

Bilberry Extracts As An Adjunct To Scaling And Root Planing.

Aakriti Sharma *, Shivjot Chhina ** Sanjeev Sharma***

*Post graduate , **Professor & Head , Department of Periodontics, I.T.S. Dental College, ***Private practitioner, Greater Noida.

Abstract: Background: Anthocyanins are antioxidant compounds which reduces lipid peroxidation and deleterious effects of ROS in vitro. The anthocyanins of bilberry can help reduce the risk of unwanted inflammation of gums. Thus, study was done to compare clinical soft tissue parameters around periodontal pockets treated with & without bilberry extracts as an adjunct to scaling & root planing. Materials & Method: 10 patients having bilateral localized periodontal pockets of 4-6 mm were chosen. Each site was randomly allocated to either group 1(test) or group 2(control), where in group 1, scaling & root planing was done while in group 2 , bilberry extracts in situ gel was delivered as an adjunct to SRP .Following parameters were recorded at baseline & after six weeks : PD, RAL, PI & GBI in both the groups. These parameters were subjected to statistical analysis to evaluate the effect of bilberry extracts, paired t test was done. Results: There was a statistically significant decrease in PD & gain in RAL at sites treated with bilberry extracts in situ gel delivered as adjunct to SRP. Conclusion: The treatment was effective in the treated patients & it was concluded that bilberry is a valuable drug clinically applicable in improving the periodontal health condition. [Sharma A Natl J Integr Res Med, 2020; 11(1):79-84]

Key Words: Bilberry extracts, Periodontal pockets

Author for correspondence: Dr. Aakriti Sharma, MDS, Department of Periodontics, I.T.S. Dental College, Hospital and Research Centre, Knowledge Park III ; Greater Noida, Uttar Pradesh ,India-201308. E-Mail: aakriti8911@gmail.com Mobile: +919811533651.

Introduction Periodontal Disease affecting the structure surrounding teeth results in inflammation initiated by bacterial aggregation & alteration in their profile .¹Inflammation is a complex series of reactions executed by the host to prevent tissue damage, activating repair processes and defense mechanisms against infectious diseases.²⁻³Inflammatory injury may be mediated by reactive oxygen species (ROS)⁴ or its reaction products and antioxidant therapy has been shown to prevent in vivo tissue injury during inflammation.^{5,6} Anthocyanins ,water-soluble red and blue flavonoid pigments are effective antioxidant compounds able to reduce lipid peroxidation and the deleterious effects of ROS in vitro. In this regard, the anthocyanins of bilberry can help reduce the risk of unwanted inflammation.^{7,8} Thus the present study aimed to assess the effect of bilberry extracts on periodontal pockets in adjunct to scaling & root planing

Materials and Method: Ten systemically healthy patients reported to the Deptt. of Periodontics ITS dental college of age group 20-55 years with twenty or more natural teeth, bilaterally symmetrical occlusion, having bilateral localized suprabony periodontal pockets of 4-6 mm were chosen for the study . Pregnant and lactating women ,patients with systemic conditions like Diabetes, hypertension, atherosclerosis etc., deleterious habits like alcohol consumption , tobacco ,smoking, and those suffering from aggressive periodontitis, or who have received

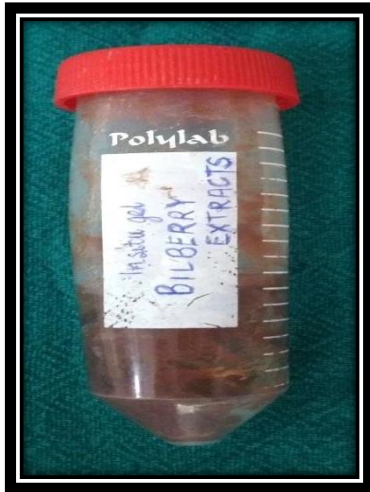
any surgical or nonsurgical periodontal therapy in past 6 months and antimicrobial therapy in past 3 months were excluded. Verbal and written informed consent was taken from each patient after explaining the procedure in patient's language along with the potential risks and benefits involved. All the patients completed the study with no fall out. The study protocol had necessary ethical committee approval and is registered with IEC Ref.No. Director-PG Studies/ITSCDSR/L/ 2018/100

Preparation of Bilberry extract gel.: 100 mg of bilberry extracts (Bilberry extracts ,HealthVit) were dissolved in 50 ml of distilled water. 50 mg of carbopol 934 was added to the solvent blend to obtain the concentration of 2%, agitated for 20 min. The dispersion was then allowed to hydrate and swell for 60 min, and finally the pH was adjusted with 0.12 ml of 98% triethanolamine (TEA) until the desired pH value was approximately reached (6.8 -7). During pH adjustment, the mixture was stirred gently with a spatula until a homogeneous gel was prepared .7.5 mg of methyl paraben and 2.5 mg of propylparaben was dissolved in 0.5 ml of ethanol as preservative. The gel was autoclaved for 30 minutes at 121 degree C. (**fig.1**)

Participants were divided into a control & test group by random sampling. After scaling and root planing ,in test sites (group 1) 1ml of bilberry extracts *in situ* gel was delivered subgingivally into the periodontal pocket with the help of a

blunt cannula and syringe in a gentle probing manner, attempting to fill the full extent of the pocket.(fig.2)

Figure 1. Prepared in-situ Bilberry gel



The gel was applied up to the gingival margin and the excess gel was removed with a sterile gauze ,after which periodontal dressing was given for seven days while in control sites (group 2) after SRP, periodontal pocket was allowed to heal with secondary intention .Both the sites were barred from probing for next 6 weeks.

Figure 2: 1 ml Bilberry extract gel carried in syringe and is locally delivered to the periodontal pocket.



After placement of the gel *in situ*, patients were instructed to follow strict oral hygiene protocol but were discouraged from using any interdental cleaning aids for 1 week after removal of periodontal dressing . They were also asked not to chew hard or sticky foods at the sites where gel is placed. All patients were recalled for follow-up measurements at 6 weeks intervals. Following parameters were recorded at baseline & after six weeks in both the groups : plaque index (Silness & Loe 1964)⁹,gingival index (Loe & Silness

1963)¹⁰, pocket probing depth , relative attachment level RAL (fig.3 &4)

Figure 3.PRE-OP RAL-8mm .



Figure 4.AFTER 6 WEEKS RAL-6mm



Statistical Analysis: Statistical analysis of the data using Statistical Package for the Social Sciences (SPSS) software 16 was performed . To test the mean changes in scores at different time points within each study group Student's T test was employed . Levene's test for equality of variances followed by t-test for Equality of Means were used to compare the mean scores between different study groups. $P < 0.05$ was considered as the level of significance in this study.

Results: This split mouth study was conducted on 10 subjects out of which 6 were females (60%) and 4 were males (40%) with the mean age group of 39.50 ± 9.07 years with bilateral localized periodontal pockets treated with scaling and root planing in each patient and bilberry extracts in one of the pockets, the sites were randomly assigned either to test or control groups.

Bilberry Extracts As An Adjunct To Scaling And Root Planing

Table 1. shows inter and intragroup comparisons of means scores of all soft tissue parameters in test and control groups at baseline and after 6 weeks.

Table 1: Inter and Intra group comparisons of test and control groups at baseline and 6 weeks

	Group	N	Mean	Std. Deviation	Std. Error Mean
Pre-op MPPD	Test	10	4.800	.6325	.2000
	Control	10	4.400	.6992	.2211
Pre-op RAL	Test	10	11.200	1.2293	.3887
	Control	10	11.400	1.1738	.3712
Pre-op GI	Test	10	1.000	.2309	.0730

Pre-op PI	Control	10	.960	.2757	.0872
	Test	10	1.120	.4158	.1315
GI after 6 week	Control	10	1.150	.3749	.1186
	Test	10	.420	.2936	.0929
PI after 6 week	Control	10	.520	.1687	.0533
	Test	10	.570	.3802	.1202
MPPD after 6 week	Control	10	.670	.3093	.0978
	Test	10	2.800	.4216	.1333
RAL after 6 week	Control	10	3.900	.5676	.1795
	Test	10	9.200	1.3166	.4163
	Control	10	10.900	.9944	.3145

Table 2: Intra group comparison of mean scores for Test group at baseline and 6 weeks.

		Paired Differences					t	P value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
					Lower	Upper		
Pair 1	Pre-op MPPD - MPPD after 6 week	2.0000	.6667	.2108	1.5231	2.4769	9.487	.000
Pair 2	Pre-op RAL - RAL after 6 week	2.0000	.6667	.2108	1.5231	2.4769	9.487	.000
Pair 3	Pre-op GI - GI after 6 week	.5800	.2700	.0854	.3869	.7731	6.794	.000
Pair 4	Pre-op PI - PI after 6 week	.5500	.5401	.1708	.1637	.9363	3.220	.010

Table 3: Intra group comparison of mean scores for Control group at baseline and 6 weeks.

		Paired Differences					t	P value
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference			
					Lower	Upper		
Pair 1	Pre-op MPPD - MPPD after 6 week	.5000	.5270	.1667	.1230	.8770	3.000	.015
Pair 2	Pre-op RAL - RAL after 6 week	.5000	.5270	.1667	.1230	.8770	3.000	.015
Pair 3	Pre-op GI - GI after 6 week	.4400	.2875	.0909	.2343	.6457	4.839	.001
Pair 4	Pre-op PI - PI after 6 week	.4800	.3615	.1143	.2214	.7386	4.199	.002

Data analysis demonstrated that the change in all the soft tissue parameters over the study period differed significantly between baseline and six week study endpoint in both groups (**Table 2 & 3**) with reduction in GI,PI,MPPD & RAL in both the

groups. However, on comparisons of means of both the groups, there was statistically significant difference reported in PD and RAL in test group as compared to control group, depicted in **Table 4**.

Table-4 :Comparison of means for test and control group at baseline and 6 weeks.

	Levene's Test for Equality of Variances							
	F	Sig.	t	P value	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
							Lower	Upper
Pre-op MPPD	.618	.442	1.342	.196	.4000	.2981	-.2264	1.0264
Pre-op RAL	.310	.585	-.372	.714	-.2000	.5375	-1.3292	.9292
Pre-op GI	.397	.536	.352	.729	.0400	.1137	-.1989	.2789
Pre-op PI	.298	.592	-.169	.867	-.0300	.1770	-.4020	.3420
GI after 6 week	1.900	.185	-.934	.363	-.1000	.1071	-.3250	.1250
PI after 6 week	.092	.765	-.645	.527	-.1000	.1550	-.4256	.2256
MPPD after 6 week	.066	.800	-4.919	.000	-1.1000	.2236	-1.5698	-.6302
RAL after 6 week	1.084	.312	-3.258	.004	-1.7000	.5217	-2.7962	-.6038

Discussion: Berries are rich in anthocyanins, water-soluble red and blue flavonoid pigments which are effective antioxidant compounds capable of reducing lipid peroxidation and the deleterious effects of ROS^{11,12}. Anthocyanins repair and protect genomic DNA integrity. Bilberries (*Vaccinium myrtillus*) contain diverse anthocyanins, like delphinidin and cyanidin glycosides bioactive molecule that improves inflammatory conditions thereby improving gingival health conditions¹³.

The transcription factor nuclear factor-kB (NF-kB) controls expression of genes involved in the inflammatory response^{14,4}, and is activated by oxidative stress and numerous other proinflammatory stimuli. Elevated levels of pro-inflammatory cytokines (IL-1, IL-12, TNF etc.) and acute phase proteins have been associated with increased risk of disease and poor outcome of chronic inflammatory diseases^{15,16}. Thus, dampening NF-kB activation, thereby limiting the inflammatory response, has been suggested as a strategy to prevent chronic periodontal inflammatory disease.

Widen et al¹⁷ observed dose dependent significant reduction in cytokine levels in the group that consumed 500 g of bilberries/day. A statistically significant reduction was observed for IL-1β, IL-6 and VEGF in GCF samples in the group that consumed 500 g of bilberries daily attributing to the anti-inflammatory effects.

Thus the following study was undertaken hypothesizing that bilberry anthocyanins may inhibit NF-kB activation reducing levels of pro-inflammatory cytokines and can help reducing risk of periodontal inflammation. The study used a novel approach in treating localized periodontal pockets by administering an in situ gel prepared from bilberry extracts capsules locally within the pocket in adjunct to conventional non-surgical periodontal treatment (SRP) and found statistically significant improvement in PD and RAL after six weeks as compared to the control group.

There was a relative decrease in PI & GI (thereby BOP) scores in both test and control groups from baseline to 6 weeks but it failed to reach statistical significance. This slight decrease could be attributed to the Hawthorne effect where study individuals devote greater attention to a given task due to the attention being focused on them. Though the results were in disagreement with the study done by Widen et al¹⁷, where the statistical reduction in BOP before and after consumption of raw bilberries over 1 week was 41% and 59% in the groups that consumed either 250 or 500 g of bilberries/day respectively, which was comparable to the standard of care reference group receiving debridement and oral hygiene instructions where BOP reduced by 58%, thus limiting use of bilberries for systemic use for periodontal treatment. However, further studies with larger sample size and longer follow up are

required to confirm the results of the present and related studies.

Conclusion: Anthocyanin bioactive molecules like delphinidin and cyaniding glycosides from Bilberry extract improves inflammatory conditions thus offers new choice of therapy as an adjunct to mechanical debridement, in chronic periodontitis patient with moderate to deep periodontitis, and can be used at a concentration of 2% as local drug delivery agent as an adjunct to scaling and root planning improving gingival health conditions.

References:

1. Shifrovitch Y, Binderman I, Bahar H, Berdicevsky I, Zilberman M. Metronidazole-loaded bioabsorbable films as local antibacterial treatment of infected periodontal pockets. *J Periodontol* 2009;80:330-337.
2. Nathan C. Points of control in inflammation. *Nature*. 2002;420: 846–52.
3. Vakkila J, Lotze MT. Inflammation and necrosis promote tumour growth. *Nat Rev Immunol*. 2004;4:641–8.
4. Barnes PJ, Karin M. Nuclear factor-kB: a pivotal transcription factor in chronic inflammatory diseases. *N Engl J Med*. 1997;336:1066–71.
5. Cuzzocrea S, Riley DP, Caputi AP, Salvemini D. Antioxidant therapy: a new pharmacological approach in shock, inflammation, and ischemia/ reperfusion injury. *Pharmacol Rev*. 2001;53:135–59.
6. Cuzzocrea S, Thiemermann C, Salvemini D. Potential therapeutic effect of antioxidant therapy in shock and inflammation. *Curr Med Chem*. 2004;11:1147–62.
7. Meiers S, Kemeny M, Weyand U, Gastpar R, von Angerer E, Marko D. The anthocyanidins cyanidin and delphinidin are potent inhibitors of the epidermal growth-factor receptor. *J Agric Food Chem*. 2001;49: 958–62.
8. Bomser J, Madhavi DL, Singletary K, Smith MA. In vitro anticancer activity of fruit extracts from *Vaccinium* species. *Planta Med*. 1996; 62:212–6.
9. Silness J, Loe H: Periodontal disease in pregnancy. II. Correlation between oral hygiene and periodontal condition. *Acta Odontol Scand*. 1984, 22: 121-135.

10. Loe H & Silness, J. (1963) Periodontal disease in pregnancy. *Acta Odontol Scand* 1963;21:533-551.
11. Meiers S, Kemeny M, Weyand U, Gastpar R, von Angerer E, Marko D. The anthocyanidins cyanidin and delphinidin are potent inhibitors of the epidermal growth-factor receptor. *J Agric Food Chem*. 2001;49: 958–62.
12. Bomser J, Madhavi DL, Singletary K, Smith MA. In vitro anticancer activity of fruit extracts from *Vaccinium* species. *Planta Med*. 1996; 62:212–6.
13. Kolehmainen, M.; Mykkanen, O.; Kirjavainen, P.V.; Leppanen, T.; Moilanen, E.; Adriaens, M.; Laaksonen, D.E.; Hallikainen, M.; Puupponen-Pimia, R.; Pulkkinen, L.; et al. Bilberries reduce low-grade inflammation in individuals with features of metabolic syndrome. *Mol. Nutr. Food Res*. 2012, 56, 1501–1510.
14. Schreck R, Albermann K, Baeuerle PA. Nuclear factor k B: an oxidative stress-responsive transcription factor of eukaryotic cells (a review). *Free Radic Res Commun*. 1992;17:221–37.
15. Libby P. Inflammation in atherosclerosis. *Nature*. 2002;420:868–74.
16. Boos CJ, Lip GY. Is hypertension an inflammatory process? *Curr Pharm Des*. 2006;12:1623–35.
17. Widén C, Coleman M, Critén S, Andersson PK, Renvert S, Rutger Persson GR. Consumption of Bilberries Controls Gingival Inflammation. *Int. J. Mol. Sci*. 2015; 16:10665-10673.

Conflict of interest: None
Funding: None
Cite this Article as: Sharma A, Chhina S, Sharma S. Bilberry Extracts As An Adjunct To Scaling And Root Planing. <i>Natl J Integr Res Me</i> 2020; Vol.11(1):79-84

