Original Article

Clinical and biochemical effects of dark chocolate in moderate chronic periodontitis

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Abstract

Introduction: Antioxidant agents such as cocoa could have some benefits in treatment of inflammation including periodontitis. The aim of this study was to investigate the effects of cocoa consumption on moderate chronic periodontitis.

Materials &Methods: This single-blind randomized clinical trial study was performed on 40 subjects who were randomly divided into two groups. Treatment group received 30 gr dark chocolate (78% cocoa) and control group received 22.5 gr white chocolate three times a day for 4 weeks. Saliva samples were collected from patients at baseline and twenty-eight days after eating chocolate. Probing pocket depth (PPD), Gingival index (GI, Silness and Loe), Modified papillary bleeding index (MPBI, Barnett), Clinical attachment loss (CAL) were recorded at baseline and 2nd, 4th, 6th, 8th weeks later in ramfjord teeth. Total antioxidant capacity (TAC) and lipid peroxidation of saliva were estimated by Ferric reducing antioxidant power (FRAP) and Tiuborbituric acid reactive substances (TBARS) methods. Data of clinical parameters were analyzed using t-test and repeated measures test. Biochemical parameters were analyzed using t-test.

Results: Intra-group comparison of clinical parameters demonstrated significant decrease in both groups ($p<0.0001$) and inter-group comparison showed significant decrease of MPBI in treatment group, ($p<0.03$). MPBI and GI were significantly decreased in treatment group compared to the control in the weeks of 4th, 6th and 8th, according t-test (GI4, P=0.008-GI6, P=0.008-GI8, P=0.009), (MPBI4, MPBI6, MPBI8, $P<0.0001$). Treatment group showed the increase in FRAP, ($p<0.00001$) and decrease in TBARS, ($P<0.015$) which were statically significant in compare with control group.

Conclusion: Consuming dark chocolate could increase TAC and decrease lipid peroxidation, gingival bleeding and inflammation.

Keywords: Chronic periodontitis, Cacao, Polyphenols, Antioxidants, Saliva, Lipid peroxidation, Clinical trials, randomized

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اثر کلینیکی و بیوشیمیایی شکلات تلخ بر پریودنتیت مزمن

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چکیده

مقدمه: ترکیبات آنتی اکسیدان از جمله کاکائو می توانند فوایدی در درمان التهاب از جمله پریودنتیت داشته باشند. هدف از این مطالعه، بررسی تاثیر مصرف کاکائو در پریودنتیت مزمن متوسط می باشد.

مواد و روش ها: یک کار آزمایی بالینی یک سویه کور بر روی ۴۰ بیمار صورت گرفته است که به صورت تصادفی به دو گروه ۲۰ نفره تقسیم گردیدند. گروه درمان ۳۰ گرم شکلات تلخ (۷۸٪ کاکائو) و گروه کنترل ۲۲/۵ گرم شکلات سفید (بدون کاکائو)، سه بار در روز، به مدت چهار هفته دریافت نمود. نمونه های بزاق در زمان شروع مطالعه و بیست و هشتمین روز پس از شروع مصرف شکلات از بیماران جمع آوری گردید. عمق پرویینگ پاکت، شاخص لثه ای(Loe and Silness)، شاخص تغییر یافته خونریزی پاپیلاری (Barnett) و میزان از دست رفتن چسبندگی کلینیکی در زمان شروع مطالعه، هفته های دوم، چهارم، ششم و هشتم پس از آن در دندانهای رمفورد اندازه گیری شد. ظرفیت تام آنتی اکسیدان و اکسیداسیون لیپیدی بزاق توسط روش Ferric reducing antioxidant power (FRAP) و Tiuborbituric acid reactive substances (TBARS) اندازه گیری شد. یافته های حاصل از پارامتر های کلینیکی با آزمون های t-test و Repeated measures test و یافته های حاصل از پارامترهای بیوشیمیایی با آزمون t-test آنالیز شده و مورد مقایسه قرار گرفته است.

یافته ها: مقایسه داخل گروهی پارامتر های کلینیکی در هر دو گروه از لحاظ آماری معنادار بود ($P<0.0001$) در مقایسه ی بین گروهی پارامتر های کلینیکی شاخص تغییر یافته خونریزی پاپیلاری در گروه درمان کاهش معنی دار از لحاظ آماری نشان داده است ($P<0.03$) تغییرات شاخص لثه ای و شاخص تغییر یافته خونریزی پاپیلاری در هفته های چهارم ($P<0.008$)، ششم ($P<0.008$) و هشتم از لحاظ آماری در گروه درمان معنی دار بوده است. گروه درمان در مقایسه با گروه کنترل افزایش معناداری در ظرفیت تام آنتی اکسیدان ($P<0.00001$) و کاهش معناداری در اکسیداسیون لیپیدی ($P<0.015$) بزاق نشان داده است.

نتیجه گیری: مصرف شکلات تلخ منجر به افزایش ظرفیت تام آنتی اکسیدان بزاق، کاهش اکسیداسیون لیپیدی بزاق و کاهش التهاب و خونریزی لثه ای می گردد.

واژگان کلیدی: پریودنتیت مزمن، کاکائو، پلی فنول، آنتی اکسیدان ها، بزاق، پراکسیداسیون لیپیدی، کار آزمایی بالینی، تصادفی

Introduction

Chronic periodontitis is a common inflammatory disease, which affects tooth-supporting tissue and is the major cause of tooth loss in adults. [1,2] The etiology of the disease is commonly related to the colonization of complex anaerobic (geram-negative) bacteria. [3] One of the mechanism distributions of periodontitis is production of reactive oxygen species (ROS). [4] For treatment of periodontal disease, mechanical approaches like scaling and root planning (SRP) and surgery have been widely used. Nowadays antibiotics and Non-steroidal anti-inflammatory drugs have been used as medicament beside the mechanical procedures. [5,6] Antioxidants agents could have some benefits in suppression of ROS production. There are some investigations which show that antioxidant agents absorb locally, systemically, in daily diet and in conjunction with dentifrices inhibits oxidative destruction of periodontal disease. [7] Lately, cocoa due to its polyphenol flavonoids content has became a material of interest as a therapeutic agent. [8] Cocoa is called the cocoa beans from the plant of Theobroma cacao. [9] Catechin and procyanidius are the main flavonoids in cocoa beans. [10, 11] More than 35% of chatechins are (-)-epicathechin. While (+)-chatechin, (+)- gallocatechin and (-)-epigallocate are the other compounds with lower amounts. [9,12,13] Some of studies investigated probable positive effects of cocoa on human health. The results show that cocoa may be used as antioxidant, ant-inflammatory, anti-carcinogenic, anti-cariogenic, anti-bacteria and anti-virus agent. [12-16] In addition, cocoa has beneficial effects on cardiovascular, immune and neural system and skin. [11,17-21] Tomofuji et al. showed that cocoa-enriched diet reduced the oxidative stress-induced periodontitis. [8] Mao et al. suggested that consumption of cocoa and dark chocolate could reduce the risk of

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periodontal disease via regulation of cytokine secretion.[12] Different studies pointed out the relationship between total antioxidant capacities (TAC), lipid peroxidation of saliva and periodontal status. [22-24] So, the aim of this study was to investigate the effects of dark chocolate consumption on these factors. Furthermore, the effects of dark chocolate consumption on improvement of periodontal clinical parameters have been evaluated.

Methods

Case selection: A single-blind randomized clinical trial study was performed on 40 subjects referred to department of periodontology, Faculty of Dentistry, Babol University of Medical Science .This investigation was approved by the Ethics Committee of Babol University of Medical Science and it was registered in Iranian Registry of Clinical Trials with IDNo:IRCT201309303813N3. Written informed consent form was signed by each patients that its included criteria were as follows: subjects who were able to cooperate with similar plaque index (silness and loe), having chronic moderate periodontitis, the age of 30 to 50 years old, average of attachment loss from 3 to 6mm, subjects without history of systemic disease, of sensitivity to cocoa, of its productive and digestive problems.

Excluding criteria were as follows: subjects who received NSAIDs and antibiotics during last one month, nutritional and vitamin supplements, cocoa and its products used in their daily nutritional regiment, subjects who smoked, were under periodontal treatment during past 6 months and menopause women.

Intervention: All patients received phase I periodontal treatment consist of oral hygiene instruction and SRP. In the next session, the patients with less than 30% plaque index participated in the current study. The patients were randomly divided into two groups. The first group and the second group received 30 gr dark chocolate 78% cocoa (Aidin Company, Tabriz, Iran) (table 1) and 22.5 gr white chocolate without cocoa (Aidin Compay, Tabriz, Iran) (table 2) three times a day for 4 weeks, respectively. Chocolates were packed in encoded pockets. Furthermore, each patient was given a daily food diary program including the list of antioxidant ingredients to determine the consumption of other antioxidants during the first 4 weeks.

Table1. Nutritive value of dark chocolate

Nutritional information of dark chocolate	100gr
Energy value	507K cal
protein	8.54gr
Fat	31.8gr
Carbohydrate	46.75gr
Calcium	345.9mgr
Sodium	44.76mgr

Table2. Nutritive value of white chocolate

Nutritional information of white chocolate	100gr
Energy value	580K cal
protein	12.5gr
Fat	38gr
Carbohydrate	43gr
Calcium	-
Sodium	840mgr

Clinical parameters measurement: Probing pocket depth (PPD), gingival index (GI, Silness and Loe), modified papillary bleeding index (MPBI, Barnett) and clinical attachment loss (CAL) were recorded at baseline and 2,4,6,8 weeks later in ramfjord teeth.

Saliva sample collection: Saliva samples were collected from patients at baseline and on 28th day of study. Patients rinsed their mouth with water for about one minute and then oral cavity was examined to insure there is not any debris or blood. Unstimulated saliva (2-3ml) was collected by using spitting. During saliva collection, patients seated comfortably and spitted saliva during 10 min. The saliva samples were collected and stored at -20 °C freezer.

Biochemical parameters measurement

Ferric reducing antioxidant power (FRAP) assay: FRAP test was used to determine TAC of saliva. The method estimates the ferric reducing ability in the presence of antioxidants. [25] FRAP reagent contains TPTZ (2, 4, 6-tripyridyl-s-triazine; sigma USA) 10 mM in HCl (40mM); $FeCl_3$ (20 mM) and buffer acetate 0.3 M (PH, 3.6) in the ratio of 1:1:10; the reagent was newly provided and warmed for 5 min at 37°C. The working FRAP reagent (1.5 ml) was mixed with 50µL of saliva. After 10 min at 37°C, the absorbance value was measured at 593 nm and compared with the standard value. $FeSO_4$ (125, 250, 500, and 1000µM) was assumed as the standard solution based on standard curve.

Tiuborbituric acid reactive substances (TBARS) assay: TBARS test was used to estimate

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Malondialdehyde of saliva that byproduct of lipid peroxidation.[26] Working TBARS reagent consisted of Trichloroacetic acid, (15%W/V, Merck Germany), TBA(375%W/V, Sigma USA) and HCl 0/25 M (Sigma USA) was prepared. Working TBARS reagent (2 mL) was mixed whit of saliva (1 mL), warmed for 15 min at 100 °C and then centrifuged at 1500 rpm for 10 min. Finally, the absorbance value was detected at 535 nm versus with the blank absorbance concentration calculated by Beer-Lambert method.[26]

Statistical analysis: Data of clinical parameters were analyzed using t-test and repeated measures tests. Biochemical parameters were analyzed using t-test. The statistical difference was significant at $p<0.05$.

Results

Forty patients participated in the present study and were divided into two groups. Treatment group (12 women and 8 men, age 38.85±5.35) received dark chocolate and control group (14 women and 6 men, age 39.85±6.16) received white chocolate. Intra-group comparison of PPD, GI, MPBI, CAL demonstrated significantly decrease in both groups ($p<0.0001$) and inter-group comparison showed significant decrease of MPBI in test group ($p<0.03$). MPBI and GI were significantly decreased in treatment group compared to the control in the 4th, 6th and 8th weeks of study, according to the t-test. (GI4, P=0.008-GI6, P=0.008-GI8, P=0.009), MPB14, MPBI6, MPBI8, P<0.0001), (table 3). Mean difference of FRAP from baseline to 4th week was 175.71±26.52µM in treatment group and 14.21±17.0µM in control group. Mean difference of TBARS from baseline to 4th week was 0.11±0.01µM in test group and 0.05±0.01µM in control group (figure 1), (figure 2). Treatment group showed the increase in FRAP ($p<0.00001$) and decrease in TBARS ($P<0.015$) were statically significant in compare with control group.

Table 3. Mean ±(SD) of clinical parameters at baseline and in the 4th ,6th and 8th weeks

Clinical parameters		Base line	week 2	week 4	week 6	week 8
PPD	Treatment group	2.54±0.34	2.46±0.36	2.35±0.38	2.31±0.39	2.29±0.40
	Control group	2.48±0.37	2.45±0.36	2.41±0.35	2.40±0.37	2.38±0.36
	P. Value	NS*	NS	NS	NS	NS
GI	Treatment group	1.52±0.29	1.29±0.28	1±0.29	0.90±0.30	0.87±0.28
	Control group	1.50±0.29	1.39±0.28	1.26±0.28	1.17±0.28	1.12±0.27
	P. Value	NS	NS	P=0.008	P=0.008	P=0.009
MPBI	Treatment group	1.70±0.25	1.39±0.26	0.99±0.30	0.84±0.32	0.72±0.32
	Control group	1.64±0.26	1.49±0.25	1.29±0.25	1.17±0.23	1.06±0.25
	P. Value	NS	NS	P<0.001	P<0.001	P<0.001
CAL	Treatment group	3.55±0.35	3.49±0.35	3.36±0.37	3.36±0.36	3.35±0.37
	Control group	3.44±0.23	3.42±0.23	3.37±0.25	3.32±0.25	3.31±0.25
	P. Value	NS	NS	NS	NS	NS

NS*: Not Significant

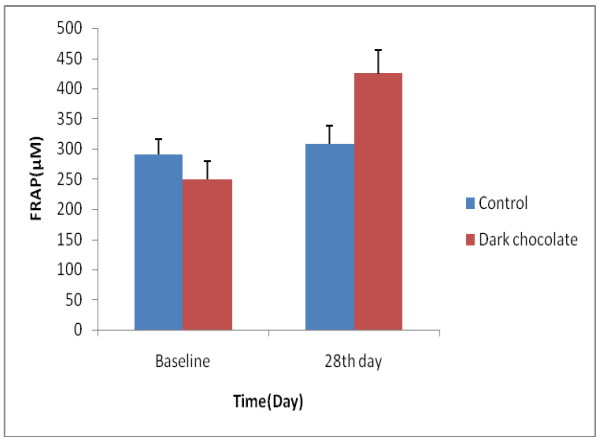


Figure1. FRAP mean±(SEM) at baseline and on 28th day of study in treatment and test groups (The number of subjects in each group was 20)

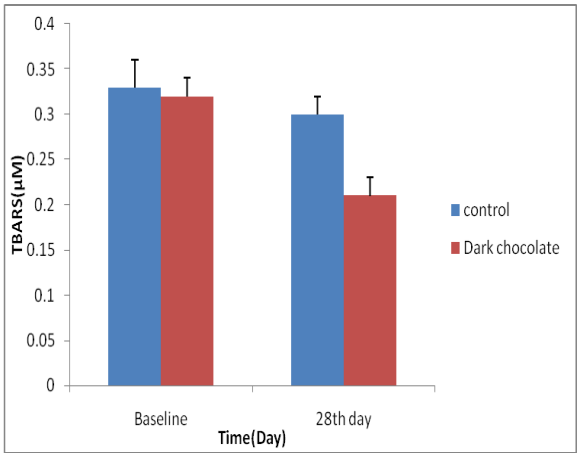


Figure2. TBARS mean±(SEM) at baseline and on 28th day of study in treatment and test groups (The number of subjects in each group was 20)

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Discussion

According to the present study, the daily consumption of 30 gr dark chocolate (78% cocoa) for 4 weeks compared to consumed 22.5 gr white chocolate could significantly increase TAC and decrease lipid peroxidation of saliva. These results may be due to the phenolic compounds of dark chocolate that works as antioxidants. There is statically significant decrease in PPD, MPBI, GI and CAL in both groups during all study periods that shows the positive effects of oral hygiene instruction and SRP on improvement of chronic moderate periodontitis. Comparing the data of clinical parameters between two groups exhibited consumption of dark chocolate caused significant decrease of gingival inflammation and bleeding. This effect is not significant in PPD and CAL that may be due to inadequate dosage and duration of cocoa consumption. Inflammation and bleeding are the first signs of periodontal disease and according to these results cocoa as an anti-inflammatory compound can decrease them.

Although similar study evaluated the effect of cocoa on TAC and lipid peroxidation in saliva was not found, there were some studies evaluated the effect of cocoa on these two factors in serum or plasma. Rein et al. [27] and Wang et al. [28] indicated that chocolate consumption was effective on the increase and decrease of plasma antioxidant capacity and plasma lipid peroxidation, respectively. In addition, the studies of Wan et al. [29] and Serafini et al. [30] displayed cocoa consumption could increase serum TAC. Mursu et al. [31] showed that long-term (3 weeks) use of dark chocolate had positive effects on one of the markers of lipid peroxidation. Fraga et al. [32] revealed that chocolate consumption decrease plasma level of MDA. Herman et al. [33] suggested that dark chocolate consumption increased FRAP in plasma. All of these findings were the same as those of present study. On the other hand, some of investigations demonstrated different results. Osakabe et al. [34] pointed out the daily consumption of 36 gr cocoa powder for 2 weeks by 15 subjects showed no significant changes in plasma level of lipid or antioxidants. Furthermore, Mathur et al. [35] demonstrated that the daily consumption of cocoa products for 6 weeks had not influenced on plasma antioxidant capacity. The research of Engler et al. [36] indicated that the daily consumption of 46 gr dark chocolate for 2 weeks could not change plasma TAC. Vlachopoulos et al. [37] research determined single dosage (100 gr) of dark chocolate use could not alter MDA and TAC in plasma. In these studies the sample size or dosage and duration of cocoa consumption were less than current study and samples were not collected from saliva. In an animal study, the preventive effects of cocoa-enriched diet on gingival oxidative stress in experimental periodontitis had been investigated. It was shown that cocoa could decrease gingival oxidative stress and inhibit bone loss and leukocyte infiltration so this pant prevented the progression of periodontitis. [8] Also, the results of this current study showed that cocoa could result in improvement and prevention of periodontitis. No study in which the effects of cocoa on periodontitis clinical parameters were evaluated was found but some other research investigated other phenolic compound such as green tea. Green tea was used in different delivery ways such as strip (Jenabian et al, 2012) and mouthwash (Kudva et al, 2011). [38,39] These studies suggested the positive effects of green tea because of its phenolic base ingredients so it can be concluded that the herbal compounds such as cocoa and green tea are useful in improving the periodontal inflammation.

The strength of this study was to assess the salivary parameters beside clinical measurements but the limitations of this study were the difficulty of managing dietary schedule of subjects and also no possibility to use the same weight for dark and white chocolate. For further knowledge, the measuring of gingival crevicular fluid antioxidant capacity and lipid peroxidation is suggested by authors Jenabian et al, 2012

Conclusion

Consuming dark chocolate could increase TAC and decrease lipid peroxidation, gingival bleeding and inflammation.

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