Abstract. – Objectives: The effect of Podophyllum hexandrum methanolic extract and \( \alpha \)-tocopherol in reducing oxidative stress in male albino rats was evaluated.

Materials and Methods: Lipid peroxidation was monitored by measuring malondialdehyde (MDA) level in different tissues of rats. Activities of free radical scavenging enzymes (superoxide dismutase, glutathione peroxidase and catalase) were determined using H\(_2\)O\(_2\) decomposition.

Results: Results showed that administration of H\(_2\)O\(_2\) (0.1%) in drinking water of the rats, for 25 weeks, increased the malondialdehyde levels in liver, kidney and lung tissues of all the rats. However, rats receiving Podophyllum hexandrum extract and \( \alpha \)-tocopherol had lower MDA levels in a dose dependent manner, which indicates decreased lipid peroxidation in these rats. Increase in the catalase activity appears to be a response to H\(_2\)O\(_2\) accumulation. The decrease in the activity of catalase and increase in the activity of superoxide dismutase and glutathione peroxidase in different organs of the rats receiving Podophyllum hexandrum extract and \( \alpha \)-tocopherol indicates the protective effect of the plant in combating oxidative stress undergone by the rats.

Objectives: Rhizome of Podophyllum hexandrum have been ethnomedically claimed to possess a wide array of biological activities including anticancer activity.

Materials and Methods: To verify the folklore claim, this study was performed in a six Human carcinoma cell lines, Lung (A-549), Prostate (PC-3), Colon (Colo-25), Breast (MCF-7), Neuroblastoma (IMR-32) and CNS (SF-295) MCF-7 and MDA-MB-231.

Results: Methanol and 70% ethanolic extracts of the rhizome of Podophyllum hexandrum showed highest cytotoxic effect on MCF-7 (Breast) and Colo-25 (Colon) cell line, as determined with sulforhodamine-B (SRB) assay.

Conclusions: These findings showed that Podophyllum hexandrum extract may ameliorate H\(_2\)O\(_2\) induced oxidative stress by decreasing lipid peroxidation via alteration of the antioxidant defense system of the rats. 2- these data also showed the anticancer activity of the plant extracts on different human cancer cell lines. However, further investigation is needed to assess the molecular mechanisms mediated anticancer activities of this plant.

Key Words: Podophyllum hexandrum, Oxidative stress, Malondialdehyde, Antioxidant enzymes, Anticancer activity.

Introduction

Podophyllum, the Himalayan Mayapple is a rhizomatous herbaceous perennial found in the high altitude wild. Podophyllum is a medicine of most extensive service; its greatest power lies in its action upon the liver and bowels. It is a gastro-intestinal irritant, a powerful hepatic and intestinal stimulant. Podophyllum is a powerful medicine exercising an influence on every part of the system, stimulating the glands to healthy action. Its most beneficial actions are obtained by the use of small doses frequently given. It is highly valuable in dropsy, biliousness, dyspepsia, liver and other medical conditions. Mayapple acts admirably upon all the secretions, removing obstructions, and producing a healthy condition of all the organs in the system. During the last twenty or thirty years, attention has be drawn by pharmacologists and medical researches to the fact that Podophyllum contains chemical agents responsible for anti-cancer activity. Many tribes...
consume or drink brew from the powder as a laxative or to treat intestinal worms. The root was also used as a tonic for liver, lung, and stomach ailments. A decoction was made by boiling the roots in water and was used to treat rheumatism. Many of these medicinal properties have been assigned to the antioxidant properties of Podophyllum.

*Podophyllum (P.) hexandrum* has been extensively exploited in traditional medicine for treatment of a number of ailments like Condyloma acuminata, Taenia capitis, monocytoid leukemia, Hodgkins disease, non-Hodgkin’s lymphoma, cancer of brain, lung, bladder and venereal warts. Utility of *P. hexandrum* has also been reported against constipation, cold, biliary fever, septic wound, burning sensation, erysipelas insect bite, mental disorders, rheumatism, plague and to provide symptomatic relief in some of the allergic and inflammatory conditions of skin. *P. hexandrum* has also been employed in treatment of cancer.

Hydrogen peroxide (H$_2$O$_2$) has been shown to induce oxidative stress in both human and animal models, leading to the generation of potent reactive oxygen species (ROS) such as hydroxyl radical (OH$^•$). Oxidative stress results when generation of reactive oxygen and or nitrogen species and activity of the antioxidant defenses are unbalanced. The increase in ROS could be due to their excessive production and/or decreased destruction. Cells exposed to severe oxidative stress may suffer degeneration of deoxyribonucleic acid (DNA), membrane lipids, Proteins and enzymes, leading to various pathological conditions. Ward have suggested that the cytotoxicity of H$_2$O$_2$ is associated with local multiplication of damaged sites on DNA. Thus, it is imperative that H$_2$O$_2$ levels in tissues be minimized. Such oxidative damage is known as one of the mechanisms leading to chronic diseases, such as atherosclerosis, aging, cancer and rheumatoid arthritis. Antioxidant enzymes, in particular superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase and glutathione-S-transfere represent cell defense mechanisms for preventing oxidative damage. Plants are known to produce various antioxidant compounds to interact with ROS in order to survive. Therapeutic properties of some of these plant extracts used in traditional medicine have linked to their antioxidant activities.

The present study was designed to investigate the efficacy of *Podophyllum hexandrum* methanolic rhizome extract in alleviating the H$_2$O$_2$ induced oxidative stress in rats; 2. to investigate the anticancer activity of methanolic and 70% ethanolic extracts of *Podophyllum hexandrum* on various human cancer cell lines.

**Materials and Methods**

*Plant Material Collection and Extraction*

The rhizome of *Podophyllum hexandrum* was collected from higher reaches of Aharbal, Shopian, J&K, India in the month of May and June 2009, identified by the Centre of Plant Taxonomy, Department of Botany, University of Kashmir, and authenticated by Dr. Irshad Ahmad Nawchoo (Department of Botany) and Akhter Hussain Malik (Curator, Centre for Plant Taxonomy, University of Kashmir). A voucher specimen has been retained in the Herbarium of the Department of Botany at the University of Kashmir under reference number KASH-bot/Ku/PH-702-SAG.

The plant material (rhizome) was dried in the shade at 30 ± 2°C. The dried rhizome material was ground into a powder using mortar and pestle and passed through a sieve of 0.3 mm mesh size. The powder obtained was extracted with methanol using a Soxhlet extractor (60-80°C). The extract was then concentrated with the help of rotary evaporator under reduced pressure and the solid extract was stored in refrigerator for further use.

**Animals**

Adult male albino rats of Wistar strain weighing 200-250 g used throughout this study were purchased from the Indian Institute of Integrative Medicine Jammu (IIIM). The animals had access to food and water *ad libitum*. The animals were maintained in a controlled environment under standard conditions of temperature and humidity with an alternating 12 hr light and dark cycle. The animals were maintained in accordance with the guidelines prescribed by the National Institute of Nutrition, Indian Council of Medical Research, and the study was approved by the Ethical Committee of the University of Kashmir.

Thirty six adult albino rats, approximately 2 months old, weighing in the range of 200-250 g were selected for the study. All the rats were healthy and not infected with virus or bacteria. The rats were housed for 25 weeks in separate...
cages, into six groups (six rats per group) and kept at 23 ± 2°C, with a 12h light dark cycle. They were provided with a standard laboratory diet and water ad libitum. The rats were randomly divided into six groups and treated for 25 weeks as follows:

**Group 1** was given only normal diet.
**Group 2** was given normal diet and 0.1% H2O2 in drinking water.
**Group 3** was given normal diet, 0.1% H2O2 and 10 mg% α-tocopherol.
**Group 4** was given normal diet, 0.1% H2O2 and 5 mg% rhizome methanolic extract of *Podophyllum hexandrum*.
**Group 5** was given normal diet, 0.1% H2O2 and 10 mg% methanolic extract of *Podophyllum hexandrum*.
**Group 6** was given normal diet, 0.1% H2O2 and 15 mg% rhizome methanolic extract of *Podophyllum hexandrum*.

At the end of the experiment, all the rats were sacrificed and the organs namely liver, kidney, lung and brain were isolated washed in ice cold 1.15% KCl and homogenized. The homogenate was centrifuged at 9,000 g for 20 minutes to remove debris. The supernatant was further centrifuged at 15,000 g for 20 minutes at 4°C to get PMS (post mitochondrial supernatant) that has been used for the estimation of lipid peroxidative indices, superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).

**Lipid peroxidation assay:** Lipid peroxidation of tissue homogenates was measured by using the thiobarbituric acid test, employing the method of Buege and Aust\(^{10}\). Tissue homogenate (0.5 ml) were mixed with 5 µl of 10mM ethylene diamine tetraacetic acid (EDTA) and 1 ml thiobarbituric acid – trichloroacetic acid – chloridic acid (TBA-TCA-HCl) solution and placed in a boiling water bath for 15 minutes. After cooling, the flocculent precipitate was removed by centrifugation at 6000 rpm for 10 minutes. The absorbance of red TBA malonaldehyde complex was then measured at 532 nm by using UV-Visible Spectrophotometer (Elico, India). Malonaldehyde formed from the degradation of poly unsaturated fatty acids, was then calculated by using an extinction coefficient of 1.56 × 10^5 M⁻¹ cm⁻¹.

**Superoxide dismutase activity (SOD):** Superoxide dismutase activity was determined according to the method of Beyer and Fridovich\(^{11}\). Enzyme activity was measured by mixing phosphate buffer, pH 7.8 containing 0.1 mM EDTA, L-methionine, Nitro blue tetrazolium and Triton X-100. Sample 100 µl was then added to the mixture, followed by addition of riboflavin (10 µl). The tubes were then illuminated for 7 min in a 100 W fluorescent lamp. The control tube, in which the sample was replaced by buffer, was also run and the absorbance measured at 560 nm.

**Catalase activity (CAT):** Catalase activity was assayed by the method of Sinha\(^{12}\). The mixture 1.5 ml containing 1.0 ml of 0.01 M phosphate buffer (pH 7.0), 0.1 ml of tissue homogenate and 0.4 ml of 2 M H2O2. The reaction was stopped by the addition of 2.0 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1: 3 ratio).

**Glutathione peroxidase (GPx):** GPx activity was assayed using the method of Sharma et al\(^{13}\). The assay mixture consists of 1.49 ml of sodium phosphate buffer (0.1 M pH 7.4), 0.1 ml EDTA (1 mM), 0.1 ml sodium azide (1 mM), 0.1 ml 1 mM GSH, 0.1 ml of nicotinamide adenine dinucleotide phosphate (NADPH) (0.02 mM), 0.01 ml of 1 mM H2O2 and 0.1 ml PMS in a total volume of 2 ml. Oxidation of NADPH was recorded spectrophotometrically at 340 nm and the enzyme activity was calculated as n moles NADPH oxidized/min/mg of protein, using € of 6.22 × 10^3 M⁻¹ cm⁻¹.

**Cell lines and Culture Medium**

The human cancer cell lines were obtained from Indian Institute of Integrative Medicine Jammu (IIIM) The cell lines namely Lung (A-549), Prostate (PC-3) Colon (Colo-25), Breast (MCF-7), Neuroblastoma (IMR-32) and CNS (SF-295) were used in this study. Cells were grown as monolayers in Minimum Essential Medium (MEM) supplemented with 10% heat-inactivated fetal calf serum and 1% Penicillin-Streptomycin mixture. Cultures were maintained at 37°C in 5% CO2 and 100% relative humidity atmosphere.

**Cytotoxicity Assay**

The in vitro cytotoxicity of extracts was determined using sulfrohydramine-B (SRB) as de-
scribed previously by Bhahwal et al\textsuperscript{14}. In brief, the stock solution (20 mg/ml) of the alcoholic, hydro-alcoholic, and aqueous extracts was prepared in dimethylsulfoxide (DMSO), dimethylsulfoxide-water (1:1) and hot water. The stock solutions were further diluted with growth medium (RPMI-1640/DMEM with 2 mM glutamine, pH 7.4, 10% fetal calf serum, 100 µg/ml streptomycin and 100U/ml penicillin) to obtain desired concentrations. The cells were grown in tissue culture flasks in growth medium at 37°C in an atmosphere of 5% CO\textsubscript{2} and 95\% relative humidity in a CO\textsubscript{2} incubator. The cells at subconfluent stage were harvested from the flask by treatment with trypsin (0.05% trypsin in phosphate buffered saline (PBS) containing 0.02\% EDTA) and suspended in the growth medium. Cells with more than 97\% viability (Trypan blue exclusion) were used for determination of cytotoxicity. An aliquot of 100 µl of cell suspension (10\textsuperscript{4} to 2 \times 10\textsuperscript{4} cells/ml depending upon mass doubling time of cells) was transferred to a well of 96-well tissue culture plate and incubated for 24h. The test materials (100 µl) were then added to the wells and incubated for another 48h. The cell growth was stopped by 50 µl of 50\% trichloroacetic acid and plates were further incubated at 4°C for an hour. The plates were washed with distilled water and air-dried. Sulforhodamine B (100 µl, 0.4\% in 1\% acetic acid) was added to each well and plates were incubated at room temperature for 30 min. The unbound SRB was removed by washing with 1\% acetic acid and air-dried. Tris-HCl buffer (100 µl, 0.01 M, pH 10.4) was added to all the wells and stirrer. The optical density was recorded on ELISA reader at 540 nm. Suitable blanks and positive controls were also included.

Each test was done in triplicate. The percentage of cell viability was calculated according to the following equation.

\[
\text{The \% of cell viability} = \frac{\text{OD of treated cells}}{\text{OD of control cells}} \times 100
\]

Statistical Analysis

The data were presented as means ± Standard Deviation and evaluated by the one way ANOVA followed by the Bonferroni t-test. Differences were considered to be statistically significant if \( p<0.05 \).

Results

Effect of Extract on MDA Levels

The localization of radical formation resulting in lipid peroxidation, measured as MDA in rat liver, kidney and lung homogenates is shown in Figures 1, 2 and 3. Malondialdehyde (MDA) contents in the liver homogenate were increased in H\textsubscript{2}O\textsubscript{2} control group (18.60 nmol/mg protein) compared to normal group (10.81 nmol/mg protein). MDA level of plant extract treated groups at the concentration of 5, 10 and 15 mg\% decreased in a dose dependent manner (Figure 1). In group VI animals treated with \( \alpha \)-tocopherol (10 mg\%) on MDA levels in H\textsubscript{2}O\textsubscript{2} control group decreased to 6.84 nmol/mg protein. Similar results were observed with lung tissue homogenates (Figure 3).

Effect of Extract on Superoxide Dismutase Activity (SOD)

The effect of \textit{Podophyllum hexandrum} methanolic extract on SOD activity in liver, kidney, lung and brain tissue is shown in Table I. SOD activity of the H\textsubscript{2}O\textsubscript{2} treated group homogenate was found to be 6.193 ± 1.15 (liver), 4.742 ± 0.66 (kidney), 2.810 ± 0.30 (lung) and 6.048 ± 0.50 U/mg protein (brain) which is lower than in normal group (38.465 ± 13.8 (liver), 30.024 ± 1.85 (kidney), 19.311 ± 1.99 (lung) and 26.600 ± 0.96 (brain). SOD activities of \textit{Podophyllum hexandrum} methanolic extract treated groups at the concentration of 5, 10 and 15 mg\% were increased by 13.27, 16.311 and 19.817 U/mg protein (liver), 9.094, 15.514 and 16.424 U/mg protein (kidney), 7.936, 9.93 and 10.240 U/mg protein (lung) and 12.378, 14.378, 17.493 U/mg protein (brain) respectively. \( \alpha \)-tocopherol (10 mg\%) restored the SOD activity in all the tested organs. At the higher concentration of plant extract (15 mg\%) the SOD activity was found comparable with group III rats treated with vitamin E in brain tissue and lung tissues.
Figure 1. Shows the effect of methanolic extract of *Podophyllum hexandrum* on lipid peroxidation in liver tissue of H$_2$O$_2$ treated rats. Each value represents the means ± SD of 6 animals. $ = p <0.001$, as compared with normal control group; $# = p <0.001$ as compared with H$_2$O$_2$ group; $@$ = $p <0.001$ as compared with Vitamin E; NS = non significant as compared with Vitamin E; b = dot not test as compared with Vitamin E; a = do not test as compared with normal control. The data were presented as means ± SD of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t-test to detect inter group differences. Differences were considered to be statistically significant if $p <0.05$.

![Figure 1](image1.jpg)

<table>
<thead>
<tr>
<th>Nmole of MDA formed/mg protein</th>
<th>Control group</th>
<th>H$_2$O$_2$ treated group</th>
<th>5% mg plant extract</th>
<th>10% mg plant extract</th>
<th>15% mg plant extract</th>
<th>Vitamin E treated group</th>
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<td>Control group</td>
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<td>H$_2$O$_2$ treated group</td>
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<td>5% mg plant extract</td>
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<td>10% mg plant extract</td>
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<td>15% mg plant extract</td>
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<td>Vitamin E treated group</td>
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</table>

Figure 2. Shows the effect of methanolic extract of *Podophyllum hexandrum* on lipid peroxidation in kidney tissue of H$_2$O$_2$ treated rats. Each value represents the mean ± SD of 6 animals. $ = p <0.001$, as compared with normal control group; $# = p <0.001$ as compared with H$_2$O$_2$ group; $@$ = $p <0.001$ as compared with Vitamin E; NS = non significant as compared with Vitamin E; ns = non significant as compared with normal control. The data were presented as means ± SD of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t-test to detect inter group differences. Differences were considered to be statistically significant if $p <0.05$.

![Figure 2](image2.jpg)

<table>
<thead>
<tr>
<th>Nmole of MDA formed/mg protein</th>
<th>Control group</th>
<th>H$_2$O$_2$ treated group</th>
<th>5% mg plant extract</th>
<th>10% mg plant extract</th>
<th>15% mg plant extract</th>
<th>Vitamin E treated group</th>
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<tr>
<td>Control group</td>
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<td>H$_2$O$_2$ treated group</td>
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<td>5% mg plant extract</td>
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<td>10% mg plant extract</td>
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<td>15% mg plant extract</td>
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<td>Vitamin E treated group</td>
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Long dose exposure of H₂O₂ in albino rats and effect of *P. hexandrum* on oxidative stress

**Effect of Extract on Catalase (CAT)**

**Group – I:** (this group was given neither H₂O₂ nor treatment). The catalase activity found in different organs are 1.349 (liver), 2.032 (kidney), 0.543 (lung) and 1.78 (brain).

**Group – II:** (the animals were given only H₂O₂). These rats were found to possess high Catalase activity of 27.336 (liver), 10.96 (kidney), 4.662 (lung) and 1.781 (brain).

**Group – III:** (treated with standard antioxidant vitamin E). There was a significant decrease in the catalase activity of 7.986 (liver), 3.498 (kidney), 1.628 (lung) and 3.290 (brain) respectively.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal control)</td>
<td>38.465 ± 13.86</td>
<td>30.024 ± 1.851</td>
<td>19.311 ± 1.998</td>
<td>26.600 ± 0.962</td>
</tr>
<tr>
<td>Group II (H₂O₂ treated)</td>
<td>6.193 ± 1.155</td>
<td>4.742 ± 0.664</td>
<td>2.810 ± 0.3077</td>
<td>6.048 ± 0.503</td>
</tr>
<tr>
<td>Group IV (5% mg plant extract)</td>
<td>13.270 ± 1.981</td>
<td>9.094 ± 1.019</td>
<td>7.936 ± 0.724</td>
<td>12.378 ± 1.236</td>
</tr>
<tr>
<td>Group V (10% mg plant extract)</td>
<td>16.311 ± 2.958</td>
<td>15.514 ± 2.945</td>
<td>9.937 ± 1.232</td>
<td>14.957 ± 0.560</td>
</tr>
<tr>
<td>Group VI (15% mg plant extract)</td>
<td>19.817 ± 3.948</td>
<td>16.424 ± 1.341</td>
<td>10.240 ± 1.530</td>
<td>17.493 ± 0.659</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of 6 animals. $ = p < 0.001$ as compared with normal control group; # = $p < 0.001$ as compared with H₂O₂ group; @ = $p < 0.001$ as compared with V.E; ns: non significant as compared with normal control. The data were presented as means ± S.D of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t-test to detect inter group differences. Differences were considered to be statistically significant if $p < 0.05$.  

Table I. Shows the effect of methanolic extract of *Podophyllum hexandrum* on SOD activity in different organs of albino rats (units/mg protein).
Group – IV, V and VI treated with methanolic extract of *Podophyllum hexandrum* at a concentration of 5, 10 and 15mg% decreases the catalase activity in a dose dependent manner (Table III). The increase in catalase activity is crucial in countering the accumulation of H$_2$O$_2$ and thus suppressing the formation of very potent and reactive OH$^\cdot$ radical. This decrease in the catalase activity of plant extract treated groups indicates free radical scavenging potential of *Podophyllum hexandrum* methanolic extract.

In vitro Cytotoxic Effect Against Human Cancer Cell Lines

In vitro cytotoxicity of the two extracts (methanolic and 70% ethanolic of P. *hexandrum* (rhizome) was evaluated at 10, 30, and 100 µg/ml against six human cancer cell lines from six different tissues origin, namely colon, prostate, breast, neuroblastoma, CNS, and lung (Table III). Growth inhibition in a dose-dependent manner was observed in all the cell lines by both the extracts. It was also observed that 70% ethanolic extract at higher concentration (100 µg/ml) was most active than methanolic extract against all the human cancer cell lines except lung (A-549) cancer cell lines where the percent growth inhibition observed was 55%, in comparison to the methanolic extract and positive control paclitaxel where we observed about 59 and 62% growth inhibition (Table IV). When compared with the different positive controls (5-FU, mitomycin C, adriamycin, and paclitaxel) specific for different cell lines the growth inhibition of 70% ethanolic extract was found higher than methanolic extract. At 100 µg/ml out of six cancer cell lines 70% ethanolic extract showed more than 50% growth inhibition against five cancer cell lines, namely 86% against Colo-205.

Table II. Shows the effect of *Podophyllum hexandrum* methanolic extract on Glutathione peroxidase activity in different organs of albino rats (units/mg protein).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal control)</td>
<td>412.181 ± 76.305</td>
<td>142.391 ± 5.426</td>
<td>55.899 ± 3.802</td>
<td>21.919 ± 0.827</td>
</tr>
<tr>
<td>Group II (<em>H$_2$O$_2$</em> treated)</td>
<td>41.036 ± 11.603</td>
<td>18.802 ± 1.985</td>
<td>6.530 ± 1.377</td>
<td>5.326 ± 0.091</td>
</tr>
<tr>
<td>Group III (α-tocopherol)</td>
<td>154.581 ± 55.460$^a$</td>
<td>53.616 ± 9.155$^a$</td>
<td>21.762 ± 0.793$^a$</td>
<td>18.129 ± 1.317$^a$</td>
</tr>
<tr>
<td>Group IV (5% mg plant extract)</td>
<td>78.141 ± 19.820$^{a,b}$</td>
<td>32.601 ± 4.843$^{a,b}$</td>
<td>9.846 ± 0.713$^{a,b}$</td>
<td>10.273 ± 0.452$^{a,b}$</td>
</tr>
<tr>
<td>Group V (10% mg plant extract)</td>
<td>107.90 ± 3.232$^{a,b}$</td>
<td>45.037 ± 8.690$^{a,b}$</td>
<td>13.613 ± 0.741$^{a,b}$</td>
<td>12.410 ± 0.686$^{a,b}$</td>
</tr>
<tr>
<td>Group VI (15% mg plant extract)</td>
<td>133.705 ± 10.593$^{a,b}$</td>
<td>52.073 ± 3.534$^{a,b}$</td>
<td>17.801 ± 2.238$^{a,b}$</td>
<td>14.603 ± 0.309$^{a,b}$</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of 6 animals. $^a = p <0.001$, as compared with normal control group; $^b = p <0.001$ as compared with H$_2$O$_2$ group; $^c = p <0.001$ as compared with VE; NS = non significant as compared with VE; $^d =$ do not test as compared with VE; $^e = do not test as compared with H$_2$O$_2$. The data were presented as means ± SD of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t-test to detect inter group differences. Differences were considered to be statistically significant if $p <0.05$.

Table III. Effect of *Podophyllum hexandrum* methanolic extract on catalase activity in different organs of albino rats (nm of H$_2$O$_2$ decomposed/min/mg protein).

<table>
<thead>
<tr>
<th>Groups</th>
<th>Liver</th>
<th>Kidney</th>
<th>Lung</th>
<th>Brain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Normal control)</td>
<td>1.349 ± 0.078</td>
<td>2.032 ± 0.289</td>
<td>0.543 ± 0.126</td>
<td>1.781± 0.137</td>
</tr>
<tr>
<td>Group II (<em>H$_2$O$_2$</em> treated)</td>
<td>27.336 ± 7.17</td>
<td>10.961 ± 2.272</td>
<td>4.662 ± 0.436</td>
<td>12.44± 1.158</td>
</tr>
<tr>
<td>Group III (α-tocopherol)</td>
<td>7.986 ± 2.261$^a$</td>
<td>3.498 ± 0.318$^a$</td>
<td>1.628 ± 0.257$^a$</td>
<td>3.290 ± 0.335$^a$</td>
</tr>
<tr>
<td>Group IV (5 mg plant extract)</td>
<td>19.800 ± 1.474$^{a,b}$</td>
<td>5.340 ± 0.673$^{a,b}$</td>
<td>3.145 ± 0.253$^{a,b}$</td>
<td>10.550 ± 0.307$^{a,b}$</td>
</tr>
<tr>
<td>Group V (10 mg plant extract)</td>
<td>18.163 ± 0.714$^{a,b}$</td>
<td>5.554 ± 0.569$^{a,b}$</td>
<td>2.886 ± 0.440$^{a,b}$</td>
<td>15.034 ± 0.440$^{a,b}$</td>
</tr>
<tr>
<td>Group VI (15 mg plant extract)</td>
<td>16.346 ± 1.629$^{a,b}$</td>
<td>4.683 ± 0.463$^{a,b}$</td>
<td>2.050 ± 0.471$^{a,b}$</td>
<td>5.038 ± 0.254$^{a,b}$</td>
</tr>
</tbody>
</table>

Each value represents the mean ± SD of 6 animals. $^a = p <0.001$, as compared with normal control group; $^b = p <0.001$ as compared with H$_2$O$_2$ group; $^c = p <0.001$ as compared with VE; NS = non significant as compared with VE; $^d = dot not test as compared with VE; $^e = non significant as compared with normal control. The data were presented as means ± SD of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t-test to detect inter group differences. Differences were considered to be statistically significant if $p <0.05$.  

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Table IV. In vitro Cytotoxic effect of Podophyllum hexandrum methanolic and 70% ethanolic extracts against human cancer cell lines.

<table>
<thead>
<tr>
<th>Tissue Cell line type</th>
<th>Lung A-549</th>
<th>Prostate PC-3</th>
<th>Colon Colo-205</th>
<th>Breast MCF-7</th>
<th>Neuroblastoma IMR-32</th>
<th>CNS SF-295</th>
</tr>
</thead>
<tbody>
<tr>
<td>Extracts/drugs</td>
<td>Conc. (µg/ml)</td>
<td>% Growth inhibition</td>
<td>Conc. (µg/ml)</td>
<td>% Growth inhibition</td>
<td>Conc. (µg/ml)</td>
<td>% Growth inhibition</td>
</tr>
<tr>
<td>70% ethanolic extract</td>
<td>10</td>
<td>40 ± 2</td>
<td>36 ± 1</td>
<td>70 ± 4</td>
<td>68 ± 4</td>
<td>45 ± 2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>42 ± 4</td>
<td>43 ± 2</td>
<td>78 ± 4</td>
<td>74 ± 3</td>
<td>49 ± 3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>55 ± 2</td>
<td>45 ± 2</td>
<td>86 ± 3</td>
<td>79 ± 3</td>
<td>55 ± 4</td>
</tr>
<tr>
<td>Methanolic extract</td>
<td>10</td>
<td>47 ± 3</td>
<td>23 ± 2</td>
<td>71 ± 3</td>
<td>68 ± 3</td>
<td>36 ± 4</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>48 ± 4</td>
<td>26 ± 1</td>
<td>77 ± 4</td>
<td>74 ± 4</td>
<td>44 ± 3</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>59 ± 3</td>
<td>30 ± 3</td>
<td>85 ± 5</td>
<td>77 ± 4</td>
<td>51 ± 5</td>
</tr>
<tr>
<td>Paclitaxel</td>
<td>1 × 10^4 M</td>
<td>62 ± 6</td>
<td>59 ± 4</td>
<td>54 ± 3</td>
<td>70 ± 6</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>Mitomycin-c</td>
<td>1 × 10^4 M</td>
<td>62 ± 6</td>
<td>59 ± 4</td>
<td>54 ± 3</td>
<td>70 ± 6</td>
<td>79 ± 6</td>
</tr>
<tr>
<td>5-FU</td>
<td>2 × 10^4 M</td>
<td>59 ± 4</td>
<td>54 ± 3</td>
<td>70 ± 6</td>
<td>79 ± 6</td>
<td>72 ± 4</td>
</tr>
<tr>
<td>Adriamycin</td>
<td>1 × 10^4 M</td>
<td>59 ± 4</td>
<td>54 ± 3</td>
<td>70 ± 6</td>
<td>79 ± 6</td>
<td>72 ± 4</td>
</tr>
</tbody>
</table>

Data is means ± SD of three independent experiments.

Long dose exposure of H2O2 in albino rats and effect of P. hexandrum on oxidative stress

Discussion

H2O2 has been shown to induce the oxidative stress in animal models when provided in a long term study, leading to the generation of potent reactive oxygen species (ROS), such as hydroxyl radical (OH·). Most of the deleterious effects of H2O2 on tissues, including lipid peroxidation, depend on its conversion into OH· which is catalyzed by iron and copper15-17. Cells exposed to H2O2 may suffer degeneration of DNA, membrane lipids, proteins and enzymes leading to various pathological conditions5.

Lipid peroxidation is an auto-catalytic free radical mediated, destructive process, whereby poly unsaturated fatty acids in cell membranes undergo degradation to form lipid hydroperoxides. These later compounds then decompose to form a wide variety of products, including low molecular mass hydrocarbons, hydroxyl aldehydes, fatty acids, ketones, alkenols and alkanals, in particular malonaldehyde (MDA)18. Thus the reduction of MDA production would indicate inhibition of lipid peroxidation. Figures 1, 2, and 3 reveals that malonaldehyde contents in liver, kidney, lung tissue homogenates of the rats significantly increased in H2O2 receiving group. However, MDA levels of normal diet treated rats were seem to increase only to a limited extent, but the levels were still significantly lower than that of H2O2 treated groups at week 25. Malonaldehyde content was evaluated as an end point, indicative of the extent of lipid peroxidation, since MDA is known to be one of the most abundant aldehydes formed as a byproduct of lipid peroxidation19.

The significant increase of MDA level in H2O2 treated rats indicates the possibility of increased radical production, and higher rate of lipid peroxidation. However, the MDA increase, experienced by normal rats may also be associated with aging20. Rodriguez and Ruiz21 indicate that plas-
ma MDA levels were increased with age healthy subjects and Nohl\textsuperscript{22} reported accumulation of lipid peroxidation products during aging. The reduction of MDA in \textit{Podophyllum hexandrum} methanolic extract fed groups at the end of the study might represent antioxidant augmentation and prove that \textit{Podophyllum hexandrum} has excellent antioxidative activity. The results of the study suggested that \textit{Podophyllum hexandrum} and α-tocopherol pretreatment may decrease lipid peroxidation and hence protect the rats undergoing H\textsubscript{2}O\textsubscript{2} treatment from possible oxidative damage.

We also observed that H\textsubscript{2}O\textsubscript{2} induced a significant decrease in SOD, CAT and GPx activities in liver, kidney, lung and brain tissue homogenates. Biological effects of ROS are controlled \textit{in vivo} by a wide spectrum of enzymatic and non enzymatic defense mechanisms, in particular superoxide dismutase, which catalyzes dismutation of superoxide anions to hydrogen peroxide and catalase, which then converts H\textsubscript{2}O\textsubscript{2} in to molecular oxygen and water\textsuperscript{20}. Their roles as protective enzymes are well known and have been investigated extensively both \textit{in vivo} and in model systems\textsuperscript{23}. Superoxide dismutase represents the front line of defense against oxidative damage\textsuperscript{24} and provides defense against toxicity of dioxygen\textsuperscript{25,26}. Catalase on the other hand has been suggested to provide an important pathway for H\textsubscript{2}O\textsubscript{2} decomposition which must be rapidly removed due to its toxicity\textsuperscript{27-29}. In this study, significant decrease in superoxide dismutase, catalase and glutathione peroxidase activity were observed in group II rats treated with H\textsubscript{2}O\textsubscript{2}. However the rats treated with methanolic extract of \textit{Podophyllum hexandrum} increases the enzyme activity in a dose dependent manner in all the tested organs.

A large variety of phytochemicals that have been reported from natural product research has been proven successful as anticancerous agents\textsuperscript{30}. This study was undertaken to scientifically prove the traditional claim of \textit{Podophyllum hexandrum} possessing anti-cancerous property. The findings from this study observed that methanolic and 70% ethanolic extracts of \textit{Podophyllum hexandrum} inhibits the proliferation of six human cancer cell lines. The cytotoxicity effect found was highest with Colo-205 (colon) and MCF-7 (breast) cell lines. The effect was analyzed at different concentration levels via 10, 30 and 100 µg mL\textsuperscript{-1} and the inhibitory effect was concentration-dependent and cell line specific. This clearly indicates the presence of potent bioactive princi-
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