

## Therapeutic effects of proanthocyanidins on the pathogenesis of periodontitis— An overview

Jayamathi Govindaraj<sup>1</sup>, Pamela Emmadi<sup>2</sup> & Rengarajulu Puvanakrishnan<sup>3\*</sup>

Department of <sup>1</sup>Biochemistry and <sup>2</sup>Periodontics, Meenakshi Ammal Dental College, Alapakkam Main Road,  
Maduravoyal, Chennai 600 095, India

<sup>3</sup>Department of Biotechnology, Central Leather Research Institute (CLRI), CSIR, Chennai 600 020, India

Periodontitis is a bacterially induced chronic inflammatory disease that destroys the connective tissue and bone that support teeth. Bacteria initiates periodontitis and destruction of the alveolar bone and periodontal connective tissue is clearly observed. But, the events occurring between these two points of time remain obscure and this study focusses on these aspects. The proanthocyanidins (PC) have variable pharmacological and nutraceutical benefits including improvement of ischemic cardiovascular disease, prevention of atherosclerosis and antiarthritic, anticancer and antimicrobial activities. The benefits associated with the antioxidant activity of PC have been evaluated both *in vivo* and *in vitro*. But, reports on the ameliorative effects of PC on oral diseases and specifically on periodontitis are very few. Hence, a novel attempt is made to review the possible protective effects of PC and its mechanism of action in periodontitis and also to show whether PC could be developed as a therapeutic agent for periodontitis.

**Keywords:** Antioxidant property, Nitric oxide, Periodontitis, Proanthocyanidin, Reactive oxygen species

Periodontitis, a complex infection of bacterial origin, is characterized by an inflammatory host response against microorganisms of the bacterial plaque and their products<sup>1</sup>. Most of these microorganisms can produce tissue destruction in two ways: (i) directly, through invasion of the tissues and the production of harmful substances that induce cell death and tissue necrosis, and (ii) indirectly, through activation of inflammatory cells which can produce and release mediators that act on effectors, with potent proinflammatory and catabolic activity<sup>2</sup>. Some bacteria also interfere with the normal host defense mechanism by deactivating specific antibodies or inhibiting the action of phagocyte cells<sup>2</sup>. The pathogenesis of periodontal destruction involves the sequential activation of different components of the host immune and inflammatory response, aimed in the first place at defending the tissues against bacterial aggression, reflecting the essentially protective role of the response. The proportion of damage caused by direct effects of the bacteria and that caused by indirect host response-mediated action have yet to be established<sup>3</sup>.

### Microbiology of chronic periodontitis

Periodontal disease is marked by inflammatory injuries (called lesions) from calculus, a hard substance that forms from plaque, which is essentially bacterial overgrowth. More than 300 species of bacteria colonize in the subgingival area and their cell wall components can trigger immune activation. In periodontitis, the initial step in the disease process is the colonization of periodontal tissues by pathogenic species. Entry of the bacterium itself or of bacterial products into the periodontal tissues may be important in the disease process. Further, inherent in successful colonization of host tissues is the ability of the bacterium to evade host defense mechanisms<sup>2</sup>.

Periodontitis is a bacterially induced chronic inflammatory disease that destroys the connective tissue and bone that support teeth<sup>4</sup>. The role of bacteria in the initiation of periodontitis is well documented and the end result viz., destruction of the alveolar bone and periodontal connective tissue is clearly observed; but the events occurring between these two points of time remain obscure and are the focus of this review. Bacteria induce tissue destruction indirectly by activating host defense cells, which in turn produce and release mediators that stimulate the effectors of connective tissue breakdown. A central feature of periodontitis is the

\*Correspondent author  
Telephone: 0 9444054875  
Fax: 91 (44) 24911589  
E-mail: puvanakrishnan@yahoo.com

remodeling of connective tissue that leads to a net loss of local soft tissues, bone and the periodontal attachment apparatus. Mediators including proteinases, cytokines and prostaglandins are produced as a part of the host response that contribute to tissue destruction<sup>4</sup>.

### Pathogenesis of chronic periodontitis

The role of host immune system in periodontal pathogenesis involves the following in response to bacterial infection<sup>5</sup> (Fig. 1):

- (i) Innate factors such as complement, resident leukocytes and especially mast cells play an important role in signaling endothelium, thus initiating inflammation.
- (ii) Acute inflammatory cells (i.e., neutrophils) protect local tissues by controlling the periodontal microbiota within the gingival crevice and junctional epithelium.
- (iii) Chronic inflammatory cells, macrophages and lymphocytes protect the entire host from within the subjacent connective tissues and do all that is necessary to prevent a local infection from becoming systemic and life threatening, including the sacrifice of local tissues<sup>6</sup>.

### Innate factors and initiation of inflammation

The onset of inflammation involves the development of edema and erythema which are signs of vascular changes. Activation of the complement system in response to bacterial infection results in cleavage of the third and fifth components of the complement system (C3 and C5 respectively), generating the peptides C3a and C5a, both of which are potent anaphylatoxins. C3a and C5a can trigger contraction of smooth muscle, increase the permeability of small blood vessels and regulate vasodilation. In addition, C3a and C5a can stimulate respiratory burst in macrophages<sup>7</sup>, neutrophils<sup>8</sup> and eosinophils<sup>9</sup>. C3a can also stimulate serotonin release from platelets<sup>10</sup> and modulate synthesis of IL-6 (Interleukin-6) and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) by B- lymphocytes and monocytes<sup>11</sup>. C5a is a potent chemotactic molecule for macrophages<sup>12</sup>, neutrophils<sup>13</sup> and T-lymphocytes<sup>14</sup>.

### Primary role of neutrophils

The strong evidence linking reactive oxygen species (ROS) to the pathological destruction of the connective tissue during periodontal disease rests on neutrophils infiltration as the main event in the host's response to bacterial invasion<sup>15</sup>. If complement is not

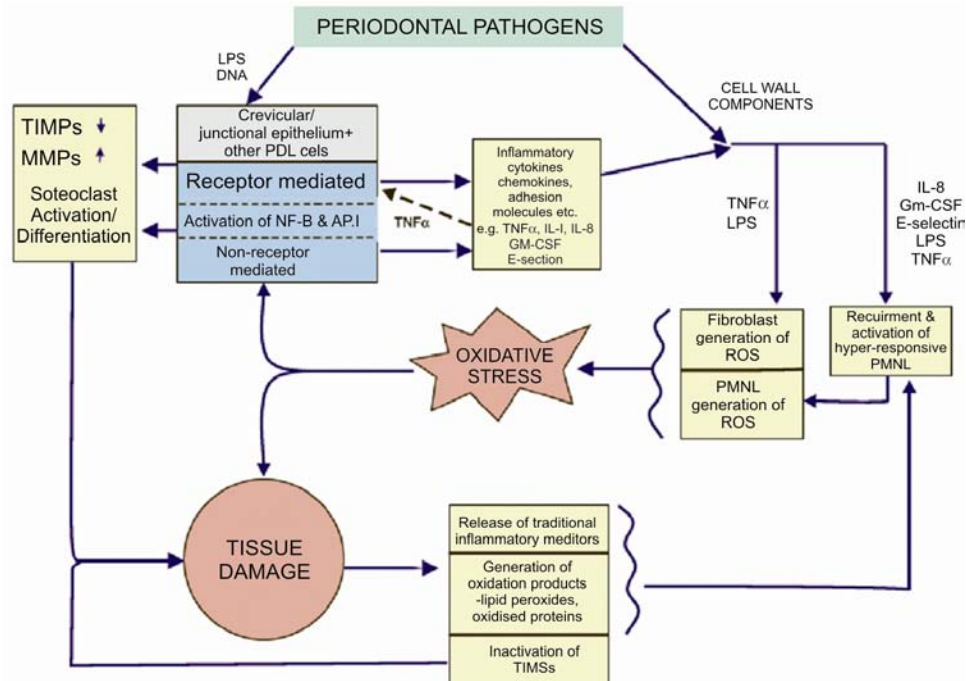


Fig. 1—Diagram illustrating a central role of ROS in generating chronic inflammation and tissue damage in response to periodontal pathogens<sup>5</sup>. Matrix metalloproteinase (MMP); tissue inhibitor of matrix metalloproteinase (TIMP); nuclear factor kappa B (NF- $\kappa$ B); activating protein-1 (AP-1); periodontal ligament (PDL); tumor necrosis factor (TNF); interleukin (IL); granulocyte-macrophage colony-stimulating factor (GM-CSF); lipopolysaccharide(LPS) reactive oxygen species ROS

successful in controlling the pathogens, neutrophils are recruited into the gingival crevice (a hypoxic environment) and they provide the first cellular host mechanism to control periodontal bacteria<sup>16</sup>. In periodontitis, bacteria triggers polymorphonuclear leukocytes (PMN) to release ROS, which includes hydroxyl radical, superoxide anion and hydrogen peroxide. ROS, in smaller quantity, play a necessary role in metabolic processes. Increased cellular production of ROS is linked to most degenerative conditions including heart diseases and periodontal diseases. ROS are also implicated in the destruction of periodontium during chronic stages<sup>5</sup>.

### Role of macrophages

Macrophage is the dominant cellular player in chronic inflammation and it is a component of the mononuclear phagocyte system. From the blood, monocytes migrate into various tissues and differentiate into macrophages. Differentiation of monocyte/macrophage system is directed by cytokines. One primary function of macrophage is the phagocytosis of large particles<sup>17</sup>. Activated macrophages secrete a wide variety of biologically active products that, if unchecked, result in tissue injury and fibrosis. The products of activated macrophages serve to eliminate injurious agents such as microbes and to initiate the process of repair. Some of these products are toxic to microbes and host cells (reactive oxygen and nitrogen intermediates) or extracellular matrix (proteases); some cause influx of other cell types (cytokines, chemotactic factors)<sup>18</sup>. This impressive arsenal of mediators makes macrophages powerful allies in the body's defense against unwanted invaders, but the same weaponry can also induce considerable tissue destruction when macrophages are appropriately activated<sup>19</sup>. Thus, tissue destruction is one of the hallmark of chronic inflammation.

### Role of lymphocytes

T- and B-lymphocytes are produced by lymphokines in response to inflammation. Lymphocytes and macrophages interact in a bidirectional way and these reactions play an important role in chronic inflammation. T-cells play an important immunoregulatory role in the pathogenesis of periodontal diseases<sup>20</sup>. Immunoglobulins are glycoproteins synthesized by B-lymphocytes and plasma cells that have the property

of specific binding to the antigen. The production of antibodies, especially IgG and IgA, is considered to play a protective role against periodontal disease pathogenesis<sup>2</sup>.

### Matrix metalloproteinases (MMPs)

The major matrix metalloproteinases (MMPs) in neutrophils are MMP-8 (collagenase-2) and MMP-9 (gelatinase-B). While MMP-8 potently degrades interstitial collagens, MMP-9 is a gelatinolytic enzyme degrading several extracellular matrix proteins, including basement membrane (type IV) collagen. Both these enzymes can inactivate 1-proteinase inhibitor thereby increasing the tissue serine proteinase activity. MMP-8 and MMP-9 are the main collagen-degrading enzymes in gingival crevicular fluid (GCF) and saliva<sup>21</sup> and they are believed to be mainly responsible for collagen degradation in inflamed tissue during gingivitis and adult periodontitis. They induce fibroblasts and macrophages to produce neutral metalloproteinase such as procollagenase.

The major MMPs produced by fibroblasts are MMP-1 (collagenase-1), MMP-13 (collagenase-13), MMP-2 (gelatinase-A), MMP-3 (stromelysin-1), and MT1-MMP (MMP-14). Fibroblast type collagenase MMP-1 can be detected in the GCF of periodontitis patients<sup>22</sup>.

### Cytokines

Cytokines are also inflammatory mediators produced by non-lymphocytic cells viz., macrophages, fibroblasts and keratinocytes. Three proinflammatory cytokines viz., interleukin-1 (IL-1 which includes, IL-1 $\alpha$  and IL- $\beta$ ), IL-6 and tumor necrosis factor (TNF-1 $\alpha$  and TNF- $\beta$ ) appear to have a central role in periodontal tissue destruction<sup>3</sup>. They are found in significant concentrations in periodontal diseased sites. Microbial components, especially lipopolysaccharide (LPS), have the capacity to activate macrophages to synthesize and secrete a wide array of molecules including IL-1 and TNF- $\alpha$ , prostaglandins E<sub>2</sub> (PGE<sub>2</sub>) and hydrolytic enzymes. These cytokines manifest potent proinflammatory and catabolic activities and they play key roles in periodontal tissue breakdown<sup>18</sup>.

### Prostaglandins

Prostaglandins are arachidonic acid metabolites generated by cyclooxygenases (COX-1, COX-2). The

host response to the pathogens which induce the production of inflammatory molecules including cytokines and prostanoids, is involved in the initiation and progression of periodontal disease<sup>23</sup>. Numerous studies have indicated that prostanoids, in particular PGE<sub>2</sub>, are involved in the pathogenesis of periodontal disease<sup>5</sup>. Elevated PGE<sub>2</sub> levels are detected in the gingiva and gingival crevicular fluid of patients with periodontal diseases, compared to periodontally healthy subjects. It is partly responsible for the bone loss associated with periodontitis. It is clear that prostaglandins are involved in the pathogenesis of periodontal disease because studies have indicated that, in both animal and human models, traditional non-steroidal anti-inflammatory drugs inhibit progression of the disease<sup>6</sup>. According to recent researches, COX-2 plays a crucial role in prostaglandin production in periodontal disease and selective COX-2 inhibitors are as efficacious as traditional non-steroidal anti-inflammatory drugs for the inhibition of progression of periodontal disease in animal models<sup>24</sup>.

#### Acute phase reactants

The acute phase reaction refers to physiological and metabolic alterations that ensue immediately after onset of infection or tissue injury. In contrast with the specificity of cellular and humoral immunity, the acute-phase changes are nonspecific and occur in response to many conditions<sup>25</sup>. Acute phase proteins viz., fibrinogen, C-reactive protein (CRP),  $\alpha$ -1-acid glycoprotein,  $\alpha$ -2-macroglobulin,  $\alpha$ -1-antitrypsin and  $\alpha$ -1-antichymotrypsin<sup>26</sup> serve important functions in restoring homeostasis after infection or inflammation. Elevated levels of CRP in periodontitis patients have been demonstrated when compared with non-diseased control subjects<sup>27</sup>. Excessive fibrinogen production may play a role in upregulating host immune responses<sup>28</sup>.

#### Role of ROS

Reactive oxygen species (ROS) are capable of damaging cell membranes and a wide variety of biomolecules. The direct role of oxygen radicals in microbial killing is well established<sup>29</sup>. Free radicals are important in the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NFkB)<sup>30,31</sup>, a factor in the important transcription of a number of genes of IL-2, TNF- $\alpha$  and IL-2 receptor and MHC class I genes. Free radicals have been implicated in IL-1 induced osteoclastic bone resorption<sup>32</sup>. Free radicals damage may account not

only for the production of altered IgG but also for many autoantibodies, their production not depending on a polyclonal activation of B cells but on the sensitivity of individual molecules to different radical generating systems<sup>33</sup>. ROS can also modify aminoacids such as methionine, histidine, cysteine, proline and lysine<sup>34</sup>, which cause alterations in the protein structure, conformation and antigenicity. Fragmentation of proteins by ROS can also cause crosslinking and aggregation of proteins and proteins are more susceptible to proteolysis<sup>35</sup>.

Hyaluronic acid forms the central axis of proteoglycans and it maintains the viscosity of synovial fluid within joints. Following exposure to free radical systems, this polymer fragments<sup>36</sup> leading to destabilization of connective tissue and loss of synovial fluid viscosity. A number of natural defense mechanisms exist for limiting oxidative damage<sup>5</sup>.

#### Role of nitric oxide (NO)

Nitric oxide (NO) is an inorganic, gaseous free radical<sup>37</sup>. Identified as the "endothelium relaxing factor" (EDRF), with its role in regulating blood pressure<sup>38</sup>, the molecule now has several other functions. NO is a potent vasodilator *in vitro*, thus contributing to the cardinal signs of inflammation, heat, redness and swelling<sup>39</sup>. Other cellular sources of NO include macrophages, neutrophils, T-lymphocytes, chondrocytes and synoviocytes<sup>40</sup>. NO, a pleiotropic mediator of inflammation, plays an important role in the vascular and cellular components of inflammatory responses<sup>41</sup>. NO and its derivatives are microbicidal and thus NO is also a mediator of host defense against infection.

#### Antioxidant mechanisms

In recent years, there has been a tremendous stress on free radicals and antioxidant defense mechanisms. The human system contains a number of protective antioxidant mechanisms, whose specific role is to remove harmful oxidants as they form, or to repair damage caused by ROS *in vivo*<sup>5,42</sup>. There are, however, several nonenzymatic and enzymatic systems to contribute to inactivation of free radical reactions. Antioxidants either block the initiation of free radical formation or scavenge free radicals and terminate radical damage.

#### Periodontal medicine

Periodontal diseases result from susceptible hosts having their periodontal tissues colonized by specific

oral pathogens sufficient to overwhelm their tissue defenses. Clinical parameters in the treatment of periodontal diseases are the reduction of the bacterial load or enhancement of the host tissues and ability to defend load or repair itself. Most dental infections are caused by mixtures of facultative and anaerobic bacteria but the anaerobic bacteria usually dominate. In certain types of periodontal diseases including chronic advanced periodontitis and periodontitis as a manifestation of systemic diseases, adjunctive chemotherapeutic agents may be necessary to control the disease process<sup>43</sup>.

### Chemotherapeutic agents

Some of the commonly used antimicrobial agents are illustrated in Table 1. They can be administered locally, orally or parenterally. In either case, their purpose is to reduce the number of bacteria present in the periodontal pocket. Systemic antibiotics may be necessary adjunct in controlling bacterial infection, because bacteria can invade periodontal tissues making mechanical therapy alone sometimes ineffective.

Local administration of antimicrobial agents generally directly into the pocket has the potential to provide greater concentration directly to the infected area and reduce possible systemic side effects.

The most significant disadvantage of chemotherapy is the development of treatment-related side effects. Though chemotherapy has been proven effective in the resolution of diseases, there is always a risk that the disease may reemerge after the treatment has ended.

### Proanthocyanidins (PC)—A novel approach

In order to improve the success rate in periodontitis, a novel new treatment aimed at early intervention, the enhancement of host resistance and the inhibition of the biological and mechanical irritants involved in the onset of gingivitis and the progression to periodontal disease has been developed. This functional approach employs the use of biological plant extracts, selected coenzymes and specific vitamins to strengthen and support the tissues of the oral cavity and the resistance of the host.

An ever increasing number of pharmacological effects have become known through the discovery of new plant flavanoids with variations in chemical structure and related derivatives. A new group of phytochemicals that has been attracting much attention from both the general public and health professionals is PC. PC are found in leaves, fruits, bark, seeds, flowers and roots of many plants and it is predominantly present in tea, honey, wines, grape seed, vegetables, nuts, olive oil, cocoa, pine bark and cereals. Grape seed extract (GSE), the richest source of PC, has potent antioxidants and exhibits numerous pharmacological activities. PC, one of the most abundant flavanoids in the plant kingdom, are extracted generally from grape seeds and they have antioxidant<sup>44</sup>, free radical scavenging<sup>45</sup>, anticarcinogenic<sup>46</sup> and antiinflammatory properties<sup>47</sup>. Govindaraj *et al.*<sup>47</sup> have shown that an effective dose (30mg/kg body weight for 30 days) of PC exerts a protective effect against *E.coli* endotoxin induced experimental periodontitis (EP) in rats. Since oxidative stress is encountered in periodontitis, the effect of PC on periodontal inflammation is worth studying.

Table 1—Commonly used antimicrobial agents for treating periodontitis

Antimicrobial agent	Mechanism of action	Antibacterial action	References
Tetracycline	inhibits action of the prokaryotic 30S ribosome, by binding aminoacyl -tRNA	broad spectrum antibiotic — effective against gram (-) bacteria.	86
Doxycillin	inhibits protein synthesis by reversibly binding to the 30S ribosomal subunit of susceptible organisms	broad spectrum antibiotic — Antiprotozoal, Antibacterial and Antihelminthic	87
Metronidazole	disrupts bacterial DNA synthesis	Effective against anaerobic bacteria and protozoa	88
Ciprofloxacin	inhibits bacterial DNA synthesis	broad spectrum antibiotic — effective against gram - negative rods including all facultative and some anaerobic putative periodontal pathogens	89
Amoxicillin	inhibits the synthesis of bacterial cell wall	extended spectrum antibiotic — effective against both gram (+) and gram(-) bacteria	90

### Chemistry of PC

PC, also known as condensed tannins, are widely distributed in the plant kingdom and it represents a ubiquitous group of plant phenolics which take the form of oligomers or polymers of polyhydroxy flavan-3-ol units such as (+)-catechin, (-)-epicatechin and (-)-epicatechin-3-gallate. The fundamental structural unit of PC is the phenolic flavan-3-ol nucleus. The flavan-3-ol consists of a C15 (C6-C3-C6) structure characterized by a phenylbenzopyran moiety. Proanthocyanidins consist of flavanol units linked by two C-4 C-8 interflavan bonds<sup>48</sup> (Fig. 2).

### PC in oral diseases

Despite the recently reported drop in the overall death rate from cancer, the estimated survival rate and number of deaths from oral cancer remain virtually unchanged. PC administration may induce apoptosis in cervical and oral cancer cell lines, while acting merely to suppress proliferation of the normal cell line control<sup>49</sup>. Administration of grape seed PC is shown to have a beneficial effect on physical health, specifically the health of bone. The effects of PC on mandibular bone are assessed by examining trabecular and cortical bone density, mineral content and noninvasive bone strength in low calcium diet

rats and an increase in both bone formation and bone strength in rat mandibles has been observed after PC administration<sup>50</sup>. Houde *et al.*<sup>51</sup> have demonstrated that PC have potent antioxidant properties and it should be considered as a potential agent in the prevention of periodontal diseases. Green tea catechin (monomeric unit of PC) shows a bactericidal effect against gram negative anaerobic rods and is effective in improving periodontal status<sup>52</sup>. Alveolar bone resorption is a characteristic feature of periodontal diseases and it involves removal of both the mineral and the organic constituents of the bone matrix, a process mainly carried out by multinucleated osteoclast cells.

(-)-Epigallocatechin gallate (EGCG, monomeric unit of PC), the main constituent of green tea polyphenols, has been reported to induce the apoptotic cell death of osteoclasts and to modulate caspase activation in various tumor cells<sup>53</sup>. These results indicate that the inhibitory effect of EGCG on the production of toxic end metabolites of *Porphyromonas gingivalis* can be attributed to the presence of the galloyl moiety, which is ester-linked with the 3-OH of the catechin moiety in the polyphenolic compounds. This study has shown that continuous application of tea polyphenols on a daily basis can be considered as a useful and practical method for the prevention of periodontal diseases<sup>54</sup>. Green tea is a very popular beverage and *in vitro* studies have shown that green tea polyphenols inhibit the growth and cellular adherence of periodontal pathogens and their production of virulence factors. The epidemiological relationship between the intake of green tea and periodontal disease is a modest inverse association<sup>55</sup>. MMPs produced by resident and inflammatory cells in response to Gram (-) periodonto-pathogens play a major role in the tissue destruction observed during periodontitis. Also, GSE dose-dependently inhibits the activity of MMP-1 and MMP-9 and this study suggests that GSE may be potentially used in the development of novel host-modulating strategies for the treatment of MMP-mediated disorders such as periodontitis<sup>56</sup>.

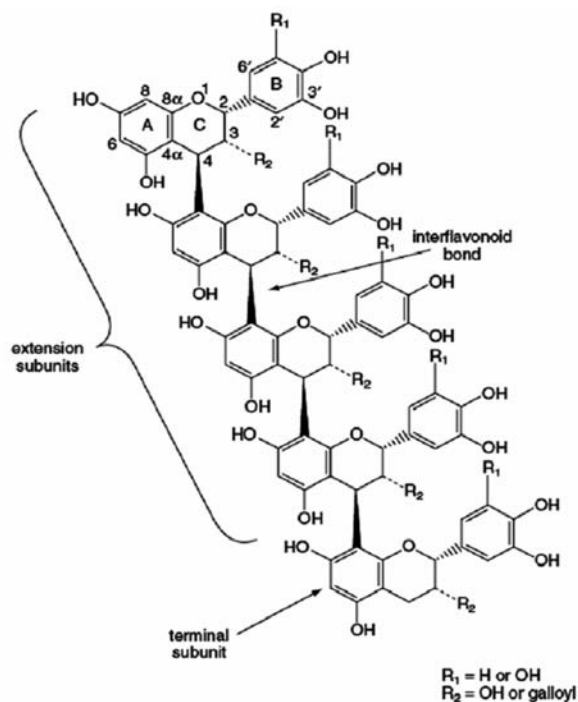


Fig. 2—Generalized proanthocyanidin structure indicating subunit type (extension or terminal) and interflavanoid bond location ( $4\beta \rightarrow 8$ )<sup>91</sup>

### Mechanism of action of PC

**Antioxidant property** — Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes, periodontitis etc. and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. PC have been shown as

antioxidants through the following mechanisms viz., (i) free radicals scavenging property and (ii) metal chelating activity.

*Free radicals scavenging property* — The scavenging capacity of catechin and epicatechin molecules depends on the number of ortho-dihydroxyl and ortho-hydroxyketol groups and C2-C3 double bonds due to their hydrogen donating ability<sup>57,58</sup>. It is also proposed that the higher antioxidant activity is related to the greater number of hydroxyl groups on the flavonoid nucleus<sup>59</sup>. The dimeric PC are more effective than vitamin C in trapping oxygen radicals<sup>45</sup>. The protective role of PC through its free radical scavenging property both *in vitro* and *in vivo* has also been demonstrated by Ye *et al.*<sup>60</sup>. It is demonstrated that PC have inhibitory effect on the ROS generation as well as the lysosomal enzymes release<sup>47</sup>. The electronic configuration of PC allows for easy release of electrons to free radical species (R<sup>•</sup>) such as superoxide anion, hydroxyl, peroxy and nitric oxide radicals. By release of electrons, the radical character of the ROS is transferred to PC (P<sup>•</sup>). PC structure determines relative ease of oxidation and free radical scavenging activity<sup>61,62</sup>. PC have been suggested to be superior to flavonols in their antioxidant capacity since oxidation of PC predominantly produces semiquinone radicals that couple to produce oligomeric compounds through nucleophilic addition. In other words, the presence of electron-donating groups attached to the aromatic ring such as -CH<sub>3</sub> and -OH ought to increase the ease of hydrogen atom abstraction and, consequently, antiradical performance, whereas groups with electron-withdrawing properties such as -COOH, -CHO and -COOR should have the opposite effect<sup>63</sup>. Although electron-donating -OH groups are attached to the aromatic ring in PC, the hydrogen atom is more easily abstracted. This could be the reason why PC shows antiradical activity at the concentrations employed as shown earlier<sup>47</sup>. Chemically, the important features of flavonoids, are their remarkable antioxidant properties. The hydrogen donating substituents (hydroxyl groups) attached to the aromatic ring structures of flavonoids, enable 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, helps to delocalize and regulate the free radicals<sup>64</sup>. Chemical structure determines the relative ease of flavonol or PC oxidation and free

radical scavenging activity although the presence of galloyl groups and the number and position of hydroxyl groups (based on redox potential) enhance antioxidant activity<sup>64,65</sup>.

*Metal chelating activity* — The presence of free state iron and copper in biological systems catalyzes free radical reactions such as Fenton and Haber-Weiss reactions. In the Fenton reaction, iron catalyzes the generation of hydroxyl radicals. The ability of PC to bind such divalent transition metals effectively reduces the concentration of these cations and thus the extent of oxidative activity<sup>66</sup>. Facino *et al.*<sup>67</sup> have indicated that PC strongly complex iron and copper cations in the ratios of Fe<sup>2+</sup>/procyanidin (2:1) and Cu<sup>2+</sup>/procyanidin (4:1) respectively. Results of an investigation involving the effect of PC hydroxylation patterns and degree of polymerization on aluminum chelating capacity reveal that hydroxyl groups are essential sites for metal chelation, o-dihydroxyl phenyl groups of the B ring in particular, and that increasing the degree of polymerization leads to higher stability of tannin-metal complexes<sup>68</sup>.

### Antiinflammatory effects

COX and lipoxygenase (LOX) play an important role as inflammatory mediators. They are involved in the release of arachidonic acid, which is a starting point for a general inflammatory response. Selected phenolic compounds are shown to inhibit both the COX and 5-LOX pathways and this inhibition reduces the release of arachidonic acid<sup>69</sup>. The exact mechanism by which flavonoids inhibit these enzymes is not clear<sup>69</sup>. PC efficiently restrain the inflammatory response of activated neutrophils *in vitro* and when absorbed *in vivo*, they could prevent the oxidative discharge at the sites of their adhesion<sup>70</sup>.

### Nitric oxide synthase (NOS) activity

While a small amount of NO is essential to maintain normal body function (homeostasis), a significant increase of NO synthesized by inducible nitric oxide synthase (iNOS) activates inflammatory process and acts synergistically with other inflammatory mediators<sup>41</sup>. Catechin, EGCG, and other flavanoids repress NO production in macrophages and human peripheral blood mononuclear cells<sup>71</sup>. Interestingly, EGCG is shown to exert its effect on iNOS expression and reduce the activity by competitively inhibiting the binding of arginine and tetrahydrobiopterin and it has been demonstrated that the gallate structure of this catechin is important for

its action<sup>72</sup>. Li *et al.*<sup>73</sup> have conclusively shown that PC from grape seeds significantly decreases the levels of ROS and markedly lowers the NOS activity as well as the content of NO in carrageenan induced paw edema in rats. This finding is in agreement with a recent report wherein it is demonstrated that inhibition of lipid peroxidation and NO formation is an antiinflammatory mechanism of PC<sup>74</sup>.

### Antimicrobial activity

PC, well known for their high levels of antioxidants and polyphenols, have also shown promise as novel antimicrobial agents<sup>75</sup>. The polyphenol compounds may form aggregates with the toxin, in turn preventing its receptor binding and internalization into the host cell<sup>76</sup>. The antimicrobial effects of several tannin extracts on yeast, filamentous fungi, bacterial and viral toxicity have been reviewed by Chung *et al.*<sup>77</sup>. Polymeric PC may be useful as suppressors of antibiotic resistance in *Staphylococcus aureus* and they also show promise as an alternative treatment to antibiotic use against *Staphylococcus aureus* infection<sup>78</sup>.

The various biological functions of PC are diagrammatically represented in Fig. 3.

### Animal studies

Animal models in which microbiological, biochemical and immunological aspects of periodontal lesion can be studied prospectively seem well warranted. Rat, a commonly used animal in experimental periodontitis studies, bears much resemblance to man with respect to periodontal anatomy, development and composition of dental plaque, histopathology of periodontal lesions and basic immunology<sup>79</sup>. Rat is equipped with one incisor and three molars in each quadrant. The incisors are rootless, continuously growing teeth and therefore

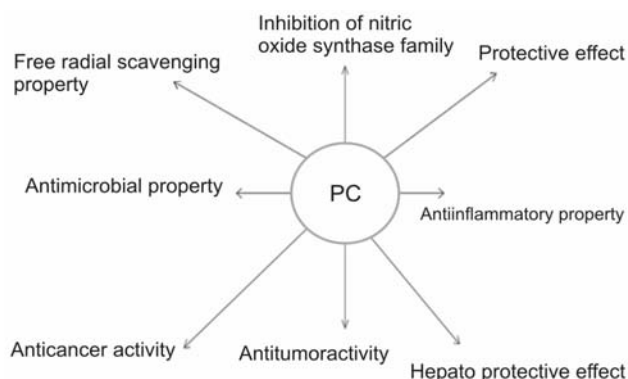


Fig. 3—Schematic representation of protective effects of PC

unsuitable as models for human periodontal disease. In contrast, the structure and organization of the periodontal tissues of the molars (oral gingival epithelium, oral sulcular epithelium, junctional epithelium, periodontal collagen fibers and alveolar bone) are similar in rats and humans. With respect to periodontal anatomy of molar teeth, development and composition of dental plaque and histopathology of periodontal lesions, the rat bears much resemblance to man that it seems reasonable to use it as a model for periodontal disease<sup>79</sup>.

### Induction of periodontitis in rats

Ligature induced periodontitis — Three methods are followed viz.,

- (i) Male Sprague-Dawley rats (each weighing from 280-400 g) are slightly anaesthetized with surgical doses of sodium pentobarbitone (35 mg/kg). Sterile 2-0 black-braided silk thread is placed around the cervix of the lower left first molar and knotted mesially<sup>80</sup>.
- (ii) Rats are anaesthetized (40 mg of ketamine / kg of body weight and 9 mg of xylazine/kg of body weight) and 3-0 silk suture is placed around the second premolar of both mandibular quadrants<sup>81</sup>.
- (iii) Sterile 9-0 silk ligatures (5 mm long) are inoculated with *P.gingivalis*, tied on the first molar in the right maxillary quadrant with sterile instruments and then carefully pushed into the gingival sulcus<sup>82</sup>.

Endotoxin induction — There are three methods viz.,

- (i) Under anaesthesia, injections are administered using *Escherichia coli* endotoxin dissolved at 1 mg/ml. The injection volume is 10 µl of endotoxin. Each rat receives three injections given every other day at 5 injection sites per animal. Three injections are given into the anterior maxillary labial gingivae and palatal incisor gingivae<sup>83</sup>.
- (ii) Experimental periodontitis is induced by repeated injection of *E.coli* endotoxin (LPS) (1mg/ml) using an insulin syringe in rats. Solutions are placed into the palatal gingivae between the first and second maxillary molars and into the labial and oral gingivae between the central incisors in the mandible and maxilla every other day on three separate days<sup>84</sup>.



- (iii) Periodontitis is induced by intragingival injection of 1 µl of LPS (10 µg/µl) derived from *Salmonella typhimurium*. A fine hypodermic needle is inserted at the mesial aspect of the first mandibular molar and the tip moves distally so that the injection is made at the interdental papilla between the first and second molars<sup>85</sup>.

### Conclusion

PC exhibits protective and therapeutic effects and it appears to have a significant potential against inflammatory disease such as periodontitis. Ingestion of the PC demonstrates a substantial antioxidant capacity for protecting cells from oxidative damage. It is shown that PC provides superior antioxidant efficacy as compared to vitamins C and E<sup>65</sup>. Dietary supplementation of PC enhances the host resistance and inhibits the oxidative stress by balancing the oxidant and antioxidant ratio of periodontal diseases. PC could be developed as a safe drug for the treatment of periodontitis.

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