Antioxidant Activity of Geraniol on n-Nitrosodiethylamine-induced Hepatocarcinogenesis in Wistar Albino Rats

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Abstract
The chemopreventive effect of Geraniol was evaluated in N-nitrosodiethylamine-induced experimental liver tumor in Wistar albino rats. In this study the animals were divided into four groups with 6 rats each and received treatments for 4 weeks. Group I served as control (untreated), Group II treated with N-nitrosodiethylamine (DEN) (200 mg/kg, i.p.) and Phenobarbital (PB) (0.05%) in drinking water; Group III received Geraniol (250 mg/kg b.wt) in addition to DEN and Group IV treated with Geraniol only for four weeks. The administration of Geraniol in rats with DEN induced HCC rats (Group III) shows a significantly reduced level of liver serum marker (aspartate aminotransferase, alanine aminotransferase, acid phosphatase, alkaline phosphatase, lactate dehydrogenase, gammaglutamyl transpeptidase, alpha fetoprotein and 5'nucleotidase). These results are evident that Geraniol possesses protective effect against N-Nitrosodiethylamine (DEN)-induced Hepatocellular Carcinogenesis (HCC).

Keywords: DEN, Carcinogenesis, HCC, Geraniol, Marker Enzymes

1. Introduction
Oxygen free radicals are generated during various metabolic processes and the antioxidant defense mechanism of the body keeps a check on the excess production of free radicals generated in the system¹,². The imbalance between the exposure to oxidants and the endogenous antioxidants results in ‘oxidative stress’ which is found to play an important role in various pathological conditions including asthma, Acute Respiratory Distress Syndrome (ARDS), Chronic Obstructive Pulmonary Disease (COPD), idiopathic pulmonary fibrosis, cardiovascular dysfunction, neurodegenerative diseases, immunosuppression, cancer, premature ageing etc.³,⁴.

The liver is a vital organ where free radicals are largely generated and a large number of plants have claimed to have hepatoprotective activity⁵,⁶.

Diethyl Nitrosamine (DEN) is one of the most important environmental carcinogens in N-nitrosamines class, which primarily induces tumours of the liver⁷. DEN is considered to be a genotoxic carcinogen⁸. It is often assumed that DEN initiates and propagates tumor development primarily by inducing DNA alterations that lead to mutations⁸.

Herbs have been used as food and for medicinal purposes for centuries. Research interest has focused on various herbs that possess hypolipidemic, antiplatelet, antitumor or immune-stimulating properties that may be useful adjuncts in helping reduce the risk of cardiovascular disease and cancer. In different herbs, a wide variety of active phytochemicals including the flavonoids, terpenoids, lignins, sulphides, polyphenolics, carotinoids, coumarines, saponins, plant sterols, curcumins and phthalides have been identified⁹.
2. Materials and Methods

2.1 Materials

2.1.1 Animals

Adult male Wistar albino rats were obtained from the King’s Institute–Guindy, Chennai and housed in clean polypropylene cages. Animals were maintained according to the principle and guidelines of the committee (No. 945/C/01/04/06/CPCSEA) under the supervision of Animal Ethical Committee. The animals were fed on commercial pelleted feed procured from Poultry Research Station, Chennai and water was provided ad libitum.

2.1.2 Chemicals

Geraniol (98% pure), Diethylnitrosamine (DEN), Phenobarbital (PB) and Reduced glutathione were purchased from Sigma Chemical Company, St. Louis, MO, USA.

2.2 Methods

2.2.1 Experimental Design

Four groups with six animals each were taken for experiment.

Group I : Control rats (untreated)
Group II : Rats administered with Diethylnitrosamine (DEN) (200 mg/kg body weight) intraperitoneally and Phenobarbital (PB) (0.05%) in drinking water.
Group III : Hepatocellular Carcinoma (HCC) bearing rats administered orally with Geraniol (250 mg/kg body weight).
Group IV : Rats administered orally only with Geraniol (250 mg/kg body weight).

Group I rats fed with pellet feed and water served as normal control rats. Group II rats were treated with DEN by a single intraperitoneal injection (200 mg/kg body weight). After two weeks, the carcinogenic effect was promoted by Phenobarbital, the promoter (0.05% PB). The Promoter was supplemented to the animals through drinking water up to 14 successive weeks. Group III animals were also treated with DEN by intraperitoneal injection (200 mg/kg body weight). After two weeks Phenobarbital was supplemented through drinking water for 14 successive weeks. At the beginning of 15th week, the cancer induced rats were treated with geraniol (250 mg/kg bodyweight) dissolved in corn oil (2.5 ml/kg body weight) orally for eight consecutive weeks. Group IV animals were treated only with Geraniol (250 mg/kg body weight) for 8 consecutive weeks to study the cytoxicity (if any) induced by Geraniol.

At the end of the experiment, by cardiac puncture blood samples were collected ad allowed to clot. From the clotted sample serum was separated by centrifugation at 3000 g for 10 min and stored at -70 °C for biochemical assays such as Aspartate Aminotransferase (AST),
Alanine Aminotransferase (ALT), Acid Phosphatase (ACP), Alkaline Phosphatase (ALP), Lactate Dehydrogenase (LDH), Gamma Glutamyl Transpeptidase (GGT), Alpha Feto-Protein (AFP) and 5’Nucleotidase levels.

2.3 Supernatant Preparation

The liver samples were harvested from the experimental animals and weighted and perfused immediately with ice-cold saline (0.85%, w/v NaCl), and homogenized with chilled phosphate buffer (0.1M, pH 7.4) containing KCl (1.17%, w/v). The homogenate was then centrifuged (800 g, 5 min, 4 °C) for removing the cell debris. The supernatant was collected and centrifuged at 10,000 g for 20 min at 4 °C. Then the resulting supernatant is then assayed for CAT, SOD, and GPx activities.

2.4 Determination of the Extract Effect on Lipid Peroxidation in Liver

Lipid peroxidation was estimated by Thiobarbituric Acid (TBA) reaction with Malondialdehyde (MDA) as reported earlier. Lipid peroxidation was expressed as nanomoles of MDA per milligram of protein.

2.5 Determination of the Extract Effect on Antioxidant Enzyme Activities in the Liver

2.5.1 Superoxide Dismutase Activity

The SOD activity was measured according to the method used by Marklund and Marklund. The enzyme activity was expressed as units/mg protein.

2.5.2 Catalase Activity

The protein content of the supernatant was determined using the method with copper sulphate as reported earlier.

2.5.3 Glutathione Peroxidase Activity

To estimate the GPx activity, the reaction mixture consisted of 1.65 mL phosphate buffer (0.1 M, pH 7.4), 0.1 mL EDTA (0.5 mM), 0.05 mL oxidized glutathione (1 mM), 0.1 mL NADPH (0.1 mM), and 0.1 mL supernatant in a total volume of 2 mL. The disappearance of NADPH at 340 nm was recorded at 25 °C. The enzyme activity was calculated as nano mol of NADPH oxidized/min/mg protein using molar extinction coefficient.

3. Results

The activities of enzymatic antioxidants (SOD, CAT and GPx) level in the liver hemogenate of the experimental animals in each group are shown in Table 1.

The activities of SOD, CAT and GPx level were significantly decreased in hemogenate of tumor bearing animals (Group II) as compared to control animals. Geraniol administrated rats showed significant increase in the enzymatic antioxidants levels (Group III and Group IV).

The activities of enzymatic antioxidants (SOD, CAT and GPx) level in liver tissue of tumor bearing animals (Group II) as compared to control animals. Geraniol administrated rats showed significant increase in the enzymatic antioxidants levels (Group III and Group IV).

The activities of non-enzymatic antioxidants (GSH, Vit C and Vit E) level in haemolysate of the experimental animals in each group are shown in Table 3.

Table 1. Effect of geraniol on the enzymatic antioxidants in liver hemogenate of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Superoxide dismutase (SOD)</th>
<th>Catalase (CAT)</th>
<th>Glutathione peroxidase (GPx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>3.72 ± 0.08</td>
<td>65.92 ± 4.23</td>
<td>6.52 ± 0.25</td>
</tr>
<tr>
<td>Group II (DEN)</td>
<td>2.34 ± 0.33**</td>
<td>42.44 ± 2.42***</td>
<td>3.81 ± 0.32***</td>
</tr>
<tr>
<td>Group III (DEN + Geraniol)</td>
<td>3.38 ± 0.03***</td>
<td>57.25 ± 4.49***</td>
<td>4.40 ± 0.33***</td>
</tr>
<tr>
<td>Group IV (Geraniol)</td>
<td>3.59 ± 0.19NS</td>
<td>64.68 ± 2.76NS</td>
<td>6.42 ± 0.28NS</td>
</tr>
</tbody>
</table>

Values represent mean ± SD for 6 rats in each group.

Units: SOD – units /mg protein
CAT – μ moles of H₂O₂ utilized/min/mg protein.
GPx – μ moles of GSH oxidised /min/mg protein.

**p<0.01; ***p<0.001; †p<0.05
NS Non-significant. † when compared with group I. †† when compared with group II.
The activities of GSH, Vit C and Vit E level were significantly decreased in haemolysate of tumor bearing animals (Group II) as compared to control animals. Geraniol administrated rats showed significant increase in the non-enzymatic antioxidants levels (Group III and Group IV).

The activities of non-enzymatic antioxidants (GSH, Vit C and Vit E) level in liver of the experimental animals in each group are shown in Table 4.

Table 2. Effect of geraniol on the enzymatic antioxidants in liver of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Superoxide dismutase (SOD)</th>
<th>Catalase (CAT)</th>
<th>Glutathione peroxidase (GPx)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>8.14 ± 0.47</td>
<td>60.88 ± 3.39</td>
<td>5.40 ± 0.30</td>
</tr>
<tr>
<td>Group II (DEN)</td>
<td>4.41 ± 0.34***</td>
<td>43.05 ± 1.56***</td>
<td>2.77 ± 0.42***</td>
</tr>
<tr>
<td>Group III (DEN + Geraniol)</td>
<td>7.30 ± 0.42***</td>
<td>54.23 ± 0.61***</td>
<td>4.43 ± 0.36***</td>
</tr>
<tr>
<td>Group IV (Geraniol)</td>
<td>7.87 ± 0.19NS</td>
<td>60.25 ± 1.15NS</td>
<td>5.16 ± 0.11NS</td>
</tr>
</tbody>
</table>

Values represent mean ± SD for rats in each group.
Units: SOD – units /mg protein
CAT – µ moles of H₂O₂ utilized/min/mg protein.
GPx – µ moles of GSH oxidised /min/mg protein.
*** p<0.001; ** p<0.01; * p<0.05
NS Non-significant. a when compared with group I. b when compared with group II.

Table 3. Effect of geraniol on the non-enzymatic antioxidants in haemolysate of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glutathione reduced (GSH)</th>
<th>Vitamin C</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>3.19 ± 0.29</td>
<td>1.90 ± 0.06</td>
<td>1.41 ± 0.11</td>
</tr>
<tr>
<td>Group II (DEN)</td>
<td>1.29 ± 0.04***</td>
<td>0.84 ± 0.03***</td>
<td>0.71 ± 0.04***</td>
</tr>
<tr>
<td>Group III (DEN + Geraniol)</td>
<td>2.76 ± 0.27***</td>
<td>1.79 ± 0.12***</td>
<td>1.26 ± 0.15***</td>
</tr>
<tr>
<td>Group IV (Geraniol)</td>
<td>2.85 ± 0.30NS</td>
<td>1.86 ± 0.04NS</td>
<td>1.24 ± 0.14NS</td>
</tr>
</tbody>
</table>

Values represent mean ± SD for 6 rats in each group.
Units: GSH, vitamin C, vitamin E- µg/mg protein.
*** p<0.001; ** p<0.01; * p<0.05
NS Non-significant. a when compared with group I. b when compared with group II.

Table 4. Effect of geraniol on the non-enzymatic antioxidants in liver of control and experimental animals

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glutathione reduced (GSH)</th>
<th>Vitamin C</th>
<th>Vitamin E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>4.46 ± 0.35</td>
<td>2.81 ± 0.26</td>
<td>1.83 ± 0.05</td>
</tr>
<tr>
<td>Group II (DEN)</td>
<td>2.33 ± 0.16***</td>
<td>1.33 ± 0.08***</td>
<td>0.84 ± 0.02***</td>
</tr>
<tr>
<td>Group III (DEN + Geraniol)</td>
<td>3.83 ± 0.13***</td>
<td>2.45 ± 0.16***</td>
<td>1.72 ± 0.18***</td>
</tr>
<tr>
<td>Group IV (Geraniol)</td>
<td>4.20 ± 0.16NS</td>
<td>2.60 ± 0.30NS</td>
<td>1.80 ± 0.06NS</td>
</tr>
</tbody>
</table>

Values represent mean ± SD for 6 rats in each group.
Units: GSH, Vitamin C, Vitamin E- µg/mg protein
*** p<0.001; ** p<0.01; * p<0.05
NS Non-significant. a when compared with group I. b when compared with group II.
4. Discussion

In the present study we demonstrate that the monoterpene geraniol has antioxidant activity. Geraniol, an acyclic monoterpene alcohol found in lemongrass and aromatic herb oils, has been shown to exert in vitro and in vivo antitumor activity against murine leukemia, hepatoma, and melanoma cells\textsuperscript{25–27}.

M. S. Seema Farhath et al.\textsuperscript{28} studied the Antioxidant activity of Geraniol, Geranial acetate, Gingerol and Eugenol was employed by two complementary test systems, namely, 2,2-diphenyl-1-picrylhydrazyl (DPPH) free-radical scavenging and Super Oxide Dimutase (SOD) activity. Antioxidant activity of Gingerol was found to be higher than those of the others in DPPH assay.

Geraniol, a colourless liquid, is an acyclic terpene alcohol with a flowery rose-like odour. It is found widely as a chief constituent in essential oils including orange flower oil, lemon grass oil and lavender oil. Experimental studies demonstrated several pharmacological activities including antioxidant and anticancer potential of geraniol\textsuperscript{29}.

Geraniol exerted anti-tumor activity against various cancer cells both in vitro and in vivo\textsuperscript{28–32}. It has also been reported that geraniol exhibited potent insecticidal, antimicrobial and anti-inflammatory effects\textsuperscript{33–34} reported that dietary geraniol suppressed hepatic HMG CoA reductase activity and lowered the levels of serum cholesterol in experimental animals.

Artega et al.\textsuperscript{35} performed extend study focused on the antioxidant activity of low-molecular antioxidants present in the spice and some pharmaceuticals (gallic acid, sesamol, eugenol, thymol, carvacrol, vanillin, salicylaldehyde, limonene, geraniol, 4-hexylresorcinol).

Hanaa H et al.\textsuperscript{36} studied the essential oil Dracocephahan moldavica L by TLC, GC-MS, HPLC and NMR and its constituents were gerneyl acetate, geraniol, nerol, neryl acetate, nerol and linoolool. This oil showed antimicrobial and antioxidant activity.

S. Manoharan et al.\textsuperscript{37} investigated the chemopreventive potential of geraniol, an acyclic monoterpene alcohol, by monitoring the tumor incidence and analyzing the status of phase II detoxification agents, lipid peroxidation by products and antioxidants in 7,12-dimethylbenz(a)anthracene (DMBA) induced mouse skin carcinogenesis and found that geraniol might have inhibited abnormal cell proliferation occurring in skin carcinogenesis by modulating the activities of phase II detoxification agents and through its free radical scavenging potential.

5. Conclusion

From this study Geraniol shows to exhibited chemopreventive potential against in N-Nitrosodiethylamine induced hepatocarcinogenesis in Wistar Albino Rats. Thus concluding that the chemopreventive potential of Geraniol could be due to its enzymatic antioxidant and non-enzymatic properties against N-Nitrosodiethylamine (DEN) induced Hepatocarcinogenesis (HCC).

6. References