ANTIOXIDANT ACTIVITY OF ETHANOLIC EXTRACTS OF *DAUCUS CAROTA* LEAF

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**ABSTRACT**

The antioxidant properties in carrots were investigated in this study. Methanolic extract of *daucus carota* is used for antioxidant activity. Oxidative stress were induced in rats by thioacetamide 100mg/kg s.c in four groups of rats (2 test, control, standard). The test groups received daucus carota leaf extract at doses of 200 and 400mg/kg. Standard group received silymarin (25mg/kg) and control only thioacetamide on the animal were sacrificed and brain like superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase (GRD) and lipid Peroxidation were estimated in brain homogenate. The antioxidant enzyme a significant (P<0.001) increase in SOD, CAT, GRD was observed in all drug treated groups as compared with thioacetamide group. But in case of LPO a significant (P<0.001) reduction was observed as compared to toxic control group. DCLE has contributed to the reduction of oxidative stress of brain.

**KEYWORDS:** *Daucus carota* leaf extract, Silymarin, Lipid Peroxidation, ROS.

**1. INTRODUCTION**

In oxidation process highly reactive and harmful chain reactions of oxygen species are generated, causing damage to living organism. The oxygen centered free radicals and other reactive oxygen species (ROS), which are continuously produced has resulted in cell death or tissue damage. The oxidative damage cause d by free radical is related to pathogenesis of many chronic degenerative disease like cancer, diabetes, neurodegenerative disease, atherosclerosis, cirrhosis, malaria and AIDS.¹ Reactive oxygen species including superoxide free radical, hydrogen peroxide, hydrogen free radical and singlet oxygen play a key role in
the oxidative damage of these disease. This in turn resulted in DNA mutation, protein inactivation, rapid peroxidation and cell death.[2]

Antioxidant is a molecule which terminate the chain reaction by removing free radical intermediates. Plants and animals maintain complex system of multiple type of antioxidant. The natural plant based antioxidants have played an important role in the maintenance of human health for the past three decades.[3]

Daucus carota (D.carota) Linn commonly known as “carrot” belong to family Apiaceae and is cultivated almost all over the world as a useful vegetable. The plant has undergone extensive phytochemical studies and a large number of active ingredients have been isolated. These include volatile oils, steroids, triterpenes, carbohydrates, glycerides, tannins, flavonoids, aminoacid carotene and hydro carotene.[4] Pharmacological studies showed that D.carota exhibit antifertility, hypoglyacemic,hepatoprotective and aphrodisiac activity.[5,6] D.carota is also used as a noval model to evaluate the effect of light on carotenogenic gene expression isolated animal organ studies.[7,8]

The objective of present study was to evaluate the antioxidant activity of ethanolic extract of D. carota leaf extract in rats.

2. MATERIALS AND METHODS

2.1. Plant material
Carrot leaf were collected from Lucknow. The plant was identified by a CDRI. After authentication, leaves were cleaned and milled into coarse powder by a mechanical grinder.

2.2. Preparation of leaf extract
Powdered leaves (2 kg) were extracted with 95% ethanol using a Soxhlet apparatus. The ethanolic extract was filtered and concentrated by distillation process. A green colored residue was obtained (yield 6.79% w/w) and was kept in a desiccators. This ethanolic extract of D. carota leaf extract (DCLE) was used for further experimants.

2.3. Experimental animals
Healthy, adult male rats of Wister strain, weighing (180 ± 5) g were obtained from animal house, CDRI lucknow India. The animals were kept in a well-ventilated room and they were exposed to 12 hours day and 12 hours night cycle with a temperature between (20 ± 2)c. The animals were housed in spacious, hygienic polypropylene cages during the course of the
experiment. The animals were fed with water and mice pellet feed. All the experimental procedures and protocols used in this study were reviewed by institutional animal ethics committee and were in accordance with the guidelines of the CPCSEA.

2.4. Drugs and chemicals
Silymarin (Silybin 140) was purchased from Microlabs Limited, Goa and Thioacetamide from Lova Laboratories Pvt. Ltd., Mumbai. All others chemicals used in this study were of analytical grade.

2.5. Experimental design
Rats were randomly divided into five groups of four animals each and each group was kept in a separate cage. All the groups were treated orally for 7 days. Group I served as negative control and was treated with vehicle (0.5% carboxyl methyl cellulose). Group II served as positive control and treated with vehicle water. Group III served as standard and was treated with silymarin 25 mg/kg. Group IV was treated with 200 mg/kg DCLE by suspending in 0.5% carboxyl methyl cellulose. Group V was treated with 400 mg/kg DCLE by suspending in 0.5% carboxyl methyl cellulose.

2.6. Biochemical estimation
The biochemical Parameters of oxidative stress were estimated in all groups after the completion of behavioral studies. These estimations were done in whole brain homogenate. Rat were decapitated under ether anesthesia. the skull was cut, opened and the brain was exposed from its dorsal side. The whole brain was quickly removed and cleaned with chilled normal saline on ice. The isolated whole brain was homogenized to prepare 10% (W/V) brain homogenate samples. Further these homogenized sample were centrifuged at 4000 rpm, -22°C for 10 minutes using a refrigerated centrifuge and the supernatant was collected to estimate the biochemical parameters such as superoxide dismutase (SOD), Catalase (CAT), Glutathione reductase(GRD) and lipid peroxidation activity in brain homogenate.

2.7. Statistical analysis
The collected data were subjected to appropriate statistical test like one-way ANOVA (Analysis of variance), followed by an appropriate turkey test. P values of less than 0.01 were considered as significant. The analysis was carried out using Graph pad prism software of Version 4.
3. RESULTS
The DCLE dose at 200 mg/kg and 400 mg/kg dose significantly decrease the level (P< 0.001) in blood serum as compared to thioacetamide treated animals. Silymarin treated group showed significant decrease (P< 0.001) when compared to thioacetamide treated animals. The dose of 400 mg/kg treated group was found to be more effective similar to silymarin treated group (Table 1).

Enzymatic antioxidants like SOD, CAT, GRD, GPX and GST activity in brain was found significantly reduced (P< 0.001) in thioacetamide treated animals when compared to control.

200 mg/kg and 400 mg/kg DCLE treated group significantly increased (P< 0.001) the level of enzymatic antioxidants when compared to thioacetamide treated animals. Silymarin treated animals also increases more significantly (P< 0.001) the level of enzymatic antioxidants in the liver homogenate when compared with thioacetamide treated animals. The dose of 400 mg/kg treated group was found to be more effective similar to silymarin treated group (Table 2.)

LPO activity was significantly increased (P< 0.001) in thioacetamide treated group when compared to control. The dose of 200 mg/kg and 400 mg/kg treated group was found significantly decreased (P< 0.001) the level of LPO in liver homogenate when compared to thioacetamide treated animals. Silymarin treated group was found to be more significant (P< 0.001) when compared to thioacetamide treated animal.

<table>
<thead>
<tr>
<th>S.N.</th>
<th>Group(n=4)</th>
<th>SOD (µ/mg protein)</th>
<th>CAT(µ/mg protein)</th>
<th>GRD(µ/mg protein)</th>
<th>LPO(µ/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Negative control(Water)</td>
<td>---</td>
<td>---</td>
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</tr>
<tr>
<td>2.</td>
<td>Positive control(CMC Suspension)</td>
<td>10.11±0.07***</td>
<td>63.24±1.68***</td>
<td>7.08±0.06***</td>
<td>16.47±0.09***</td>
</tr>
<tr>
<td>3.</td>
<td>Standard(silymarin,25mg/kg)</td>
<td>9.65±0.11***</td>
<td>58.46±1.44***</td>
<td>4.12±0.08***</td>
<td>12.67±0.07***</td>
</tr>
<tr>
<td>4.</td>
<td>DCLE(200mg/kg)</td>
<td>6.15±0.09***</td>
<td>52.23±1.64***</td>
<td>3.09±0.01***</td>
<td>10.76±0.02***</td>
</tr>
<tr>
<td>5.</td>
<td>DCLE(400mg/kg)</td>
<td>4.13±0.13***</td>
<td>49.12±1.46***</td>
<td>2.87±0.02***</td>
<td>9.89±0.04***</td>
</tr>
</tbody>
</table>

4. DISCUSSION
Thioacetamide is a potent hepatotoxin and carcinogen for rats. It is also known to produce marked liver damage in exposed animals. Toxicity experienced by liver during thioacetamide poisoning results from the production of metabolite, thioacetamide S- oxide, which is direct hepatotoxin. It has also been observed that thioacetamide causes changes in nucleolus and
increased synthesis of guanine and cytosine rich RNA, with concomitant decrease in ribosomal RNA in the cytoplasm.\textsuperscript{[18-27]}

In the present study pretreatment with extract was found to significantly reverse the thioacetamide rise in the biochemical parameters like SGPT, SGOT and ALP level, thereby demonstrating the membrane stabilizing activity of the extract. The activities of SGPT and SGOT were almost brought down to normal suggesting the membrane stabilizing effect of the extract. The difference between group II and group V was found to be more statistically significant. The level of ALP, which was elevated was also brought down in the rats pretreated with the extract followed by thioacetamide.

In case of antioxidant enzyme, DCSE was found to significantly increase the level of SOD, CAT, GRD, GPX and GST. The level of LPO which was elevated due to thioacetamide also came to normal.

DCSE has contributed to the reduction of oxidative stress and showed hepatoprotective activity in experimental rats.

\textbf{Conflict of interest statement}

We declare that we have no conflict of interest.

\section*{ACKNOWLEDGMENTS}

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