EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 9
Issue: 7
335-343
Year: 2022

EFFECTIVENESS OF BARBERRY EXTRACT DENTAL GEL IN CONTROLLING GINGIVITIS, DENTAL PLAQUE AND GLYCEMIC PROFILE IN TYPE II DIABETES: A DOUBLE BLINDED RANDOMISED PLACEBO CONTROLLED TRIAL

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Article Received on 10/05/2022 **Article Revised on 31/05/2022** **Article Accepted on 21/06/2022**

ABSTRACT

Introduction: Periodontitis is a common oral manifestation in diabetes. Barberry is a herb which has been used in traditional medicine due to its anti-inflammatory, febrifugal, hypotensive and immunomodulatory effects. Thus the aim of the present study was to evaluate the effectiveness of a dental gel containing Barberry extract in controlling gingivitis, dental plaque, as well as glycosylated haemoglobin levels in type II diabetes. **Materials and Method:** The present study was a double blinded randomised parallel arm placebo controlled trial carried out among 80 subjects. Participants in Group A received a placebo gel with oral hygiene instructions and those in group B received barberry extract dental gel with oral hygiene instructions. Plaque index, gingival index, gingival bleeding indices were assessed at baseline, one and two months of follow up where as HbA1c levels were assessed at baseline and six months of follow up. **Results:** It was observed that barberry extract gel was more effective than placebo gel in the reduction of HbA1c levels as well as in the reduction of gingival bleeding index values. Barberry extract gel was more effective than the placebo gel in the first month in reduction of plaque. However at two months the reduction did not show any significant difference. Both, barberry extract gel as well as placebo gel performed equally in reducing the gingival index. **Conclusion:** Within the limitations of the study it can be concluded that the application of a dental gel containing the extract of barberry might prove to be highly beneficial in type 2 diabetic patients in controlling both gingivitis as well as glycaemic index.

KEYWORDS: Barberry, Diabetes, Gingivitis, Glycosylated Haemoglobin, Plaque.

1 INTRODUCTION

Barberry, also known as Berberis vulgaris (B. vulgaris) Linn belongs to the family Berberidaceae and is a common herb in Iran as well as other parts of South Asia, Central and Eastern Europe and north of America.[1] Various parts of this plant such as the stem, dried leaves, fruit, root and flower have been used in traditional medicine due to its anti-inflammatory, febrifugal, hypotensive and immunomodulatory effects.[2] Berberine, oxyacanthine, berbamine and palmatine are the principal alkaloids present in the plant which contribute to its therapeutic effects. Berberine, which is the most active alkaloid extracted from the root and stem of the plant, is a quaternary benzylisoquinoline alkaloid that belongs to the structural class of protoberberines.[3,4] Along with berberine, other components such as acanthine, berberrubine, bargustanine, bervuleine, beriambine, columbamine, magnoflorine, jatrorrhizine, lambertine, thaliemidine, ascorbic acid, vitamin K, flavonoids, flavanols, tannins, triterpenes, and coumarins that have been identified in *B. vulgaris* might have an additional synergistic effect.[1]

Diabetes mellitus is a global non communicable disease characterised by hyperglycemia. Type II diabetes which is caused due to defective insulin secretion, resistance to insulin action or a combination of both, is a highly prevalent form. The condition of chronic glycaemia has severe clinical implications such as diabetic retinopathy, renal disease, neuropathy, cardiovascular disease, microvascular and macrovascular complications.[5] Currently available pharmacotherapy for diabetes include insulin and various oral drugs such as metformin, meglitinides and sulfonylureas, alpha glucosidase inhibitors, thiazolidines, Dipeptidyl peptidase-4 inhibitors and Sodium glucose co-transporter-2 inhibitor which are used as monotherapy or in combination to achieve better glycemic regulation. However many of these medications have a number of side effects.[6] Periodontitis is often a common manifestation observed

in diabetic patients. The risk of periodontitis is directly proportional to the glycaemic control which can be assessed by measuring the level of glycated haemoglobin (HbA1c) in blood. HbA1c is the measure of the percentage of haemoglobin that has glucose molecules absorbed onto the haemoglobin molecule which is typically in the range of 8-9% (64-75 mmol/mol) or higher in patients with poor glycaemic control.[7] Phytotherapy has gained attention in the treatment of periodontal diseases due to the anti inflammatory, antimicrobial and antioxidative effect of herbs.[8] Aqueous extract of *B. vulgaris* has been recommended in the management of diabetes based on a survey conducted by herbalists. Further, a recent study has demonstrated the hypoglycemic effect of barberry in induced diabetic rats.[9] The use of barberry extract dental gel for the control of inflammatory periodontal disease has also been documented in literature.[10] However, there has been no randomised trial till date to analyse the combined effect of application of barberry extract gel in controlling gingival and periodontal disease as well as HbA1C levels. Thus the aim of the present study was to evaluate the effectiveness of a dental gel containing Barberry extract in controlling gingivitis, dental plaque, as well as glycosylated haemoglobin levels in type II diabetes. The null hypothesis of the study was that there is no difference in the effectiveness of barberry extract gel and placebo gel in controlling gingivitis, dental plaque and glycosylated haemoglobin levels in type II diabetes.

2 MATERIALS AND METHOD

2.1 Study Design, Setting and Location

The present study was a double blinded randomised parallel arm placebo controlled trial, carried out among 80 subjects at a recognised dental college at Navi Mumbai, satellite centres of the college during the months February to November 2021.

2.2 Ethical Approval

The protocol was reviewed by the Institutional review board (IRB) and Ethical clearance was obtained from the institutional ethics committee (IEC) of a recognised dental college at Navi Mumbai. (YMTDC/IEC/2021/18).

2.3 Study Groups

The study comprised of two groups
Group A: Placebo gel with oral hygiene instructions.
Group B: Barberry extract gel with oral hygiene instructions.

2.4 Eligibility Criteria

The study included participants between the age group of 35 to 44 years with type II diabetes and giving an informed signed consent, having glycosylated haemoglobin (HbA1C) Value <10%.

The study excluded those with Systemic diseases such as autoimmune disorders, ischemic heart problems, renal, liver and thyroid disorders. Patients with pregnancy and those not giving an informed signed consent.

2.5 Sample Size Determination

Sample size was determined using the estimates of mean and standard deviation values from literature[11] and using the formula

$$n = \frac{2 (Z_{\alpha} + Z_{\beta})^2 \lceil s \rceil^2}{d^2}$$

where Z_{α} is the z variate of alpha error which is a constant with value 1.96, Z_{β} is the z variate of beta error which is a constant with value 0.84.[12]

Approximate estimates

1. 80% power
2. Type I error to be 5%
3. Type II error to be 20%
4. True difference of atleast 1.1 units between the groups
5. Pooled standard deviation of 1.68

Substituting the values,

$$n = \frac{2 (2.8)^2 [1.68]^2}{(1.1)^2}$$

n = 36.57

Taking into consideration the attrition to be 7%

$$n = N / (1-0.07)$$

$$n = \frac{37}{(1-0.07)}$$

= 39.78

Hence 40 subjects / patients per group were recruited in the present study.

2.6 Randomization Sequence Generation Allocation Concealment and Blinding

Each patient was randomly assigned using an internet generated block randomization sequence to obtain the same number of patients in each arm. Allocation of the participants of each group was concealed using the SNOSE (Sequentially numbered, opaque, sealed envelope) technique. The laminate tubes of the dentifrices procured were without any labels. Labelling was done by wrapping a paper which had letters A and B. Double blinding was done where both the primary investigator and subjects were not aware of the intervention.

2.7 Procurement of material and preparation of extract[13]

Branches of the barberry plant were collected and dried outdoors for three weeks. The degree of dehydration was verified by measuring the weight of the branches periodically. The branches were ground to a particle size of 1000 +/- 250 microns. A total of 200 g of ground was extracted using the reflux protocol using 700 ml of 96% ethanol over 24 hours using the soxhlet instrument. UV spectroscopy was used to standardise the extract at 340 nm on the basis of concentration of berberin of the primary plant alkaloid that consisted of 0.005% of total

dried plant weight. Five grams of the extract was triturated with 95 gm of gel base using mortar and pestle to obtain a 5% aqueous gel specimen. Aqueous solution of 5% polyvinyl alcohol was used as the gel base. The placebo gel contained the same base without the berberine extract. 15 gms of the samples were packed into aluminium tubes.

2.8 Intervention

Prior to the intervention, 5ml blood was collected from the patients to assess the HbA1c levels. The baseline plaque index and gingival index were recorded.

Once the patients were allocated to the groups, the patients were provided with the intervention gel and oral hygiene instructions. The patients were asked to apply a pea size quantity of the gel twice a day (morning and night) after brushing and rinse it after 5 minutes using water. The patients were asked to fill a compliance form and produce the record on the day of follow up one month, two months and six months interval.

2.9 Outcome Assessment

Plaque Index was assessed using the Loe and Silness technique. The teeth that were examined were maxillary right first molar, maxillary right lateral incisor, maxillary left first bicuspid, mandibular left first molar, mandibular left lateral incisor and mandibular right first bicuspid. The scoring was done as follows: When there was no plaque, a score of 1 was given. A film of plaque adhering to the free gingival margin and adjacent area of the tooth had a score of 2. The plaque may be seen in situ only after application of disclosing solution or by using the probe on the tooth surface. A score of 3 was given for moderate accumulation of soft deposits within the gingival pocket, or on the tooth and gingival margin which can be seen with the naked eye. A score of 4 was given for abundance of soft matter within the gingival pocket and/or on the tooth or the gingival margin.[14]

For the assessment of gingival bleeding index, the technique introduced by GBI Ainamo & Bay in 1976 was used. The gingival crevice was probed gently. A positive finding was recorded if bleeding occured in 10 seconds.[15]

The gingival index was assessed using the technique by Loe and Silness 1963 which had the following scoring criteria: A score of 0 was given for normal gingiva. Slight change in colour, slight edema and no bleeding on probing was considered as mild inflammation and a score of 1 was given. Redness, edema and glazing along with bleeding on probing was considered as moderate inflammation and had a score of 2. Severe inflammation was characterised as marked redness and edema, tendency to bleed spontaneously and ulceration. Severe inflammation had a score of 3. The gingival index score was obtained by adding the value for each tooth and dividing by the total number of teeth examined.[16]

For analysis of HbA1c levels, whole blood was collected in EDTA anticoagulant tubes and sent to the laboratory.

2.10 Statistical Analysis

Data obtained was compiled on a MS Office Excel Sheet (2016) and was subjected to statistical analysis using the Statistical package for social sciences (SPSS v 23.0, IBM). Descriptive statistics like frequencies and percentage for categorical data, Mean & SD for numerical data were calculated. Inter group comparison for demographic data was done using t test. Comparison of frequencies of categories of variables with groups was done using chi square test.

Normality of data was checked using Shapiro-Wilk test and it was found that data did not follow a normal curve hence non parametric tests were used. Inter group comparison was done Mann-Whitney U test and intra group comparison was done using Friedman's test (for more than two observations) followed by pairwise comparison using Wilcoxon Signed rank test.

For all the statistical tests, $p<0.05$ was considered to be statistically significant, keeping α error at 5% and β error at 20%, thus giving a power to the study as 80%.

RESULTS

Based on the results computed from the present study, it was observed that the highest values of PI, GBI and GI were observed at baseline and the lowest values were observed at two months of follow up for both the groups. This reduction in the values of the indices were statistically highly significant ($p<0.001$) (Table 1 and 2). Upon pairwise comparison of different time intervals, a highly significant reduction in the values of the indices was observed from baseline to one month, baseline to two months as well as from one month to two months of follow up for both the groups ($p<0.001$) except for the PI in group B which did not show a significant reduction from one month to two months ($p = 0.097$). The HbA1c levels showed a highly significant reduction from baseline to six months of follow up in both the groups ($p<0.001$) (Table 3).

An intergroup comparison was done to compare the efficacy of barberry extract gel to that of placebo gel and it was observed that at baseline, the PI and GBI values in both the groups did not show any statistically significant difference. However the GI values at baseline were significantly higher in group A than in group B. At one month's follow up, the PI and GBI values were significantly lower in group B than in group A. Although the GI values at one month's follow up were lower in group B than group A, the difference was not statistically significant. At two months follow up, there was no statistically significant difference observed for the PI and GI values between both the groups, however the GBI values were significantly lower in group B than in group A The HbA1c values recorded at six months of follow up were significantly lower in group B than in group A (Table 4).

Further, it was observed that group B showed a significantly higher reduction in PI values from baseline to one month whereas group A showed a significantly higher reduction in values from one month to two months of follow up. When analysing the overall reduction in PI values from baseline to two months, there was no significant difference in the performance of barberry extract gel and placebo gel. The reduction in GBI value from baseline to one month was significantly higher in group B whereas there was no significant difference observed in the reduction of gingival bleeding index values from one month to two months of follow up. However, the overall reduction in gingival bleeding index from baseline to two months significantly higher in group B than in group A. The comparison of reduction in GI values between different time intervals showed no statistically significant difference between both the groups. The reduction in HbA1c levels from baseline to six months of follow up was significantly higher in group B than in group A (Table 5).

TABLES

Table 1: Comparison of plaque index, gingival bleeding index and gingival index for group A (Placebo) at baseline, one month and two months of follow up. ** = highly statistically significant.

	N	Mean	Std. Deviation	Minimum	Maximum	Median	p value of Friedman Test
PLAQUE INDEX							
Baseline	40	2.502	.2202	2.1	2.9	2.485	0.000**
One month	40	2.334	.2917	1.5	3.0	2.345	
Two months	40	1.6852	.30916	1.04	2.80	1.6600	
GINGIVAL BLEEDING INDEX							
Baseline	40	13.70	1.728	10	17	14.00	0.000**
one month	40	12.03	1.349	8	14	12.00	
two months	40	9.70	1.757	6	14	9.00	
GINGIVAL INDEX							
Baseline	40	1.4365	.22174	1.08	2.12	1.4100	0.000**
One month	40	.8343	.26482	.33	1.39	.8800	
Two months	40	.623	.2410	.1	.9	.705	

Table 2: Comparison of plaque index, gingival bleeding index and gingival index for group B (Barberry) at baseline, one month and two months of follow up. ** = highly statistically significant.

	N	Mean	Std. Deviation	Minimum	Maximum	Median	p value of Friedman Test
PLAQUE INDEX							
Baseline	40	2.454	.2017	2.1	2.8	2.465	0.000**
One month	40	1.995	.2590	1.2	2.5	2.000	
Two months	40	1.8365	.51337	1.01	2.65	1.8350	
GINGIVAL BLEEDING INDEX							
Baseline	40	14.18	1.693	10	17	14.00	0.000**
One month	40	10.70	1.786	7	14	10.00	
Two months	40	8.75	1.149	6	12	9.00	
GINGIVAL INDEX							
Baseline	40	1.2700	.19622	1.00	1.79	1.2550	0.000**
One month	40	.7998	.22769	.36	1.25	.8400	
Two months	40	.533	.2467	.1	.9	.590	

Table 3: Pairwise comparison of the values of plaque index (PI), gingival bleeding index (GBI), gingival index (GI) and HbA1c levels between different time intervals for group A (placebo) and group B (Barberry) ** = highly statistically significant.

Time pairs	p value of Wilcoxon Signed Ranks Test
GROUP A	
PI one month - PI baseline	0.002**
PI two month - PI baseline	0.000**
PI two months - PI one month	0.000**
GBI one month - GBI baseline	0.000**
GBI two month - GBI baseline	0.000**
GBI two month - GBI one month	0.000**
GI one month - GI baseline	0.000**
GI two months - GI baseline	0.000**
GI two months - GI one month	0.000**
HbA1C After 6 months - HbA1C baseline	0.000**
GROUP B	
PI one month - PI baseline	0.000**
PI two months - PI baseline	0.000**
PI two months - PI one month	0.097#
GBI one month - GBI baseline	0.000**
GBI two months - GBI baseline	0.000**
GBI two months - GBI one month	0.000**
GI one month - GI baseline	0.000**
GI two months- GI baseline	0.000**
GI two months - GI one month	0.000**
HbA1C After six months - HbA1C baseline	0.000**

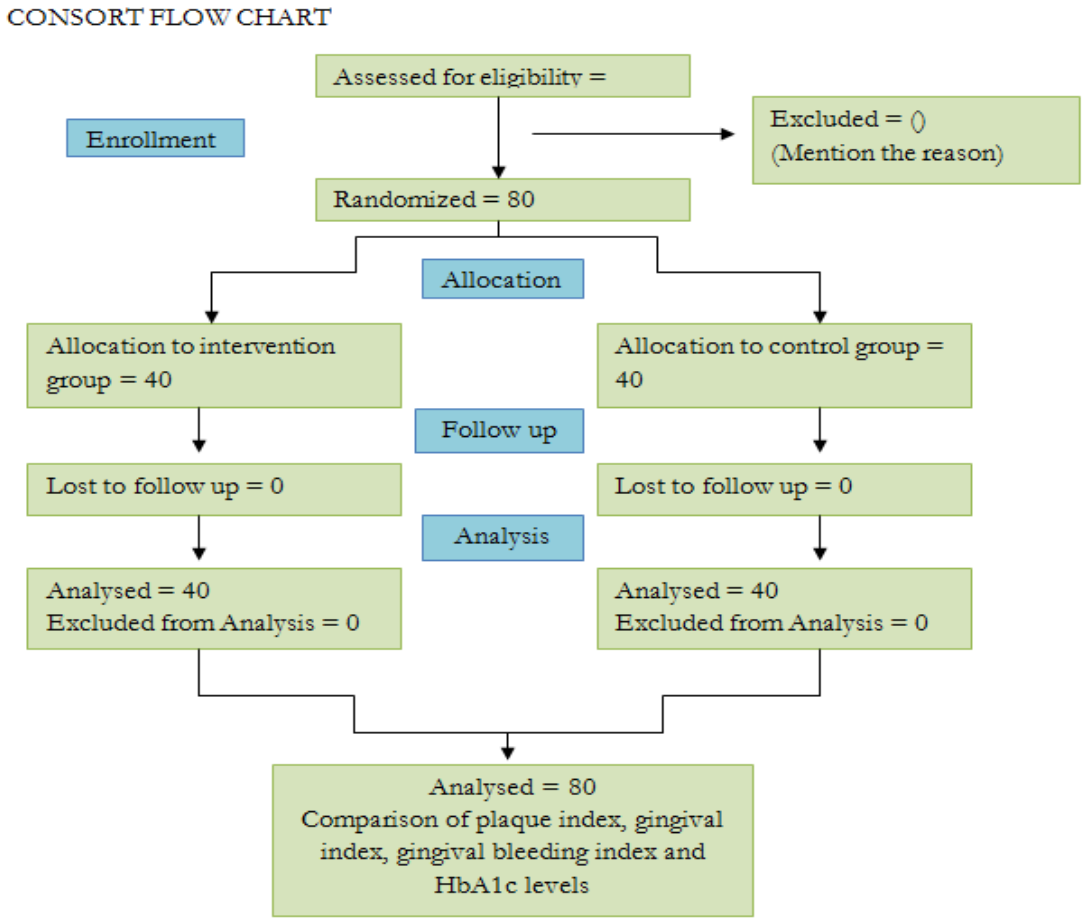
Table 4: Inter group comparison of plaque index (PI), gingival bleeding index (GBI) and gingival index (GI) between group A (placebo) and group B (Barberry) at baseline, one months and two months of follow up. ** = statistically highly significant # = no significant difference observed.

Group	Mean	Std. Deviation	Median	p value of Mann-Whitney U test
PI baseline	2.501	.2202	2.485	0.333#
A				
B	2.454	.2017	2.465	
PI one month	2.334	.2917	2.345	0.000**
A				
B	1.995	.2590	2.00	
PI two months	1.6852	.30916	1.660	0.229#
A				
B	1.8365	.51337	1.835	
GBI baseline	13.70	1.728	14.00	0.189#
A				
B	14.18	1.693	14.00	
GBI one month	12.03	1.349	12.00	0.001**
A				
B	10.70	1.786	10.00	
GBI two month	9.70	1.757	9.00	0.004**
A				
B	8.75	1.149	9.00	
GI baseline	1.4365	.22174	1.41	0.001**
A				
B	1.2700	.19622	1.255	
GI one month	.8342	.26482	0.880	0.444#
A				
B	.7997	.22769	0.840	
GI two months	.622	.2410	0.705	0.091#
A				
B	.533	.2467	0.590	

HbA1C baseline A B	11.90 11.65	1.842 1.622	12.30 11.45	0.630#
HbA1C After six months A B	10.587 9.550	1.6726 1.6002	10.35 9.15	0.008**

Table 5: Intergroup comparison of differences between the values of plaque index (PI), gingival bleeding index (GBI), gingival index (GI) from baseline (B) to one month (1M), one month to two months (2M) and baseline to two months and the difference between the values of HbA1c levels from baseline to six months (6M). Group A (placebo) and Group B (barberry). ** = statistically highly significant # = no significant difference observed.

Group	N	Mean	Std. Deviation	Median	p value of Mann-Whitney U test
PI B to 1M A B	40 40	.287 .489	.2037 .2814	0.250 0.485	0.001**
PI B to 2M A B	40 40	.8393 .6685	.33189 .46487	0.825 0.640	0.088#
PI 1M to 2M A B	40 40	.6813 .4702	.39224 .30482	0.630 0.440	0.017*
GBI B to 1M A B	40 40	1.93 3.73	1.492 2.501	1.500 3.00	0.001**
GBI B to 2M A B	40 40	4.00 5.43	2.207 1.907	4.000 6.00	0.002**
GBI 1M to 2M A B	40 40	2.68 2.55	1.639 1.535	2.50 2.00	0.814#
GI B to 1M A B	40 40	.6023 .4703	.29579 .19225	0.540 0.460	0.063#
GI B to 2M A B	40 40	.8140 .7368	.30724 .16029	0.710 0.740	0.547#
GI 1M to 2M A B	40 40	.2118 .2665	.14443 .17041	0.170 0.250	0.164#
Diff HbA1C A B	40 40	1.313 2.103	.8269 .7399	1.100 2.000	0.000**



DISCUSSION

The null hypothesis of the study was partly rejected since the barberry extract gel proved to be more effective than placebo gel in the reduction of HbA1c levels as well as in the reduction of gingival bleeding index values. Barberry extract gel was more effective than the placebo gel in the first month in reduction of plaque. However at two months the reduction did not show any significant difference. Both, barberry extract gel as well as placebo gel performed equally in reducing the gingival index values since no statistical significant difference was observed at follow up.

Previously Makaren et al demonstrated that the use of barberry dental gel brought about a 56% reduction in plaque and 33% improvement in gingival index in school children which was significantly higher than placebo gel and recommended its use as a dentifrice.[11] However, when compared to a placebo gel, Moeintaghav et al observed no difference in the gingival index and plaque index scores between the two groups of patients with periodontitis needing periodontal surgery. Upon histological examination at the time of surgery, they observed a reduction in the inflammatory cell infiltrates in the mucosa of those patients who received the barberry extract gel.[13] However the follow up periods in their study were shorter compared to that in the present study.

A previous randomised clinical trial demonstrated that consumption of barberry juice helped in reducing the blood pressure, fasting blood sugar, total cholesterol and triglycerides in patients with type 2 diabetes. The study also demonstrated an increase in Paraoxonase-1 (PON1) following the consumption of barberry juice which is an important antioxidant.[17] The consumption of barberry juice has been shown to lower the levels of glycated haemoglobin in type II diabetic patients.

A systematic review and meta analysis of randomised controlled trials concluded that barberry supplementation helped in improving insulin levels, however other glycaemic indices were not significantly affected.[19] In this study it has been demonstrated that topical application of a gel containing barberry extract helped in reducing HbA1c levels in patients with type II diabetes.

The isoquinoline alkaloids extracted from the root of barberry and stem bark are the principal constituents that are responsible for its therapeutic effects. Protoberberines (barberine, berbamine, jetorrhizine and palmatine) and bisbenzlisoquinolines (oxycanthine) are the two classes of alkaloids that haven been identified of which berberine is the main active component on which extensive research has been carried out.[20] Berberine has a broad antimicrobial activity and also blocks the adherence of group A streptococci to epithelial cells.

This extract has been previously used in either the crude or pure form to treat pyogenic infections.[21] A previous study has demonstrated the role of berbamine in reducing chronic inflammation by suppressing leukocyte infiltration.[22] It has been demonstrated that berberine is a macrophage activator which may contribute to its antimicrobial and antitumour actions.[23]

The reduction in gingivitis, plaque and HbA1c levels observed in the test group of the present study can be attributed to the anti inflammatory, antioxidant and immuno modulatory effect of the alkaloids present in the extract of barberry.

Justification of the use of placebo gel with oral hygiene instructions for the control group is based on the findings in literature that demonstrated oral hygiene instructions as an effective tool in maintaining good oral hygiene as well as in control of plaque and gingivitis.[24–26] The indices used for assessment of plaque and gingivitis are gold standard references and commonly used in studies on gingival inflammation in children and young adults. The gingival index by Ainamo & Bay 1976 is easy to interpret and insensitive to examiner difference.[27]

One of the drawbacks of a study where a participant is asked to carry out a procedure at home is that the clinical trial participants may experience some improvements that are not associated with the therapeutic properties of the test agent, but rather due to behavioural modifications as a consequence of participating in the trial. This is known as the Hawthron effect[28] which might have masked the efficacy of the test agent in comparison to that of the control such as that which was seen in relation to the gingival index and the plaque index levels at the second follow up period.

CONCLUSION

Within the limitations of the present study, it can be concluded that application of barberry extract gel reduced the levels of plaque and gingivitis in patients with type II diabetes. In comparison to placebo, the significant difference in the better performance of barberry was seen with respect to gingival bleeding index and one month follow up for plaque index. Barberry was highly effective in reducing the HbA1c levels as compared to placebo. Application of a dental gel containing the extract of barberry might prove to be highly beneficial in type 2 diabetic patients in controlling both periodontitis as well as glycaemic index.

However, further studies are required to standardise the concentration and methods to commercially incorporate the extract into a dental gel.

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