

# **Evaluation of Antioxidant effect of Tea in Patients with Periodontitis - A Spectrophotometric Analysis of Saliva**

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## INTRODUCTION

Periodontal disease is a chronic condition common in adults. It had long been thought that the pathological events leading to the destruction of the periodontium during inflammatory periodontal diseases are likely to represent complex interactions involving an imbalance in enzymatic and non-enzymatic degradative mechanisms. Although enough research had been done in the field of periodontics to list out the factors that would eventually lead to periodontal diseases, it was only in 1997 that Halliwell studied the role of Reactive Oxygen Species in the pathogenesis of periodontal disease<sup>21</sup>.

Periodontal diseases have been proved to be associated with an imbalance between oxidants and anti-oxidants, due to both an increase in free radical production and a defect in the total antioxidant activity of saliva<sup>6,53</sup>. There is at present ample evidence that proves the role of Reactive Oxygen Species (ROS) in destruction of periodontal tissues<sup>6,53</sup>. Polymorphonuclear leukocytes (PMN) are recognized as a particularly rich source of ROS, which in the absence of suitable antioxidants can lead to tissue damage. ROS is a collective term which includes oxygen derived free radicals (ODFR), such as the superoxide radical ( $O_2^-$ ), Hydroxyl radical ( $^{\cdot}OH$ ) and the nitric oxide radical ( $NO^{\cdot}$ ) species, and the non radical derivatives of oxygen, such as hydrogen peroxide( $H_2O_2$ ) and hypochlorous acid( $HOCl$ ). The presence of one or more unpaired electrons in the outer orbitals of ODFR makes such species, especially the  $^{\cdot}OH$  species extremely reactive in nature<sup>10</sup>.

Reactive oxygen species cause tissue damage by different mechanisms: DNA damage, Lipid Peroxidation, protein damage, and oxidation of important

enzymes, and by stimulation of pro-inflammatory cytokine release by monocytes and macrophages<sup>10</sup>.

To counteract the detrimental effects of ROS in vivo, a variety of antioxidant defense mechanisms exist. An antioxidant is defined as any substance that, when present in low concentrations compared to those of an oxidisable substrate, significantly delays or inhibits the oxidation of that substrate. Thus, to decrease the harmful effects of ROS, there is the need for administration of antioxidants to patients with periodontal diseases. Since the commercially available anti-oxidants are not very economical and have a variety of side effects, and with the paradigm shifting towards natural products, there evolved the need to study the effect of natural herbs on the ROS.

Tea has been propagated for quite sometime for having numerous medicinal properties<sup>11,14,19</sup>, but its use in the prevention of periodontal diseases still remains unexplored. To study the phenomena of tea in prevention of periodontal diseases knowledge of the antioxidants present in tea as well as Reactive Oxygen Species causing periodontal disease is mandatory.

Although Hoe and co workers<sup>25</sup> came up with the idea of evaluating the antioxidants present in tea extracts, which they found were effective and caused inhibition of ROS (DPPH radical, lipooxygenase, lipid peroxidation and superoxide), it has been proved by numerous studies that tea dilutions work better than the extracts<sup>28</sup>.

The rationale for this study is the presence of polyphenols in tea, namely, catechins and theaflavins that have antioxidant properties, and are therefore effective against free radicals<sup>12</sup>. This study was done to evaluate the antioxidant effect of Black, Green, and Oolong teas, by both qualitative and quantitative analysis of the inhibition of ROS, in saliva, of patients with periodontal diseases.

## REVIEW OF LITERATURE

### *REACTIVE OXYGEN SPECIES AND PERIODONTAL DISEASES*

It is known that periodontitis is a multifactorial disease characterized by the presence of a variety of molecular species, among which are free radicals and the Reactive Oxygen Species (ROS)<sup>5,8,10</sup>. Reactive Oxygen Species are essential in a number of metabolic pathways but their excessive production can result in cell damage, as during the “respiratory burst” of phagocytosing leukocytes (PMN). Such PMN activity is physiological and usually results in mild oxidative damage, perfectly controlled by the defense mechanisms of the host tissues. Specific factors promoting periodontitis can also alter this equilibrium. The strongest evidence implicating Reactive Oxygen Species in the pathological destruction of connective tissues during periodontal diseases arises in considering PMN infiltration as a key event of host response against microbial invasion. If this infiltration is numerically consistent, it is likely to lead to an increase in Reactive Oxygen Species level<sup>10</sup>.

The implication of reactive oxygen species (ROS) in the pathogenesis of a variety of diseases, in addition to providing an important function in normal metabolic reactions has been well established. Numerous diseases like AIDS, cancer<sup>12</sup>, atherosclerosis, chronic inflammatory conditions<sup>8</sup>, aging process<sup>14</sup>, have been associated with ROS and periodontal disease is no exception<sup>17</sup>. The fact that neutrophils produce Reactive Oxygen Species responsible for tissue destruction in the oral cavity was first studied in the late 70's. In **1977, Tauber AI and Bablor BM** were the first to study the **hydroxyl radical production by neutrophils**<sup>49</sup>. They examined the ability of these cells to support the release of ethylene from methional. According to their study, ethylene production required the presence of neutrophils, opsonized zymosan, and methional, indicating that it was formed from methional but not resting neutrophils. Superoxide Dismutase reduced ethylene

production to 21% of control levels, but catalase had no significant effect in the system. Their findings indicated that stimulated neutrophils produce a highly reactive oxidizing radical, which they thought was OH<sup>-</sup>, which released ethylene from methional. They also formulated that O<sup>2-</sup> generated during the respiratory burst was involved in the production of this reactive species.

Neutrophils increase tissue destruction by releasing Reactive Oxygen Species by blocking  $\alpha$ 1- proteinase inhibitor, which inhibits serum elastase<sup>23</sup>. **Harvey Carp and Aaron Janoff (1979)** studied the in-vitro suppression of serum elastase-inhibitory capacity (EIC) by Reactive Oxygen Species generated by phagocytosing polymorphonuclear leukocytes. Their results suggested that H<sub>2</sub>O<sub>2</sub> and O<sup>2-</sup>, through generation of more potent oxidant (perhaps •OH), are capable of suppressing the EIC of serum, probably by inactivating  $\alpha$ 1-Pi. Their results proved that oxidative inactivation of  $\alpha$ 1-Pi by these cells may contribute to connective tissue damage observed in chronic inflammatory processes.

**R.D.Paola, S.Marzocco, E.Mazon et al<sup>39</sup>**, evaluated the **nitric oxide and Reactive Oxygen Species in ligature induced periodontitis in rat models** in the year **1983**. Periodontitis was induced in rats by placing a piece of 2/0-braided silk around the left 1<sup>st</sup> premolar. According to their study, ligation significantly increased inducible nitric oxide synthetase activity and expression, and damaged tissue revealed increased neutrophil infiltration, lipid peroxidation and positive staining for nitrotyrosine formation and polymerase (ADP- ribose) activation. Ligation significantly increased Evans blue extravasation in gingivomucosal tissue and alveolar bone destruction.

Among the stimuli for Respiratory Burst are the following:

- 1). Opsonized microbes; 2). Complement C5a; 3). Leukotriene B<sub>4</sub>; and 4). N- formulated oligopeptides released from lysed microorganisms<sup>10</sup>.

Respiratory Burst results in the production of reactive oxidants such as superoxide, hydrogen peroxide, and hydroxyl radicals. Such potent oxidizing agents destroy not only microbes, etc for which they are intended, but also the surrounding normal cells as well as the components of the extracellular matrix (collagen, hyaluronic acid, etc.). Thus, normal tissue destruction is part of the typical inflammatory response. Thus free radical scavenging enzymes (Superoxide Dismutase) and hydrogen peroxide degrading enzymes (catalase) are essential to protect normal cells and matrix components from oxidation<sup>10</sup>. Interestingly the anti-inflammatory nature of Superoxide Dismutase was known long before the specific function of the protein was appreciated.

In **1991**, a study was conducted on the electron microscopic immunolocalization of a Copper- zinc Superoxide Dismutase in association with collagen fibers of periodontal soft tissues, by **Bennet H. Jacoby and Walter L. Davis**<sup>29</sup>. They cleaved periodontal soft tissues from freshly extracted human teeth and prepared them for subsequent biochemical and morphological studies under Electron Microscopy, by immersion into liquid nitrogen and fixation. Biochemical analysis showed that human periodontal ligament contained about twice as much Superoxide Dismutase activity as human skin (dermis), but considerably less enzyme activity than seen in red blood cells. Immunohistochemistry localized the enzyme activity to the periphery of matrix collagen fibrils and to the glycocalyx of tissue fibroblasts.

The most important species implicated in inflammatory injuries to the tissues are the hydroxyl ( $\bullet\text{OH}$ ) radical, the superoxide anion ( $\text{O}^{2-}$ ) the nitrous oxide radical and hypochlorous acid, hydrogen peroxide and singlet oxygen which are Reactive Oxygen Species<sup>10</sup>. Superoxide Dismutase is an enzyme extensively used as a biochemical indicator of pathological states associated with oxidative stress<sup>5</sup>. At the 1<sup>st</sup> step of the defense system against oxidative stress, it catalyzes

dismutation of the superoxide anion ( $O_2^-$ ) into hydrogen peroxide ( $H_2O_2$ ). At the second step of antioxidant defense system, Glutathione Peroxidase (GSH-Px) and catalase independently degrade  $H_2O_2$  to water. Any increase in Superoxide Dismutase activity should therefore, produce excess  $H_2O_2$  that must be efficiently neutralized by either GSH-Px or catalase. In patients with periodontal disease, excess  $H_2O_2$  cannot be neutralized efficiently, due to exhaustion of enzyme by  $H_2O_2$  and lipid peroxides. If the amount of free radicals that cannot be efficiently neutralized after an oxidative stress reaches a critical level, then lipid peroxidation occurs subsequently, indicating an increased risk of cell membrane damage<sup>5</sup>. Among the several oxidative markers available, lipid hydroperoxides are among the most reliable<sup>5</sup>. Taking all this data into account, we studied the effect of three different tea types on Superoxide Dismutase<sup>51</sup>, Glutathione and Lipid Peroxidation<sup>5</sup>.

In the year **1994, Asman B, Wijkander P, and Hjerpe A**<sup>1</sup> suggested that free radicals may play a role in the collagen destruction by granulation tissues, as in periodontitis.

In **1996, A. Gustafsson and B. Asman**<sup>20</sup> proved the host response in adult periodontitis to be specifically associated with neutrophils. In their study, the release of free oxygen radicals and degranulation was studied in neutrophils from 14 patients with adult periodontitis and 14 healthy controls. Fcγ – receptor stimulation, using *Staphylococcus aureus* opsonised with gamma globulin, activated the neutrophils. Release of oxygen radicals was measured as luminol enhanced chemiluminescence. Degranulation was assessed as release of elastase, measured with a specific substrate and as release of lactoferrin, measured with ELISA. Their study showed a more than 2-fold higher release of free oxygen

radicals from Fc $\gamma$  – receptor stimulated neutrophils compared with healthy controls.

**ILC Chapple (1997)**<sup>10</sup> reviewed the role of Reactive oxygen species and antioxidants in inflammatory diseases. They clearly defined a substantial role for free radicals or reactive oxygen species in periodontitis, but little research had been done in this area. The discovery of transcription factor nuclear factor  $\kappa$ B (NF- $\kappa$ B) was reviewed and several potential pathways for cytokine-induced periodontal tissue damage, mediated by NF- $\kappa$ B were discussed. Emphasis was placed on cytokines studied in periodontics, principally TNF- $\alpha$ , IL-1, IL-6, IL-8, and  $\beta$ -interferon.

In **1998**, another study by **A. Gustafsson and B. Asman**, along with **Fredriksson M and Bergstrom K**<sup>20</sup>, confirmed the previous results and elucidated the mechanism of this phenomenon by measuring chemiluminescence in parallel with the intracellular production of hydrogen peroxide, after stimulation with opsonised bacteria. Venous blood was obtained from 17 treated periodontitis patients and 17 controls. To determine whether the higher chemiluminescence was associated with altered responsiveness to priming, the cells were pre-incubated with TNF- $\alpha$  and lipopolysaccharides. This study showed a Fc $\gamma$  – receptor mediated chemiluminescence of peripheral neutrophils from adult patients with periodontitis. The authors attributed this hyperactivity of increased tissue destructive peripheral neutrophils to reactive oxygen species in periodontal lesion.

**Halliwell et al (2000)**<sup>21</sup> was first to propose **the role of Reactive Oxygen Species in pathogenesis of periodontal disease, as a missed opportunity**. Reactive Oxygen Species, in having a potential role in the pathogenesis of periodontal diseases, was reviewed in the year **2000** by **RJ Waddington, R Moseley, and G Embery**<sup>53</sup>. According to the data gathered by them ROS are generated predominantly by polymorphonuclear leukocytes (PMN) during an

inflammatory response and are regarded as being highly destructive in nature. Detection of ROS oxidation products, the elevation of iron and copper ions, which catalyze the production of the most reactive radical species, and the identification of an imbalance in the oxidant/antioxidant activity within periodontal pockets suggests a significant role in periodontal tissue destruction. The identification and characterization of connective tissue metabolites in Gingival Crevicular Fluid (GCF) resulting, from the degradation of periodontal tissues, notably alveolar bone, provides further evidence for a role for ROS in tissue destruction associated with inflammatory periodontal diseases.

According to professor **ILC Chapple (2001)**<sup>10</sup>, low anti-oxidant levels are both local and systemic. This study investigated the possibility of differences in GCF anti-oxidant capacity between periodontal health and disease. The results from this cross-sectional study show that GCF total anti-oxidant capacity is significantly decreased in patients with periodontal disease. Furthermore, this local decrease was reflected systemically by lower mean anti-oxidant capacity in plasma from patients with periodontitis. In **another study** by the same authors, they proved that free radicals could destroy periodontal tissues and facilitate bone resorption. In aggressive and chronic forms of periodontitis, the predominant inflammatory cells within the periodontal tissues appear to be functionally activated and exhibit increased production of free radicals. These molecules are capable of inducing periodontal tissue destruction, and are associated with osteoclastic bone resorption.

**Maurizio Battino, Maria-Soledad Ferreiro et al (2001)**<sup>34</sup> suggested that a specific antioxidant therapy could be a promising approach in treating some Papillon-Lefevre Syndrome subjects. They studied a group of patients belonging to 3 generations of a family with different degrees of severity of Papillon-Lefevre Syndrome. They evaluated Coenzyme Q, vitamin E, Glutathione, and uric acid by

high-performance liquid chromatography techniques, and hydroperoxides by a spectrophotometric method. The affected individuals presented lower antioxidant levels with very high hydroperoxide concentrations.

**Margareta I.Fredriksson, Anders K.Gustafsson, Kurt G.Bergstrom and Bjorn E.Asman<sup>17</sup>** studied constitutionally hyperreactive neutrophils in periodontitis. The peripheral neutrophils from venous blood of 15 patients was taken and activated with IgG-opsonized bacteria, complement-opsonized zymosan, or phorbol myristate acetate. The total release of oxygen radicals was measured with luminol-enhanced chemiluminescence (CL) and the extracellular release of oxygen radicals with isoluminol-enhanced CL. They reported increased chemiluminescence peripheral neutrophils in periodontitis and related it to greater responsiveness of Fc $\gamma$ - receptor.

#### **SALIVARY ANTIOXIDANTS AND PERIODONTAL DISEASE STATUS**

Saliva, a heterogeneous fluid comprising proteins, glycoproteins, electrolytes, small organic molecules and compounds transported from the blood, constantly bathes the teeth and oral mucosa. Its components exert a well-documented role in health and disease. In addition to its lubricant properties, saliva contains many biochemical systems known to be involved in soft-tissue repair, and many antibacterial components including lysozyme, lactoferrin and salivary peroxidase. Saliva contains various antioxidants<sup>4,43</sup>, and hence could constitute a first line of defense against free radical mediated oxidative stress. With the historical prejudice towards saliva as a potential fluid for periodontal diagnosis, the ease of availability, non-invasiveness of the procedure and centrality of saliva in defense of the mouth, whole saliva offers numerous possibilities for marking disease activity, for development of techniques suitable for salivary antioxidant evaluation and evaluating treatment outcome<sup>31</sup>.

Previous research considering salivary antioxidant status and periodontal

disease<sup>13,31,43,44,48</sup> is sparse and has yielded conflicting data. This may be a result of the different methodology employed by the authors, though there are other factors that may cause discrepancies.

**Moore *et al* (1994)**<sup>37</sup> measured the antioxidant capacity of saliva in diseased and healthy individuals using the Trolox equivalent antioxidant capacity assay. Measurements were made using stimulated and unstimulated saliva samples. No difference was found between the antioxidant capacity of saliva in diseased and healthy patients. However, the diseased sample size was small (seven subjects) and their disease status was not categorized, having been defined only as needing dental treatment. Similarly, definition of the healthy cohort was ambiguous, being described as 'apparently healthy'. No clinical examination was made on the control group. In addition, saliva samples were stored at -20°C; that may have allowed degradation of antioxidant capacity.

**Salivary antioxidants and periodontal disease status** studied by **Dean V. Sculley, Simon C. Langley-Evans (2002)**<sup>43</sup> revealed that the bacterium *Porphyromonas gingivalis* has been implicated in the etiology of periodontal disease, which causes destruction of the connective tissue and bone around the root area of the tooth. It has been observed that invading *P.gingivalis* bacteria trigger the release of cytokines such as interleukin 8 and tumour necrosis factor  $\alpha$ , leading to elevated numbers and activity of polymorphonuclear leukocytes (PMN).

**The decrease in the total antioxidant activity of saliva in patients with periodontal disease** was evaluated by **Diab-Ladki R, Pellat B, Chahina R (2003)**<sup>13</sup>. The study examined the role of free radical-induced tissue damage and the anti-oxidant defence mechanism of saliva in periodontal disease. This study measured the scavenging capacity of saliva against free radicals generated in vitro by electrolysis, xanthine-xanthine oxidase, or stimulated polymorphonuclear leukocytes. Total protein content and total anti-oxidant capacity of saliva was also

determined. They found a decrease in salivary antioxidant activity of patients with periodontal disease as compared to healthy individuals. The saliva of healthy individuals was found to be 40- 50% more effective than saliva of patients with periodontal disease in scavenging a wide variety of free radicals generated in vitro. They concluded that periodontal diseases are associated with an imbalance between oxidants and antioxidants, due to both an increase in free radical production and a defect in the total antioxidant activity of saliva.

A cohort study was done to determine whether periodontitis and gingivitis are associated with impaired salivary antioxidant status and increased oxidative injury<sup>43</sup>. 129 patients' periodontal status was determined using CPITN system. Total salivary antioxidant capacity and salivary ascorbate, urate and albumin were determined in a sample of whole unstimulated saliva. Protein carbonyl concentrations were determined as an index of oxidative injury. In this study, **D.V.Sculley and S.C.Langley-Evans (2003)** found poor periodontal health to be associated with increased concentrations of protein carbonyls in saliva. They concluded that periodontal disease is associated with reduced salivary antioxidant status and increased oxidative damage within the oral cavity. The main finding of their study was that subjects with worst periodontal health status tended to have greater oxidative injury, as indicated by the presence of protein carbonyls in saliva.

In **2004**, local and systemic antioxidant capacity in periodontitis and health was studied in saliva, plasma, serum and gingival crevicular fluid<sup>6</sup>. **Brock GR, Butterworth CJ, Matthews JB, and Chapple ILC**, collected whole saliva (both stimulated and unstimulated) after overnight fasting from 20 patients, and total antioxidant capacity was determined using Chemiluminescence method. Salivary total antioxidant capacity was found to be lower in patients with periodontitis.

## ***ANTI-OXIDANT SUPPLEMENTATION***

Whilst most ROS have extremely short half-lives, they can cause substantial damage by initiating free radical chain reactions. The role of antioxidant mechanisms is therefore specific which is to remove harmful oxidants (or ROS) as soon as they form, or to repair the damage caused by ROS in vivo. Important antioxidants include the ‘chain-breaking’ vitamin E, vitamin C, vitamin A, urate, bilirubin, and those substances containing sulphhydryl (thiol/SH) groups<sup>30</sup>.

Antioxidants are classified according to their mode of action<sup>10</sup>. ‘Scavenging antioxidants’ prevent oxidative stress by literally scavenging radicals as they form. ‘Preventive antioxidants’ function largely by sequestering transition metal ions and preventing Fenton reactions, they are therefore largely proteins by nature. ‘Enzyme antioxidants’ are systems that function by catalyzing the oxidation of other molecules.

Numerous antioxidants have been tried and tested both by systemic administration and as mouthwashes. These include synthetic products like vitamins<sup>30</sup> to natural products like triphala, wine, rosemary, turmeric, tea, cocoa powder<sup>36</sup>.

**Dr.Mariam Sheikhi et.al** proved that antioxidant **vitamins could counteract free radicals. Fusobacterium nucleatum (FN) is associated with periodontal disease**<sup>32</sup>. They challenged the neutrophils with fusobacterium nucleatum. FN strains stimulated neutrophils to produce a large amount of reactive oxygen species (ROS) or free radicals. Then the inhibition of Lipid Peroxidation by Vitamin E was estimated, and they found Vitamin E to have a 50% reduction in Lipid Peroxidation.

In 1994, Asman B, Wijkander P, and Hjerpe A observed a reduction of collagen degradation in experimental granulation tissue by vitamin E and selenium<sup>1</sup>. In this in vivo animal trial, degradation of homologous H-collagen

powder by experimental granulation tissue induced by cellulose sponges in the rat was monitored as the radioactivity excreted in urine. By administering pharmacological doses of both vitamin E and selenium subcutaneously and by injection into sponges implanted subcutaneously, this breakdown of collagen was reduced. Injections in the sponges also arrested the maturation of the granulation tissue. Thus they proved that Vitamin E and selenium are potential inhibitors of the free oxygen radicals from phagocytic inflammatory cells.

**Effects of three dietary phytochemicals from tea, rosemary and turmeric on inflammation-induced nitrite production** was studied by **Chan MM, Ho CT, Huang HI (1995)<sup>9</sup>**. In chronic inflammation, cytokines induce the production of nitric oxide (NO.) that is converted to DNA damaging and carcinogenic peroxynitrite and nitrite. The compounds epigallocatechin gallate (EGCG), carnosol, and curcumin are non-vitamin phytochemicals contained in commonly consumed dietary plants. They inhibited lipopolysaccharide (LPS) and interferon-gamma (IFN gamma) induced nitrite production by mouse peritoneal cells by more than 50% at 2.5-10  $\mu$ M.

The in vitro antioxidant activities of mouthrinses and their components were evaluated by **Battino M, Ferreiro MS, Fattorini D, Bullon P, in 2002<sup>2</sup>**. They evaluated the anti-oxidant activity of 11 mouth rinses and their active principles, regardless of their efficacy as anti-microbial agents. They found that methyl-salicylate containing mouth rinses were most effective.

With the shift of health consciousness towards natural products, tea has been tried for its antioxidant properties in innumerable conditions.

## **NATURAL ANTIOXIDANTS FROM TEA**

Tea, a beverage originating from a single species of plant, *Camellia sinensis*, is widely cultivated around the world in both tropical and subtropical regions and has become the second most popular drink after water. There are three major forms

of manufactured teas: Green tea, Oolong tea, and Black tea. Green tea is prepared in such a way as to preclude the oxidation of green leaf polyphenols<sup>25</sup>. The manufacture of black tea is carried out so that a high degree of oxidation of the tealeaf polyphenols is ensured, and Oolong tea is a partially oxidized product. There are two major polyphenols in teas: catechins, theaflavins and thearubigens. Catechins are major constituents of green tea leaves, and are also present in oolong and black teas, whereas thearubigins and theaflavins are found only in black and Oolong teas. Due to the presence of antioxidants in tea, the catechins and theaflavins, tea can reduce the ROS in the oral tissues.

Green tea is rich in Catechins, and contains Theaflavins and Thearubigens in small amounts. Black tea is richest in Theaflavins and Thearubigens and contains minimal Catechins. Oolong tea contains both catechins and theaflavins and thearubigens in equal quantities due to its processing and way of manufacture.

Tea has been used as a medicinal herb for a long time, and numerous studies have been done both on tea extracts and tea in its crude form. A lot of research work has been done in the past on the effects of Green tea, as compared to Oolong and Black teas.

Tadashi Ishigami and Yukihiro Hara evaluated the anti-cariogenic and bowel modulating actions of tea<sup>28</sup>. In this study, firstly the anti-cariogenic property of tea drinking was explicitly manifested. This was subsequently proven to be due to the anti-plaque and antibacterial activities of tea or tea polyphenols. Secondly, the bactericidal effects of tea polyphenols on the enteric, pathogenic bacteria were examined. Tea polyphenols inhibited the growth of food borne pathogenic bacteria at much lower concentrations than are contained in a normal cup of tea and inhibited none of the enteric acidophilus bacteria at the concentration of a normal brew.

## **GREEN TEA**

According to a study by **Dr.Masatomo Hirasawa et al<sup>24</sup>** **catechins compounds from green tea kill p.gingivalis**. Green tea catechin showed a bactericidal effect against p.gingivalis. His experiment showed a marked reduction in the probing depth and proportion of Bacteria Producing Residue (mainly the gram negative bacteria). BPR produce tissue destructive enzymes such as collagenase and peptidase. These enzymes play a role in destroying the gingival tissues, in osteoclast breakdown, and in the progress and development of periodontitis.

**Lin YL, Lin JK (1997)<sup>33</sup>** investigated the effect of various tea polyphenols and caffeine on the induction of NO synthase (NOS) in thioglycollate-elicited and lipopolysaccharide (LPS)-activated peritoneal macrophages. Gallic acid (GA), -epigallocatechin (EGC), and -epigallocatechin-3-gallate (EGCG), the major tea catechin, were found to inhibit inducible NOS (iNOS) protein in activated macrophages, and the reduction could occur through prevention of the binding of nuclear factor-kappaB to the iNOS promoter, thereby inhibiting the induction of iNOS transcription.

The **Indian-US Head and Neck Cancer Cooperative Group** evaluated the **effect of green tea on leukoplakia in 1997<sup>50</sup>**. Two doses of green tea were utilized: 3.6g per day and 5.4g per day. Relevant to oral carcinogenesis, the levels of EGCG following the intake of a single cup of tea were found to be higher in the saliva than in the blood, suggesting additional rationale for the use of tea as a preventive agent against tobacco induced oral cancers. The results provided basis for continuing international collaborative efforts in chemoprevention trials in head and neck cancer.

**Sakanaka S et al<sup>54</sup>** carried out a study on the **green and its effect on cellular adherence**. They reported inhibition of cellular adherence by green tea

polyphenols. **Dr. John Weisburger** reported a **reduction in bacterial cell membrane fluidity caused by catechins in green tea**<sup>54</sup>. This study concluded that it was this property of green tea that led to its anti-plaque activity.

**Polyphenolic antioxidant (-)-epigallocatechin-3-gallate from green tea reduces UVB-induced inflammatory responses and infiltration of leukocytes in human skin.** In several mouse skin models, topical application as well as oral consumption of green tea has been shown to afford protection against chemical and UVB-induced carcinogenesis and inflammatory responses. In this study, **Katiyar SK, Matsui MS, Elmets CA, Mukhtar H.(1999)** investigated in human skin, whether topical application of (-)-epigallocatechin-3-gallate (EGCG), the major polyphenolic constituent in green tea, inhibits UVB-induced infiltration of leukocytes (macrophage /neutrophils), a potential source of generation of reactive oxygen species (ROS), and generation of prostaglandin (PG) metabolites. Their results suggested that EGCG has the potential to block the UVB-induced infiltration of leukocytes and the subsequent generation of ROS in human skin. This may explain the possible mechanism involved in anti-inflammatory effects of green tea.

To determine the usefulness of green tea catechin for the improvement of periodontal disease, a clinical pilot study on **Improvement of periodontal status by green tea catechin using a local delivery system** was carried out by **Hirasawa M, Takada K, Makimura M, Otake S (2002)**<sup>24</sup>. The minimum inhibitory concentration (MIC) and bactericidal activity of green tea catechin against black-pigmented, Gram-negative anaerobic rods (BPR) were measured. Hydroxypropylcellulose strips containing green tea catechin as a slow release local delivery system were applied in pockets in patients once a week for 8 weeks. Green tea catechin showed a bactericidal effect against Bacteria Producing Residue and the combined use of mechanical treatment and the application of green tea

catechin using a slow release local delivery system was effective in improving periodontal status.

A study was conducted on **Green tea protection against age-dependent ethanol-induced oxidative stress** by **Luczaj et al (2004)**<sup>33</sup>. The aim of this study was to investigate the influence of green tea as a source of water-soluble antioxidants on the ability to prevent oxidative stress in aged rats sub-chronically intoxicated with ethanol. Ethanol intoxication produced age-dependent decrease in the activity of serum superoxide dismutase, glutathione peroxidase, and reductase and in levels of glutathione (GSH), vitamins C, E, and A, and beta-carotene. They found that Green tea partially protected against changes in antioxidant enzymatic as well as nonenzymatic parameters produced by ethanol and enhanced by aging. Administration of green tea significantly protects cellular components such as lipids and proteins against oxidative modification, and that green tea effectively protects blood serum against oxidative stress produced by ethanol as well as aging.

**Inhibitory effects of green tea polyphenols on the production of a virulence factor of the periodontal-disease-causing anaerobic bacterium *Porphyromonas gingivalis*** was proved by **Sakanaka S, Okada Y (2004)**<sup>41</sup> Green tea polyphenols completely inhibited the production of n-butyric acid and propionic acid at a concentration of 1.0-2.0 mg/mL in general anaerobic medium (GAM). (-)-Epigallocatechin gallate (EGCg), which is a major component of tea polyphenols also inhibited the production of phenylacetic acid at 0.5 mg/mL in GAM broth. In their experiment using resting cells of *P. gingivalis*, phenylacetic acid was produced from l-phenylalanine and phenylpyruvic acid, but this reaction was also inhibited by EGCg, (-)-epicatechin gallate, and (-)-gallocatechin gallate. Their study showed 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.

## **OOLONG TEA**

In a study by **Ooshima T, et al**<sup>39</sup>, on Oolong tea extracts, they reported a reduction of dental plaque deposition in humans by Oolong tea extract.

**Qin Yan Zhu et al (2003)**<sup>40</sup> evaluated the effect of antioxidative activities of Oolong tea water extract. The in vitro effect for inhibitory effect on Fenton Reaction System-induced DNA damage and erythrocyte hemolysis were evaluated. They also tested for the reducing power of the extract and its DPPH radical scavenging activity. They found that Oolong tea showed high antioxidative activities in all of the model systems tested.

## **BLACK TEA**

**Theaflavin-3,3'-digallate from black tea blocks the nitric oxide synthase by down-regulating the activation of NF-kappaB in macrophages.** Nitric oxide (NO) plays an important role in inflammation and also in multiple stages of carcinogenesis. In this study **Lin YL, Tsai SH, Lin-Shiau SY, Ho CT, Lin JK (1999)**<sup>32</sup> investigated the effects of various tea polyphenols, including theaflavin, a mixture of theaflavin-3-gallate, theaflavin-3,3'-digallate, thearubigin, and (-)-epigallocatechin-3-gallate on the induction of NO synthase in lipopolysaccharide-activated murine macrophages, RAW 264.7 cells. Their results affirm that theaflavin-3,3'-digallate decreases the protein levels of inducible NO synthase by reducing the expression of inducible NO synthase mRNA, and the reduction could be via preventing the activation of NF-kappaB, thereby inhibiting the induction of inducible NO synthase transcription. It was also demonstrated that the gallic acid moiety of theaflavin-3,3'-digallate is essential for their potent anti-inflammation activity.

**Prof. Christian Wu & Dr. Min Zu (2003)**<sup>54</sup> evaluated the **anti-plaque activity of black tea**, and found a significant reduction in plaque formation, in

subjects who rinsed their mouths for one minute eight times a day with black tea. In another study by the same authors, they reported inhibition of plaque formation by black tea by virtue of its anti-microbial activity.

The most cited example to estimate the antioxidants in the various types of teas i.e black, green and oolong, and their effect on reactive oxygen species is the work of **C.T.HOE et al<sup>25</sup>** who evaluated **the effect of polyphenolic fractions in vitro, on DPPH radical, superoxide, lipooxygenase and Rancimat test**. They found theaflavins and catechins to have antioxidant properties against the Reactive Oxygen Species observed.

Reactive oxygen Species may have beneficial as well as detrimental role in periodontitis. To counteract the harmful effect of Reactive Oxygen Species with antioxidants, our study was designed to evaluate the in vivo antioxidant activity of Black, Oolong and Green teas in saliva of patients with periodontal diseases, by a spectrophotometric analysis, to find its usefulness in prevention of periodontal disease, by measuring the levels of superoxide dismutase, glutathione and lipid peroxidation.

## **SUMMARY AND CONCLUSION**

The implication of Reactive Oxygen Species (ROS) in the pathogenesis of periodontal diseases has been well established<sup>53</sup>. The ROS (free radicals), though present for a very short duration, causes detrimental effect that may lead to substantial tissue damage. To prevent this tissue damage, antioxidants are administered to counteract the ROS<sup>1,30</sup>.

Tea contains the antioxidants catechins and theaflavins, and the difference in the three types of tea<sup>25</sup>, Green, Oolong and Black, is based on the difference in manufacture and polyphenolic (antioxidant) content. Though tea as an antioxidant

has been used in various systemic diseases<sup>12,14,19,26,28</sup>, its use in the field of periodontics<sup>33,42,54</sup> has been very limited. The therapeutic benefits of tea in various diseases, the ease of availability and the affordability, make tea an interesting subject to investigate with regards to periodontal disease.

Our study aimed to evaluate the antioxidant effect of Green, Oolong and Black tea in patients with periodontitis. Ninety patients with periodontitis were selected for our study, and their saliva was collected (baseline). The patients were randomly divided into three groups, and each group was given one type of tea. After 15 minutes of tea administration, the saliva of these patients was collected again. The saliva at baseline and after tea administration was subjected to spectrophotometric analysis for Superoxide Dismutase, Lipid Peroxidation and Glutathione levels.

The data obtained was subjected to SPSS package for statistical analysis. Our observations revealed that:

- Ⓢ Green, Oolong and Black tea showed a reduction in Lipid Peroxidation.
- Ⓢ There was a definite increase in Superoxide Dismutase levels seen after administration on Green, Oolong and Black tea.
- Ⓢ An increase in Glutathione levels after administration of all three teas.

On comparing the effect of these three tea types, we observed that,

- Ⓢ Black tea seemed to be more effective for Lipid Peroxidation, as compared to Green tea.
- Ⓢ For Superoxide Dismutase, Black tea was found to be more efficacious than Oolong and Green tea, and Oolong tea proved to be better than Green tea.
- Ⓢ As far as Glutathione is concerned, all types of tea showed equal reduction.

Compiling the data obtained from our study, all types of tea were effective in inhibiting Reactive Oxygen Species. Black tea showed better antioxidant effect as compared to Oolong and Green tea. Therefore we conclude by saying that

drinking tea, in particular Black tea could be an effective and economical method of ROS reduction.

Whether this decrease in ROS can effectively cause a decrease in tissue destruction remains to be seen. Further studies are necessary to establish the therapeutic effect of tea in periodontal diseases.

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