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Original Article

Impact of Consumption of Chicory Leaf Extract in Adjunct with Non-surgical Periodontal Therapy on Serum Antioxidant and Lipid Status in Patients with Periodontal Disease: Preliminary Study

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ABSTRACT

Background and Objectives: Periodontal disease is a chronic disorder with a high prevalence. There are few studies about the role of diet in prevention and treatment of periodontal disease. The aim of this study was to evaluate the effect of consumption of Chicory leaf extract in adjunct with non-surgical periodontal therapies on serum antioxidant and lipid status.

Materials and Methods: This study was a double-blind, randomized controlled clinical trial conducted on 40 patients in Sina Hospital of Ahvaz Jundishapur University of Medical Sciences (Iran) in 2014. The intervention (n=20) and control groups (n=20) were allocated using blocked randomization. The intervention group received 2 capsules (2 g) of Chicory leaf extract daily for 8 weeks. All subjects underwent non-surgical periodontal therapy during the intervention period. Anthropometric indices, 24-hour diet records, total antioxidant capacity, malate di-aldehyde (MDA), uric acid, total cholesterol, triglyceride (TG) and HDL-c was measured before and after intervention.

Results: The mean level of total antioxidant capacity (1.89 ± 0.49; 1.20 ± 0.25, respectively; P<0.001) and uric acid (7.15±1.98; 4.48±1.34, respectively; P<0.001) increased in the intervention group compared with the control group post intervention. The mean level of MDA decreased in the intervention group compared with the control group post-intervention (3.01±1.15; 3.97±1.19, respectively; P.d<0.001). Cholesterol difference was not significant pre- and post-intervention between the two groups (P=0.35). The mean level of serum triglyceride (TG) was significantly lower in the intervention group compared with the control group post intervention (149.50±97.88; 109.35±58.00, respectively; P.d<0.001). The mean level of HDL-c was also significantly lower in the intervention group compared with the control group post treatment (42.25±8.47; 39.80±8.94, respectively; P.d<0.001).

Conclusions: It seems that consumption of Chicory leaf in adjunct with non-surgical periodontal treatment has beneficial effect against periodontal disease.

Keywords: Periodontal disease, Chicory, Total antioxidant capacity, Inflammation, Lipid

Introduction

Periodontal disease with high prevalence is one of the most chronic dental diseases in the world (1). An epidemiological study indicated that 66% of people suffer from periodontal disease (2). This disease is a

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chronic inflammatory and infective disease in which the supportive tissue of teeth is destroyed. Periodontal disease is diagnosed by gum bleeding, plaque formation, damaging alveolar bone, damaging connecting tissue and loosing the affected teeth (3, 4). It is indicated that about 10-15% of young people suffer from severe periodontal disease with pocket depth of 6 mm (5). It has been shown that 80% of young people in England and 51% of Americans have some symptoms of periodontal disease during their life (6).

Gram-negative, anaerobic *bacteria* are responsible for periodontitis (7). Several studies suggest that using antibiotics, mouthwashes, removing tartars and root planning may be useful for treating periodontal disease (8). However, using antibiotics in the treatment of periodontal disease may lead to bacterial resistance. Therefore, lack of a certain therapeutic strategy is felt. Natural compounds and herbal extracts such as polyphenols may influence the pathology of periodontal disease (9).

Some researchers have indicated a relationship between diet and periodontitis. According to some studies, fruits, vegetables and whole grains reduce the prevalence of periodontitis (6, 10, 11). Jenzsch and colleagues found that consuming fruits, vegetables, beans and dairy during 12 months could reduce symptoms of periodontal disease (11). Furthermore, recent studies suggest that nutrients with high antioxidant activity may protect tissues in the mouth against oxidative stress (12, 13).

Van der Velden U and co-workers found that using antioxidants, vitamin D and calcium or consumption of vegetables, berries and fruits may have effective role in prevention of periodontal disease (14). Deficiency of antioxidants and polyphenols may lead to increase in the inflammation occurring in periodontitis (15, 16). Some studies have indicated that cytokines have a key role in inflammation and can increase photolytic and osteoclastic enzymes in periodontal disease (17).

Oxidative stress is considered as an important factor involved in pathogenesis of periodontal disease. According to several studies, ROS produced by the PMNs (the first defense system against periodontal pathogenic bacteria) in periodontal disease can result in further damage to periodontal ligaments and tissues supporting the affected teeth. It has also been shown that ROS level is high in periodontal disease. It is difficult to evaluate ROS levels due to their short lifetime; therefore, evaluation of MDA levels would be an alternative approach (16). Several studies have reported the role of antioxidant in prevention of periodontal disease, but no effect was seen in the treatment (18).

In periodontitis, serum antioxidant level may be decreased and free radical may be increased (19). According to some studies, concentration of ascorbic acid, carotenoid, bilirubin and some saliva antioxidants such as albumin, uric acid and ascorbic acid is reduced in periodontal disease. Patients with periodontal disease have higher levels of inflammatory markers such as IL-1β, TNFα and IL-6. These inflammatory factors may adversely alter lipid metabolism (16, 20).

Cichorium intybus L. is a herbal plant (30-120 cm height) from Asteraceae family growing in Europe (21). This plant is also found in some mountainous regions of Iran such as Khorasan, Zanjan, Tehran, Guilan, Mazandaran, Tabriz, and Fars. About 64 compounds have been extracted from Chicory (21). Chicory leaves contain anthocyanin, Vitamin A, Vitamin C, potassium, calcium, phosphorus, phenolic acid and flavonoids. Furthermore, derivations of hydroxy-cynamid acid, 8-mono dicaffeolquinic acid, 3-tartaric acid can be extracted from Chicory (22).

It is suggested that chicoric acid extracted from Chicory may reduce inflammation and bacterial infections (22). Along with some non-surgical therapies in periodontal disease, some dietary supplements are suggested as valuable therapy in the treatment process of this disease (23).

There are few *in vitro* studies about the protective role of Chicory against periodontal disease (23-25), to our knowledge, there are no clinical studies in this regard. Polyphenols found in Chicory leaf extract have antibacterial properties (24, 25). Moreover, the effect of Chicory leaf on serum antioxidant levels in periodontal disease has not been clearly proven. Therefore, the aim of this study was to investigate the effect of Chicory leaf extract supplementation along with non-surgical periodontal treatments on serum antioxidant and lipid in patients with periodontal disease.

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Materials and Methods

Participants: This study is a double-blind, randomized controlled clinical trial carried out in Sina Hospital and Perio Clinic of Jundishapur University of Medical Sciences, Ahvaz, Iran. Some 40 patients (19 females and 21 males) were participated. Inclusion criteria included males and females aged between 20-65 years old, body mass index (18.5<BMI<30) and moderate periodontal disease (pocket depth of minimum 4 mm in ¾ of the mouth) diagnosed by a dentist.

Exclusion criteria included pregnancy, breast-feeding, traveling for more than 2 weeks, smoking, using any dietary supplements or antioxidants, receiving immune-suppressors, history of any treatment for periodontal disease, losing weight more than 10% in the last two months, hyperlipidemia, and using lipid lowering medication. The study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Sciences, Iran (REC. 2013, 177). A written consent was obtained from all subjects.

The patients were randomly (block design) allocated into intervention group (20 patients) and control group (20 patients). The intervention group received 2 capsules of methanolic extract of Chicory leaf daily for 8 weeks. In the control group, patients received 2 placebo capsules (containing 2 gram wheat flour) daily for 8 weeks. All subjects had non-surgical periodontal therapy including tartar removing and flattening the root for two times during the study.

Evaluation of anthropometric indices and dietary intake: Anthropometric indices including weight (by a Seca scale) and height were assessed pre- and post-intervention. In this study, dietary intakes of the subjects were recorded using 24-hour dietary recall. Intakes of calorie, carbohydrate, lipid, protein, saturated fat, vitamin A, vitamin C, vitamin E and beta-carotene were analyzed by the Nutritionist IV program.

Measurement of biochemical indices: A blood sample (10 ml) was collected after a 12-hour fasting at baseline and post- intervention. After centrifugation process, the serum samples were separated and stored at −80°C until analyzing.

Biochemical indices including total antioxidant capacity, MDA, TC, TG, HDL-c and uric acid were measured before and after intervention. Total antioxidant capacity was measured by Randox detection kit (England). MDA was assessed by thiobarbituric acid reactive substances (TBARS) and N-botanol (26). Total cholesterol, HDL-c and TG were detected by Pars Azmoon kit (Iran).

Chicory leaf extraction: Chicory sample was collected in summer from Kandovan, Tabriz and the process of preparing the extract was conducted and approved in the Drug Applied Research Center, School of Pharmacy, Tabriz University of Medical Sciences, Iran. For obtaining Chicory leaf extraction, the leaves were infused by water and then dried by mill. Afterward, 1000 gr of Chicory powder was dissolved in methanol 70% over 72 hours. The extract was filtered by Whatman paper-1 and then put in Rota-rod evaporator (Heidolph, Germany). The high-gradient extract was frozen in freeze-drier (Christ Alpha 1-4, Germany) and then kept in 4 °C until usage (27).

Statistical analysis: As there was no *in vivo* study about the effects of receiving Chicory extract in periodontal disease in humans, this study was designed as a preliminary and pilot study. Therefore, the sample size of 20 subjects in each study group would be enough to show the possible changes in the main variables. Statistical analyses were carried out using the SPSS software, ver. 22 (SPSS Inc., Chicago, IL, USA). All data were expressed as mean ± standard deviation (SD). In order to determine whether the data have a normal distribution, a visual examination of the data and a goodness of fit test (Kolmogorov-Smirnov) were conducted. Independent-sample **t**-test and paired sample t-test were used for statistical analysis. P<0.05 was considered to be significant.

Results

Anthropometric indices and general characteristics are shown in Table 1. The mean age of subjects was 39.45±11.71 and 42.05±15.38 years old in the intervention and control groups, respectively. The mean BMI of patients was 27.66±5.68 and 25.95±4.84 kg/m^2 in the intervention and control group, respectively. There was no significant difference between the intervention and control groups for age and BMI at baseline (P>0.05).

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Table 1. Basic Characteristics of the participants in the intervention and control groups before the treatment

Variables	Intervention (n=20)	Control (n=20)	P
Age (years)	39.45± 11.71	42.05± 15.38	0.551
Sex			
Female	(8)%40	(11)%55	
Male	(12)%60	(9)%45	
BMI(kg/m2)	27.66± 5.68	25.95± 4.84	0.313

Values are expressed as means ± SD. P <0.05 was considered as statistically significant.

P <0.05 was considered as significant using Independent T-test between the two groups at baseline.

There were 21 males and 19 females with 55% (n=11) females and 45% (n=9) males in the intervention group, and 40% (n=8) females and 60% (n=12) males in the control group. There was no significant difference in gender, total antioxidant capacity, uric acid and total cholesterol between the two groups at baseline (P>0.05, Tables 1 and 2). However, there were significant differences in MDA, TG and HDL-c between the intervention and control groups at baseline (P<0.05, Table 2). Furthermore, there was no significant difference in energy intake, saturated fat, vitamin A, vitamin E, vitamin C and beta-carotene between the study groups pre- and post-intervention (P>0.05).

The mean serum total antioxidant capacity was significantly (P<0.001) greater in the intervention group (1.89±0.49 mg/dl) compared with the control group (1.20±0.25 mg/dl) post-intervention. In the intervention group, the mean level of total antioxidant capacity was increased significantly (P<0.001) post-intervention (1.89±0.49 mg/dl) compared with baseline (1.50±0.35 mg/dl). On the other hand, the mean level of total antioxidant capacity reduced significantly (P=0.044) in the control group post-intervention (1.20±0.25 mg/dl) compared with baseline (1.43±0.44 mg/dl).

The mean level of uric acid was also significantly (P<0.001) greater in the intervention group (7.15±1.98 mg/dl) compared with the control group (4.48±1.34 mg/dl) post-intervention.The mean level of serum uric acid increased significantly (P<0.001) post-intervention (7.15±1.98 mg/dl) compared with baseline (5.77±2.16 mg/dl) in the intervention group. In addition, the mean level of uric acid decreased significantly (P=0.045) in the control group post-intervention (4.48±1.34 mg/dl) compared with baseline (5.28±1.70 mg/dl) (Table 2).

As there was a significant difference in the mean level of serum MDA between the study groups (p=0.002) at baseline, we used mean differences in comparison analysis. So, the mean level of MDA was significantly (P.d<0.001) lower in the intervention group (3.01±1.15mmol/l) compared with the control group (3.97±1.19 mmol/l) post-intervention. In the control group, MDA concentration was significantly (P<0.001) increased post-intervention (3.97±1.19 mmol/l) compared with baseline (3.29±1.16 mmol/l) (Table 2).

Table 2. Serum antioxidant factors in the intervention and control groups before and after intervention

Variables	Time	Control (n=20)	Intervention (n=20)	P*
Total antioxidant capacity(mg/dl)	Pre-intervention	1.50± 0.35	1.43±0.44	0.584
	Post-intervention	1.89±.49	1.20±0.25	<0.001
P		<0.001	0.044	
Uric acid(mg/dl)	Pre-intervention	5.77±2.16	5.28±1.70	0.422
	Post-intervention	7.15±1.98	4.48±1.34	<0.001
P		<0.001	0.045	
MDA(mmol/l)	Pre-intervention	4.53±1.22	3.29±1.16	0.002
	Post-intervention	3.01±1.15	3.97±1.19	<0.001#
P†		<0.001	<0.001	

†Paired t-test in intra-group comparison. *Independent t-test of means in the intervention and control groups. #Independent t-test of difference means between the intervention and control groups. Data are shown as mean ± SEM. P<0.05 was expressed as statistically significant.

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There was no significant difference (P=0.353) observed in the mean level of serum total cholesterol between the intervention (168.00±48.59mg/dl) and control groups (180.70±35.92mg/dl) post-intervention. On the other hand, total cholesterol concentration was significantly (P<0.001) reduced in the intervention group post-intervention (168.00±48.59 mg/dl) compared with baseline (201.65±58.22mg/dl). However, in the control group, total cholesterol concentration was significantly (P=0.158) reduced post-intervention (174.80±38.88 mg/dl) compared with baseline (180.70±35.92mg/dl) (Table 3).

Regarding with TG, as there was a significant (p=0.002) difference between the intervention and control groups pre-intervention, therefore, we used mean differences. The mean level of TG was significantly (P.d<0.001) attenuated in the intervention group (149.50±97.88mg/dl) compared with the control group (109.35±58.00mg/dl) post-intervention. In the control group, the mean concentration of TG increased significantly (P=0.026) post-intervention(109.35±58.00mg/dl) (93.70±47.84 mg/dl) compared with baseline (93.70±47.84mg/dl) (Table 3).

Regarding HDL-c, there was a significant difference in the mean levels between the two groups (p =0.001) at baseline. So, we used mean differences for comparison analysis. It was found that the mean level of HDL-c was significantly (P.d<0.001) higher in the intervention group (42.25±8.47mg/dl) compared with the control group (39.80±8.94mg/dl) post-intervention. Within the control group, HDL-c concentration was not changed significantly (P=0.144) post-intervention.

Table 3. Lipid factors in the intervention and control group before and after treatment

Variables	Time	Control (n=20)	Intervention (n=20)	* P
Total cholestrol (mg/dl)	Pre-intervention	174.80±38.88	201.65±58.22	0.094
	Post-intervention	180.70±35.92	168.00±48.59	0.353
P		0.158	<0.001	
TG(mg/dl)	Pre-intervention	93.70±47.84	190.05±119.07	0.002
	Post-intervention	109.35±58.00	149.50±97.88	<0.001#
P		0.026	0.001	
HDL(mg/dl)	Pre-intervention	41.30±9.45	32.15±6.41	0.001
	Post-intervention	39.80±8.94	42.25±8.47	<0.001#
†P		0.144	<0.001	

†Paired t-test in intra-group comparison.
*Independent t-test of means in the intervention and control groups.
#Independent t-test of difference means between intervention and control groups.

Discussion

In the current study, we found that receiving eight weeks of Chicory leaf extract increased serum total antioxidant capacity and uric acid. In addition, the intervention could reduce serum MDA in the patients with periodontal disease.

It has been indicated that periodontal disease is influenced by nutritional factors, and that nutritional intervention (specifically with anti-inflammatory and antioxidant effects) improves the status in periodontal disease. There are few studies about the role of nutritional intervention in adjunct with non-surgical periodontal therapies in the prevention and treatment of periodontal disease. Previous studies have shown that dietary supplements with anti-inflammatory and antioxidant properties such as vitamin C, alpha-tocopherol, beta-carotene, biotene, omega-3, green tea, probiotics and cranberry could improve periodontal disease (28).

Oxidative stress is considered as the elevator of the intracellular levels of reactive oxygen species (ROS) causing damage to lipids, proteins and DNA (29). Oxidative stress may lead to several pathological diseases. Chapple et al. suggested a negative correlation between the prevalence of

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periodontitis (30) and serum concentration of antioxidants and total antioxidant capacity. Several studies agree that total antioxidant capacity is decreased in sever periodontal disease (31). Zhang MF and co-workers reported a significant difference in dietary vitamin C, flavonoids and beta-carotene between the control and intervention groups (32). Chicory leaf possess high level of antioxidants and phytochemical. Regarding with the beneficial effects of Chicory leaf, it may be a good alternative for using any medication. Chicory leaf may increase the antioxidant defensive system, reduce ROS, and finally, enhance the body health (33).

Oxidative stress is an important risk factor in the pathogenesis of periodontal disease (16). Some investigations revealed that antioxidants may prevent periodontal disease but they are not involved in periodontal therapy (19).

Recent publications suggest that a diet with high antioxidant content can improve periodontal disease (14). Deficiency of antioxidant and polyphenols may result in increased oxidative stress and inflammation, so may enhance the risk of developing periodontitis (16). In the study of Base U (2015), reductions found in total antioxidant capacity amplified periodontitis and subsequently damaged gum tissue (34). Zhiqiang Liu and colleagues showed that patients with periodontitis had low level of total antioxidant capacity (35). Another study by Biju Thomas et al. in 2014 confirmed the previous reports that total antioxidant capacity reduced the prevalence of periodontal disease (36). Moreover, in a study by Zare Javid and coworkers, it was found that increasing the consumption of fruits and vegetables in patients with periodontitis increased saliva and serum total antioxidant capacity (5). On the other hand, Aboi Sulaiman AE and co-workers showed that vitamin C could not improve total antioxidant capacity in patients with periodontitis (37).

Wang Q et al. reported that Chicory leaf contains saccharides, organic acids, alkaloids, triterpenes, coumarins, etc. (38). These compounds can reduce serum levels of lipids, glucose and uric acid. Also it was observed that Chicory extract had beneficial role on cardiovascular diseases (38).

Chicory contains inulin, and it has been claimed inulin could reduce uric acid, TG and fat in Quail. Chicory regulated the expression of acetyl-CoA carboxylase, xanthine oxidase and fatty acid synthase (39). Furthermore, Chicory inhibited xanthine oxidase, and consequently, abolished uric acid synthesis; so the researchers suggested that this herb might have beneficial role against gout disease, while our current results are in contrast with Lin Z's conclusion (39). Cristina Mourão et al. (2015) reported that the level of LDL, glucose and uric acid increases in chronic periodontitis (40). Aditi Mathur and co-workers observed that patients suffering from periodontal disease have high level of uric acid, indicating the elevation of antioxidant function (41); our results confirm the previous findings of elevation in uric acid in periodontal disease. However, there is no evidence to report the role of dietary intervention on the serum levels of uric acid.

Enhanced level of MDA (a peroxidase enzyme in liver) is a main indicator of oxidative stress (42). Our findings suggest that receiving Chicory extract for eight weeks reduced MDA level. Yasser S. El-Sayed et al. showed that consuming Chicory root (100mg/kg) for 2 weeks inhibited MDA (43). It was suggested that Chicory can reduce ROS and increase antioxidant defensive system through increasing some antioxidant compounds such as anthocyanin, flavonoids, polyphenols and vitamin C (44). Likewise, Guo-Yu Li et al. reported that intake of 54 g/kg of Chicory extract for 8 weeks could decrease MDA (45).

Chicory comprises glycosides, estrols and polyphenols, which may diminish inflammation by inhibiting prostaglandins, nitric oxide, TNF-alpha, interleukin-6 (IL-6) and interleukin-1 (IL-1) (46, 47). In the present study, we found that the level of TG decreased and HDL-c increased but serum level of cholesterol remained unchanged during the intervention.

There is a positive correlation between serum cholesterol level and sever periodontal disease. In this disease, bacterial lipopolysaccharides can enter circulation, increase hydroxyl- methyl-glutaraldehyde CoA, and subsequently, increase cholesterol synthesis (48). In periodontal disease, infection and bacterial pathogens can elevate IL-6 and TNF-alpha and thus may lead to dyslipidemia and high triglyceride level (49). A cohort study by Tu et al. (2013) reported a connection between metabolic syndrome and

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periodontal disease (50). Behfarnia and colleagues showed that reduction of serum lipid level and LDL-c and elevation of HDL-c had advantageous role on periodontal disease (51). In addition, another study suggested that reduction of cholesterol and LDL-c improved periodontal disease (52). Ghamarian and co-workers indicated that Chicory extract (125 mg/kg body weight) for 27 days decreased total cholesterol and TG in diabetic rats (53). It was suggested that Chicory could ameliorate lipid synthesis and inhibit adipogenesis (54, 55). Several studies reported that probiotic carbohydrates and phenolic compounds in herbal medicine might attenuate cholesterol level (56).

Jerzy Jus´kiewicz et al. indicated that dietary supplementation of Chicory root or leaf extract (containing chicoric acid and polyphenols) could decrease peroxidation process in lung. Also they found that Chicory leaf extract had strong effect on reduction of cholesterol and HDL (57). Jerzy Jus´kiewicz proposed that chicoric acid and polyphenols are high in leaves than in roots (57). The diverse findings among our results and previous reports may relate to some conditions such as intervention duration, diet and different experimental models. In agreement with our results, it was shown that inulin consumption reduced TG (58, 59). Also confirming our findings, Pazola and Cieslak found that Chicory solution had no effect on serum cholesterol level (60).

Totally, investigators have revealed that periodontal disease can be prevented before 50 years old (61). So, it is suggested that Chicory leaf extract may be a beneficial dietary supplement with antibacterial and antioxidant properties in periodontal disease. We suggest that the local vegetables like Chicory may be a good candidate to assist the treatment of periodontal disease by non-surgical periodontal therapy in short time.

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