# Susceptibility of Ozonated Water Against *P. Endodontalis* and *P. Gingivalis* and *S. Mutans* an Invitro Study

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**Abstract:**

**Background:**Ozone is a very unstable and extremely oxidizing compound, that quickly returns to nascent oxygen molecule, and has been in use as an antibacterial, antifungal, antioxidant, anticancer, and antiviral agent. Due to these antimicrobial properties, ozonated water has also been in dentistry as a disinfectant. Furthermore, studies also revealed that aqueous ozone has better biocompatibility when compared with chlorhexidine, sodium hypochlorite, and hydrogen peroxide considering its antibacterial properties the objective of our study was to evaluate the susceptibility of ozonated water against *Porphyromonasendodontalis, Porphyromonasgingivalis,* and *Streptococcus mutans*.

**Methodology:** It was an in-vitro preclinical experimental study conducted at the dental OPD of a tertiary care hospital in Karachi.The samples of the organisms were collected from the microbiology lab and the organisms*S. mutant,P. gingivalis and, P. endodontalis* were grown in their appropriate media for further experiment.The ozonated water 1µl was used to wet the paper disc and placed in the organism's growth plates. And sensitivity analysis was performed.

**Results:** The growth of colonies was calculated on growth media plates in samples. The comparison of samples showed a significant (p-value <0.05) decrease in number of colonies in ozonated water growth plates when compared to control plates. The sensitivity analysis showed that the most of the strains of *S. mutant,P. gingivalis and, P. endodontalis*were sensitive to ozonated water (p=0.002)

**Conclusion:** The ozonated water reduced the number of colonies on agar plates and it showed sensitivity towards *S. mutant,P. gingivalis and, P. endodontalis.*

**Keywords:** Ozonated water, Sensitivity*S. mutant,P. gingivalis and, P. endodontalis.*

**Tob Regul Sci. ™ 2022;8(1): 2961-2968**
**DOI: doi.org/10.18001/TRS.8.1.224**

**Introduction:**

The periodontal diseases belong to the category of most prevalent diseases that are having a significant global burden of disease. The Association of periodontal infections with multiple other systemic diseases, including cardiovascular diseases, has also been reported(1). The initiation of periodontitis is dependent upon the accumulation of various gram-positive and gram-negative bacterial species in the subgingival area. Further colonization of these bacteria in the subgingival area will progress the periodontal disease that may lead to the destruction of tooth-supporting tissues (2). Such bacterial infections are the major culprits of periapical lesions and pulp necrosis which in the future may lead to endodontic failure(3). The initiation of dental decay is carried out mainly by gram-positive bacteria like *streptococcus mutans* and *lactobacilli*(4). While the chronic progression of periodontal disease is associated with gram-negative, facultative, and strict anaerobic bacteria including *Porphyromonasgingivalis*, *Porphyromonasendodontalis, Treponema denticola* and *Tannerella forsythia*(5).

*P. gingivalis* is reported as a 'keystone' pathogen in periodontitis due to its ability of biofilm formation over the gingival epithelial cells and calcified hard tissues. This resistant biofilm formation leads to a chronic inflammatory response that leads to the destruction of periodontal tissues and tooth loss (6). The establishment of *P. gingivalis* in the oral cavity is mainly due to its virulent secretions and structural fimbriae that help the pathogen to invade (7). *P. endodontalis* is a black-pigmented and rod-shaped microbe that has a critical association with endodontic infections and pulp necrosis. The periapical lesions and bone resorption caused by *P. endodontalis*are mainly promoted by the release of lipopolysaccharides-induced inflammatory cytokines from the pathogen (8). *S. mutans* affect the hard calcified tissue of periodontium, mainly enamel, by fermentation of sucrose leading to lactic acid formation (9).

Ozone is a very unstable and extremely oxidizing compound, that quickly returns to nascent oxygen molecule,andhas been in use as an antibacterial, antifungal, antioxidant, anticancer, and antiviral agent(10, 11). Due to these antimicrobial properties, ozonated water has also been in dentistry as a disinfectant (12). Furthermore, studies also revealed that aqueous ozone has better biocompatibility when compared with chlorhexidine, sodium hypochlorite, and hydrogen peroxide(13). Ozone is now also being widely used as a remarkable antibacterial and a preservative agent, with no chemical residues, in the food industry (14). Ozone had been used as a disinfectant in water treatment and waste in sewage networks (15). In gaseous form, ozonated water is unstable and may damage respiratory tissues while ozonated water is much more stable and easier to handle (16).

The objective of our study is to evaluate the susceptibility of ozonated water against *Porphyromonasendodontalis, Porphyromonasgingivalis,*and *Streptococcus mutans* bacteria in an in-vitro study.

**Methodology:**

It was an in-vitro preclinical experimental study conducted at the dental OPD of a tertiary care hospital in Karachi from June 2022 to August 2022. The samples of the organisms were collected from the microbiology lab and the organisms were grown further for the experiment. For the culture of *S. mutant* samples were inoculated in Columbia Agar with 5% sheep blood and incubated for 48 h at 37 °C and increased levels of $CO_2$. *P. gingivalis* were grown in Wilkins-Chalgren and *P. endodontalis* was inoculated on blood agar in anaerobic broth under anaerobic conditions of 5% CO2, 10% H2, and 85% N2 at 37 °C. All the bacteria were sub-cultured twice and were grown to the early stationary phase. The ozonated water 1µl was used to wet the paper disc and placed in the organism's growth plates. The comparative groups were not been administered any drug to observe the normal growth of the organisms and to identify the sensitivity of ozonated water towards these organisms. Student t-test was applied as test of significance for experimental comparison, <0.05 p-value was considered as significant at 95% confidence interval.

**Results:**

The growth of colonies was calculated on growth media plates in samples. The comparison of samples showed a significant (p-value <0.05) decrease in number of colonies in ozonated water growth plates when compared to control plates. All three organisms seemed to be sensitive to ozonated water discs. The grown colonies were further analysed to report the sensitivity of the organisms towards ozonated water and 25 strains of each organism were evaluated that showed that the most of the strains of *S. mutant,P. gingivalis and, P. endodontalis*were sensitive to ozonated water (p=0.002) as shown in figure 1.

**Table. 1 Student t-test analysis showing the number of colonies before and after intervention**

| | Control growth plates | Ozonated Water Growth Plates | p-value |
|---|---|---|---|
| ***S.mutans*** | $10 \pm 2 \times 10^4$ | $6 \pm 1 \times 10^4$ | 0.002* |
| ***P. gingivalis*** | $17 \pm 2 \times 10^3$ | $10 \pm 2 \times 10^3$ | 0.001* |
| ***P. endodontalis*** | $15 \pm 4 \times 10^3$ | $9 \pm 3 \times 10^3$ | 0.001* |
| *significant p-value | | | |

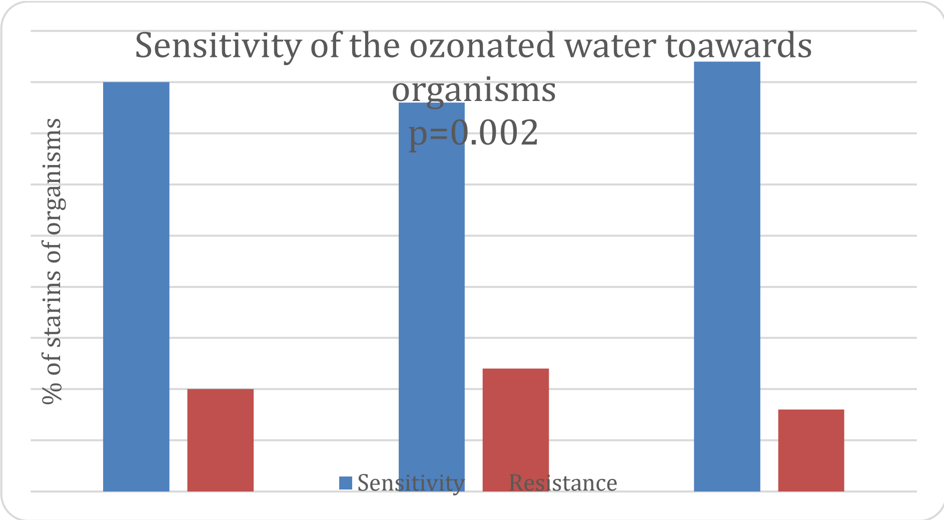


**Figure 1. Sensitivity of the ozanoated water towards *S. mutant,P. gingivalis and, P. endodontalis***

**Discussion:**

Ozone is a highly reactive inorganic gas composed of three oxygen atoms(17). Ozone has potent oxidative properties that have been evaluated extensively in various studies for their commercial and industrial uses(18). The gaseous form of ozone is being used for deodorization, disinfection, and decontamination in various commercial, industrial, and medical settings(19). The liquid formulations of ozone mainly include ozonated water and ozonated oils(20). Liquid forms of ozone are also widely used majorly for the decontamination of water pipes, disinfection, wound care, and food preservation(21). Literature has reported promising clinical effects of ozone in the betterment of chronic wounds, burns, dermatitis, and diabetic foot ulcers(22). Various instruments can not be sterilized at high temperatures and require alternate methods. Ozone is also being used for sterilizing such medical or dental instruments for having strong antibacterial properties(23). The antibacterial mechanism of ozone is thought to be the blockage of the enzymatic activity of bacteria by oxidizing glycoproteins and glycolipids (24).

The results of our study reported a significant antibacterial effect of ozonated water against *S. mutans* when compared with the control group. S. mutans is a major etiological agent for dental caries and an important component of dental plaque (25). Multiple other studies support our results and exhibit potent antimicrobial effects of ozone against *S. mutans*(26-28). Preventive measures for dental caries do not only require mechanical removal of plaque but the application of antibacterial mouth rinses,which mainly contain chlorhexidine as an antimicrobial agent, is also important(29). Chlorhexidine has multiple adverse effects including burning sensation, taste alteration, teeth, and soft tissue discoloration, and loss of sensation of oral soft tissues(30). These adverse effects widely contribute to non-compliance by patients that leads to progression of dental caries and periodontitis. Therefore, ozonated water can be a good alternative for its use as an antimicrobial mouth rinse to prevent dental caries.

Our results also reported remarkable suppression and inhibition of *P. gingivalis* growth when compared with the control group. *P. gingivalis* is a potent mediator of local inflammatory response in periodontitis that invades the epithelial cells of gingiva and its growth leads to gingivitis and periodontitis (31). Similar results were observed in previous literature that reported antibacterial effects of ozone against P. gingivalis(32-34).Therefore, ozone formulations can be used for various preventive and treatment applications,such as during scaling and root planning, against oral microbes.

Similar antibacterial effects were also exhibited against *P. endodontalis* when we compared the ozone group with the control group. Other studies also reported antimicrobial effects of ozonated water against *P. endodontalis*(35, 36).*P. endodontalis* is known for its bacterial colonization in the root canals of the tooth(37). *P. endodontalis* is predominantly found in infected root canals and has a primary association with the progression and development of apical periodontitis and endodontic treatment failures (38). One of the major reasons for endodontic failures is ineffective and weak irrigation of canals during the treatment that leads to re-growth of endodontic bacteria(39). Therefore, ozonated water can be used as a potent root canal irrigation agent to minimize the possibilities of endodontic failures.

**Conclusion:**

The ozonated water reduced the number of colonies on agar plates. The ozonated water seemed to be sensitive and can inhibit the growth the three vulnerable organisms of the oral cavity. Further to this ozonated water showed sensitivity towards *S. mutant,P. gingivalis and, P. endodontalis* however the sensitivity profile was greater for *P. endodontalis* hence it is recommended to use this liquid as a root canal irrigation agent and clinical trials should be carried out to endorse the finding of current study.

**Conflict of interest: No any**

**Ethical approval:** Not applicable as the samples were collected from microbiology laboratory.

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