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A COMPARATIVE EVALUATION OF THE ANTI-OXIDANT EFFECTS OF DENTIFRICES IN SMOKERS WITH PERIODONTAL DISEASE – A PILOT PROJECT

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ABSTRACT

Background & Objectives: Smoking, an important risk factor for periodontitis, induces oxidative stress in the body and causes an imbalance between reactive oxygen species (ROS) and anti-oxidants. No known evidence exists regarding the role of dentifrices on the antioxidant levels in the oral cavity as against evidence with regard to their antimicrobial activity. The present study was conducted to compare the antioxidant & antibacterial effects of herbal and conventional dentifrices in smokers with chronic gingivitis & periodontitis.

MATERIALS AND METHOD: 150 male patients, (34±9) years with smoking habit were randomly assigned to two groups- one was instructed to use herbal antioxidant dentifrice (n=75) and the other group, conventional dentifrice for 1 month (n=75). Changes in clinical parameters and in addition, saliva samples collected were assessed for antioxidant and antibacterial activity at baseline, 1week, 15 days and 1 month.

RESULTS: The clinical parameters showed statistically significant change within both test and control group (P 0.001) whereas, the change in antioxidant and antimicrobial levels were significant only in the test and not in the control group.(P>0.05)

CONCLUSION: Both dentifrices may be equally effective in improving both the clinical parameters in smokers, however, the herbal dentifrice holds a slight edge over its regular counterpart with regard to antibacterial and antioxidant effects.

KEYWORDS : Dentifrices, Antioxidant, Antibacterial

INTRODUCTION

Periodontal tissues are vulnerable to various types of reactive oxygen species (ROS) from dental restorative procedures and the environment. One of the most common chemical insults to the oral tissue is nicotine (Nic). Studies [1,2] have demonstrated that Nic decreased gingival fibroblast migration, signalling molecules, and altered the response to transforming growth factor-beta 1 (TGF-b1) by decreasing the morphologic change from fibroblast to myofibroblast cells.

Smoking is associated with an increased risk for poor oral health and dental problems. A recent study [3] showed that cigarette smoking was one of the risk factors involved in the development and progression of periodontal disease. Smoking promotes a high degree of ROS release that results in heightened oxidative damage to gingival tissues, periodontal ligaments (PDLs), and alveolar bone.[4] Previous studies have documented the clinical effects of smoking including persistent gingival bleeding, [5] vertical bone loss, [6] and poor treatment outcomes.[7] Similarly, an in vitro study [8] showed that Nic had inhibitory effects on the attachment and growth of gingival and PDL fibroblasts. A clinical study [9] showed that chemical substances in tobacco can slow down the healing process and affect periodontal treatment surgical outcomes. All tobacco products can increase the growth and development of oral cancer, halitosis, stained teeth, bone loss, taste loss, periodontal treatment failure, dental implant failure, gingival recession, mouth sores, and facial wrinkling.[10]

These studies [3-10] evaluated the mode of action that tobacco smoking uses to affect oral health. In addition to an increase in ROS formation, smoking may also decrease antioxidant (AO) levels. It was theorized that treatment with AOs may block the production of ROS or block their effects and may be therapeutically valuable in reducing the risk for many dental maladies.[11] A dose-response decrease in the salivary and gingival crevicular fluid superoxide dismutase levels was found in light smokers and heavy smokers compared to non-smokers.[12]

A significant reduction in the serum levels of vitamin C and other nonenzymatic AOs such as vitamin A and E and coenzyme Q10 was found among smokers. Saliva being the first biological fluid met by external substances ingested such as food, drinks, inhaled volatile cigarette smoke (CS), microorganisms; it represents the first line of defence against OS [13]. OS represents the imbalance between the production of highly reactive molecular species (ROS, reactive nitrogen species [RNS]) and antioxidant defense systems [14] Antioxidants represent one of the defense mechanisms against OS which are present in all body fluids and tissues [14]. ROS have been reviewed to be implicated in the pathogenesis of periodontitis [15]. It has been suggested that PMN produce and release a big quantity of ROS, culminating in increased oxidative damage to gingival tissue, periodontal ligament and alveolar bone [16]. It was therefore decided to evaluate and compare the antioxidant and antimicrobial activity of a herbal antioxidant and conventional dentifrice in smokers.

MATERIALS AND METHODS

150 male patients with an average age of 34±9 years with a habit of smoking visiting dental institutes and various dental clinics were selected for the study based on the following:-

Inclusion Criteria

- Patients with a history of smoking.
- Number of teeth present ≥ 20.
- Patients with chronic gingivitis or chronic periodontitis (localised/generalised).

Exclusion Criteria

- Presence of any systemic or debilitating diseases .
- A recent history or presence of any acute or chronic infections.
- Patients with history of any drug intake including antibiotics, analgesics or any other drugs 3 months prior to the study.
- Patients who have undergone periodontal therapy in the last 6 months.
- Patients who are physically or mentally challenged.

The 150 patients were randomly assigned to :

test group (n=75); where the patients were instructed to use herbal

dentrifices.*

and control group(n=75), where the patients were instructed to use regular dentifrices#.

*** herbal dentifrice** – Himalaya antioxidant tooth paste, Himalaya drug company, Bangalore, India.

 # regular dentifrice – Colgate strong teeth, Colgate- Palmolive pvt ltd, Mumbai, India.

Clinical parameters such as simplified oral hygiene index (OHIS), gingival bleeding index (GBI), plaque index (PI), gingival index (GI), were recorded at baseline. Saliva samples were also collected for assessment of anti-bacterial and anti-oxidant effect.

This was followed by scaling and root planning in all the patients of both the groups after which the patients were instructed to use the dentifrices.

Changes in clinical parameters of all the patients were evaluated at baseline and at the end of 1week, 15 days and 1 month, 3 months & 6 months. In addition, saliva samples were also assessed for anti-bacterial and anti-oxidant effect at various time intervals.

Procedure for antioxidant assay

Saliva samples were stored at -70˚C which was diluted with phosphate buffer solution at ph 7.0 (Trolox) + 2.0 ml ethanol, following the vial was vortexed for 30-60 sec, and samples were diluted at 1:40 and 200 ml of dilute samples were placed in each well of the kit. Further the plate was read at 450 nm and 50 µl copper solution was added to each well. This was then incubated for 30 mins at room temperature & 50 µl of stop solution was then added and the plate was read for the second time at 450 nm.

Antimicrobial Assessment

Microbial culture were carried out and the microbial counts were expressed as Colony forming units.(CFUs)

STATISTICAL ANALYSIS

Statistical test used was done was done using SPSS soft ware version "t-test". For antimicrobial levels comparison, the $\log_{10}(x)$ transformation was done.

RESULTS

The clinical parameters viz OHI(S), GI, PI and GBI (except at 1 week) showed statistically significant change within both the test group ($P< 0.001$) and in the control group (P 0.001) at various time intervals. However, there was no significant difference with regards to the clinical parameters when both the groups were compared with each other ($P\geq0.05$) at the various time intervals, except for the plaque score at 1 month interval which showed a significant change. (TABLES 1,2&3)

The mean antioxidant level showed statistically significant change within the test group ($P< 0.001$) at the end of 1 month when compared to the various time intervals in contrast to the control group where the changes were not significant. ($P > 0.05$). However, comparison between the two groups revealed no other relevant significant differences at various time intervals ($P\geq0.05$). In addition, the antioxidant activity in test group showed significant correlation with antibacterial activity at 15 days. ($P< 0.05$).(TABLE 4A & 4B)

The mean antimicrobial levels showed statistically significant change within the test group (P 0.001) at the end of 1 month when compared to the various time intervals in contrast to the control group where the changes were not significant. ($P > 0.05$). However, comparison between the two groups revealed no other relevant significant differences at various time intervals ($P\geq0.05$). Additionally, the antibacterial activity of the test group showed significant correlation with plaque index at 1 week and with antioxidant activity at 15 days ($P < 0.05$). No such correlation was observed with the control group. (TAB;E 5A & 5B)

DISCUSSION

Herbal Toothpastes have been shown to be effective and safe to use in the prevention and management of dental plaque and other common dental problems including gingival bleeding and periodontal diseases[17].

Smoking, which is an important risk factor for periodontitis, induces oxidative stress in the body and causes an imbalance between reactive oxygen species (ROS) and antioxidants, such as superoxide dismutase (SOD). A progressive reduction in SOD levels has been seen from healthy non -smokers to light smokers to heavy smokers, thus highlighting the role of oxidative stress in increasing the risk of periodontal disease in smokers.[12]

The reactive oxygen species are known to cause periodontal tissue damage by,

1. Ground substance degradation
2. Collagenolysis either directly or indirectly or as a result of oxidation of proteases
3. Stimulation of excessive pro-inflammatory cytokine release through NF-κB activation
4. PG-E2 production via lipid peroxidation and superoxide release, both of which have been linked with bone resorption[18]
5. Since IL-1 & TNF-α positively regulate their own production, the additive effects of endotoxin mediated cytokine production and that arising from respiratory burst of PMNLs in response to the same organisms, lead to periodontal inflammation and subsequent attachment loss.[19] While most ROS have extremely short half- lives , they can cause substantial tissue damage by initiating free radical chain reactions.

Smoking is known to adversely affect the antioxidant mechanisms thereby increasing the ROS. The role of smoking in periodontal disease is well documented and cessation of smoking may indeed be a great benefit to enhance the periodontal treatment outcome. Nevertheless, this being a herculean task the present study was carried out to ascertain whether an antioxidant dentifrice can suitably counteract the adverse effects of smoking induced oxidant activity and at the same time, be able to effectively exert its antimicrobial and clinical effects on the periodontally diseased tissues.

As was evident in the results, both the herbal and conventional dentifrice users showed a significant improvement in gingival index ,plaque index scores within the respective groups from baseline to 1 month which is in accordance with a number of recent and past evidences[17,20-22] wherein dentifrices effectively improved clinical parameters.

However, there was no significant differences between the 2 groups which was in line with previous findings [22-25], wherein a significant reduction in plaque and gingivitis score in the test group(with dentifrice) when compared with control group(placebo) was observed. The greater inhibition of plaque formation and protection of gingival health was also in accordance with previous studies [26-28].

With regard to the antimicrobial effects, the significant findings observed within the test group are in tandem with the results of A.R. Pradeep et al (2012)[25] wherein significant improvement in gingival and plaque index scores as well as microbiologic counts were observed compared with placebo dentifrice. Various other studies evaluating antimicrobial effects of dentifrices, include a study by Manupati P(2011)[29] , who demonstrated that triclosan containing toothpastes formulations are more effective in control of oral microflora compared to non-triclosan containing synthetic toothpastes. In addition, Patel ED et al(2012)[30] found that dentifrices containing triclosan and fluoride significantly improved oral health by a reduction in plaque, gingivitis and calculus along with

reduction in P. Gingivalis over a period of 6 weeks. However, the lack of significant antibacterial activity in the control group are in contrast to the above findings but concur partly with Manupati P[29] as already stated above. To the authors knowledge, there is no available evidence suggesting limited or no antibacterial activity of dentifrices. Most dentifrices exert favourable antibacterial activity as well.

Oxidative stress induced by smoking was reflected by the reduced antioxidant levels in both the groups at baseline as reported by various researchers.[12,31-34] Successful non-surgical periodontal therapy has shown to reduce oxidative stress during periodontal inflammation and can restore plasma total antioxidant capacity at 1 month post-therapy[35]. Locally delivered antioxidant gel as an adjunct to non surgical therapy has also been shown to be effective in reducing oxidative stress and periodontal disease[21]. Whether these therapeutic interventions exert the same effects in patients with the chronic smoking habit is yet unknown.

The present study was thus undertaken to establish whether anti-oxidant dentifrices may have beneficial effects on the oxidative stress in smokers. There is a definite lack of evidence in literature, with regard to this, due to which, the authors are unable to draw comparisons.

Within the limitations of the study design, evaluation of the baseline data revealed superior antioxidant activity in patients in the test group compared with control group specially in the 15 day and 1 month time interval which was in accordance with evidence [20] which showed significant improvement in clinical parameters and super oxide dismutase levels when vitamin E supplementation as an adjunct to SRP was compared with SRP alone thereby concluding that vitamin E may be effective as an adjunct to SRP in the management of periodontitis and in improving systemic antioxidant status. In addition, researchers[21,35] have also observed that plasma total antioxidant capacity(TAOC) levels after 1 month post-treatment of patients with chronic periodontitis were significantly higher than the baseline values with the adjunctive use of vitamin C and SRP and with SRP alone.

Till date, no studies have evaluated whether the antioxidant activity is enhanced specifically with the use of dentifrices. The herbal dentifrice used in this study had been specifically formulated with enhanced antioxidant effects. The inclusion of a regular dentifrice as part of the study design was to assess the same, especially in smokers. Rehabilitation of smokers is an important part of periodontal therapy, and use of appropriate antioxidants could well be a suitable contributing agent for the same.

CONCLUSION: The use of natural herbal preparations in oral health care continues to be popular. Their clinical efficacy is comparable to conventional dentifrices; therefore, they could be used for the improvement of plaque and gingival status. Nevertheless, this study also showed enhanced antimicrobial and antioxidant efficacy with their use when compared to the conventional dentifrice. Therefore, although it can be suggested that the herbal antioxidant dentifrice has a slight edge over its regular counterpart in smokers, further long-term prospective studies with a larger sample size are needed to authenticate the results achieved in this study.

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TABLE 1: TEST GROUP

Parameter	Time interval	Mean	Std Dev	SE of Mean	Mean Diff	t	P-Value
OHIS	Baseline	3.19	0.68	0.18	0.793	4.837	<0.001*
	1 Week	2.39	0.69	0.18			
	Baseline	3.19	0.68	0.18	1.207	5.612	<0.001*
	15 Days	1.98	0.74	0.19			
	Baseline	3.19	0.68	0.18	1.547	6.188	<0.001*
	1 Month	1.64	0.83	0.21			
	1 Week	2.39	0.69	0.18	0.413	4.004	0.001*
	15 Days	1.98	0.74	0.19			
	1 Week	2.39	0.69	0.18	0.753	5.693	<0.001*
	1 Month	1.64	0.83	0.21			
	15 Days	1.98	0.74	0.19	0.340	3.523	0.003*
	1 Month	1.64	0.83	0.21			
Parameter	**Time interval**	**Mean**	**Std Dev**	**SE of Mean**	**Mean Diff**	**t**	**P-Value**
GI	Baseline	2.03	0.46	0.12	0.313	6.714	<0.001*
	1 Week	1.71	0.39	0.10			
	Baseline	2.03	0.46	0.12	0.520	5.610	<0.001*
	15 Days	1.51	0.37	0.10			
	Baseline	2.03	0.46	0.12	0.820	7.968	<0.001*
	1 Month	1.21	0.29	0.07			
	1 Week	1.71	0.39	0.10	0.207	3.250	0.006*
	15 Days	1.51	0.37	0.10			
	1 Week	1.71	0.39	0.10	0.507	6.368	<0.001*
	1 Month	1.21	0.29	0.07			
	15 Days	1.51	0.37	0.10	0.300	5.809	<0.001*
	1 Month	1.21	0.29	0.07			
Parameter	**Time interval**	**Mean**	**Std Dev**	**SE of Mean**	**Mean Diff**	**t**	**P-Value**
PI	Baseline	1.94	0.48	0.12	0.307	3.570	0.003*
	1 Week	1.63	0.40	0.10			
	Baseline	1.94	0.48	0.12	0.533	5.342	<0.001*
	15 Days	1.41	0.29	0.07			
	Baseline	1.94	0.48	0.12	0.747	6.032	<0.001*
	1 Month	1.19	0.30	0.08			

	1 Week	1.63	0.40	0.10	0.227	5.013	<0.001*
	15 Days	1.41	0.29	0.07			
	1 Week	1.63	0.40	0.10	0.440	6.264	<0.001*
	1 Month	1.19	0.30	0.08			
	15 Days	1.41	0.29	0.07	0.213	4.904	<0.001*
	1 Month	1.19	0.30	0.08			

* denotes significance
SOHI – simplified oral hygiene index
GI- gingival index
PI – plaque index

Parameter	Time interval	Mean	Std Dev	SE of Mean	Mean Diff	Z	P-Value
GBI	Baseline	1.00	0.00	0.00	0.000	0.000	1.000
	1 Week	1.00	0.00	0.00			
	Baseline	1.00	0.00	0.00	0.667	-3.162	0.002*
	15 Days	0.33	0.49	0.13			
	Baseline	1.00	0.00	0.00	0.933	-3.742	<0.001*
	1 Month	0.07	0.26	0.07			
	1 Week	1.00	0.00	0.00	0.667	-3.162	0.002*
	15 Days	0.33	0.49	0.13			
	1 Week	1.00	0.00	0.00	0.933	-3.742	<0.001*
	1 Month	0.07	0.26	0.07			
	15 Days	0.33	0.49	0.13	0.267	-2.000	0.046*
	1 Month	0.07	0.26	0.07			
Parameter	**Time interval**	**Mean**	**Std Dev**	**SE of Mean**	**Mean Diff**	**t**	**P-Value**
AO	Baseline	0.16	0.10	0.03	-0.004	-0.173	0.865
	1 Week	0.16	0.08	0.02			
	Baseline	0.16	0.10	0.03	-0.012	-0.288	0.777
	15 Days	0.17	0.11	0.03			
	Baseline	0.16	0.10	0.03	-0.050	-1.206	0.248
	1 Month	0.21	0.11	0.03			
	1 Week	0.16	0.08	0.02	-0.008	-0.245	0.810
	15 Days	0.17	0.11	0.03			
	1 Week	0.16	0.08	0.02	-0.046	-1.556	0.142
	1 Month	0.21	0.11	0.03			
	15 Days	0.17	0.11	0.03	-0.038	-2.375	0.032*
	1 Month	0.21	0.11	0.03			
Parameter	**Time interval**	**Mean**	**Std Dev**	**SE of Mean**	**Mean Diff**	**Z**	**P-Value**
AB	Baseline	10966.67	15546.87	4014.19	1811.333	-0.175	0.861
	1 Week	9155.33	7875.31	2033.40			
	Baseline	10966.67	15546.87	4014.19	-2666.667	-0.028	0.977
	15 Days	13633.33	17374.52	4486.08			
	Baseline	10966.67	15546.87	4014.19	8267.067	-2.442	0.015*
	1 Month	2699.60	4827.39	1246.43			
	1 Week	9155.33	7875.31	2033.40	-4478.000	-0.057	0.955
	15 Days	13633.33	17374.52	4486.08			
	1 Week	9155.33	7875.31	2033.40	6455.733	-2.443	0.015*
	1 Month	2699.60	4827.39	1246.43			
	15 Days	13633.33	17374.52	4486.08	10933.733	-2.354	0.019*
	1 Month	2699.60	4827.39	1246.43			

* denotes significance
GBI – Gingival bleeding index
AB – antibacterial activity
AO- antioxidant activity

TABLE 2: CONTROL GROUP

Parameter	Time interval	Mean	Std Dev	SE of Mean	Mean Diff	t	P-Value
OHIS	Baseline	2.83	0.66	0.17	0.367	4.083	0.001*
	1 Week	2.46	0.46	0.12			
	Baseline	2.83	0.66	0.17	0.713	4.534	<0.001*
	15 Days	2.11	0.48	0.12			
	Baseline	2.83	0.66	0.17	1.000	5.423	<0.001*
	1 Month	1.83	0.53	0.14			
	1 Week	2.46	0.46	0.12	0.347	4.711	<0.001*
	15 Days	2.11	0.48	0.12			
	1 Week	2.46	0.46	0.12	0.633	6.008	<0.001*

	1 Month	1.83	0.53	0.14			
	15 Days	2.11	0.48	0.12	0.287	5.375	<0.001*
	1 Month	1.83	0.53	0.14			
Parameter	**Time interval**	**Mean**	**Std Dev**	**SE of Mean**	**Mean Diff**	**t**	**P-Value**
GI	Baseline	2.01	0.59	0.15	0.253	7.875	<0.001*
	1 Week	1.76	0.57	0.15			
	Baseline	2.01	0.59	0.15	0.480	6.743	<0.001*
	15 Days	1.53	0.50	0.13			
	Baseline	2.01	0.59	0.15	0.753	7.725	<0.001*
	1 Month	1.26	0.44	0.11			
	1 Week	1.76	0.57	0.15	0.227	4.603	<0.001*
	15 Days	1.53	0.50	0.13			
	1 Week	1.76	0.57	0.15	0.500	6.560	<0.001*
	1 Month	1.26	0.44	0.11			
	15 Days	1.53	0.50	0.13	0.273	6.348	<0.001*
	1 Month	1.26	0.44	0.11			
Parameter	**Time interval**	**Mean**	**Std Dev**	**SE of Mean**	**Mean Diff**	**t**	**P-Value**
PI	Baseline	1.83	0.21	0.05	0.247	7.046	<0.001*
	1 Week	1.58	0.25	0.06			
	Baseline	1.83	0.21	0.05	0.453	7.179	<0.001*
	15 Days	1.37	0.34	0.09			
	Baseline	1.83	0.21	0.05	0.680	9.056	<0.001*
	1 Month	1.15	0.40	0.10			
	1 Week	1.58	0.25	0.06	0.207	5.568	<0.001*
	15 Days	1.37	0.34	0.09			
	1 Week	1.58	0.25	0.06	0.433	8.293	<0.001*
	1 Month	1.15	0.40	0.10			
	15 Days	1.37	0.34	0.09	0.227	7.549	<0.001*
	1 Month	1.15	0.40	0.10			

* denotes significance
SOHI – simplified oral hygiene index
GI- gingival index
PI – plaque index

Parameter	**Time interval**	**Mean**	**Std Dev**	**SE of Mean**	**Mean Diff**	**Z**	**P-Value**
GBI	Baseline	1.00	0.00	0.00	0.067	-1.000	0.317
	1 Week	0.93	0.26	0.07			
	Baseline	1.00	0.00	0.00	0.600	-3.000	0.003
	15 Days	0.40	0.51	0.13			
	Baseline	1.00	0.00	0.00	0.933	-3.742	<0.001*
	1 Month	0.07	0.26	0.07			
	1 Week	0.93	0.26	0.07	0.533	-2.828	0.005*
	15 Days	0.40	0.51	0.13			
	1 Week	0.93	0.26	0.07	0.867	-3.606	<0.001*
	1 Month	0.07	0.26	0.07			
	15 Days	0.40	0.51	0.13	0.333	-2.236	0.025*
	1 Month	0.07	0.26	0.07			
Parameter	**Time interval**	**Mean**	**Std Dev**	**SE of Mean**	**Mean Diff**	**t**	**P-Value**
AO	Baseline	0.15	0.10	0.02	-0.009	-0.389	0.703
	1 Week	0.16	0.08	0.02			
	Baseline	0.15	0.10	0.02	-0.020	-0.527	0.606
	15 Days	0.17	0.12	0.03			
	Baseline	0.15	0.10	0.02	-0.008	-0.246	0.809
	1 Month	0.16	0.12	0.03			
	1 Week	0.16	0.08	0.02	-0.011	-0.635	0.536
	15 Days	0.17	0.12	0.03			
	1 Week	0.16	0.08	0.02	0.001	0.076	0.941
	1 Month	0.16	0.12	0.03			
	15 Days	0.17	0.12	0.03	0.012	0.893	0.387
	1 Month	0.16	0.12	0.03			
Parameter	**Time interval**	**Mean**	**Std Dev**	**SE of Mean**	**Mean Diff**	**Z**	**P-Value**
AB	Baseline	38714.13	103991.88	26850.59	-54639.200	-0.511	0.609
	1 Week	93353.33	334277.98	86310.20			
	Baseline	38714.13	103991.88	26850.59	28647.467	-1.647	0.100
	15 Days	10066.67	21482.27	5546.70			

	Baseline	38714.13	103991.88	26850.59	35474.800	-1.817	0.069
	1 Month	3239.33	3003.06	775.39			
	1 Week	93353.33	334277.98	86310.20	83286.667	-0.596	0.551
	15 Days	10066.67	21482.27	5546.70			
	1 Week	93353.33	334277.98	86310.20	90114.000	-0.384	0.701
	1 Month	3239.33	3003.06	775.39			
	15 Days	10066.67	21482.27	5546.70	6827.333	-0.384	0.701
	1 Month	3239.33	3003.06	775.39			

* denotes significance
GBI – Gingival bleeding index
AB – antibacterial activity
AO- antioxidant activity

TABLE 3: 37. Comparison of various parameters between test group and control group

Parameter	Time Interval	Group	Mean	Std Dev	SE of Mean	Mean Difference	t	P-Value
OHIS	Baseline	Test	3.19	0.68	0.18	0.360	1.468	0.153
		Control	2.83	0.66	0.17			
	7 days	Test	2.39	0.69	0.18	-0.067	-0.313	0.757
		Control	2.46	0.46	0.12			
	15 days	Test	1.98	0.74	0.19	-0.133	-0.587	0.562
		Control	2.11	0.48	0.12			
	1 Month	Test	1.64	0.83	0.21	-0.187	-0.737	0.467
		Control	1.83	0.53	0.14			
GI	Baseline	Test	2.03	0.46	0.12	0.013	0.069	0.945
		Control	2.01	0.59	0.15			
	7 days	Test	1.71	0.39	0.10	-0.047	-0.262	0.795
		Control	1.76	0.57	0.15			
	15 days	Test	1.51	0.37	0.10	-0.027	-0.167	0.869
		Control	1.53	0.50	0.13			
	1 Month	Test	1.21	0.29	0.07	-0.053	-0.390	0.699
		Control	1.26	0.44	0.11			
PI	Baseline	Test	1.94	0.48	0.12	0.113	0.842	0.407
		Control	1.83	0.21	0.05			
	7 days	Test	1.63	0.40	0.10	0.053	0.439	0.664
		Control	1.58	0.25	0.06			
	15 days	Test	1.41	0.29	0.07	0.033	0.291	0.773
		Control	1.37	0.34	0.09			
	1 Month	Test	1.19	0.30	0.08	0.047	0.363	0.719
		Control	1.15	0.40	0.10			
GBI	Baseline	Test	1.00	0.00	0.00	0.000	---	---
		Control	1.00	0.00	0.00			
	7 days	Test	1.00	0.00	0.00	0.067	1.000	0.326
		Control	0.93	0.26	0.07			
	15 days	Test	0.33	0.49	0.13	-0.067	-0.367	0.716
		Control	0.40	0.51	0.13			
	1 Month	Test	0.07	0.26	0.07	0.000	0.000	1.000
		Control	0.07	0.26	0.07			
Anti Oxidant Levels	Baseline	Test	0.16	0.10	0.03	0.005	0.127	0.900
		Control	0.15	0.10	0.02			
	7 days	Test	0.16	0.08	0.02	0.000	-0.002	0.998
		Control	0.16	0.08	0.02			
	15 days	Test	0.17	0.11	0.03	-0.003	-0.081	0.936
		Control	0.17	0.12	0.03			
	1 Month	Test	0.21	0.11	0.03	0.047	1.097	0.282
		Control	0.16	0.12	0.03			
Anti Microbial Levels§	Baseline	Test	109.67	155.47	40.14	-286.475	-0.805	0.428
		Control	396.14	1036.87	267.72			
	7 days	Test	87.59	81.88	21.14	-847.740	0.825	0.416
		Control	935.33	3342.25	862.97			
	15 days	Test	136.33	173.75	44.86	35.667	1.846	0.075
		Control	100.67	214.82	55.47			
	1 Month	Test	26.99	48.28	12.47	-34.206	-1.637	0.113
		Control	61.19	119.61	30.88			

§ log-transformed data used for significance testing
No significant difference was observed between test and control group for any other parameter at any of the time intervals (P≥0.05).

TABLE 4 A:- Correlation between Antibacterial Activity and other parameters in TEST Group

Group 1	OHIS	GI	PI	GBI	PPD	AO	
Baseline	r	-0.060	-0.296	-0.457	---	0.258	0.222
	P-Value	0.832	0.284	0.087	---	0.353	0.426
1 Week	r	-0.037	-0.324	-0.620	---	-0.032	-0.092
	P-Value	0.895	0.238	0.014*	---	0.910	0.745
15 Days	r	0.046	0.205	-0.087	-0.204	-0.213	-0.532
	P-Value	0.871	0.463	0.758	0.467	0.445	0.041*
1 Month	r	-0.066	-0.048	-0.030	-0.109	0.116	-0.167
	P-Value	0.816	0.866	0.916	0.699	0.681	0.551

*denotes significant correlation

38. TABLE 4 B - Correlation between Antibacterial Activity and other parameters in CONTROL Group

Group 2	OHIS	GI	PI	GBI	PPD	AO	
Baseline	r	0.236	0.369	0.041	---	0.290	0.153
	P-Value	0.398	0.177	0.885	---	0.295	0.586
1 Week	r	-0.390	-0.074	0.146	0.077	-0.310	0.103
	P-Value	0.150	0.792	0.603	0.785	0.261	0.715
15 Days	r	-0.193	0.445	-0.258	-0.020	0.189	-0.058
	P-Value	0.491	0.097	0.354	0.943	0.501	0.838
1 Month	r	0.055	-0.343	0.419	0.402	0.044	-0.143
	P-Value	0.845	0.211	0.120	0.138	0.877	0.612

TABLE 5A - Correlation between Antioxidant and other parameters in TEST Group

Group 1	OHIS	GI	PI	GBI	PPD	AB	
Baseline	r	-0.236	-0.227	0.030	---	0.254	0.222
	P-Value	0.397	0.416	0.916	---	0.361	0.426
1 Week	r	0.173	-0.107	0.202	---	0.018	-0.092
	P-Value	0.538	0.705	0.471	---	0.950	0.745
15 Days	r	0.047	0.068	0.160	-0.217	-0.105	-0.532
	P-Value	0.868	0.809	0.568	0.436	0.709	0.041*
1 Month	r	0.135	0.016	0.144	-0.078	-0.311	-0.167
	P-Value	0.632	0.955	0.608	0.783	0.259	0.551

*denotes significant correlation

TABLE 5 B - Correlation between Antioxidant and other parameters in CONTROL Group

Group 2	OHIS	GI	PI	GBI	PPD	AB	
Baseline	r	-0.572	-0.348	-0.071	---	0.084	0.153
	P-Value	0.026*	0.203	0.803	---	0.765	0.586
1 Week	r	-0.194	-0.200	-0.149	0.141	-0.035	0.103
	P-Value	0.489	0.475	0.596	0.615	0.901	0.715
15 Days	r	-0.051	-0.111	-0.261	-0.380	0.030	-0.058
	P-Value	0.855	0.693	0.348	0.162	0.916	0.838
1 Month	r	-0.270	-0.277	-0.323	-0.333	0.139	-0.143
	P-Value	0.331	0.318	0.241	0.225	0.622	0.612

*denotes significant correlation

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