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ANTIOXIDANT PROPERTY OF A NOVEL LEMONGRASS OIL MOUTH WASH: AN EXPERIMENTAL STUDY

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Abstract

Oxidative stress is believed to be in part responsible for the inflammatory conditions affecting the periodontium manifesting as gingivitis and periodontitis. Antioxidants are those substances which when present in minimum quantities prevents the oxidation of a substrate. Recently, there has been a considerable interest in finding natural antioxidants from plants. Natural antioxidants are presumed to be safe since they occur in plant foods. These natural antioxidants occur in all higher plants, and in all parts of plants.

The aim of our study was to compare the efficacy of lemongrass oil mouthwash for anti oxidant property by estimation of thiol levels before and after administration of lemongrass oil mouthwash.

A total of 40 subjects were included in this study. Subjects were divided into 4 groups i.e. 3 test groups and one control group. Initially, saliva and gingival crevicular fluid (GCF) were collected and sent for analyzing the thiol levels. After scaling, lemongrass oil mouthwash that was prepared indigenously was administered at three different concentrations. Subjects were recalled on the 15th day; saliva and GCF sample were collected and sent for estimation of thiol levels. Statistical analysis was done using SPSS 16.0 software and results were analyzed.

There was no statistical difference in the thiol levels within the case groups whereas the subjects in the case group showed increased thiol levels when compared to the control group.

The lemongrass oil mouthwash was found to have anti oxidant activity at all the three concentrations levels.

Introduction

The mouth similar to other surfaces of the body is subject to numerous biologic stresses. This could be due to the environmental or pathological microorganisms that reside in niches in the oral cavity. The mode of stress which has been a speculative subject of interest in the recent times is "oxidative stress phenomenon" this is believed in part to be responsible for the inflammatory conditions affecting the periodontium and manifest as gingivitis and periodontitis.

In health a judicious balance exist within the pro-oxidant and anti-oxidant mechanism but in disease it is tipped in favor of the former which is due to oxidative stress. The antioxidants are those substances which when present in minimum quantities prevents the oxidation of a substrate.

Recently, there has been a considerable interest in finding natural antioxidant from plant materials to replace synthetic additives. Natural antioxidants are presumed to be safe since they occur in plant foods, and are seen as more desirable than their synthetic counter parts. These natural antioxidants occur in all higher plants, and in all parts of plants. Typical compounds that exhibit antioxidant activity include vitamins, carotenoid and phenolic compounds [1]. Plants also possess enzymatic systems that protect them against H2O2 and other harmful reactive oxygen species; these include superoxide dismutase (SOD) and catalase [2].

The concept of developing dental care products by simultaneously incorporating antioxidant micronutrients which can nourish and protect the periodontium are currently being investigated. Based on important features of the herb an attempt to incorporate antioxidant micronutrient in the form of mouthwash a small trial was done by using lemongrass oil at various concentrations.

Aim of the study

The purpose of the study was to investigate the role of intrinsic thiol antioxidants in periodontal environment in gingivitis and after nonsurgical treatment, using lemongrass oil mouthwash with various concentrations (0.1%, 0.25% and 0.5%).

Materials and Methods

The study was done in Department of Periodontics, Manipal. A total of 40 subjects were recruited for the study after signing the informed consent. 30 subjects were in case group, out of which it was again divided

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into 3 groups based on the concentration of the lemongrass oil used in the mouthwash and 10 were in the control group. Lemongrass oil mouthwash was prepared using the standard protocol at various concentrations (0.1%, 0.25% and 0.5%) in Department of Pharmaceutics, MCOPS, Manipal.

The patients were clinically examined and subjects with moderate to severe gingivitis were included in the study. Exclusion criteria were regular user of mouthwash, patients who have undergone antimicrobial therapy and scaling within past three months. The age of the patients ranged between 20 - 25 years. There were 25 male and 15 female included in the study. Initially saliva and Gingival crevicular fluid was collected using the standard protocol and sent for thiol antioxidant estimation after which the scaling was done. The case group patient received the lemongrass oil mouthwash of any one concentration. The patients were advised to use 15ml mouthwash twice daily for 15 days. On 15th day the patients were recalled, saliva and gingival crevicular fluid is collected and sent for the estimation of thiol anti oxidant.

Sampling of the saliva was done by collecting the whole saliva in glass beakers and transferred into salivette / eppendorf tubes and centrifuged at 3000 rpm at 4° C for 5 min, the supernatant was stored at -80°C until analysis. Gingival crevicular fluid sampling was performed between 8:00 and 10am. The area was isolated with cotton rolls and gently air dried. Care was taken to eliminate salivary contamination. The samples were collected by standardized periopaper strips using intra crevicular method given by Loe and Holm Pedersen (1965). Total 12 strips were placed successfully for 1 minute each at the entrance of the sulcus or pocket and the fluid seeping out was collected. Any paper contaminated with blood was discarded and collection was repeated. To ensure sufficient assay sensitivity 12 strips were used, six for each antioxidant thus standardizing the procedure and keeping them at the pocket or sulci for equal duration (i.e. 1 minute each). The GCF strips were pooled with 1ml Tris-HCL buffer (PH 6.5) and eluted for 30 minutes and stored till analysis for SOD assay. 600 microliter phosphate buffer saline eluted for 30 minutes and stored till analysis for protein thiol assay. Thiol was analyzed in samples by DTNB (dithio nitrobenzoic acid) which reacts with accessible thiol groups and reduces them to stable compounds. DTNB is reduced to MNB (mercaptonitrobenzoate). It was measured in units/ ml. Statistical analysis was done using the statistical package SPSS/PC+ 16.0.

Results

Thiol levels in saliva were analyzed and the results showed that group 1, 2, 3 and group 4 had the median score 128.12 micromol/l, 75.12 micromol/l, 48.69 micromol/l, -13.50 micromol/l respectively. When group 1 was compared with group 2 there was no statistical difference between the group (p= 0.247). When group 1 was compared with group 3 there was statistical significant difference between the groups (p=0.023). When group 1 was compared with group 4 there was statistical significant difference between the groups (p=0.000). When group 2 was compared with group 3 there was no statistical significance difference between the groups (p=0.353). When group 2 was compared group 4 there was statistical significant difference between the groups (p=0.000). When group 3 was compared with group 4 there was statistical difference between the groups (p=0.000). (table 1)

Thiol levels in gingival crevicular fluid were analyzed and the results showed that group 1, 2, 3 and 4 had the median score of 56.125 micromol/l, 74.875 micromol/l, 45.125 micromol/l, -12.37 micromol/l respectively. When group 1 was compared with group 2 there was no statistical difference between the groups (P=0.684). When group 1 was compared with group 3 there was no statistical significant difference between the groups (P=0.143). When group 1 was compared with group 4 there was statistical significant difference between the groups (P=0.000). When group 2 was compared with group 3 there was no statistical difference between the groups (P= 0.089). When group 2 was compared with group 4 there was statistical difference between the groups p=0.005. When group 3 was compared with group 4 there was statistical difference between the groups P=0.000. (table 2).

Table 1: Comparison of thiol levels in saliva between groups

Comparison of Thiol levels in Saliva between groups							
Group	NO.	Median	Std Deviation	Std error	IQR	95% confidence interval	
						Lower bound	Upper bound
Group 1	10	128.12	116.95	36.983	169.94	60.71	228.03
Group 2	10	75.12	112.31	35.51	102.38	10.38	171.06
Group 3	10	48.69	30.15	9.534	41.06	26.64	69.78
Group 4	10	-13.50	21.61	6.835	14.81	-24.74	6.18
Comparision	1 v/s 2	1v/s 3	1 v/s 4	2 v/s 3		2 v/s 4	3 v/s 4
Mann – Whitney U	34.000	20.500	2.000	37.000		6.000	4.000
Wilcoxon W	89.000	75.500	57.000	92.000		61.000	59.000
Asymp sig (2 -tailed)	0.226	0.026	0.000	0.326		0.001	0.000
Exact sig[2*(1 -tailed sig.)]	0.247[a]	0.023[a]	0.000[a]	0.353[a]		0.000[a]	0.000[a]

Table 2: Comparison of thiol levels in gingival crevicular fluid between groups

Comparison of Thiol levels in gingival crevicular fluid between groups							
Group	NO.	Median	Std Deviation	Std error	IQR	95% confidence interval	
						Lower bound	Upper bound
Group 1	10	56.125	114.73	36.28	125.31	25.37	189.52
Group 2	10	74.875	136.26	43.09	174.12	20.52	215.47
Group 3	10	45.125	18.91	5.98	30.94	27.67	54.73
Group 4	10	-12.37	15.74	4.97	33.56	-24.78	-2.26
Comparision	1 v/s 2	1v/s 3	1 v/s 4	2 v/s 3		2 v/s 4	3 v/s 4
Mann – Whitney U	44.00	30.50	0.00	27.5		14.0	0.000
Wilcoxon W	99.00	85.5	55.00	82.5		69.0	55.0
Asymp sig (2 -tailed)	0.650	0.14	0.00	0.089		0.006	0.00
Exact sig[2*(1 -tailed sig.)]	0.684	0.143	0.00	0.089		0.005	0.00

Discussion

Inflammation represents the response of the tissues to a noxious stimulus, whether mechanical or infectious. The inflammation is acute or chronic dependent upon the regulated humoral and cellular response and a variety of molecules are considered to mediate inflammation. Polymorphonuclear leukocytes are widely believed to be the initial and predominant defense cells present during host response the host response against bacterial pathogens in a variety of pathological conditions including periodontal disease.

Polymorphonuclear leukocyte infiltration in periodontitis leads to phagocytosis, which results in non mitochondrial oxygen consumption which may be 10 -20 times that of resting potential. This is recognized as respiratory burst which ultimately ends in generating free radicals and reactive oxygen species. Reactive oxygen species are highly reactive molecules that can promptly modify lipids, carbohydrates, proteins, nucleic acids and even lipoproteins and membranes which lead to lipid peroxidation and this can contribute to periodontal disease. Periodontal damage by reactive oxygen species is due to ground substance degradation and collagenolysis either directly or as a result of oxidation of antiproteases.

In the present study the subjects selected were all with moderate gingivitis. The thiol levels increased when compared with the initial values in all the 4

groups. The increase may be due to the initial periodontal therapy in group 4 and in group 1,2 and 3 the increase in thiol was due to initial therapy and use of lemongrass oil mouthwash. This indicates that as the periodontal disease reduces, antioxidant levels increase and oxidative stress reduces which is in correlation to the reported literature by Bartold PM et al 1986 [8], Battino M et al 2002 [9], Halliwel lB 1994 [10], Van dyke TE et al 1994 [11], Waddington RJ 2000 [12]. In group 4 there was increase in the antioxidant level, but it was lesser than that of the other groups. This also implies that the lemongrass oil mouthwash is having additive effect on the treatment outcome, when used along with nonsurgical treatment. The antioxidant activity of the lemongrass oil was also studied by Rabbani SI et al. (2006)[13] studied the antioxidant activity of citral in vitro by superoxide scavenging method. The result showed that citral significantly inhibited the formation of micronuclei induced by nickel.

The thiol antioxidants has gained attention in relation to oral disease is because it is reduced glutathione. Both gingival crevicular fluid and saliva expressed an increase in the thiol antioxidant level after treatment. This could be protective strategy at the epithelial interface of the gingival sulcus which is in accordance with the study done by Chapple et al 2002 [14], who observed that the reduced glutathione was expressed 1000 fold in the GCF and was reduced in chronic periodontal disease.

In the present study the thiol antioxidants were detected in saliva which is in accordance with the study done by Hojo Y et al 1987 [15]. Thiols levels were reduced before treatment and were increased after the non surgical treatment and also by using lemongrass oil mouthwash. The study is in accordance with the study done by Diab –Ladki et al 2003 [16] who showed reduced thiol antioxidant level in gingivitis and periodontitis group when compared with the healthy group. Saliva is a glandular secretion unlike GCF which is an interstitial fluid but since saliva has protein in its composition thiol anti oxidants will be expressed as a part of its protein system. GCF will also contribute antioxidants to saliva but they may be diluted due to presence of high water content.

Thiol antioxidants are more sensitive to the reduction of inflammatory load even in minor amounts. Thiol antioxidants orchestrate many biologic responses to inflammation and immunity, they function as signaling mechanisms for redox regulation, even minimal levels of oxidative stress is highly sensed and the protective antioxidant mechanism is set into action which is essential for the maintenance of the structural integrity of proteins thus explaining why their levels must have increased in the present study.

As the lemongrass oil has antibacterial property[17] it reduces the bacterial load, it is also anti-inflammatory[18],[13], all the above property may contribute to the antioxidant effect of the lemongrass oil mouthwash. Lemongrass oil mouthwash can be used as an adjunct along with the non surgical therapy. Further investigations to emphasize the relevance of these intrinsic antioxidants and the beneficial properties of lemongrass oil mouth wash need to be done.

Conclusion

The lemongrass oil mouthwash was found to have anti oxidant activity at all the three concentrations levels. Lemongrass oil mouthwash can be used as an adjunct along with the non surgical therapy. Further studies are required with a larger sample size.

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