Inhibition of Benzo(a)pyrene Induced Lung Adenoma by Panax ginseng Extract, EFLA400, in Swiss Albino Mice

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In brief ginseng extract EFLA400 (Panax ginseng) is an exciting pharmaceutical anticancer agent that has been shown to possess pharmacological properties including “antifatigue” and “anti-stress” actions, normalizing effects on carbohydrate metabolism, and stimulating effect on the central nervous system. It has also adaptogenic property. In the present investigation an attempt has been made to evaluate the anticancer potential of Panax ginseng extract, EFLA400, against lung adenoma induced by benzo(a)pyrene using Yun’s medium term anticarcinogenicity test model in Swiss albino mice.

MATERIALS AND METHODS

Animals Newborn Swiss albino mice (less than 24 h old) were used for experiments. These animals with their mothers were maintained in the animal house at temperatures of 24±3°C and a light (14 h light : 10 h dark). They were housed in polypropylene cages and fed standard mouse feed (to mothers) from Hindustan Lever Ltd., India. Tap water was provided to the animals.

Ginseng Extract The ginseng extract EFLA400 (Panax ginseng) (Batch 303298) prepared according to the published procedure, (Reference: Korean Patent 0425022, PCT/KR2003/000003) was supplied from Lotte Group R&D Center (Seoul, Korea).

In brief ginseng extract EFLA400 (Phoenix ginseng) is produced by decoction of Panax ginseng (approx. 90%) and Hawthorn berry (approx. 10%) under the patented specific manufacturing process. The nature of Hawthorn berry promotes conversion of ginsenosides present in natural Panax
ginseng to therapeutically more active ginsenosides. The content of ginsenoside Rg3, a pharmacologically active ingredient of the EFLA400 was $>3.0\%$ (w/w), as determined by HPLC, modified method of Kwon et al.$^{10}$ The quality control is being carried out with the contents of Rg3 by HPLC using C18 reverse phase column and gradient solvent system. The ginsenosides are very labile under acidic condition. Therefore it is necessary to standardize ginseng extract in order to expect constant quality and efficacies.

**Experimental Design. Medium Term Anticarcinogenicity Test Model (Yun's Model)** Newborn Swiss albino mice less than 24 h old were subcutaneously injected once in the scapular region with 0.02 ml of benzo(a)pyrene (BP, 0.5 mg suspension in 1% aqueous gelatin). After weaning, test material was administered for 6 weeks through oral gavage. All animals were sacrificed at the 9th week after birth.$^{1}$

- **Group I** (No BP, no EFLA400): After weaning, vehicle Tween 80 (volume equal to EFLA400) was administered for 6 weeks through oral gavage.
- **Group II** (EFLA400 Alone): After weaning, EFLA400 (10 mg/kg body weight) was administered for 6 weeks through oral gavage.
- **Group III** (BP Alone): This group of animals was subcutaneously injected once in the scapular region with 0.02 ml of BP.
- **Group IV** (BP+EFLA400): The animals of this group were subcutaneously injected once in the scapular region with 0.02 ml of BP. After weaning, EFLA400 (10 mg/kg body weight) was administered for 6 weeks through oral gavage.

Lungs were excised and fixed in Tellyesniczky’s solution (100 ml of 70% ethanol, 3 ml formalin, 5 ml glacial acetic acid), and the number of the adenoma were counted by the naked eyes. To obtain an index of tumor incidence, the percentage of tumor bearing mice per total number of mice in each group was calculated. Tumor multiplicity was defined as the average number of tumors per mouse obtained, by dividing the total number of tumors by the total number of mice per group including nontumor-bearing animals. Statistical comparison was made using the Student’s ‘$t$’ test.

**Cytogenetic Study. Chromosomal Aberration Analysis** Cytogenetic damage in the bone marrow cells was studied by chromosomal aberration analysis at the end of the experiments. All the animals were injected i.p. with 0.025% colchicine and sacrificed 2 h later by cervical dislocation. Both femurs were dissected out. Metaphase plates were prepared by the air drying method.$^{11}$ Bone marrow from the femur was aspirated, washed in saline, treated hypotonically (0.6% sodium citrate), fixed in 3 methanol: 1 acetic acid, dried and stained with 4% Giemsa (Sigma, U.S.A.). Different types of aberration like chromatid breaks, chromosome breaks, fragments, rings, exchanges and dicentrics were scored. When breaks involved both the chromatids, it was termed “chromosome type” aberration, while “chromatid type” aberration involved only one chromatid. If the deleted portion had no apparent relation to a specific chromosome, it was called a fragment.$^{12}$ Chromosomal aberrations were scored under a light microscope. A total of 400 metaphase plates were scored per animal and each aberration type was calculated in percentage.

**Micronuclei Assay** The method of Schmid$^{13}$ was employed for the micronucleus assay. The femurs were dissected out and the bone marrow was flushed out, vortexed and centrifuged. The pellet was resuspended in a few drops of fetal calf serum. Smears were made on pre-cleaned, dry slides, air dried and fixed in absolute methanol. The slides were stained with May-Grünwald’s and Giemsa stain. At least 2000 erythrocytes were observed and the number of polychromatic erythrocytes and normochromatic erythrocytes were counted. The micronuclei in them were recorded and micronuclei per 1000 cells were calculated.

### RESULTS

In Group III, in which a newborn Swiss albino mice less than 24 h old were subcutaneously injected once in the scapular region with 0.02 ml of benzo(a)pyrene, a significant reduction in body weight and weight of lungs was observed at the termination of experiment (9 weeks). The lung adenoma incidence was $68.3\%\pm2.96\%$ and tumor multiplicity was $0.91\pm0.08$ in this group (Tables 1, 2).

In treatment group (Group IV, BP+EFLA400), animals showed significantly less number of lung adenomas also significant increase ($p<0.05$) was also observed in the weight of lungs. In this group the percentage of lung adenoma incidence and inhibition rate was also less ($p<0.001$) as compared to the BP-alone group. The average weight of mice in Group III (BP-alone) was $19.3\pm0.4$ g whereas it was significantly increased in EFLA400-treated group ($24.0\pm0.47$). A significant decrease in the number of adenomas was ob-

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Number of mice</th>
<th>Average weight of mice (g)</th>
<th>Number of mice with lung adenoma</th>
<th>Number of adenomas</th>
<th>Average weight of lung (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No BP, no EFLA400</td>
<td>30</td>
<td>27.54±0.60</td>
<td>0</td>
<td>0</td>
<td>0.28±0.005</td>
</tr>
<tr>
<td>Group II</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>EFLA400 alone (10 mg/kg b. wt)</td>
<td>30</td>
<td>28.48±0.62</td>
<td>0</td>
<td>0</td>
<td>0.29±0.007</td>
</tr>
<tr>
<td>Group III</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BP alone (0.5 mg/mice s.c.)</td>
<td>53</td>
<td>19.3±0.4, $p&lt;0.001$</td>
<td>36</td>
<td>14.6±5.46, $p&lt;0.001$</td>
<td>0.20±0.004, $p&lt;0.001$</td>
</tr>
<tr>
<td>Group IV</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>BP+EFLA400</td>
<td>71</td>
<td>24.0±0.47, $p&lt;0.0005$</td>
<td>14</td>
<td>6.00±1.69</td>
<td>0.24±0.009, $p&lt;0.05$</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. Significance levels: $p<0.05$, $p<0.005$ and $p<0.001$. Statistical comparison: Group I vs. Group II; Group III vs. Group I; Group III vs. Group IV.
served in EFLA400-treated group (Table 1). The lung adenoma incidence was 68.3 ± 2.96% in control group whereas it was 19.1 ± 1.64 in EFLA400-treated group (Table 2). The inhibition rate was 72.05 ± 1.36% in EFLA400-treated group with respect to reference (Group III). The tumor multiplicity was 0.91 ± 0.08 in mice treated with 0.5 mg of BP. However, when treated with EFLA400 it was 0.25 ± 0.01 (p < 0.001).

Chromosomal aberrations analysis showed single subcutaneously injected once in the scapular region with 0.02 ml BP in newborn mice was able to induce significantly increased chromosomal anomalies in bone marrow cells (Group III). In this group, significant increase in chromosomal aberrations in the form of chromatid breaks, chromosome breaks, centric rings, dicentrics, exchanges and acentric fragments were observed (Table 3). Also significant increase in percentage of pulverized cells, polyploids, aberrant cells and aberrations/damaged cell were recorded in this group of animals. The frequencies of induced micronuclei in control group of animals were observed as 20.82 ± 1.81%. Whereas, in the treatment group (Group-IV), significant decrease in chromosomal aberrations and micronuclei frequencies were observed as compared to control group (Tables 4, 5).

**DISCUSSION**

The present study demonstrated that the oral administra-
The effects of scavengers of free radicals. It has been suggested that tumor formation in mouse skin and other systems proceeds in discrete, genetically determined steps, initiation is considered to be due to the induction of point mutations in a critical target site in the cell.

In the present study, EFLA400-treatment showed significant decreases in chromosomal aberrations and micronuclei frequencies. Several findings concerning the action of tumor promoters and the process of tumor promotion in the mouse skin system have been discussed and interpreted such as the frequency, reversibility and transient nature of conversion, dependence of tumor promotion on DNA synthesis, induction of DNA breaks by tumor promoters, and the protective effect of scavengers of free radicals. It has been suggested that tumor formation in mouse skin and other systems proceeds in discrete, genetically determined steps, initiation is considered to be due to the induction of point mutations in a critical target site in the cell.

The inhibitory action of the these compounds is related to factors such as the prevention of formation of the carcinogen, induction of coordinated enzyme response and scavenging of active metabolites of the carcinogen. These agents modify or suppress the carcinogen action on critical target sites in the cell.

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Cancer chemoprevention is defined as the use of chemicals or dietary components to block, inhibit or reverse the development of cancer in normal or preneoplastic tissue. A large number of potential chemopreventive agents have been found to function by a variety of mechanisms directed at all major stages of carcinogenesis. Previous studies have demonstrated that one avenue for chemoprotection against toxic mutagenic chemicals and anticarcinogenicity is induction of the drug-metabolizing enzymes that interfere with bioactivation and accelerate the detoxification process. Many carcinogens and mutagens are not active per se and must be activated into reactive electrophiles before exerting their biologic effects. Accumulating evidence supports the hypothesis that several naturally occurring inducers present in common edible plant parts exhibit an inhibitory effect on carcinogenesis. Accumulating evidence supports the hypothesis that several naturally occurring inducers present in common edible plant parts exhibit an inhibitory effect on carcinogenesis.

Certain fractions or purified individual ingredients of ginseng have been shown to exert cytotoxic or cytostatic activities against tumor cells in culture. The growth inhibitory or anti-proliferative effects of certain ginsenosides appear to be associated with their capability to induce apoptosis and inhibit the reverse transcriptase enzyme telomerase, oncogenes, and loss of tumor suppressor genes. Since telomeres are the guardians of the chromosomes and, therefore, of the genome, their erosion resulting may cause telomere dysfunction.

Table 5. Frequency of Micronuclei Induced by Benzo(a)pyrene in Newborn Mice with and without Panax Ginseng Extract, EFLA400 Treatment

<table>
<thead>
<tr>
<th>Treatment and dose</th>
<th>Number of micronuclei per 1000 cells</th>
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<tbody>
<tr>
<td>Group I</td>
<td>0.28±0.02</td>
</tr>
<tr>
<td>Group II</td>
<td>0.26±0.02</td>
</tr>
<tr>
<td>Group III</td>
<td>20.82±1.81, p&lt;0.001</td>
</tr>
<tr>
<td>Group IV</td>
<td>3.48±0.82, p&lt;0.001</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. Significance levels: p<0.05, p<0.005 and p<0.001. Statistical comparison: Group I vs. Group II; Group III vs. Group I; Group III vs. Group IV.
of ginsenoside on cancer preventive effects. It has been shown that methanolic extract of heat processed *Panax ginseng* attenuates the lipid peroxides in rat brain homogenates and is capable of scavenging superoxide generated by xanthine oxidase or by 12-O-tetradecanoylphorbol-13-acetate (TPA) in differentiated human promyelocytic leukemia (HL-62) cells.20 Further EFLA400 contains 3 percent Rg3. It has been reported that Rg3 pretreatment abrogated the expression of cyclooxygenase-2 in TPA stimulated mouse skin. It also inhibited the TPA induced activation of eukaryotic transcription factor NF-kB in both mouse skin and of promyelocytic leukemia (HL-62) cells.21

**Acknowledgement**

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**REFERENCES**