Original Article

Comparative evaluation of alkaline ionized water and normal water on oral microbial flora: An *in-vitro* study

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Abstract

Background: Various artificial chemical agents have been evaluated over many years with respect to their antimicrobial effect in oral cavity. The gold standard for removal of plaque is usage of chlorhexidine, but it can cause alteration in taste sensation and staining of teeth. Electrolytes and oxidizing water may be useful against microbes, but its clinical application has still not been evaluated. Hence this present study was conducted to evaluate the effectiveness of the alkaline ionized water on oral microbial flora.

Materials and Methods: Ten non-carious, un-restored and intact freshly extracted human teeth were collected and sectioned using a round bur. Each tooth was sectioned longitudinally in two parts and stored in closed sterile containers which was filled with alkaline ionized water (Group 1) and normal water (Group 2), respectively for 15 days. The microbial growth was analyzed prior to dipping in the solutions, 3 days, 7 days and 15 days. The pH of alkaline ionized water and normal water was evaluated using pH meter before placing teeth in different solutions. Results were analyzed using *t*-test and the level of significance was set at ≤ 0.05.

Results: No difference in bacterial colony was observed before test and after 3 days among Group 1 and Group 2, respectively. After 7 days and 15 days, statistically significant decrease in bacterial colony count was seen among Group 1 as compared to Group 2 ($P \leq 0.05$).

Conclusion: It was then concluded that alkaline ionized water can be effective in reduction of oral microbial flora.

Keywords: Alkaline ionized water, normal water, oral microbial flora

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INTRODUCTION

Health is a common theme in most cultures. In fact, all communities have their concepts of health, as part of their culture. According to WHO, health is a state of complete physical, mental and social well-being and not merely an absence of disease or infirmity. Health is a condition or quality of the human organisms expressing the adequate functioning of the organisms in given conditions, genetic and environment. The philosophy of health has been quite focused on the problem of determining the nature of the concepts of health, illness and disease.[1]

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Modern life is abreast on the rapid advancement in technology and life extension. Thus, there is an escalating prevalence of people who suffer from the so-called diseases of civilization such as senile diseases, lifestyle-related diseases, and immune-related allergic diseases.[2]

Lifestyle such as rising consumption of Western food is positively correlated to the accumulation of fats and cholesterol in the body that could lead to burst in reactive oxygen species.[3] Although newly developed drugs for a therapeutic approach are rapidly growing. However, drugs are often inadequate and are usually accompanied with side effects.[4]

Dental diseases are recognized as major public health problem throughout the world. Numerous epidemiological studies showed that diseases such as tooth decay and diseases of the periodontium are among the most common afflictions of mankind. Dental plaque plays a major role in the etiology of periodontal disease. The mainstay of preventing periodontal disease is the control of plaque and thus the prevention of plaque-induced gingivitis.[5]

Various synthetic chemical agents have been evaluated over the years with respect to their antimicrobial effect in oral cavity. The benchmark control in the removal of plaque is chlorhexidine. But it cannot be used for a long duration because it has many side-effects like altered taste sensation and staining of tongue. Chemical plaque control agents are used as an adjuvant because they have the ability to inhibit growth and metabolism as well as colonization of bacteria; however, all are associated with various side effects. Thus,

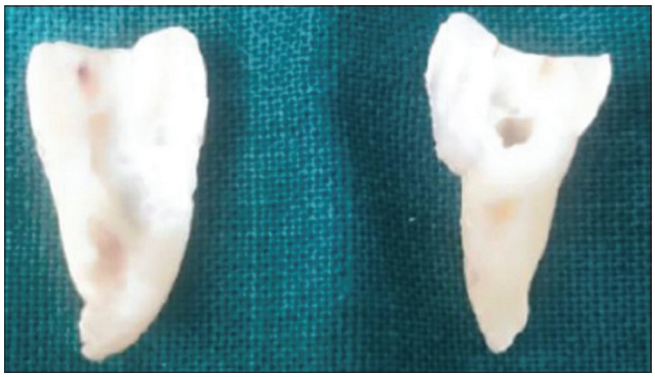

Figure 1: Sectioned Tooth

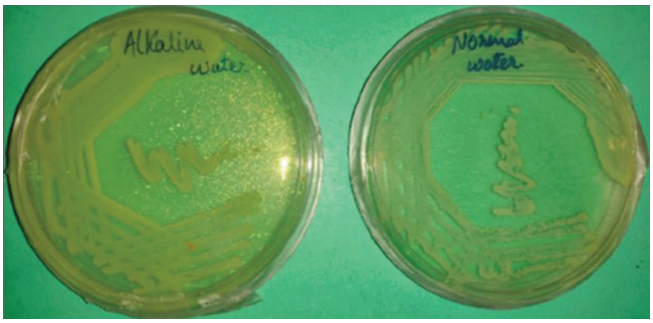

Figure 2: Bacterial growth at baseline among alkaline ionized water and normal water

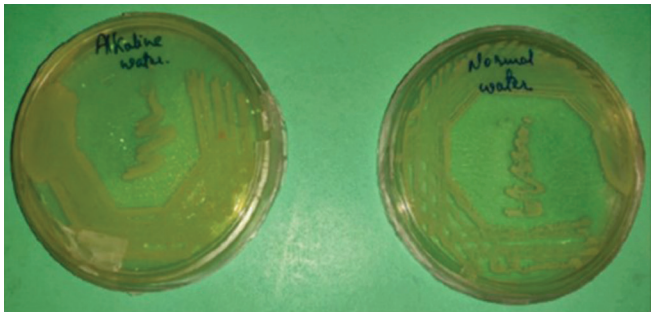

Figure 3: Bacterial growth at 3 days among alkaline ionized water and normal water

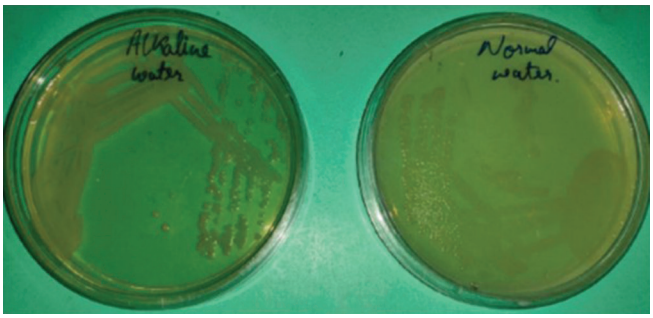

Figure 4: Bacterial growth at 7 days among alkaline ionized water and normal water

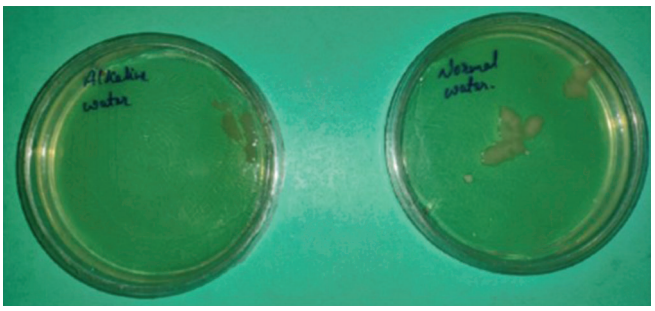

Figure 5: Bacterial growth at 1 days among alkaline ionized water and normal water

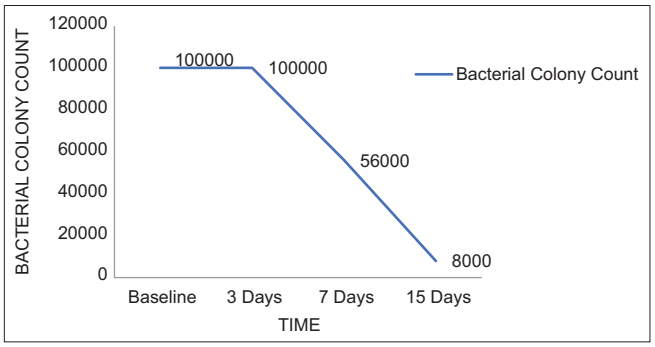

Figure 6: Bacterial colony count in alkaline ionized water

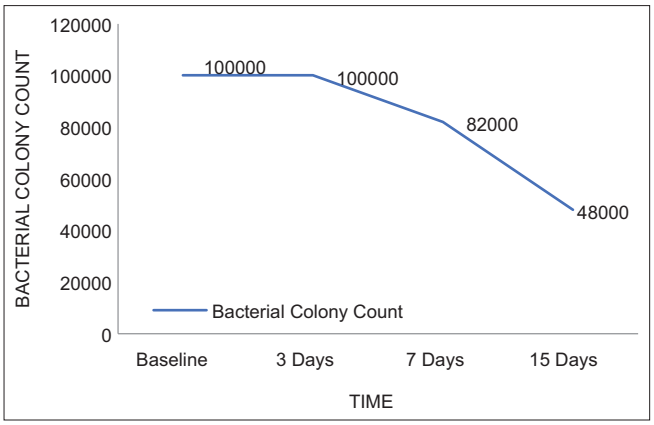

Figure 7: Bacterial colony count in Normal water

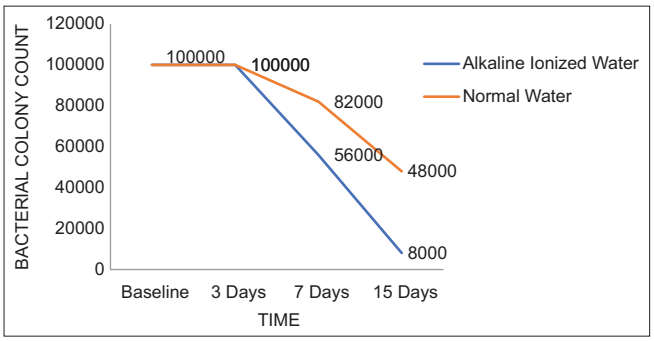

Figure 8: Comparison of Bacterial colony count among Alkaline Ionized Water and Normal water

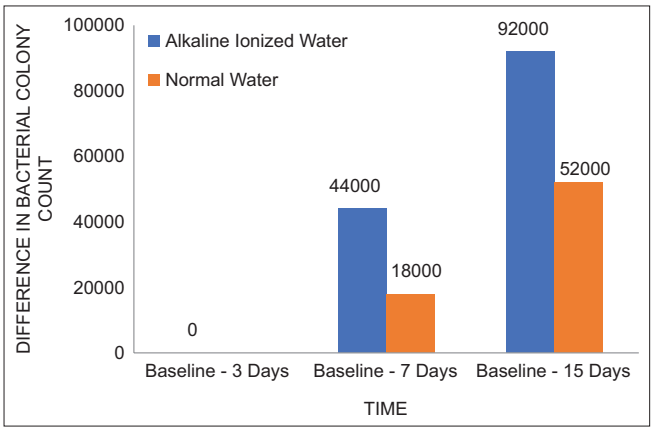

Figure 9: Comparison of differences in Bacterial colony count from baseline to 15 days among Alkaline Ionized Water and Normal water

patients are going away from modern-day medicines, and they prefer using alternate preparations which are efficient without causing any side effects.[6]

Therefore, approaches on discovering effective and safer alternative medicine are still necessary to meet both requirements. Advocates on developing novel therapeutics against continuous rising number of various diseases had led to the use of alkaline ionized water (AIW). AIW exhibits special properties such as alkaline pH, micro-clustered water molecules, extremely negative ORP value, and high contents of dissolved hydrogen.[7]

Alkaline ionized water has a variety of applications; from disinfection to improving digestive functions and enhancing the quality of agricultural products in the food industry. AIW is prepared by electrolysis of tap water by ionizer machines. Depending on the electrolysis process conditions, five types of EW are produced, namely basic (pH: 10–12), mildly basic (pH: 8–10), neutral (pH: 6.5–7.5), slightly acidic (pH: 5–6.5), and acidic (pH: 3–5). Acidic electrolyzed water (AEW) is produced in the anode chamber of an ionizer machine and is mainly used in the medical field (wound cleaning and disinfection of instruments and surfaces). Whereas basic electrolyzed water (BEW) is produced in the cathode chamber and, because of its health benefits often applied to suppress oxidative stress-related diseases as well as known for its anti-cancer and anti-diabetes properties. It has been reported that these water types have the ability to destroy all types of anerobic and aerobic bacteria that cause dental decay. Some mechanisms have been postulated for this antibacterial activity such as the effect of a high or low pH, oxidation-reduction potential, chlorine concentration, and AIW's high concentrations of molecular hydrogen. However, some researchers have also reported negative effects such as the cytotoxicity of acidic water and growth retardation in high basic water. Acidic water with low chlorine concentrations also has a lower rate of tissue destruction compared with water with higher chlorine concentrations at the same pH value.[8]

People began to use AIW as an option to help in the intervention of several diseases as anti-diabetic, benefits in the body's metabolic process.[9] Ionizer alkaline water searches for free radicals and converts them into oxygen that the body can apply as the push to develop cells and tissues.[10]

Cancer and most diseases cannot live in alkaline environments and oxygenation. Some studies suggest that some countries like Korea and Japan acknowledged the efficacy of AIW as a novel material for the progress of abnormal intestinal fermentation, chronic diarrhea, gastric hyperacidity, and dyspepsia.[11]

Salivary secretion is one of the mechanisms that maintain homeostasis against acid-induced changes in the oral environment. Salivary buffering capacity is particularly important for this purpose. However, it has been reported that elderly people who take medication have low salivary

Table 1: Bacterial colony count in alkaline ionized water

Time	Number	Bacterial colony count		Confidence interval		*P*
		Mean	SD	Upper	Lower	
Baseline	10	100000	0.00	100000	100000	-
3 Days	10	100000	0.00	100000	100000	-
7 Days	10	56000	8432.74	49967.58	62032.41	0.001*
15 Days	10	8000	2581.98	6152.96	9847.04	

Level of significance ≤0.05; *Statistically Significant; **Statistically Non-Significant

Table 2: Difference in bacterial colony count among group in alkaline ionized water

Time	Mean difference	*P*
Baseline		
3 Days	0	-
7 Days	44000	0.001*
15 Days	92000	0.001*
3 Days		
7 Days	44000	0.001*
15 Days	92000	0.001*
7 Days		
15 Days	48000	0.001*

Level of significance ≤0.05; *Statistically Significant; **Statistically Non-Significant

Table 3: Bacterial colony count in normal water

Time	Number	Bacterial colony count		Confidence interval		*P*
		Mean	SD	Upper	Lower	
Baseline	10	100000	0.00	100000	100000	-
3 Days	10	100000	0.00	100000	100000	-
7 Days	10	82000	10327.95	74611.82	89388.17	0.001*
15 Days	10	48000	7888.10	42357.18	53642.81	

Level of significance ≤0.05; *Statistically Significant; **Statistically Non-Significant

secretion. Moreover, dehydration due to insufficient fluid consumption and sweating during exercise, regardless of age, leads to decreasing secretion and loss of the buffer capacity of saliva, which results in an oral environment that promotes the initiation and progression of acid erosion.[12]

Alkaline beverages are thought to be effective for quickly neutralizing the oral cavity that has been acidified by acidic beverages; however, few such beverages have been confirmed to be safe to drink. Various functional water products have been recently investigated, including several types of electrolytically generated hypochlorite water, ozonated water, and ozone ultrafine bubble water. Of these, alkaline electrolyzed water (AEW) produced by electrolysis of purified tap water (PTW) is effective in improving gastrointestinal symptoms, and have confirmed in a large-scale clinical study that AEW is suitable for drinking. As potable AEW has a pH of 9.5–10.0, mouth rinsing with AEW, including nonpotable water with a pH of 10.0–10.5, may be useful for neutralizing the acidic environment in the oral cavity and restoring it to a neutral environment. Mouth rinsing with AEW may also be effective for individuals at childcare and nursing care homes who are unable to clean their mouths by themselves. Alkaline water may help reduce the number of bacteria in the mouth by neutralizing the acidic environment that promotes bacterial growth. In addition to this, alkaline water may help to improve oral health by reducing inflammation and oxidative stress in the body.[13]

Hence this present study was conducted to evaluate the effectiveness of the AIW on oral microflora.

MATERIALS AND METHODS

This *in vitro* double blind experimental study was carried out at 150 ft ring road, Rajkot city, Gujarat, during the period of August–September 2020.

The study was done to compare the antimicrobial effects of AIW with normal water.

Prior to conducting the experimental study, ethical clearance from the institutional review board of the college was obtained.

Pre-study procedures

In this study, the following procedure was adopted/carried out.

Determination of pH of water

Alkaline ionized water was collected from a renowned alkaline water manufacturer. Normal drinking water was collected from the RO purifier. The pH of the water was determined using pH indicator. The pH indicator is a halochromic chemical compound which is added in small amount to any solution so that the pH of the solution can be determined visually. It is a chemical indicator for hydrogen ions.

Collection of teeth

The teeth were collected from the Department of Oral and Maxillofacial Surgery of Narsinhbhai Patel Dental College and Hospital, Visnagar, Gujarat. The teeth were washed initially using normal saline. The teeth were then stored in a sterile container containing 10% formalin for 5 days. After 5 days, the teeth were washed again with normal saline followed by plain water. The 10% formalin was later discarded off as per proper waste disposal method.

Study procedures

Ten non-carious, unrestored, and intact extracted human tooth were used for the study. The teeth were then sectioned using a round bur. In all, 20 sectioned teeth were stored individually in closed sterile containers. The sterile

containers were then labelled as normal water and AIW container respectively. Prior to dipping in the water, a dry dacron swab was used and swabbed several times across the buccal side of the sectioned tooth [Figure 1]. The swab was then placed in a nutrient agar plate. The bacterial count in the sectioned tooth, prior to dipping in the water were analyzed [Figure 2]. After the bacterial count was analyzed, AIW and normal water were filled in the sterile container containing the sectioned tooth respectively.

Group-1: Ten sectioned teeth were immersed in 20 mL of AIW in 10 sterile containers.

Group-2: Ten sectioned teeth were immersed in 20 mL of normal water in 10 sterile containers.

All the sectioned teeth were then kept immersed separately in 20 mL of AIW and normal water, respectively, for 3 days at room temperature. After 3 days, the teeth were taken from the water by sterile tooth forceps; the swab was taken from the buccal side and the teeth were then placed into the same water. The bacterial count after 3 days was analyzed [Figure 3]. After 7 days and 15 days, the teeth were again taken off from the water, underwent the same procedure, and bacterial count was analyzed [Figure 4 and Figure 5 respectively]. The bacterial count at the beginning of the test, after 3 days, after 7 days and after 15 days were then compared.

Table 4: Difference in bacterial colony count among group of normal water

Time	Mean difference	*P*
Baseline		
3 Days	0	-
7 Days	18000	0.001*
15 Days	52000	0.001*
3 Days		
7 Days	18000	0.001*
15 Days	52000	0.001*
7 Days		
15 Days	34000	0.001*

Level of significance ≤0.05; *Statistically Significant; **Statistically Non-Significant

Table 5: Comparison of bacterial colony count among alkaline ionized water and normal water

Time	Groups	Number	Bacterial colony count		*P*
			Mean	SD	
Baseline	Alkaline Ionized Water	10	100000	0.00	-
	Normal Water	10	100000	0.00	
3 Days	Alkaline Ionized Water	10	100000	0.00	-
	Normal Water	10	100000	0.00	
7 Days	Alkaline Ionized Water	10	56000	8432.74	0.001*
	Normal Water	10	82000	10327.95	
15 Days	Alkaline Ionized Water	10	8000	2581.98	0.001*
	Normal Water	10	48000	7888.10	

Level of significance ≤0.05; *Statistically Significant; **Statistically Non-Significant

Bacterial colony count

Oral bacterial species such as *Streptococcus salivarius* (*S. salivarius*), *Staphylococcus aureus* (*S. aureus*), and *Lactobacillus casei* (*L. casei*) were analyzed. Advanced microbiological techniques were used for isolation and detection of bacterial count. To inoculate the anerobic bacteria, bacterial swabs were used and cultured in nutrient agar media for 3 days at 37°C to permit the detection of very slow growing strains. Bacterial colony was counted after 3 days of culturing. Identification of bacteria was done through electron microscope after staining process. Preliminary identification of bacterial species was based on their colonies formed and stereoscopic lens at 16 × magnification was used for visualization. Isolates were Gram stained, purified by subculture, tested for catalase production, and their gaseous requirements established by incubation for 3 days anerobically. The resultant data were collected and subjected for statistical analysis.

Statistical analysis

All the data collected were then entered into excel spread sheet and then the result was analyzed using the software Statistical Package for the Social Sciences (SPSS) version 20.0. Student's *t*-test was performed to show the efficacy of alkaline water. $P \leq 0.05$ was kept as a level of significance.

RESULTS

The mean bacterial colony count was 100000 ± 0.00 at baseline, 100000 ± 0.00 at 3 days, 56000 ± 8432.74 at 7 days, and 8000 ± 2581.98 at 15 days. Statistically

Table 6: Comparison of differences in bacterial colony count from baseline to 15 days among alkaline ionized water and normal water

Time	Groups	Number	Bacterial colony count		*P*
			Mean difference	SD	
Baseline - 3 Days	Alkaline Ionized Water	10	0	0.00	-
	Normal Water	10	0	0.00	
Baseline - 7 Days	Alkaline Ionized Water	10	44000	8944.27	0.001*
	Normal Water	10	18000	10954.45	
Baseline - 15 Days	Alkaline Ionized Water	10	92000	2738.61	0.001*
	Normal Water	10	52000	8366.63	

Level of significance ≤0.05; *Statistically Significant; **Statistically Non-Significant

significant difference was observed in bacterial colony count from baseline to 15 days in AIW group (*P* value: ≤0.05) [Table 1 and Figure 6].

Statistically significant difference was observed among mean bacterial colony count reduction from baseline to 7 days and 15 days in AIW group. Statistically significant difference was observed among mean bacterial colony count reduction from 3 days to 7 days and 15 days in AIW group. Statistically significant difference was observed among mean bacterial colony count reduction from 7 days to 15 days in AIW group (*P* value: ≤0.05) [Table 2].

The mean bacterial colony count was 100000 ± 0.00 at baseline, 100000 ± 0.00 at 3 days, 82000 ± 10327.95 at 7 days, and 48000 ± 7888.10 at 15 days. Statistically significant difference was observed in bacterial colony count from baseline to 15 days in Normal Water group (*P* value: ≤0.05) [Table 3 and Figure 7].

Statistically significant difference was observed among mean bacterial colony count reduction from baseline to 7 days and 15 days in Normal Water group. Statistically significant difference was observed among mean bacterial colony count reduction from 3 days to 7 days and 15 days in normal water group. Statistically significant difference was observed among mean bacterial colony count reduction from 7 days to 15 days in Normal Water group (*P* value: ≤0.05) [Table 4].

Mean bacterial colony count was less in AIW (56000 ± 8432.74) than in Normal Water (82000 ± 10327.95) at 7 days. Statistically significant difference was present in mean bacterial count between AIW and Normal Water at 7 days. Mean bacterial colony count was less in AIW (8000 ± 2581.98) than in Normal Water (48000 ± 7888.10) at 15 days. Statistically significant difference was present in mean bacterial count between AIW and Normal Water at 15 days (*P* value: ≤0.05) [Table 5 and Figure 8].

Mean reduction in bacterial colony count was more in AIW (44000 ± 8944.27) than in Normal Water (18000 ± 10954.45) from baseline to 7 days. Statistically significant difference was observed in mean bacterial count reduction between AIW and Normal Water from baseline to 7 days. Mean reduction in bacterial colony count was more in AIW (92000 ± 2738.61) than in Normal Water (52000 ± 8366.63) from baseline to 15 days. Statistically significant difference was observed in mean bacterial count reduction between AIW and Normal Water from baseline to 15 days (*P* value: ≤0.05) [Table 6 and Figure 9].

DISCUSSIONS

In this study, we demonstrated the antimicrobial effect of AIW *in vitro*. There is a continuous fluctuation of pH that is being altered in the body in order to maintain homeostasis. The scale of pH ranges from zero to fourteen. A pH of seven is considered neutral, and a number below and above seven is acidic and alkaline, respectively. It has been shown that the diet whether alkaline or acidic has different effects on the body. Water has varying degrees of pH and benefits, depending on the source. BEWE, or Basic Erosive Wear Examination for dental care professionals can have a positive influence on patient care and can help promote a state of well-being.[14]

Electrolyzed ionized water is prepared by the electrolysis of aqueous solution of sodium chloride (NaCl) in an electrolytic cell that has a semi-permeable separation membrane between the cathode and anode. Alkaline electrolyzed water from the cathode side has been demonstrated to exert antibacterial activity and has been used in medical and food applications.[15]

The average mouth had a pH of 6.3 but when the pH of the oral cavity decreases below 5.5 demineralization of enamel begins; lower the pH of ingested substances, there will be increased rate of demineralization. In our study, compared to normal water, electrolyzed alkaline water showed significant reduction in the colony count of aerobes, anerobes, and other microbial flora of tooth in culture. However, this was contrary to the findings of Tanaka H *et al.* who found that a strong acidic solution of super oxidized water (pH 2.3–2.7) with a high oxidation-reduction potential, showed antibacterial activity against a variety of Gram-positive and Gram-negative bacteria. Its bactericidal activity was superior to that of, the leading oral antiseptic, 0.1% chlorhexidine.[16]

In our study, significant relation was observed among reduction of microbial count by AIW. However, this was contradictory to the findings ofPark H *et al.,* Itoh K *et al*., and Nakagawara S *et al.* who showed that there is no significant relation between utilization of alkaline water and reduction of microbial flora. They showed that the use of acidic EW was effective for the prevention of infection caused by methicillin resistant *Staphylococcus aureus*, and killing *Campylobacter jejuni* on chicken and *Escherichia coli* O157:H7 on lettuce. The bactericidal effect of acidic EW is due to the combined action of concentration of hydrogen ion, high oxidation-reduction potential, and the presence of highly bactericidal concentration of hypochlorous acid (HOCl).[17-19]

The antibacterial activity of electrolyzed alkaline water is due to the combined action of short-lived reactive oxygen species (ROS) such as singlet oxygen, superoxide free radicals (O2–), hydroxyl radicals (OH–), and free chlorine. This was similar to the findings of Nakajima *et al*., who showed that alkaline water had antimicrobial effects. They suggested that free chlorine is a bactericidal substance and its concentration in alkaline water leads to its increased antibacterial effects.[20]

Compared to normal water, AIW showed significant inhibition in the growth of aerobic as well as anerobic periodontopathogens. The number of bacteria both after 7 days and 15 days was significantly lower as compared to that of bacterial count at baseline. This was similar to the findings of Nelson P *et al.,* Sato S *et al.,* Quirynen M *et al.,* and Lee S *et al.* They showed that there is reduction in bacterial count as time passes by usage of AIW. They showed that several parameters related to oral AIW administration can be used for senile disease treatment and hospitalized patients.[21-24]

Many other studies have shown the basic advantages of EW apart from its bactericidal effects and showed its application in medicine, translational medicine, and the food industry.[25-27] However, many reports have shown that there is unknown systemic effects and growth retardation in rats due to the drinking normal water.[28] In addition, recent reports have showed that the presence of hydrogen molecule, rather than pH, is the main cause of antibacterial effect of EW.[29]

This study was conducted *in vitro* to evaluate the antimicrobial effects of AIW. As this study was conducted *in vitro*, its exact effect on oral cavity in reduction of bacterial count was not evaluated. Further *in vivo* studies involving large number of sample size must be conducted in future to determine the antibacterial effects and anti-plaque effects in oral cavity.

CONCLUSIONS

Nowadays, our eating habits have changed due to better standard of living. People consume too much of acidic beverages. These acidic beverages can damage our oral cavity. We Indians are more extravagant on consumption of acidic water as compared to the people from other Western countries. Bacteria causes an acidic environment, which is responsible for causing oral cavities. The alkaline water, which has a basic pH, is 100 times more alkaline than spring water. The basicity of the alkaline water neutralizes the acid produced by bacteria which promotes stronger teeth and bones. Alkaline water also stimulates saliva production, which helps get rid of the excess bacterial production in mouth and helps alleviate dry mouth and bad breath.

Due to their lifestyle habits, many people suffer from acidification that leads to diabetes, heart diseases, cancer, liver, and kidney diseases due to consumption of acidic water. On the contrary, in comparison with normal water, the alkaline water contains an abundance of calcium ions and other minerals that are required for enhancement of the body. Hence, the usage of alkaline water is a savior for those suffering from oral health conditions, as well as many other systemic diseases and provide good assistance in enhancing good oral health condition. However, not much of the research has been carried out in pertaining to the importance of alkaline water.

In this study, we demonstrated that AIW markedly inhibited the growth of salivary bacteria like *Streptococcus salivarius* (*S. salivarius*), *Staphylococcus aureus* (*S. aureus*), and *Lactobacillus casei* (*L. casei*) as well as cultured periodontopathogens. It can be made easily in a small scale and could be useful for daily oral hygiene if used as a mouthwash. It may be especially useful for people wearing orthodontic apparatus as well as physically and mentally challenged people.

It is important to note that while some studies suggest that alkaline water may have benefits for oral health, more research are needed to fully understand its effects.

Clinical relevance

Scientific Rationale for the Study: People are more implied towards the usage of normal tap water than alkaline water because of its easy availability. Acid contained in food and drinks are involved in the initiation and progression of dental diseases. By using the alkaline water, the acidic condition in the oral cavity can be reduced and the oral diseases can be controlled.

Principal Findings: In the present study, it was found that alkaline water reduces a greater number of oral microbes as compared to that of normal water.

Practical Implications: By using the alkaline water in our daily life, the bacterial count in our oral cavity can be controlled and the proper oral hygiene can be maintained.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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