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Anti Inflammatory Effects of Proanthocyanidns in Endotoxin Induced Experimental Periodontitis in Rats

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Introduction

Periodontitis, a complex infection of bacterial origin, is characterized by an inflammatory host response against microorganisms of the bacterial plaque and their products. The inflammatory and immune reactions induced by the bacterial plaque represent the main characteristics of periodontitis [1]. Periodontally associated inflammatory processes contribute to an increase in the levels of local and systemic inflammatory mediators, including tumor necrosis factor alpha (TNF- α), interleukin-1β (IL-1 β), Interleukin-6 (IL-6), and other cytokines, C-reactive protein (CRP) etc. considered as major mediators of inflammation. The continuous high secretion of various cytokines including IL-1α, IL-6, IL-8, and TNF α by host cells following stimulation by periodontal pathogens modulates periodontal tissue destruction [2]. Destructive periodontal diseases, when left untreated, become a chronic inflammatory condition [1]. But, studies on the anti-inflammatory effects 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, catechin, epicatechin and procyanidin dimers and trimers composed of flavan-3-ol units with C4-C8 or C4-C6 interflavan linkages [3]. In vitro and in vivo studies have shown the protective effects of proanthocyanidins on endotoxin induced experimental periodontitis (EP)[4]. This study is designed to assess whether proanthocyanidins could exert antiinflammatory effect on endotoxin induced experimental periodontitis in rats.

Materials and Methods

PC 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 , for the use of laboratory animals.

Extraction of endotoxin preparations and induction of EP was induced by injecting E.coli endotoxin as described by Jayamathi et al [4]. The animals were broadly divided into two groups: Group 1: control; Group 2:EP. Animals of group 2 were further divided into group 3 and received 30mg PC / kg body weight, for 30 days After the experimental period, the animals were sacrificed by cervical decapitation and blood was collected.

Biochemical assays

Assay of IL-1 β [5], IL-6, [6], TNF- α [7] will be determined in serum with little modification. Assay of high sensitivity CRP in serum by Latex- enhanced immune turbidimetric assay for the quantitative *in vitro* determination of CRP in serum was carried out [8].

Histopathological analysis

Bone and teeth of the right maxillary halves were dissected out and the histopathological evaluation was performed on right maxillary halves were analysed by light microscopy.

Statistical analysis

All values used in analysis will be represented as mean ± SE of 6 rats. Multiple comparison of POST HOC Tukey's test will be applied to find out the statistically significant groups. ap < 0.001 as compared to Group 1. bp < 0.001 as compared to Group 2.

Results

Cytokines like IL-1β, IL-6 and TNF- α

was presented in Table 1. The levels of IL-1β, IL-6 and TNF- α was significantly increased in endotoxin induced EP rats (Group 2) as compared to control groups (Group 1). PC treatment (Group 3) was shown to significantly inhibit the cytokine levels, were found to have significant (p < 0.001) protective effect. A significant increase in the levels of acute phase proteins such as C-reactive proteins in plasma was noticed in EP rats. PC (Groups 3) treated groups were observed to have a significant (P < 0.001) decrease in the levels of acute phase proteins (Table 2)

Table 1. Effect of PC on the levels of IL-1β, IL-6 and TNF-α in endotoxin induced EP

Groups	IL-1β	IL-6	TNF- α
1	133	243	15.5
2	160 [a]	279 [a]	38.7 [a]
3	136 [b]	249 [b]	13.4 [b]

All values are Mean ± SE of 6 animals. ap < 0.001 as compared to Group –1 by POST HOC Tukey HSD, bp < 0.001 as compared to Group 2 by POST HOC Tukey HSD. The levels of IL-1β, IL-6 and TNF- α is expressed as pg/ml.

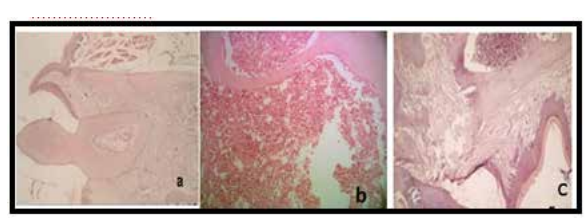
Table 2. Effect of PC on the levels of acute phase proteins, CRP in endotoxin induced EP

Groups	CRP
1	0.2
2	2.7 [a]
3	0.4 [b]

All values are Mean ± SE of 6 animals. ap < 0.001 as compared to Group –1 by POST HOC Tukey HSD, bp < 0.001 as compared to Group 2 by POST HOC Tukey HSD. CRp expressed as mg / dl of blood

Some typical photomicrographs were shown in Fig. Fig a represents normal architecture of rat maxillae on histopathological evaluation and indicated that progressive disease was associated with the presence of cellular infiltration of inflammatory cells (Fig b) in EP rats. PC treated groups (Fig c) exhibited scattered, diffused inflammatory cells and blood vessels.

Fig (a-c) - Histopathological changes observed in endotoxin induced rats EP treated with PC



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 diffuse chronic inflammatory cellular infiltrate;

Discussion

A great body of evidence demonstrated that the human neutro-

phil was both a target and a source of various proinflammatory cytokines, chemokines, and growth factors, and therefore it exerted its proinflammatory functions through an autoregulatory pathway [9]. Neutrophils were exquisite targets of proinflammatory cytokines viz., IL-1 β and TNF- α, chemokines such as IL-8, and growth factors such as granulocyte/ monocyte colony stimulating factor. The presence of bacteria adjacent to the gingival crevice and the intimate contact of bacterial lipopolysaccharide with the host cells trigger monocytes, polymorphonuclear leukocytes, macrophages, and other cells to release inflammatory mediators such as IL-1β, TNF-α, and prostaglandin E2. Macrophages and polymorphonuclear leukocytes, in response to the chemoattractant effect of bacterial lipopolysaccharide [10], are activated to produce important inflammatory mediators — notably, TNF-α, IL-1, IL-6, and other cytokines related to the host response and tissue destruction [10].

The continuous high secretion of various cytokines including IL-1, IL-6, IL-8, and TNF α by host cells following stimulation by periodontopathogens modulates periodontal tissue destruction. Our finding appears to agree with the previous study of Yang et al. [11], who demonstrated that green tea polyphenol-rich epigallocatechin gallate blocked mouse macrophage production of TNF- α. In this study, it was found that treating PC in endotoxin induced EP rats resulted in significant reduction in the secretion of both IL-1 β and TNF- α cytokines.

PC appears to be a promising candidate for the development of such therapies due to the demonstrated ability to inhibit secretion of inflammatory cytokines[4]. However, additional studies are required to identify the exact mechanisms by which the PC exert its beneficial properties. Identification of histological manifestation is a necessary step to assess the pathological changes in EP and also in groups treated with PC. Mononuclear cell infiltration were important characteristics of chronic gingival inflammation with resultant increased numbers of inflammatory cells[12] and the observations of this study were in agreement with this report.

Pro-inflammatory cytokines originating at the site of local pathology activate hepatocytes to produce acute phase proteins including CRP [13] and this forms part of the non specific response. A number of reports demonstrated elevated levels of C-reactive protein in periodontitis patients compared with CRP in non-diseased control subjects[4]. Several proinflammatory molecules, TNF α and IL-6, have been shown to be synthesized and secreted by adipose tissue. Both TNF α and IL-6 induce acute-phase hepatic protein production such as CRP [14]. The findings of the present study showed a trend toward a better systemic response to PC resulted in significant reduction of CRP levels.

Conclusion

Thus, the findings of the present study clearly demonstrated that dietary supplementation of proanthocyanidins enhanced the host resistance and immunity in periodontal diseases.

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