A Novel Anti-Oxidant Lemon grass Oil Mouth Wash-a clinical trial

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INTRODUCTION
A growing number of consumers are embracing the philosophy that natural products are better for their health and the environment. As such, they are seeking products they perceive to be safer, healthier and without toxic chemical or synthetic ingredients. Research has also proven its efficacy, breathing new life into it. Laboratory analysis has shown that herbs contain vital vitamins, minerals, and natural chemicals that may be essential to curing a diseased body. Natural antioxidants are presumed to be safe since they occur in plant foods, and are seen as more desirable than their synthetic counter parts. Plants also possess enzymatic systems that protect them against H2O2 and other harmful reactive oxygen species; these include superoxide dismutase (SOD) and catalase. 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. Human body is subject to numerous biologic stresses. This may be due to the environmental or pathological. 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. This may affect the periodontium which manifests as gingivitis and periodontitis.

The botanical genus name cymbopogan for lemongrass is derived from Greek Cymbo – boat and pogon – beard. The essential oil of lemon grass consists mainly of citral. Citral is a mixture of two stereoisomeric monterpene aldehydes.
in lemon grass oil, the trans isomer geranial and isomer neral (25 to 38%). Further terpenoids in lemon grass oil are nerol, limonene, linalool and \( \beta \)-caryophyllene. The content of myrcene is low, but still enough to make the oil susceptible to oxidative polymerization. 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 AND OBJECTIVE:**
The purpose of the present study was to investigate the role of intrinsic superoxide dismutase antioxidants in periodontal environment in gingivitis, after nonsurgical treatment, nonsurgical treatment with using lemongrass oil mouthwash with various concentrations (0.1%, 0.25% and 0.5%) respectively.

**MATERIALS AND METHODS:**
The present study was done in Department of Periodontics, Manipal in collaboration with department of biochemistry Manipal. A total of 40 subjects were recruited for the study after taking the informed consent. Subjects were divided into 2 groups cases and controls. 30 subjects were included in case group, which was again divided into 3 groups based on the concentration of the lemongrass oil used in the mouthwash. A total of 10 subjects were included in 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.

All the patients were examined clinically, subjects with moderate to severe gingivitis were included in the study. Exclusion criteria were regular users of mouthwash, patients who had undergone antimicrobial therapy and scaling in past three months. The age of the patients ranged between 20-35 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 superoxide dismutase 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 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 fluidsampling 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 [3]. 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 (dithionitrobenzoic acid) which reacts with accessible thiol groups and reduces them to stable compounds. DTNB is reduced to MNB (mercaptanitrobenzoate) Superoxide dismutase activity was analyzed by the reduction of NBT (nitrobluetetrazolium) by Xanthin / xanthin oxidase system. The fromedformazan was detected spectrophotometrically at 560nm and compared to standard. It was measured in units/ ml. Statistical analysis was done collecting the data which was fed into a computer and analyzed using the statistical package SPSS/PC+.

**RESULTS:**
Superoxide dismutase levels in saliva were analyzed and the results showed that group one two three and group four had the median score 28.98 /ml, 23.42 /ml, 26.69 /ml, 13.01 /ml respectively. (Table1). The superoxide dismutase levels group 1 when it was compared with group2 there was no statistical difference between the group (p= 0.24).

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=0.35).

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)
Superoxide dismutase in gingival crevicular fluid were analyzed and the results showed that group one two three and group four had the median score and 30.21 /ml, 23.80 /ml, 16.59 /ml and 3.71 /ml respectively. (Table 2) The superoxide dismutase levels of gingival crevicular fluid when group 1 was compared with group 2 there was no statistical difference between the groups (p=0.35). When group 1 was compared with group 3 there was no statistical significant difference between the groups (p=0.015). 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=0.43). When group 2 was compared with group 4 there was statistical difference between the groups (p=0.002). When group 3 was compared with group 4 there was statistical difference between the groups (p=0.023). (Table 2)

**DISCUSSION:**
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. In the present study the subjects selected were having gingivitis. Superoxide dismutase 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 1,2and 3 the increase in superoxide dismutase was due to initial therapy and use of lemongrass oil mouthwash. This indicates that as the gingivitis reduces, antioxidant levels increase and oxidative stress reduces which is in correlation to the reported literature by Bartold PM *et al* [4], Battino, M *et. al* [5], Halliwell,B.[6], Van dyke, T.E *et al.* [7], Waddington, R.J[8] In group 4 there was increase in the
antioxidant level, but it was lesser than that of the other groups. This may imply that the lemongrass oil mouthwash may have an additive effect on the treatment outcome, when it was used along with scaling.

The antioxidant activity of the lemongrass oil was also studied by Rabbani, S.I et al. [9] studied the antioxidant activity of citral by in vitro, which showed its anti oxidant activity by superoxide scavenging method. The result showed that citral significantly inhibited the formation of micronuclei induced by nickel. Superoxide scavenging activity of the citral suggests that the antioxidant action could be responsible for anti-clastogenic effect of citral against nickel chloride.

The isoform CuZnSOD which is extracellular form of superoxide dismutase enzyme is reported to be surface bound to collagen fibrils of gingival and periodontal fibroblasts and protects against the powerful oxidizing agent superoxide. During oxidative stress like states of inflammation the superoxide dismutase gets temporarily inactivated or depleted. On reducing the bacterial load, neutrophil induced release and fibroblast induced release of superoxide is reduced thus reactivating and replenishing the suppressed superoxide dismutase enzyme [10].

Saliva is a glandular secretion unlike GCF which is an interstitial fluid but since saliva has protein in its composition anti-oxidants will be expressed as a part of its protein system. In the present study the superoxide dismutase antioxidants were present in saliva. Superoxide dismutase 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. [11] who showed reduced antioxidant level in gingivitis and periodontitis group when compared with the healthy group.

Lemongrass oil has antibacterial[12], anti inflammatory[13], and also superoxide scavenging property[9]. Reduction in the bacterial load, decrease in inflammation and reduction of the oxidative stress will bring about the overall health of the tissues. Based on above property, lemongrass oil mouthwash can be used as an adjunct along with the non surgical therapy.

**CONCLUSION:**

Lemongrass oil mouthwash can be used as an adjunct along with the non surgical therapy. Further investigations are required to emphasize the relevance of these intrinsic antioxidants and beneficial properties of lemongrass oil mouthwash need to be done.
REFERENCES:


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