Oxidative stress associated alterations in lysosomal enzymes & modulatory effect of Petroselinum crispum (mill) NYMAN EX A. W. HILL leaf extract on mouse brain

Shreya R. Vora, Rahul B. Patil & Meena M. Pillai

ABSTRACT

Reactive oxygen species are one of the causative factors for the stress in post-mitotic cells. Mitochondria are the major source of the free radicals in the cells. In the present research work it has been aim to examine the effect of ethanolic extract of Petroselinum crispum (Parsley) leaves on the D-galactose induced oxidative stress in the brain regions of mouse. The activities of Lysosomal enzymes like Acid phosphatase & Non-specific esterase were measured in conjunction with lipid peroxidation in mitochondrial fraction of various regions of brain. A significant decrease in acid phosphatase & Non-specific esterase activity was observed in D-galactose stressed mice, whereas treatment of D-galactose with ethanolic extract of parsley group of mice showed protection against increased oxidative stress in all brain regions. Thus it is postulated that, parsley possess an antioxidant properties, that increases the level of enzyme activities in brain regions.

1. Introduction

Reactive oxygen species are one of the causative factors for the stress in the post-mitotic cells [1]. Mitochondria are supposed to be the chief source of reactive oxygen species (ROS) [2]. During enzymatic reduction of oxygen to produce energy, free radicals form as a byproduct [3]. A free radical is an oxygen molecule containing one or more unpaired electrons. Normally, molecular oxygen has two unpaired electrons and this electronic structure makes oxygen especially susceptible to radical formation e.g., addition of an extra electron to molecular oxygen (O2) forms a superoxide anion radical (O2-), the primary form of ROS. Then it is directly or indirectly converted into secondary ROS like hydroxyl radical (OH), peroxy radical (ROO-) or hydrogen peroxide (H2O2) [4]. Free radicals induce cellular damage when they pass this unpaired electron onto nearby cellular structures, resulting in the oxidation of cell membrane lipids, amino acids in proteins or nucleic acids [5]. As the age progresses damage due to ROS in post-mitotic cells like heart, brain also increases [6].

To scavenge & neutralize these free radicals, the cells are endowed with antioxidant defense system of enzymes such as superoxide dismutase (SOD), Glutathione peroxidase (GPx) & Catalase (CAT). But imbalance between ROS production & antioxidant defense mechanism of cells leading to excessive production of free radicals, creates a condition termed as oxidative stress [7,8]. Such a continuous oxidative stress induces the leakage of lysosomal enzymes into the cytosol. ROS also bring about cross linking in macromolecules, which greatly diminish the lysosomal degradative capacity by preventing lysosomal enzymes from targeting to functional autosomes. This leads to progressive accumulation of non-degradable, autofluorescent age pigment, lipofuscin. All these changes irreversible lead to functional decay & death of post-mitotic cells [2]. Lysosomes secrete more than 40 different hydrolytic enzymes including proteases, lipases & phosphatases. In the present investigation we have carried out estimation acid phosphatase & Non-specific esterase.

When such overwhelming condition of ROS over self antioxidant defense system occurs, there is a need of antioxidant supplementation in the form of dietary supplements. Extracts of some Ayurvedic plant have been shown to be strong antioxidant properties. Some of them are Bacopa monniera, Lactuca sativa, Petroselinum crispum, Withania sominifera, Asparagus roseomus, Evolulus alsinoides & Emblica officinalis[9-13]. The choice of plant
for present investigation was used is Petroselinum crispum, rich in flavonol glycosides like quercetin, apiin, apigenin etc. [14], belongs to family Umbelíferae, native of Europe. The leaf contain essential oils including 20% myristicin, 18 % apiole & other terpenes, phthalides, coumarins like bergapten, starch, oils including 20% myristicin, 18 % apiole & other terpenes, phthalides, coumarins like bergapten, starch, and sucrose and 1mM EDTA (2 mg/ml). The uniform homogenates were centrifuged at 1500 rpm for 10 min. The supernatants A obtained were again subjected to centrifugation at 10000 rpm for 20 min. the supernatants B, thus obtained were treated as cytosolic fraction containing microsomes, used for estimation of microsomal acid phosphatase activity. The pellets obtained after high centrifugation contained lysosomes mitochondria. Acid phosphatase is not present in mitochondria. Therefore, these pellets containing lysosomes are the only source of acid phosphatase. These pellets were suspended in 0.8 ml. distilled water and 0.2 ml 20% Triton X-100 and centrifuged at 10 000 rpm for 20 min. the supernatants thus obtained were used as samples for lysosomal acid phosphatase estimation. To 0.2 ml of the sample 0.8 ml of acid buffer substrate solution was added (5.5 x 10⁻⁶ M p-nitrophenyl phosphate in 0.05 M sodium citrate buffer, pH 4.8). The mixture was incubated at 37°C for 30 min. and 4 ml0.1N NaOH was added to inhibit the reaction. The absorbance was read at 405 nm.

2.3.1. Estimation of Acid phosphatase Activity

Acid phosphatase activity was estimated by the method of Linhardt and Walter [19]. The tissue was homogenized in 0.25M sucrose at 1mMEDTA (2 mg/ml). The uniform homogenates were centrifuged at 1500 rpm for 10 min. The supernatants A obtained were again subjected to centrifugation at 10000 rpm for 20 min. the supernatants B, thus obtained were treated as cytosolic fraction containing microsomes, used for estimation of microsomal acid phosphatase activity. The pellets obtained after high centrifugation contained lysosomes mitochondria. Acid phosphatase is not present in mitochondria. Therefore, these pellets containing lysosomes are the only source of acid phosphatase. These pellets were suspended in 0.8 ml. distilled water and 0.2 ml 20% Triton X-100 and centrifuged at 10 000 rpm for 20 min. the supernatants thus obtained were used as samples for lysosomal acid phosphatase estimation. To 0.2 ml of the sample 0.8 ml of acid buffer substrate solution was added (5.5 x 10⁻⁶ M p-nitrophenyl phosphate in 0.05 M sodium citrate buffer, pH 4.8). The mixture was incubated at 37°C for 30 min. and 4 ml0.1N NaOH was added to inhibit the reaction. The absorbance was read at 405 nm.

2.3.2. Non-specific esterase activity

It was measured by the method of Bier [20]. The separated brain and heart regions were homogenized in 0.66 M phosphate buffer pH 7.0. 1 ml of homogenates were added to 5 ml ice cold water and 2 ml phosphate buffer previously taken into the test tubes. 2 ml of working substrate solution was added to it and all the sample tubes were centrifuged at 2000 rpm for 10 min.at 4°C. After incubation of the tubes, readings of assay mixtures were taken at 400 nm. The optical densities were converted to µM of p-nitrophenol from the p-nitrophenol standard curve.

2.3.3. Estimation of protein

The total protein was performed by the method of Lowry [21]. The weighed and thawed tissues were homogenized in distilled water 2 mg/ml), centrifuged at 3000 rpm for 10 min. the supernatants were used for the estimation. 0.5 ml of all the samples were added to 3.5 ml distilled water and reagent C previously taken into the test tubes. After 10 min. 0.5 ml Folin reagent was added. After 30 min. the optical densities were read at 660 nm.

2.4. Statistical analysis

Statistical analysis was performed by Student‘t’-test. P<0.001 is considered highly significant.

3. Results

Acid Phosphatase: Injection of D-galactose reduced the activity of enzyme in all the studied brain regions significantly compared to control. Reduction was found maximum in hippocampus. The activity was found significantly increased in cerebellum & hippocampus of Parsley extract along with D-galactose injected group (Table 1 & 2).

Non-Specific Esterase: Aging accelerated group shows significantly decreased activity of enzyme in all brain regions. After administration of Petroselinum crispum along with D-galactose, Corpora quadrigemina shows higher activity than other brain regions. Treatment of leaf extract of Parsley along with D-galactose, increased the enzyme activity with similar degree of significance in all regions of brain (p<0.001) when compared to accelerated aging group (Table 3).
**Protein:** Protein content is found high in Cerebral cortex & hippocampus in control group of mice. Injection of D-galactose reduced the amount of protein content in all tissues. This fall was significantly in Corpora quadrigemina & highly significant in rest of tissues. Administration of Parsley with D-galactose increased amount of protein in all tissues (Table 4).

**Table 1: Effect of *Petroselinum crispum* leaf extract on Microsomal Acid Phosphatase activity of Brain of D-galactose induced aged mice (µmols/mg protein)**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Normal</th>
<th>D-galactose Injected</th>
<th>D-galactose + Parsley</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex (5)</td>
<td>8.21±0.02</td>
<td>6.54±0.15</td>
<td>7.95±0.03</td>
<td>t=24.19p&lt;0.001 t=20.30p&lt;0.001</td>
</tr>
<tr>
<td>Hippocampus (5)</td>
<td>6.08±0.08</td>
<td>4.90±0.05</td>
<td>5.87±0.1</td>
<td>t=26.95p&lt;0.001 t=19.26p&lt;0.001</td>
</tr>
<tr>
<td>Cerebellum (5)</td>
<td>5.79±0.25</td>
<td>3.17±0.03</td>
<td>5.70±0.1</td>
<td>t=22.90p&lt;0.001 t=53.27p&lt;0.001</td>
</tr>
<tr>
<td>Corpora quadrigemina (5)</td>
<td>7.07±0.07</td>
<td>5.88±0.07</td>
<td>6.22±0.05</td>
<td>t=25.69p&lt;0.001 t=8.56p&lt;0.001</td>
</tr>
</tbody>
</table>

Values in the paranthesis denote number of animals. Values are Mean ± S.D. p<0.001 highly significant

**Table 2: Effect of *Petroselinum crispum* leaf extract on Lysosomal Acid Phosphatase activity of Brain of D-galactose induced aged mice (µmols/mg protein)**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Normal</th>
<th>D-galactose Injected</th>
<th>D-galactose + Parsley</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex (5)</td>
<td>7.18±0.10</td>
<td>6.29±0.06</td>
<td>6.86±0.04</td>
<td>t=15.83p&lt;0.001 t=15.56p&lt;0.001</td>
</tr>
<tr>
<td>Hippocampus (5)</td>
<td>5.19±0.05</td>
<td>4.60±0.06</td>
<td>5.04±0.06</td>
<td>t=15.98p&lt;0.001 t=11.30p&lt;0.001</td>
</tr>
<tr>
<td>Cerebellum (5)</td>
<td>5.43±0.47</td>
<td>3.05±0.03</td>
<td>3.94±0.02</td>
<td>t=11.18p&lt;0.001 t=42.31p&lt;0.001</td>
</tr>
<tr>
<td>Corpora quadrigemina (5)</td>
<td>6.08±0.05</td>
<td>5.55±0.04</td>
<td>6.00±0.07</td>
<td>t=16.09p&lt;0.001 t=22.06p&lt;0.001</td>
</tr>
</tbody>
</table>

Values in the paranthesis denote number of animals. Values are Mean ± S.D. p<0.001 highly significant

**Table 3: Effect of *Petroselinum crispum* leaf extract on Non-specific Esterase activity of Brain of D-galactose induced aged mice (µmols/mg protein)**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Normal</th>
<th>D-galactose Injected</th>
<th>D-galactose + Parsley</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex (5)</td>
<td>36.82±0.43</td>
<td>28.16±1.47</td>
<td>36.75±0.40</td>
<td>t=11.50p&lt;0.001 t=12.55p&lt;0.001</td>
</tr>
<tr>
<td>Hippocampus (5)</td>
<td>30.63±1.23</td>
<td>22.62±1.05</td>
<td>28.83±1.18</td>
<td>t=9.67p&lt;0.001 t=8.74p&lt;0.001</td>
</tr>
<tr>
<td>Cerebellum (5)</td>
<td>41.17±1.20</td>
<td>31.71±0.95</td>
<td>38.34±1.13</td>
<td>t=9.99p&lt;0.001 t=6.98p&lt;0.001</td>
</tr>
<tr>
<td>Corpora quadrigemina (5)</td>
<td>51.42±0.98</td>
<td>45.15±1.35</td>
<td>52.08±0.63</td>
<td>t=8.35p&lt;0.001 t=10.33p&lt;0.001</td>
</tr>
</tbody>
</table>

Values in the paranthesis denote number of animals. Values are Mean ± S.D. p<0.001 highly significant

**Table 4: Effect of *Petroselinum crispum* leaf extract on Total Protein activity of Brain of D-galactose induced aged mice (µmols/mg protein)**

<table>
<thead>
<tr>
<th>Organ</th>
<th>Normal</th>
<th>D-galactose Injected</th>
<th>D-galactose + Parsley</th>
<th>Statistical Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cerebral cortex (5)</td>
<td>0.06±0.002</td>
<td>0.053±0.002</td>
<td>0.056±0.007</td>
<td>t=24.19p&lt;0.001 t=20.30p&lt;0.001</td>
</tr>
<tr>
<td>Hippocampus (5)</td>
<td>0.06±0.003</td>
<td>0.051±0.001</td>
<td>0.053±0.0012</td>
<td>t=26.95p&lt;0.001 t=19.26p&lt;0.001</td>
</tr>
<tr>
<td>Cerebellum (5)</td>
<td>0.057±0.001</td>
<td>0.051±0.001</td>
<td>0.054±0.0007</td>
<td>t=22.90p&lt;0.001 t=53.27p&lt;0.001</td>
</tr>
<tr>
<td>Corpora quadrigemina (5)</td>
<td>0.049±0.0015</td>
<td>0.045±0.0012</td>
<td>0.047±0.0013</td>
<td>t=25.69p&lt;0.001 t=8.56p&lt;0.001</td>
</tr>
</tbody>
</table>

Values in the paranthesis denote number of animals. Values are Mean ± S.D. p<0.001 highly significant
4. Discussion

A reducing sugar, D-galactose, is actually present in the body but when its level increases above normal, it goes oxidized into aldehydes and hydrogen peroxide [22] and stimulates some degenerative diseases like diabetes mellitus induces premature aging with increased serum AGE content and decreases motor activity [17]. D-galactose also reduces immune responses & increases oxidative stress by increasing lipid peroxidation & decreases antioxidant enzyme activities & mitochondrial function by inducing degeneration [23].

Data derived from present investigation showed marked alterations in acid phosphatase and non-specific esterase activity of various regions of brain in D-galactose induced group of mice as compared to controls. Insufficiency of lysosomal enzyme activity is further responsible for lipofuscin granules formation. Excessive generation of ROS during oxidative phosphorylation in mitochondria, attack membrane bound lipids, proteins & nucleic acids. Due to this mitochondrial metabolism may negatively affect resulting into progressive decrease in the efficiency of mitochondrial enzymes like and antioxidant defense system as well. These alterations are prominent in brain [24].

Parsley has shown to overcome the oxidative stress due to the presence of flavonol glycoside in it [25]. Administration of parsley along with D-galactose significantly elevated the level of acid phosphatase in brain region in the present investigation. To reduce the risk of oxidative stress and age related pathologies. Several dietary antioxidants are preferred like vitamin C, E & plant phytochemicals like flavonoids. They increases immunity power, dietary antioxidants are preferred like vitamin C, E & plant phytochemicals like flavonoids. They increases immunity power, increases oxidative stress by increasing lipid peroxidation & decreases antioxidant enzyme activities & mitochondrial function by inducing degeneration [23].

Results reveal that D. crispum leaves of parsley have many phytochemical constituent which may be responsible for many antioxidant activities. Present investigation suggest that parsley leaf extract, increased the level of protein as well as Acid phosphatase and Non-specific esterase enzyme activities in brain of Galactose treated mice.

Author’s Contribution

RP collected plant, prepared plant extract, carried out enzymatic activities and drafted manuscript. SV & MP have equal contribution in carrying out all enzymatic assays.

Acknowledgement

The authors are thankful to Dr. S. G. Nanaware, Head, Department of Zoology, (Shivaji University), Kolhapur (M.S.) India, for offering facilities to carry out this study. The authors are also grateful to Fumes Chemicals, Kolhapur; for providing D-galactose, other chemicals required for present investigation and make Petroselinum crispum available to carry out analysis on enzymatic activities.

6. References