level of 50 mg/kg body weight for 21 days. On 14th day DMBA (0.05mg/0.05ml acetone) was topically applied to these animals in the shaved area after a gap of minimum 6 h after treatment with the modulator followed by croton oil treatment as given to positive control group I.

Group-III
All animals of this group were treated topically with 0.1ml of Methanolic extract of *P. odoratissimus* leaf suspended in the control vehicle for 21 days at high dose of 100 mg/kg body weight. On 14th day DMBA (0.05mg/0.05ml acetone) was topically applied to these animals in the shaved area after a gap of minimum 6 h after treatment with the modulator followed by croton oil.

Papillomas appearing in the shaved area were recorded at weekly intervals and the papillomas measure greater than 1 mm in diameter was considered as significant and included in data analysis only if they persisted for 2 weeks or more.

Table- 1: Effect of two different doses of pandanus odoratissimus on DMBA induced skin papillomagenesis in mice.

<table>
<thead>
<tr>
<th>Group &amp; Treatment</th>
<th>Body weight (g)</th>
<th>Total tumor incidence (%)</th>
<th>Tumor burden (tumor/mice)</th>
<th>% inhibition of tumor burden</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
<td>final</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Only vehicle + DMBA</td>
<td>27.5±3.0</td>
<td>33.75±2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>+ croton oil</td>
<td>69.85%</td>
<td></td>
<td>3.88±2.69</td>
<td>42.13%</td>
</tr>
<tr>
<td>MEPO (50mg kg/ body wt) DMBA</td>
<td>28.2±2.2</td>
<td>34.5±2.9</td>
<td>69.85%</td>
<td>3.88±2.69</td>
</tr>
<tr>
<td>+ croton oil</td>
<td>78.16%</td>
<td></td>
<td>3.12±2.4</td>
<td>52.47%</td>
</tr>
<tr>
<td>MEPO (100mg kg/ body wt) DMBA</td>
<td>28.2±2.9</td>
<td>33.9±2.5</td>
<td>78.16%</td>
<td>3.12±2.4</td>
</tr>
<tr>
<td>+ croton oil</td>
<td>78.16%</td>
<td></td>
<td>3.12±2.4</td>
<td>52.47%</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.D. of 12-15 animals. *P<0.01 and *P<0.005 represent significant changes against control.

Fig-1. Effect of methanolic extracts of *Pandanus odoratissimus* on DMBA induced and Croton oil promoted skin papilloma on mice skin

RESULT:
The results of skin papillomagenesis obtained from treatment of test material during peri-initiation period. No adverse effect has been noticed on body weight gain, during the experimental period. Moreover no evidence of development of spontaneous tumor was observed including skin lesions in the colony of Swiss mice. In the control group the animals treated with DMBA and croton oil have developed 100% skin papillomas and the average number of tumors per tumor bearing animals (tumor burden) was 6.46 ± 2.61. In the low dose 50 mg methanolic extract of *P. odoratissimus* leaf treated group have shown 69.85% tumour development was observed (28.41 % reduction) and the tumor burden was reduced to 3.88 ± 2.69 (p <0.01). In the high dose 100 mg methanolic extract of *P. odoratissimus* leaf treatment the tumor incidence observed was 55.16% and the tumor burden was 3.12 ±2.4 (p <0.005). There was also a significant inhibition of tumor multiplicity was observed due to *P. odoratissimus* leaf extract of methanol treatment it is being 42.13% and 52.74% respectively in the low and high dose treated groups (Table-1, Graph-1, Fig-1).
DISCUSSION:
The cancer chemopreventive effects of polyphenolic antioxidants are specifically important. Since environmental pollutants, radiation, pesticides, certain medications, contaminated water and deep fried and spicy foods and UV radiations, as well as physical stress. All these exhibit the ability to produce enormous amount of free radicals which cause many diseases, including tumor promotion and cancer (Stadman, 1992).

Carcinogenesis in mouse skin and other animal tumour bioassay system and possibly in humans is a stepwise process. The mouse skin has been used as a conventional model to study the mechanism of carcinogenesis and modulation of sequential steps involved in process (Sporn.,2000). The mouse skin carcinogenesis model, which provides a conceptual framework to study the carcinogenesis process has also been used extensively to assess whether chemical or physical agents carry a carcinogenic hazard to humans. To evaluate the cancer chemopreventive effects of different agent and define the mechanism involved with their protective effects (kellen ,.1999).

Preeti et al., (2009) have reported several chemopreventive activity of resvererol on DMBA induced mouse skin tumourgenesis. The chemopreventive potential of resveratrol was also evident by increase in tumour free survival of animals. The average number of tumours bearing mouse for typical treatment of resveratrol resulted in 5.6 ± 1.63 and 3.6 ± 0.46 respectively.

Nayana et al., (2004) havestudied antitumorgenic activity of methanolic extracts of Pleurotus florid and P plurotus. The application of extract on mouse skin after tumor initiation with DMBA resulted in significant protection against skin tumour promotion in a dose dependent manner. The animal treated with concentration of 2 mg and 10 mg of Pleurotus florid and P plurotus extract treatment, results have shown the number of tumours 4.3 ± 1.2 and 3.9 ± 1.36 respectively, whereas the control showed 6.1 ± 2.1 of tumour.

Several investigators have shown that many mutagens and carcinogens exert their effect via the generation of oxygen and other radicals that play a major role in the causation of cancer, specifically at the promotion stage of carcinogenesis. A wide range of studies have shown that many naturally occurring compounds posses significant anti tumour promoting activity due to their antioxidant property.

In the present study the result indicate that the methanolic extract of Pandanus odoratissimus is having a strong antitumorgenic activity on skin papilloma. In low dose 50 mg/kg body weight the average number of tumour per tumour (tumour burden) was reduce to 3.88 ± 2.69 whereas in high dose 100 mg/kg body weight the tumour per tumour (tumour burden) 3.12 ± 2.4 was observed. Whereas in the control group the average number of tumour per tumour (tumour burden) was 6.46 ± 2.61.

These above experimental results indicates that methanolic extract of Pandanus odoratissimus has significant protection against DMBA induced skin papillomagenesis formation in a dose dependent manner. There the P. odoratissimus leaf extract of methanol contains a profound anticarcinogenic compound.

CONCLUSION:
Pandanus odoratissimus impart a significant protection against DMBA induced skin papillomagenesis formation in a dose dependent manner. The experimental findings suggest that the profound antitumorgenic activity, having significant antioxidant and anti-inflammatory properties of the extract might be one of the contributing factor for its anticarcinogenic activity.

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