Protective effect of proanthocyanidins on endotoxin induced experimental periodontitis in rats

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Received 5 August 2009; revised 17 November 2009

The pathogenesis of periodontitis involves anaerobic oral bacteria as well as the host response to infection and several drugs have been developed which can curtail these deleterious effects. Proanthocyanidin, a novel flavanoid extracted from grape seeds, has been shown to provide a significant therapeutic effect on endotoxin (Escherichia coli) induced experimental periodontitis in rats. In this study, protective action of different doses of proanthocyanidins was investigated in blood by assaying the reactive oxygen species such as hydrogen peroxide, superoxide anion, myeloperoxidase and lipid peroxides, lysosomal enzyme activities such as cathepsin B, cathepsin D, β-glucuronidase and acid phosphatase, nonenzymatic antioxidants such as ascorbic acid, α-tocopherol, ceruloplasmin, reduced glutathione and antioxidant enzymes such as catalase, superoxide dismutase, glutathione peroxidase and glutathione-s-transferase. Experimental periodontitis rats showed a reduction in body weight and body weight gain could be noticed when they were administered proanthocyanidins. The levels of reactive oxygen species and lysosomal enzymes were found to increase whereas antioxidant levels were decreased significantly in experimental periodontitis. Proanthocyanidins at an effective dose of 30mg / kg body weight, sc, for 30 days effected a decrease in serum reactive oxygen species, lipid peroxides, lysosomal enzymes, acute phase proteins and an increase in antioxidant levels. Histopathological evidence of experimental periodontitis showed cellular infiltration of inflammatory cells while proanthocyanidin treated groups demonstrated only scattered inflammatory cells and blood vessels. Thus, the results showed that dietary supplementation of proanthocyanidin enhanced the host resistance as well as the inhibition of the biological and mechanical irritants involved in the onset of gingivitis and the progression of periodontal disease.

Keywords: Acute phase proteins, Antioxidants, Experimental periodontitis, Lysosomal enzymes, Metronidazole, Proanthocyanidin, Reactive oxygen species

The inflammatory and immune reactions induced by the bacterial plaque represent the main characteristics of periodontitis¹. Invading bacteria trigger the release of cytokines leading to elevated activity of polymorphonuclear leukocytes (PMN) which, in turn, produce reactive oxygen species (ROS). Increased production of ROS contributes to oxidative stress which is reported to have significant influence in many diseases including periodontitis². The phagocytes and the release of lysosomal enzymes by PMN and macrophages can lead to severe periodontitis³.

The antioxidant mechanisms are the evolutionary designs that avidly react and annihilate ROS before they inflict oxidative damage to tissues and cells⁴. ROS can cause DNA and protein damage, initiate lipid peroxidation, oxidize α1-antitrypsin and stimulate the release of proinflammatory cytokines⁴. But, studies on the defense mechanism against oxidative stress in the pathogenesis of periodontal disease have received little attention.

Proanthocyanidins (PC), a type of flavanoids, are extracted generally from grape seeds. PC are known to consist largely of gallic acid (GA), catechin (Cat), epicatechin (Epi) and procyandin dimers and trimers composed of flavan-3-ol units with C4-C8 or C4-C6.
interflavan linkages. In vitro studies have shown that grape seed proanthocyanidins have antioxidant property, free radical scavenging property, anti-tumor, and anticarcinogenic property. But, in vivo biochemical studies on the protective effect of proanthocyanidins in periodontal pathology have not yet been reported. Since oxidative stress is encountered in periodontitis, the effect of PC on periodontal inflammation is worth studying.

Hence, this study is designed to assess whether proanthocyanidins could exert protective as well as therapeutic effect on endotoxin induced experimental periodontitis in rats. Protective action of different doses of proanthocyanidins is investigated by assaying the free radicals, lysosomal enzyme activities, acute phase proteins, enzymatic and nonenzymatic antioxidants, body weight and also by histopathological studies in comparison with a standard antibiotic ‘metronidazole’.

Materials and Methods

Proanthocyanidins were purchased from “Terravita” Brampton, Ontario, Canada. Synthetic substrates were obtained from M/s Sigma Chemical Co., USA. All other chemicals used were of analytical grade.

Male Wistar rats weighing approximately 250g were housed in solid-bottomed polypropylene cages under strict veterinary supervision and maintained in control rooms with 12 h light/dark cycle. The animals received commercial rat diet and water ad libitum. This study conformed to the guiding principles of Institutional Animal Ethical Committee (IAEC), Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Guide for the care and use of laboratory animals.

Extraction of endotoxin preparations was carried out according to Westphal and Jann. To state briefly, water saturated phenol was used for separation. E.coli organism was grown on nutrient agar in a Roux flask overnight and washed with phosphate buffered saline. The culture wash was thoroughly mixed with phenol: water in a separating funnel. The water phase was collected and again extracted with phenol. Likewise, the procedure was repeated thrice in a separating funnel. The supernatant was precipitated with ethanol and sodium acetate. The precipitate (endotoxin) was separated by centrifugation at 10,000 g for 10 min and air dried.

Experimental design and treatment protocol

Induction of experimental periodontitis (EP)—EP was induced by injecting E.coli endotoxin as described by Ramamurthy et al. Under anesthesia (ketamine 50 mg / kg body weight, ip), 1mg endotoxin dissolved in 1ml of saline was injected into the labial and palatal aspects of maxillary anterior gingivae, buccal and palatal aspects of maxillary molars to induce periodontitis. On the 10th day of endotoxin induction, the animals were divided into 15 groups of 6 animals each.

The animals were broadly divided into two groups: Group 1: control; Group 2: experimental periodontitis (EP). Animals of group 2 were further divided into following 14 sub groups and received proanthocyanidin, sc, as follows:

- Group 3: 10mg/kg body weight for 10 days;
- Group 4: 20mg/kg body weight for 10 days;
- Group 5: 30mg/kg body weight for 10 days;
- Group 6: 40mg/kg body weight for 10 days;
- Group 7: 10mg/kg body weight for 20 days;
- Group 8: 20mg/kg body weight for 20 days;
- Group 9: 30mg/kg body weight for 20 days;
- Group 10: 40mg/kg body weight for 20 days;
- Group 11: 10mg/kg body weight for 30 days;
- Group 12: 20mg/kg body weight for 30 days;
- Group 13: 30mg/kg body weight for 30 days;
- Group 14: 40mg/kg body weight for 30 days;
- Group 15: 20mg metronidazole / kg body weight for 30 days (administered orally). Each group comprised 6 animals.

After the experimental period, the animals were sacrificed by cervical decapitation and blood was collected. Bone and teeth of the right maxillary halves were dissected out and the histopathological evaluation was performed on right maxillary halves.

Biochemical assays—Hydrogenperoxide (H$_2$O$_2$), superoxide anion (O$_2^-$), myeloperoxidase (MPO) activity and lipid peroxides (LPD) in terms of malondialdehyde were assayed. The activity of cathepsin B, cathepsin D, β-glucuronidase and acid phosphatase was determined. C-reactive protein (CRP) in serum was carried out according to Kuller et al. and Thompson et al. Assay of fibrinogen was carried out using a standard assay kit (Fibriquik Organon Teknika, USA) and fibrinogen assay was based on a method described by Clauss. Ascorbic acid, α-tocopherol, ceruloplasmin and reduced glutathione (GSH) were estimated in...
serum. Superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx) and glutathione-s-transferase (GST) were estimated in serum. Protein in serum was estimated by the method of Lowry et al. using crystalline bovine serum albumin as the reference standard.

Histopathological studies— Bone and teeth of the right maxillary halves were dissected out, fixed in 10% buffered formalin solution for 24 h, decalcified, processed and embedded in paraffin. Thin sections (7µm thickness) were cut and stained with hematoxylin and eosin and they were analysed by light microscopy.

Statistical methods— One way analysis of variance was applied to evaluate any significant difference in the mean values. All values used in analysis represented as mean ± SE of 6 rats. Multiple comparison of post hoc Tukey’s test was applied to find out the statistically significant groups and Independent T test was applied to compare only group-15 with group 2.

**Results**

Levels of ROS pertaining to \(H_2O_2\), \(O_2^-\), myeloperoxidase and the levels of lipid peroxides were significantly increased in endotoxin induced experimental periodontitis rats as compared to PC and metronidazole treated groups (Table 1). PC treatment showed significant inhibition of reactive oxygen species and lipid peroxides \((P < 0.001)\). Figure 1 showed the reduction in body weight in rats with experimental periodontitis and it could be shown to gain towards normality \((P < 0.001)\) in metronidazole (Group 15) and proanthocyanidin treated groups (Groups 13 and 14).

The activities of lysosomal hydrolases are presented in Table 2. The lysosomal hydrolases viz. cathepsin B, cathepsin D, \(\beta\)-glucuronidase and acid

<table>
<thead>
<tr>
<th>Groups</th>
<th>(H_2O_2) (\mumol/dmg)</th>
<th>(O_2^-) (nmol/min/dmg)</th>
<th>Myeloperoxidase (\mumol/H_2O_2/min/dmg)</th>
<th>Lipid peroxides (nmol/ml)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>2.1± 0.08</td>
<td>4.5 ± 0.15</td>
<td>2.51 ± 0.09</td>
<td>2.9 ± 0.02</td>
</tr>
<tr>
<td>2</td>
<td>8.3 ± 0.24 (\text{a,e})</td>
<td>9.2 ± 0.16 (\text{a,e})</td>
<td>4.5 ± 0.09 (\text{a,e})</td>
<td>7.8 ± 0.03 (\text{a,e})</td>
</tr>
<tr>
<td>3</td>
<td>8.2 ± 0.23 (\text{NS})</td>
<td>9.1 ± 0.15 (\text{NS})</td>
<td>4.4 ± 0.08 (\text{NS})</td>
<td>7.7 ± 0.03 (\text{NS})</td>
</tr>
<tr>
<td>4</td>
<td>8.0 ± 0.22 (\text{NS})</td>
<td>9.0 ± 0.15 (\text{NS})</td>
<td>4.0 ± 0.08 (\text{NS})</td>
<td>7.6 ± 0.04 (\text{NS})</td>
</tr>
<tr>
<td>5</td>
<td>7.8 ± 0.21 (\text{NS})</td>
<td>8.9 ± 0.13 (\text{NS})</td>
<td>3.8 ± 0.08 (\text{NS})</td>
<td>7.5 ± 0.02 (\text{NS})</td>
</tr>
<tr>
<td>6</td>
<td>7.7 ± 0.23 (\text{NS})</td>
<td>8.4 ± 0.14 (\text{c})</td>
<td>3.6 ± 0.06 (\text{d})</td>
<td>7.3 ± 0.05 (\text{d})</td>
</tr>
<tr>
<td>7</td>
<td>8.1 ± 0.21 (\text{NS})</td>
<td>9 ± 0.08 (\text{NS})</td>
<td>4.3 ± 0.08 (\text{NS})</td>
<td>7.7 ± 0.03 (\text{NS})</td>
</tr>
<tr>
<td>8</td>
<td>7.8 ± 0.23 (\text{NS})</td>
<td>8.8 ± 0.098 (\text{e})</td>
<td>3.9 ± 0.06 (\text{e})</td>
<td>7.4 ± 0.06 (\text{e})</td>
</tr>
<tr>
<td>9</td>
<td>7.7 ± 0.22 (\text{NS})</td>
<td>8.6 ± 0.05 (\text{d})</td>
<td>3.5 ± 0.05 (\text{d})</td>
<td>7.0 ± 0.06 (\text{d})</td>
</tr>
<tr>
<td>10</td>
<td>7.3 ± 0.18 (\text{c})</td>
<td>7.6 ± 0.03 (\text{c})</td>
<td>3.1 ± 0.04 (\text{c})</td>
<td>6.6 ± 0.04 (\text{c})</td>
</tr>
<tr>
<td>11</td>
<td>7.9 ± 0.2 (\text{d})</td>
<td>8.1 ± 0.04 (\text{d})</td>
<td>4.1 ± 0.06 (\text{d})</td>
<td>7.2 ± 0.04 (\text{d})</td>
</tr>
<tr>
<td>12</td>
<td>6.4 ± 0.16 (\text{d})</td>
<td>6.6 ± 0.04 (\text{d})</td>
<td>3.5 ± 0.05 (\text{d})</td>
<td>5.7 ± 0.17 (\text{d})</td>
</tr>
<tr>
<td>13</td>
<td>3.7 ± 0.14 (\text{d})</td>
<td>5.2 ± 0.09 (\text{d})</td>
<td>3.1 ± 0.03 (\text{d})</td>
<td>3.4 ± 0.13 (\text{d})</td>
</tr>
<tr>
<td>14</td>
<td>3.2 ± 0.08 (\text{f})</td>
<td>5.0 ± 0.09 (\text{f})</td>
<td>2.8 ± 0.05 (\text{f})</td>
<td>3.3 ± 0.10 (\text{f})</td>
</tr>
<tr>
<td>15</td>
<td>3.3 ± 0.18 (\text{f})</td>
<td>4.0 ± 0.07 (\text{f})</td>
<td>3.5 ± 0.08 (\text{f})</td>
<td>4.9 ± 0.26 (\text{f})</td>
</tr>
</tbody>
</table>

\(P\) values: \(\text{a}< 0.001\) as compared to Group 1 by POST HOC Tukey HSD; \(\text{b}< 0.05\); \(\text{c}< 0.01\); \(\text{d}< 0.001\) as compared to Group 2 by POST HOC Tukey HSD; \(\text{e}< 0.001\) as compared to Group 1 by Independent T test and \(\text{f}< 0.001\) as compared to Group 2 by Independent T test.

H\(_2\)O\(_2\) level is expressed as \(\mu\)mol of hydrogen peroxide oxidized / min/mg protein; \(O_2^-\) level is expressed as nmol of superoxide liberated / min/mg protein; Myeloperoxidase level is expressed as \(\mu\)mol of \(H_2O_2\) oxidized / min/mg protein; lipid peroxide level is expressed as nmol/ml.
phosphatase were found to be elevated significantly ($P < 0.001$) in endotoxin induced EP rats. It was found to be decreased in proanthocyanidin treated rats (Groups 13 and 14) and also a significant inhibition was observed after metronidazole treatment (Group 15).

A significant increase in the levels of acute phase proteins such as C-reactive proteins and fibrinogen in plasma was noticed in EP rats. PC (Groups 13 and 14) as well as metronidazole treated groups (Group 15) were observed to have a significant ($P < 0.001$) decrease in the levels of acute phase proteins (Table 3).

Nonenzymatic antioxidants such as ascorbic acid, α-tocopherol, ceruloplasmin, GSH and antioxidant enzymes such as catalase, SOD, GPx and GST in serum were decreased significantly in EP rats whereas proanthocyanidins (Groups 13 and 14) and metronidazole (Group 15) treated groups were found to have significant ($P < 0.001$) protective effect as reflected by an increase in the levels of antioxidants as shown in Table 4.

The results on the levels of ROS, lipid peroxides, lysosomal enzymes, acute phase proteins and antioxidants demonstrated that 30 mg PC treatment for 30 days showed significant protective effect.

Some typical photomicrographs are shown in Fig. 2(a-o). Figure 2a represented normal architecture of rat maxillae on histopathological evaluation. The

<table>
<thead>
<tr>
<th>Groups</th>
<th>Fibrinogen</th>
<th>C-reactive protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>265 ± 8.16</td>
<td>0.28 ± 0.022</td>
</tr>
<tr>
<td>2</td>
<td>479 ± 9.1</td>
<td>2.6 ± 0.21</td>
</tr>
<tr>
<td>3</td>
<td>NS</td>
<td>2.3 ± 0.24</td>
</tr>
<tr>
<td>4</td>
<td>NS</td>
<td>2.1 ± 0.2</td>
</tr>
<tr>
<td>5</td>
<td>436 ± 9.61</td>
<td>1.9 ± 0.17</td>
</tr>
<tr>
<td>6</td>
<td>404 ± 7.35</td>
<td>1.6 ± 0.09</td>
</tr>
<tr>
<td>7</td>
<td>438 ± 9.8</td>
<td>1.9 ± 0.24</td>
</tr>
<tr>
<td>8</td>
<td>390 ± 6.8</td>
<td>1.8 ± 0.21</td>
</tr>
<tr>
<td>9</td>
<td>295 ± 3.8</td>
<td>1.4 ± 0.27</td>
</tr>
<tr>
<td>10</td>
<td>372 ± 5.9</td>
<td>1.1 ± 0.08</td>
</tr>
<tr>
<td>11</td>
<td>401 ± 6.5</td>
<td>1.2 ± 0.16</td>
</tr>
<tr>
<td>12</td>
<td>333 ± 7.6</td>
<td>1.1 ± 0.12</td>
</tr>
<tr>
<td>13</td>
<td>294 ± 4.6</td>
<td>0.58 ± 0.07</td>
</tr>
<tr>
<td>14</td>
<td>290 ± 4.83</td>
<td>0.54 ± 0.05</td>
</tr>
<tr>
<td>15</td>
<td>307 ± 4.9</td>
<td>0.7 ± 0.02</td>
</tr>
</tbody>
</table>

Fibrinogen expressed as mg / 100 ml of blood; C-reactive protein expressed as µ gm / ml of blood. Other details are same as in Table 1.
Discussion

It could be inferred from the observations on the levels of ROS, lysosomal enzyme activity, acute phase proteins, antioxidants levels and histopathological changes, that optimal protection was observed at a dose of 30 mg/kg body weight for 30 days of PC treatment against EP. Neutrophils were implicated to play a destructive role in the periodontal tissue breakdown process due to high levels of lysosomal enzymes and generation of reactive oxygen derivatives. MPO possessed potent antimicrobial activity, and as an indicator of neutrophilic degranulation, it was reported to play a crucial role in tissue injury. MPO activity in gingival tissues showed a significant increase in patients with periodontal diseases, when compared to the control group and the chronic inflammatory process was also shown to reflect at a systemic level. MPO and lactoferrin were considered to reflect the strength of oxidative stress and the imbalance between the levels of MPO and lactoferrin could result in tissue damage by ROS in periodontitis and oxygen derived free radicals and their products were known to play an important role in the pathogenesis of chronic inflammatory disorders. Karima et al. found that \( \text{O}_2^- \) and / or \( \text{H}_2\text{O}_2 \) were implicated in the destruction of periodontal tissues. The increased levels of ROS suppressed the antioxidant systems, leading to the damage of periodontium. It is well known that metronidazole exhibited antimicrobial activity in the treatment of periodontal diseases.

The dimERIC proanthocyanidins were more effective than vitamin C in trapping oxygen radicals. The protective role of proanthocyanidins through its free radical scavenging property both in vitro and in vivo was also demonstrated by Ye et al. It was shown that PC had inhibitory effect on the ROS generation as well as the lysosomal enzymes release. The oxygen metabolites were produced by the NADPH oxidase system and activated as part of the host response to infection especially in periodontitis.

It was reported earlier that lipids were shown to be the most susceptible macromolecules to oxidative stress and lipid peroxides may play a role in the

<table>
<thead>
<tr>
<th>Groups</th>
<th>Ascorbic acid</th>
<th>α-tocopherol</th>
<th>Ceruloplasmin</th>
<th>GSH</th>
<th>Catalase</th>
<th>SOD</th>
<th>GPx</th>
<th>GPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.30 ± 0.007</td>
<td>4.0 ± 0.06</td>
<td>30 ± 0.28</td>
<td>54 ± 0.28</td>
<td>16.11 ± 0.09</td>
<td>10.5 ± 0.07</td>
<td>1.8 ± 0.01</td>
<td>0.38 ± 0.007</td>
</tr>
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<td>16.7 ± 0.2</td>
<td>34.1 ± 0.2</td>
<td>10.7 ± 0.07</td>
<td>5.5 ± 0.16</td>
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<td>0.193 ± 0.002</td>
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<td>17.3 ± 0.2</td>
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<td>10.7 ± 0.8</td>
<td>5.7 ± 0.14</td>
<td>1.0 ± 0.02</td>
<td>0.191 ± 0.002</td>
</tr>
<tr>
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<td>2.4 ± 0.03</td>
<td>17.7 ± 0.2</td>
<td>35.6 ± 0.18</td>
<td>10.9 ± 0.7</td>
<td>6.1 ± 0.13</td>
<td>1.0 ± 0.01</td>
<td>0.190 ± 0.002</td>
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<td>18.1 ± 0.1</td>
<td>36.1 ± 0.18</td>
<td>11.03 ± 0.07</td>
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<td>0.188 ± 0.002</td>
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<td>36.6 ± 0.17</td>
<td>11.43 ± 0.08</td>
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<td>1.2 ± 0.01</td>
<td>0.181 ± 0.002</td>
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<td>17.9 ± 0.17</td>
<td>35.6 ± 0.18</td>
<td>10.9 ± 0.05</td>
<td>6.0 ± 0.14</td>
<td>1.1 ± 0.03</td>
<td>0.19 ± 0.002</td>
</tr>
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<td>8</td>
<td>0.64 ± 0.007</td>
<td>2.5 ± 0.02</td>
<td>19.4 ± 0.2</td>
<td>36.2 ± 0.14</td>
<td>11.1 ± 0.1</td>
<td>6.6 ± 0.1</td>
<td>1.1 ± 0.03</td>
<td>0.20 ± 0.003</td>
</tr>
<tr>
<td>9</td>
<td>0.67 ± 0.007</td>
<td>2.5 ± 0.04</td>
<td>21.2 ± 0.2</td>
<td>36.8 ± 0.11</td>
<td>12.5 ± 0.12</td>
<td>6.9 ± 0.1</td>
<td>1.2 ± 0.06</td>
<td>0.22 ± 0.004</td>
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<td>10</td>
<td>0.71 ± 0.006</td>
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<td>21.5 ± 0.2</td>
<td>37.5 ± 0.16</td>
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<td>7.2 ± 0.03</td>
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<td>19.4 ± 0.12</td>
<td>36.7 ± 0.15</td>
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<td>1.2 ± 0.02</td>
<td>0.25 ± 0.004</td>
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<td>2.96 ± 0.02</td>
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<td>43.0 ± 0.31</td>
<td>13.8 ± 0.10</td>
<td>8.0 ± 0.04</td>
<td>1.4 ± 0.05</td>
<td>2.9 ± 0.005</td>
</tr>
</tbody>
</table>

Ascorbic acid is expressed as mg/dl serum; α-tocopherol is expressed as mg/dl serum; Ceruloplasmin is expressed as mg/dl serum; GSH is expressed as mg/dl serum; catalase is expressed as moles of \( \text{H}_2\text{O}_2 \) decomposed / min/mg protein; SOD is expressed as 50% epinephrine autooxidation; GPx is expressed as moles of GSH /min/mg protein; GPT is expressed as U / min /mg protein. Other details are same as in Table 1.
inflammation and destruction of periodontium\textsuperscript{40}. The results of the present study showed that the levels of lipid peroxides were significantly increased in EP and PC were shown to decrease the lipid peroxides level by scavenging ROS. The acidic lysosomal cysteine proteinases viz. cathepsin B, H and L which participated in intracellular proteolytic degradation processes were of value in monitoring treatment results rather than detecting early inflammatory tissue changes\textsuperscript{41}. The release of granule components from infiltrating leukocytes such as lysosomal enzymes and reactive oxygen species which were normally intended to degrade ingested microbes, could also lead to tissue destruction and amplification of inflammatory response with continued recruitment of new leukocytes. A decrease in lysosomal membrane stability increased the levels of lysosomal hydrolases leading to altered metabolism of different connective tissue constituents including collagen\textsuperscript{42}.

Cathepsin D was observed to be linked to PMN secretion at the end point of inflammation and it was increased to 2.2 fold in periodontal disease\textsuperscript{41}. 

Fig.2—Histopathological changes observed in endotoxin induced rats EP treated with proanthocyanidins (PC) [(a) Group 1 - normal architecture of gingival tissues; (b) Group 2 - dense chronic inflammatory cells mostly with plasma cells and lymphocytes; (c) Group 3 - squamous epithelium overlying focal dense collections of chronic inflammatory cells; (d) Group 4 - dense chronic inflammatory cells mostly with plasma cells and lymphocytes; (e) Group 5 - squamous epithelium overlying diffuse chronic inflammatory cellular infiltrate; (f) Group 6 - diffuse chronic inflammatory cellular infiltrate; (g) Group 7 - squamous epithelium overlying diffuse chronic inflammatory cellular infiltrate; (h) Group 8 - focal collections of chronic inflammatory cells; (i) Group 9 - squamous epithelium overlying few scattered chronic inflammatory cells and blood vessels]
However, an increase in cathepsin D concentration represented the end point of periodontal tissue destruction and it served as a marker of irreversible cellular injury. Increased activity of serum lysosomal enzymes was increased concurred with an earlier report\textsuperscript{41}. β-glucuronidase was involved in the destruction of non-collagenous components of the extracellular matrix and it was also an indicator of periodontal disease activity\textsuperscript{43}. The findings of the present study confirmed the positive relationship between increased β-glucuronidase activity and infiltration of chronic inflammatory cells (plasma cells and lymphocytes) (Fig. 2) in the endotoxin induced experimental periodontitis.

Proanthocyanidins efficiently restrained the inflammatory response of activated neutrophils \textit{in vitro} and when absorbed \textit{in vivo}, they could prevent the oxidative discharge at the sites of their adhesion\textsuperscript{44}. Acid phosphatase was a marker of osteoclastic activity and bone resorption and acid phosphatase present in inflammatory cell was detected in gingival sulcular fluid\textsuperscript{45}.

The acute phase response was a non-specific process that may occur in the initial host response to injuries, infections, ischemic necrosis or malignancy\textsuperscript{46}. A number of reports demonstrated elevated levels of C-reactive protein in periodontitis patients compared with C-reactive protein in non-diseased control subjects\textsuperscript{47}. Recent studies suggested that excessive fibrinogen production may play a role in upregulating host immune responses\textsuperscript{48}. Fibrinogen levels were increased during infections and inflammatory conditions including periodontal diseases\textsuperscript{47}. Increased level of fibrinogen was also observed in the present study and it was restored to normal in proanthocyanidin treated rats.

The primary defense systems against oxidative threat such as CAT, SOD, glutathione peroxidase and glutathione-s-transferase decomposed $O_2^-\cdot$ and $H_2O_2$ before interacting to form the more reactive hydroxyl radical. The cooperative interaction between these
antioxidants in plasma is crucial for maximum suppression of oxidative stress. The secondary defense consisted of ascorbic acid, α-tocopherol, ceruloplasmin, and reduced GSH which scavenged residual free radicals escaping decomposition by the antioxidant enzymes.

The findings of the present study concurred with an earlier report which showed diminished levels of ascorbic acid in periodontitis and this reduction progressed along with advancement of the disease. In this study, GSH content was significantly reduced in EP rats and this could be explained by the assimilation of GSH by the rapidly generating free radicals. However, antioxidant property of PC imparted some degree of GSH salvation as GSH levels were shown to increase significantly in PC treated groups.

The present findings demonstrated that the reduced activities of SOD, GPx, ascorbic acid and α-tocopherol were well correlated with an increase in TBARS in periodontitis which are the products of enhanced peroxidation of arachidonic acid. Decreased activity of CAT and SOD could promote accumulation of ROS and the results of this study were in agreement with an earlier report by Ellis et al.

Identification of histological manifestations is a necessary step to assess the pathological changes in experimental periodontitis and also in various groups treated with proanthocyanidins. Mononuclear cell infiltration and transmigration of mononuclear phagocytes and lymphocytes were shown to be important characteristics of chronic gingival inflammation with resultant increased numbers of inflammatory cells composed of lymphocytes and plasma cells and the observations of this study were in agreement with this report.

Proanthocyanidins were suggested to be more potent when compared to flavanols in their antioxidant capacity due to the fact that oxidation of proanthocyanidins predominantly produced semiquinone radicals that coupled to produce oligomeric compounds through nucleophilic addition. Chemical structure determined relative ease of flavanol or proanthocyanidin oxidation and free-radical scavenging activity although the presence of galloyl groups and the number and position of hydroxyl groups (based on redox potential) was noticed to enhance antioxidant activity.

Natural antioxidants strengthened the endogenous antioxidant defense from ROS ravage and it restored the optimal balance by neutralizing ROS. Chemically, the important features of flavanoids, were their remarkable antioxidant properties: the hydrogen donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavanoids, which enabled them to undergo redox reactions scavenging free radicals more easily and the stable delocalization system, consisting of aromatic and heterocyclic rings as well as multiple unsaturated bonds, which helped to delocalize and regulate the free radicals.

Thus, the findings of the present study clearly demonstrated that dietary supplementation of proanthocyanidins enhanced the host resistance and inhibited the oxidative stress by balancing oxidant and antioxidant ratio of periodontal diseases.

Acknowledgement

Thanks are due to the Principal, Meenakshi Ammal Dental College, Dean, Meenakshi Medical College and Research Institute and the research committee members for their encouragement and support to publish this work, and to Dr. A.B. Mandal, Director, CLRI, Chennai for support. The award of CSIR Emeritus Scientist project to Dr. R. Puvanakrishnan is gratefully acknowledged.

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