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Comparison Of Total Antioxidant Efficacy Of Aloe Vera Alone, Scaling & Root Planing With Aloe Vera And Scaling & Root Planing Alone In Type 2 Diabetic Patients With Chronic Periodontitis- A Spectrophotometric Analysis

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Abstract

Objective and background: Diabetes mellitus is a group of multi system metabolic disorders characterized by hyperglycemia. It has been proposed that there is a causal 2-way relationship between insulin resistance, oxidative stress and periodontitis. Herbal medicines like Aloe vera with antioxidant, radical scavenging and anti-inflammatory actions have been tried in the treatment of diabetes and periodontal diseases. Hence, the present study is designed to evaluate and compare the total antioxidant efficacy of Aloe vera in type 2 diabetic patients with chronic periodontitis alone and as an adjunct to scaling and root planing(SRP).

Material and methods: 120 gingival crevicular fluid (GCF) samples were collected from 30 subject with type 2 diabetes mellitus and chronic periodontitis. Gingival index (GI) probing pocket depth (PPD) and clinical attachment level (CAL) at baseline were recorded. Samples were collected from sites exhibiting deepest PPD and grouped into four categories. Group 1- GCF before SRP; group 2- GCF before SRP after treating with Aloevera; group 3- GCF after SRP alone and group 4- GCF after SRP and treated with Aloevera. The samples were assessed for total antioxidant capacity (TAOC) using Spectrophotometric assay.

Results: Aloevera was found to have significant TAOC when used alone and adjunctive to SRP ($p<0.001$). Highest levels of TAOC were observed in group 4 (18.042 ± 9.672) and lowest in group 1 (4.855 ± 5.441).

Conclusion: Aloevera possessed significant antioxidant capacity and inhibited the production of reactive oxidant species when used both alone and adjunctive to SRP. Hence, it can be used as a potent antioxidant in the treatment of type 2 diabetes with chronic periodontitis.

Keywords: Total antioxidant capacity, TAOC, Aloevera; type 2 Diabetes Mellitus; Chronic periodontitis; Oxidative stress.

Introduction

Diabetes mellitus is a group of multi system metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion and insulin action or both; the more prevalent form being diabetes type 2 comprising about 90% of the total diabetic cases [1]

Oxidative stress has been proposed to reduce insulin secretion and increase insulin resistance leading to glucose intolerance [2]. Oxidative stress plays a pivotal role in cellular injury from hyperglycemia by enhancing the production of free radicals termed as reactive oxygen species (ROS). An imbalance in ROS production and antioxidant defense occurs leading to the domination of oxidative stress. Progressive intolerance to glucose contributes to many micro and macro vascular complications, [1] if left untreated.

It has been proposed that there is a causal 2-way relationship between insulin resistance, oxidative stress and periodontitis [3]. Conversely, presence of periodontitis may exert a negative impact on metabolic risk status in type 2 diabetes [4].

In most cases, periodontitis in diabetic patients goes undiagnosed which may further increase the severity of both the diseases. Recently, a variety of herbal medicines have been tried in the treatment of diabetes and periodontal diseases which have shown beneficial outcome.

Aloe vera (Aloe barbadensis Miller) has been widely acknowledged in the management of diabetes; the major component being glycosides (aloins). Animal model studies have shown that Aloe vera possesses antioxidant and radical scavenging potential along with anti-inflammatory action [5].

Hence, to the best of our knowledge, this is the first study designed to evaluate and compare the total antioxidant efficacy of Aloe vera in type 2 diabetic patients with chronic periodontitis alone and as an adjunct to scaling and root planing.

Materials and Methods

Study Design: This study is a Randomized controlled single blind trial.

Method of Collection of Data: A total of 120 samples were obtained from 30 subjects of age group 25-65 years, reporting to the Department of Periodontology, Rajarajeswari Dental College and Hospital, Bangalore, diagnosed with type 2 diabetes mellitus and chronic periodontitis.

Inclusion Criteria

1. Subjects with type 2 diabetes mellitus at least from the past 2 years- As per American Diabetes Association (ADA) criteria, i.e. fasting plasma glucose of >126mg/dl and on oral hypoglycemic drugs with no modification in therapy for the past 12 months.
2. Subjects with chronic periodontitis as per American Academy of Periodontology (AAP) guidelines (1999).
3. Subjects should have at least 20 natural teeth.
4. Subjects with probing pocket depth (PPD) ≥5mm and clinical attachment level (CAL) of ≥4mm in more than 30% of the sites.

Exclusion Criteria

1. Subjects with any systemic disease apart from type 2 diabetes mellitus.
2. Subjects who have undergone any periodontal treatment in the last 6 months.
3. Pregnant/ lactating women.
4. Tobacco or alcohol users.
5. Subjects who were on antibiotics, anti-inflammatory drugs or steroids for the past 3 months.
6. Subjects who were on vitamins, mineral supplements or antioxidants for the past 3months.

After obtaining an informed consent, the selected patients underwent a full mouth periodontal probing and charting.

Clinical Parameters Recorded

1. Gingival index (GI) (Loe H and Silness J, 1963).
2. Probing pocket depth (PPD) measured using graduated Williams periodontal probe from the crest of gingival margin to base of the pocket.

3. Clinical attachment level (CAL) measured from cementoenamel junction (CEJ) to base of the pocket.

Four samples of GCF were collected from each subject randomly by means of chit method; two before scaling and root planing and two after one week post scaling and root planing. The samples were then grouped into the following categories and were assessed for total antioxidant capacity.

Group 1: TAOC levels before scaling and root planing (SRP). (Sample size, n=30)

Group 2: TAOC levels before SRP but after treating sample with Aloe vera solution alone. (n=30)

Group 3: TAOC levels after SRP alone. (n=30)

Group 4: TAOC levels after SRP and treating sample with Aloe vera solution. (n=30)

Groups 2&4 was treated with Aloe vera solution.

Preparation and Delivery Of Aloe Vera Solution

Aloe vera powder of 99% purity was procured from Prakash Spices® (P)Ltd. Shivtolla Street, Kolkata, West Bengal. The Aloe vera solution was prepared by dissolving 20 mg of Aloe vera in 10 ml of distilled water (2000 µg/ml) and the prepared solution was added invitro into the GCF samples stored in vials at the time of processing.

Collection of GCF Samples

Pooled GCF samples were collected from sites exhibiting deepest PPD, the sample collection site was well isolated and without touching the marginal gingiva, supra gingival plaque was removed. GCF was collected by placing the micro capillary pipette (SigmaAldrich, India) at the entrance of the gingival sulcus (extrasulcular method) by gently touching the gingival margin. A standardized volume of 3 ml was collected in single capillary from the same site using the calibration on white color coded 1–5 ml calibrated volumetric microcapillary pipettes. The GCF samples were examined for any blood contamination. In case of visible blood contamination, the sample was discarded. The collected GCF samples were transferred to air tight plastic vials and stored at -70◦C until assayed.

Measurement of Total Antioxidant Capacity

TAOC of these samples was evaluated using the spectrophotometric assay. The method is based on the principle that, when a standardized solution of Fe- EDTA (prepared freshly by mixing equal volumes of solutions, Sodium benzoate: 5 mmol/litre and EDTA: 1mmol/ litre in phosphate buffer were left to stand 60 minutes at room temperature) complex reacts with hydrogen peroxide by a Fenton –type reaction, it leads to the formation of radicals. These ROS degrade benzoate, resulting in the release of thiobarbituric acid reactive substances (TBARS). Antioxidants from the added sample of human fluid/ any agent cause suppression of the production of TBARS. This reaction can be measured spectrophotometrically and the inhibition of colour development is defined as the antioxidant capacity [18]

Analytical procedure

Each sample (A1) acted as its own control (A0, sample blank) in which the Fe–EDTA mixture and H2O2 (5 mmol/litre) was added after 20% acetic acid. For each series of analysis, a negative control (K1 and K0) was prepared, containing the same reagents as A1 or A0, except that GCF sample was replaced with phosphate buffer. Standards containing 1mmol/litre uric acid (UA1 and UA0) was used for calibration. Solutions were pipetted into tubes (in millilitres) and incubated for 60 minutes. Then, Aloe vera solution (only to group 2 and group 4) and TBA were added and incubated for 10 minutes at 100 ◦C (in a boiling water bath) and cooled in an ice bath. Absorbance was measured at 532 nm against deionized water [Table 1].

Antioxidant activity (AOA) was calculated as follows:

AOA (mmol/litre) = (CUA) (K − A)/ (K − UA) where K = absorbance of control (K1 – K0); A = absorbance of sample (A1 – A0); UA = absorbance of uric acid solution (UA1 – UA0); CUA = concentration of uric acid (in mmol/litre).

The TAOC levels were analysed by measuring the suppression of thiobarbituric acid reactive substances (TBARS) produced by reactive oxidant species (ROS) which is assessed by the inhibition of colour development. More the inhibition of colour development more is the total antioxidant capacity thus indicating increased level of antioxidants in the study samples.

Statistical Analysis

Descriptive and inferential statistical analysis was carried out in the present study. The Statistical software namely SPSS 15.0, Stata 8.0, Medical 9.0.1 and Systat 11.0 were used for the analysis of the data and Microsoft word and Excel have been used to generate graphs, tables etc. The values obtained were subjected to statistical analysis. The test of significance was applied. The results were statistically analyzed using the following methods.

1. Kruskal-Wallis test- to compare values among three groups.

2. Mann Whitney U test- to compare values among two groups.

Mean value was calculated using the formula:

Standard deviation (SD) was calculated using the formula:

$$SD = \sqrt{\frac{\Sigma(X-X)^2}{n-1}}$$

X is = mean

Results

The present study was done to assess the total antioxidant capacity (TAOC) of Aloe vera in 30 subjects with type 2 diabetes mellitus patients with chronic periodontitis. The study population consisted of sixteen males and fourteen females in the age group of 25-65 years; (mean age is 51.2± 9.956 years).

Mean values of baseline clinical parameters among the study subjects revealed moderate gingival inflammation with an average PPD of 6.53± 0.96 mm and an average CAL of 5.55± 1.12 mm.

Comparison of Total antioxidant capacity (TAOC) among study groups

The TAOC was 4.855 ± 5.441 (0.20-21.10), 7.668 ± 3.930 (0.33-13.40), 8.581 ± 7.783 (0.32-37.90) and 18.042 ± 9.672 (4.00-42.00) in Group 1, Group 2, Group 3 and Group 4respectively. [Table 2]

TAOC levels in each group was statistically significant with p value < 0.001 (Table 1, kruskal wallis). The highest levels of antioxidising capacity was evident in group 4 (18.042± 9.672) and lowest for group 1 (4.855± 5.441).

When compared with group 4 (18.042± 9.672), a larger difference was noted in the mean values of total antioxidising capacity of groups 1, 2 and 3 (4.855± 5.441, 7.668± 3.930, 8.581± 7.783 respectively); the highest between groups 1 and 4. The differences among these groups were also significant statistically (p<0.001). [Graph 1].

Mean TAOC of group 3 (8.581± 7.783) was found to be higher compared to that of group 1 (4.855± 5.441); the results were also found to be statistically significant (p=0.020) but there was no statistically significant difference noted between groups 3 and 2 (p=0.994) (Graph) which showed comparable values of mean total antioxidant capacity (TAOC). (8.581± 7.783 and 7.668± 3.930 respectively).

Discussion

Oxidative stress has been proposed to reduce insulin secretion and increase insulin resistance leading to glucose intolerance. The plasma antioxidant level is significantly lower in diabetic subjects with poor glycaemic control,

while patients with good glycaemic control had plasma antioxidative values similar to controls [19, 20]. It was found that the increase in glucose concentrations can lead to tissue damage by increasing oxidative stress [21].

In our study, the mean TAOC levels of samples collected from type 2 diabetic patients with periodontitis assessed at baseline (group 1) was found to be lowest (4.855 ± 5.441) when compared to other groups [groups, 2, 3 and 4 (7.668 ± 3.930, 8.581 ± 7.783 and 18.042 ± 9.672 respectively)] where intervention was done stressing the fact that TAOC is directly proportional to oxidative stress. The oxidative stress was higher in group 1 as compared to other groups indicating reduced total antioxidant capacity in group 1.

These findings are in consistent with previous studies which assessed the total antioxidant capacity levels in diabetic patients with periodontitis. Pendyala G et al. who compared salivary total antioxidant capacity in type 2 diabetes patients with and without periodontitis found depleted total antioxidant capacity in the former category (0.40 ± 0.09) compared to the latter (1.24± 0.18) thus highlighting the negative effect of periodontitis on the compromised oxidative status in type 2 diabetics [9].

One possible source of the higher oxidative stress noticed in type 2 diabetics with periodontitis may be hyperactive neutrophils since both the inflammatory conditions are associated with neutrophil priming and altered release of ROS, correlating with the severity of periodontitis and glycemic control [22, 23, 24, 25].

Aloe vera, which has been proved to have amazing medicinal properties, belonging to Liliaceae family, has been widely acknowledged in the management of diabetes. Animal model studies have shown that Aloe vera possesses antioxidant and radical scavenging potential along with anti-inflammatory action [10, 12, 15, 16].

In our study, Aloe vera was used alone and also as adjunct to scaling and root planing to evaluate its antioxidising potential in type 2 diabetics with periodontitis at a dosage of 2000 µg/ml.

The mean total antioxidant capacity (TAOC) in the study samples collected before SRP and treated with Aloe vera solution (group 2) was 7.668 ± 3.930 and it was found to be significantly higher than that of group 1 (4.855 ± 5.441) (p=0.003). This shows that addition of Aloe vera lowered the reactive oxidative species to a considerable level thus reducing the oxidative stress and influencing the inflammatory status. It was proved that organic extracts of Aloe vera leaf possess potent in vivo antioxidant capacity due to the presence of the antioxidant polyphenols, indoles, and alkaloids [12, 13]. Polysaccharides are considered to be the active ingredients of Aloe's anti inflammation and immune modulation effects.[11] Also, Aloe vera protected against pro oxidant-induced membrane and cellular damage by a significant reduction in the levels of cytochrome P450 and cytochrome b5.

The highest TAOC level of 18.042 ± 9.672 ($p < 0.001$) was observed in group 4 (SRP + Aloe vera) indicating greatest reduction of oxidative stress in this group. The action of SRP reduced the ROS and addition of Aloe vera increased the antioxidant level together increasing the TAOC in our study.

This findings is in accordance with another clinical study by Bhat G et al. which showed subgingival administration of Aloe vera gel resulted in improvement of clinical parameters like plaque index (1.78 to 1.71), gingival index (1.57 to 1.59) and reduction in probing depth (0.92mm to 1.71mm).[17] Similarly, Saritha V et al. found that Aloe vera gel extracts possessed significant antioxidant and antibacterial activity and could be used to reduce oxidative stress and levels of lipid oxidation.[10] Similar results were obtained when antioxidant and antibacterial properties of Aloe vera were analysed which suggested the use of Aloe

vera in preventing or alleviating symptoms associated with cardiovascular diseases, cancer, diabetes etc [13].

In the present study, the mean TAOC levels of samples collected from the type 2 diabetic patients with periodontitis assessed after scaling and root planing alone (group 3) was 8.581 ± 7.783 ($p < 0.001$) which was found to be higher than that at baseline (4.855 ± 5.441); making it evident that scaling and root planing was efficient in reducing oxidative stress in the study subjects. Here, group 3 acted as the positive control.

The present study may have an important therapeutic implication in terms of the use of antioxidants in diabetics as well as in periodontal therapy to prevent tissue destruction.

To the best of our knowledge, this is the first study showing the positive effect of Aloe vera on TAOC with type 2 diabetes and periodontitis. The difference in the mean values of TAOC levels from other studies could be attributed to the variation in biochemical analyses done in those researches. To reduce the confounding factors, strict matching in terms of gender, periodontal status and exclusion of smokers, those with other systemic illness, on anti-inflammatory, antibiotic or antioxidant therapy were done in our study.

Although this study has potential implications, it also has few limitations. Most of the oral hypoglycemic agents consumed by diabetic patients are known to have potent antioxidant effects [26] which could have influenced the results. Further studies with inclusion of periodontally healthy and controlled diabetics who are not on hypoglycemic agents may prove better in the identification of oxidative stress markers. The antioxidising potential of Aloe vera can vary with the shelf life and dosage. The antioxidising potential was found to be reduced with older samples of Aloe vera [14]. Since we have used the commercially available powdered form of aloevera, it can also contribute to alteration in antioxidising potential. Further research with fresh extract samples might provide unambiguous results.

An interesting finding noticed in the present study was that there was no significant difference between groups, 2 and 3 in the mean TAOC values obtained ($p < 0.224$). This suggests that Aloe vera alone was capable of reducing the inflammatory level and hence can be used as an effective antioxidant agent even in the absence of oral prophylaxis. This can be beneficial in diabetic patients where oral hygiene maintenance is difficult as in cases of debilitating patients.

Above data sheds light on the fact that an early diagnosis and treatment of periodontitis in patients with type 2 diabetes can minimize the pathogenic effects of severe oxidative stress upon B cell function and glycemic control [9]. Thus treating this aspect of oxidative stress by nonsurgical therapy may improve symptoms of both the diseases.

Tables

	A_1	A_0	K_1	K_0	UA_1	UA_0
Serum	0.01	0.01	-	-	-	-
Uric Acid	-	-	-	-	0.01	0.01
Buffer	0.49	0.49	0.50	0.50	0.49	0.49
Na-BENZOATE	0.50	0.50	0.50	0.50	0.50	0.50
Acetic Acid	-	1.00	-	1.00	-	1.00
Fe-EDTA	0.20	0.20	0.20	0.20	0.20	0.20
H_2o_2	0.20	0.20	0.20	0.20	0.20	0.20
Incubate For 60 Minutes At 37^0 C, Then Add						
Acetic Acid	1.00	-	1.00	-	1.00	-
TBA	1.00	1.00	1.00	1.00	1.00	1.00

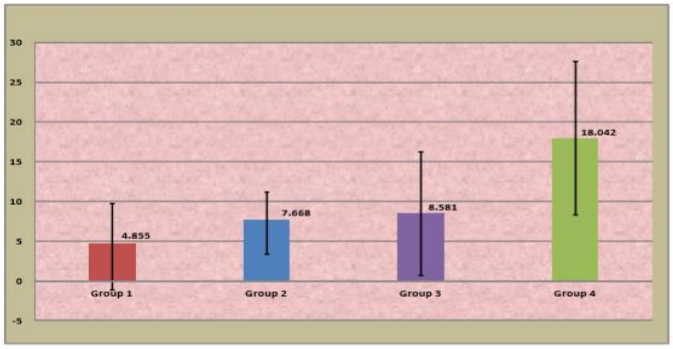
Table 1

Table 2: Intergroup Comparison Of Mean Values Of Total Antioxidant Capacity (TAOC)

Group	N	Mean	SD	Median	Min.	Max.	Chi-square*	P value
Group 1	30	4.855	5.441	2.840	0.20	21.10	42.582	*<0.001*
Group 2	30	7.668	3.930	8.300	0.33	13.40		
Group 3	30	8.581	7.783	7.660	0.32	37.90		
Group 4	30	18.042	9.672	16.750	4.00	42.00		

Kruskal Wallis test

Graph 1: Intergroup Comparison Of Mean Values of Total Antioxidant Capacity (TAOC)



Conclusion

Hence, from the present clinical trial, it can be concluded that Aloe vera was found to have potent total antioxidant potential and is effective in reducing the oxidizing stress in type 2 diabetics with chronic periodontitis. Further in vivo studies are required to assess their efficacy clinically. Due to its effect on oxidation stress and periodontal inflammation, together which may influence insulin resistance, future prospective lies in exploring the positive effect of Aloe vera in glycemic control in patients with type 2 diabetes mellitus and periodontitis.

Abbreviations

AOA-ANTI OXIDIZING AGENT

CAL- CLINICAL ATTACHMENT LEVEL

EDTA- ETHYLENE DIAMINE TETRA ACETIC ACID

GCF- GINGIVAL CREVICULAR FLUID

GI- GINGIVAL INDEX

PPD- PROBING POCKET DEPTH

SRP- SCALING AND ROOT PLANING

TAOC- TOTAL ANTIOXIDANT CAPACITY

TBA- THIOBARBITURIC ACID

TBARs- THIOBARBITURIC ACID REACTIVE SUBSTANCES

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