ISSN: 1812–1217
E- ISSN: 1998-0345

Antioxidant Effect of Folic Acid and its Relation to Salivary Proteins and Oral Health

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الخلاصة

الأهداف: تهدف الدراسة الى تقييم تأثير العلاج الجهازي بحمض الفوليك على قدرة اللعاب على انتاج مضادات الأكسدة وانتاج البروتين الكلي وعلاقة ذلك بمؤشرات صحة الفم للمرضى الذين يعانون من التهاب اللثة المزمن. **المواد وطرائق العمل**: تم تصنيف أربعين مريضا إلى مجموعتين: - المجموعة ١: (٢٠) مرضى بالتهاب اللثة المزمن ، لم يتلقوا أي دواء (مجموعة مراقبة) ، المجموعة ٢: (٢٠) مرضى بالتهاب اللثة المزمن (مجموعة العلاج) ، تناولوا قرصاً واحداً باليوم ، بجرعة ١ ملغم من حمض الفوليك يومياً على مدار ٤٢ يوماً. في بداية الدراسة تم تنفيذ عمليات ازالة التكلسات السنية والتلميع لكل متطوع للوصول إلى نقطة الاساس لمؤشر اللثة ومؤشر صحة الفم. في اليوم التالي ، تم قياس هذه المؤشرات لجميع المشاركين ، ثم تم قياسها بعد ٢١ يوماً ثم بعد ٤٢ يوماً من العلاج. وفي كل زيارة ، تم جمع خمسة ملليلتر من اللعاب غير المحفز وذلك لقياس القدرة اللعابية لإنتاج مضادات الاكسدة والبروتينات اللعابية الكاملة بواسطة مقياس الطيف الضوئي. **النتائج**: أظهرت النتائج وجود اختلاف كبير في القدرة اللعابية على انتاج مضادات الاكسدة ، وأنه لا يوجد فرق كبير في القدرة اللعابية على انتاج البروتينات الكاملة، كذلك لا يوجد فرق كبير في مؤشر اللثة ومؤشر النظافة الفموية لمجموعة العلاج بعد ٦ أسابيع من العلاج. **الاستنتاجات**: الاستخدام لحمض الفوليك خلال مدة قصيرة ممكن ان يحسن من القدرة اللعابية على انتاج مضادات الاكسدة ويقلل من مستويات البروتينات اللعابية على نحوٍ ضعيف

ABSTRACT

Aims: To assay the effect of systemic treatment with folic acid on salivary total antioxidant capacity (TAC) and total protein (TP) in relation to oral health indices of patients with chronic gingivitis. **Materials and Methods:** Forty patients were classified into two groups :- Group 1: (n:20) chronic gingivitis patients, did not receive any medication (control group), Group 2: (n:20) chronic gingivitis patients (treatment group), received 1 mg\day oral tablet of folic acid for 42 days. At the beginning of study, scaling and polishing have been carried out for each volunteer to reach the base line for gingival index and oral hygiene index. In the next day, these indices were measured for all participants , then measured after 21 days then after 42 days from treatment. At all visits, five milliliters of unstimulated saliva were collected for measurement of salivary TAC and salivary TP by spectrophotometer. **Results:** the results revealed that there is a significant difference in salivary TAC of treatment group after 6 weeks, no significant difference in salivary TP during study period, no significant difference in gingival index and oral hygiene index between treatment and control group. **Conclusions:** sub-acute using of systemic folic acid in chronic gingivitis patients improves salivary TAC and decrease salivary TP slightly .

Key words: Total antioxidant capacity, Salivary total protein , Folic acid, Oral health.

Al-Basrawi RF., Al-Mashhadane FA**.** Antioxidant Effect of Folic Acid and its Relation to Salivary Proteins and Oral Health.. Al–Rafidain Dent J. 2020 ;20 (1): 83-94.
DOI: 10.33899/rden.2020.164540

Received: 23/2/2020 ***Sent to Referees:*** 25/2/2020 ***Accepted for Publication:*** 26/4/2020

INTRODUCTION

Periodontal disease is highly prevalent in world population[1]. Gingivitis is a form of periodontal disease in which gingival tissues are inflamed but their destruction is mild and reversible. Gingival Inflammation is originated by a pathogenic microorganisms which activate the innate immunity system[2,3] causing inflammation and oxidative stress orally; therefore, eradication of excessive stress of oxidation can slow down the progression of periodontal disease[4]. Mitochondrial oxidative metabolism, cellular response to xenobiotics, cytokines, and bacterial invasion generate reactive oxygen species[5] at which many evidences have shown to be associated with periodontal disease[6-8]. The antioxidant which is useful in neutralizing oxidative injury includes enzymatic and non-enzymatic systems [9]. Enzymatic antioxidants are available in every cell of eukaryotic organisms, protecting them from oxidative harm, produced by salivary glands and play roles in balancing the periodontal inflammatory status[10]. Saliva is the ease and noninvasiveness biological liquid that reveal the health and disease status[11]. Also, saliva hold a large number of proteins that contribute in the defense of the oral tissues[12]. Estimation of total salivary proteins helps in diagnosis of gingivitis thus facilitating early prognosis and treatment[13]. Supplementing patients with antioxidants has been known to have an significant function in minimizing the degree of inflammation caused by oxidative stress[14]. At folic acid reaction with oxidizing free radicals, hydroxyl group can participate an important role in inhibiting the oxidation outcome. Researchers have describe that folic acid has a considerable antioxidant effect and lessen oxidative stress[15-17]. This study aims to investigate the effects of folic acid on gingival and oral hygiene indices of patients with chronic gingivitis and to evaluate its effect on TAC and TP in their saliva.

MATERIALS AND METHODS

This study was agreed by the scientific committee/department of Dental Basic Science/College of Dentistry/University of Mosul. The study sample included forty patients, their ages ranged between (20-40 years), recruited from the dental private clinics in Mosul city. They were classified into two groups : Group 1 consisted of 20 chronic gingivitis patients, did not receive any medication (control group), Group 2 also consisted of 20 chronic gingivitis patients (treatment group), received 1 mg\day oral tablet of folic acid (SDI Company/Iraq). The choice of each volunteer depended on inclusion criteria (systematically and orally healthy individuals except for chronic gingivitis, Non-pregnant or lactating females, no any drug or supplements, complement of more than 20 teeth, non-smoking, non-alcoholic). Scaling and polishing had been carried out for each

volunteer to reach the base line gingival index(Löe and Silness, 1963) and oral hygiene index (Greene and Vermilion, 1960). In the next day, these indices were measured for all participants and measured again after 21 days and 42 days from treatment. At the same visits, five milliliters of unstimulated saliva were collected for measurement of salivary TAC by Total Antioxidant Capacity Assay Kit (Elabscience® /USA) and measurement of salivary TP by Total Protein Assay Kit (Biolabo®/France). Statistical Analysis were done by Microsoft Excel-2010. Independent t-test and One-way Analysis of Variance test (ANOVA-test) with Tukey's Pair-wise comparisons were used. *p* value is < 0.05.

RESULTS

Demographic data:

In this study, there was no significant difference between mean age of treatment group and control group. The treatment group was consisted of 11 female (11.55%) and 9 male (9.45%), while the control group was consisted of 9 female (9.45%) and 11 male (11.55%). In this study no significant differences were observed in oral health indices between treatment and control groups throughout study visits, reductions in oral health indices at 21st and 42nd days of the study are observed, but they are not significant (Tables 1, 2 and 3)

Table (1): Comparison in oral health scores between the two groups at the beginning of the study.

Oral health scores	**Treatment group** [n = 20] Mean ± SD	**Control group** [n = 20] Mean ± SD	P-value*
Gingival index	0.458 ± 0.436	0.550 ± 0.329	0.457
Oral hygiene index	0.233 ± 0.348	0.433 ± 0.380	0.091

* Independent T-test of two means was used $p < 0.05$.

Table (2): Comparison in oral health scores between the two groups at 21st day of the study.

Oral health scores	**Treatment group** [n = 20] Mean ± SD	**Control group** [n = 20] Mean ± SD	P-value*
Gingival index	0.350 ± 0.346	0.541 ± 0.338	0.086
Oral hygiene index	0.308 ± 0.394	0.558 ± 0.493	0.085

* Independent T-test of two means was used $p < 0.05$.

Table (3): Comparison in oral health scores between treatment group and control group at 42nd day of the study.

Oral health scores	**Treatment group** [n = 20] Mean ± SD	**Control group** [n = 20] Mean ± SD	P-value*
Gingival index	0.400 ± 0.317	0.566 ± 0.406	0.156
Oral hygiene index	0.441 ± 0.307	0.624 ± 0.515	0.180

* Independent T-test of two means was used $p < 0.05$.

Comparisons between oral health scores in treatment and control groups during all the study period are showing that there is no significant difference in means of the oral health scores during the entire study period as illustrated in the (Tables 4 and 5)

Table (4): Effect of systemic folic acid treatment on oral health scores during the study period.

Oral health Scores	**Treatment group**			**P-value***
	1st day Mean ± SD	21st day Mean ± SD	42nd day Mean ± SD	
Gingival index	0.458 ± 0.436	0.350 ± 0.346	0.400 ± 0.317	0.653
Oral hygiene index	0.233 ± 0.348	0.308 ± 0.394	0.441 ± 0.307	0.175

* One-way ANOVA-test , $p < 0.05$ means no significant difference

Table (5): Comparison in oral health scores of control group during the study period.

Oral health scores	**Control group**			**P-value***
	1st day Mean ± SD	21st day Mean ± SD	42nd day Mean ± SD	
Gingival index	0.550 ± 0.329	0.541 ± 0.338	0.566 ± 0.406	0.974
Oral hygiene index	0.433 ± 0.380	0.558 ± 0.493	0.624 ± 0.515	0.427

* One-way ANOVA-test , $p < 0.05$ means no significant difference

There is no significant difference between means of treatment group and control group in salivary TAC and salivary TP at the beginning of the study, as illustrated in (Table 6).

Table (6): Comparison in mean of salivary parameters between treatment group and control group at the beginning of the study.

Salivary parameters	Treatment group [n = 20] Mean ± SD	Control group [n = 20] Mean ± SD	P-value*
Salivary TAC (U/ml)	5.02 ± 3.77	5.51 ± 4.08	0.695
Salivary TP (g/dl)	0.76 ± 0.46	0.66 ± 0.39	0.470

* Independent T-test of two means was used. $p < 0.05$

At the 21st day and 42nd day of the study there are higher levels of salivary TAC accompanied with lower levels of by salivary TP, although these differences are not significant as illustrated in (Tables 7 and 8).

Table (7): Comparison in mean of salivary parameters between treatment group and control group at the 21st day of the study.

Salivary parameters	Treatment group [n = 20] Mean ± SD	Control group [n = 20] Mean ± SD	P-value*
Salivary TAC (U/ml)	7.51 ± 4.26	6.22 ± 3.88	0.322
Salivary TP (g/dl)	0.56 ± 0.31	0.67 ± 0.34	0.278

* Independent T-test of two means was used. $p < 0.05$

Table (8): Comparison in mean of salivary parameters between treatment group and control group at the 42nd day of the study.

Salivary parameters	Treatment group [n = 20] Mean ± SD	Control group [n = 20] Mean ± SD	P-value*
Salivary TAC (U/ml)	8.16 ± 4.17	6.49 ± 4.49	0.232
Salivary TP (g/dl)	0.49 ± 0.31	0.74 ± 0.56	0.107

* Independent T-test of two means was used. $p < 0.05$

For control group there is no significant difference in salivary TAC and salivary TP at the 1st, 21st, 42nd days of the study, as illustrated in (Table 9).

Table (9): Comparison in salivary parameters levels of control group during the study period.

Salivary parameters	Control group			
	1st day Mean ± SD	21st day Mean ± SD	42nd day Mean ± SD	P-value*
Salivary TAC (U/ml)	5.51 ± 4.08	6.22 ± 3.88	6.49 ± 4.49	0.745
Salivary TP (g/dl)	0.66 ± 0.39	0.67 ± 0.34	0.74 ± 0.56	0.859

* One-way ANOVA-test $p < 0.05$ means no significant difference

During comparisons of means of salivary parameters levels in treatment during the study period one can see that there are significant difference in mean of salivary TAC level among the study days without significant difference in means of salivary protein (Table 10).

Table (10): Effect of systemic folic acid treatment on salivary levels of biochemical and antioxidant parameters during the study period.

Salivary parameters	Treatment group			
	1st day Mean ± SD	21st day Mean ± SD	42nd day Mean ± SD	P-value*
Salivary TAC (U/ml)	5.02 ± 3.77 ^{B}	7.51 ± 4.26 ^{AB}	8.16 ± 4.17 ^{A}	0.044
Salivary TP (g/dl)	0.76 ± 0.46 ^{A}	0.56 ± 0.31 ^{A}	0.49 ± 0.31 ^{A}	0.065

* Normal sig., $P \leq 0.05$. Means do not share A letter are significant difference

DISCUSSION

Saliva has buffering, lubricating, antibacterial, and remineralizing tasks, it is also an excellent source of both enzymatic and non-enzymatic antioxidants, which preserving the redox homeostasis, and stop disturbances of them in the oral environment by overproduction of free radicals or reduction in antioxidants synthesis which is called oxidative stress state. Salivary TAC is mainly associated to some proteins and enzymes (i.e., glutathione peroxidase, salivary peroxidase, superoxide dismutase, catalase, and myeloperoxidase), uric acid, ascorbic acid and finally, albumin[18]. The excessive production of free radicals or antioxidants deficiency have a role in pathogenesis of periodontal disease as gingivitis. It has been proposed, that antioxidant supplementation could decrease or slow gingival tissues injury. Up to date studies affords constructive evidences of antioxidant therapeutic of periodontal disease[19,20]. Accumulation of dental plaque and calculus, both are considered the integral parts of oral hygiene index, plays a critical role in periodontal disease as they contain various

pathogenic bacteria that induce production of free radicals which leads to inflammation or may progress to periodontium destruction[21,22].

Our study results have found that there is a significant effect of folic acid on treatment group by increasing salivary TAC value through the entire period of study. This result can be explained by the dual role (direct and indirect) of folic acid. The indirect role involves subsiding gingival inflammation by decreasing cytokines production [23-27] . In case of a mild gingivitis, the normal immune response to bacterial endotoxin (Lipopolysaccharides) involves neutrophils recruiting and production of inflammatory cytokines during inflammation as Interleukin-6 which activates Nrf2 system [28] , then production of ROS in fair level enough to return redox homeostasis of gingival tissue. If there is excessive production of ROS as in severe case of gingivitis, the neutrophils infiltrate increased together with a downward regulation of the Nrf2 pathway and following inhibition of antioxidant production, resulting in a higher synthesis of ROS leads to periodontal tissue damage that may lead to periodontitis. Nrf2 is nuclear factor erythroid 2-related factor 2, it currently recognized as one of main cellular defense mechanism against oxidative stress. It is a transcriptional factor involved in cellular redox homeostasis. In healthy condition it found in cytoplasm in inactive state, often sequestered by special molecule called Klech like- ECH-associated protein 1 (Keap-1). At inflammatory state when the cell expose to the ROS or pro-inflammatory cytokines, results in conformational changes in cysteine moiety of Keap-1 molecule leads Nrf2 to dissociates from Keap-1 and translocates into nucleus where it has a role in regulating the expression antioxidants genes by transcriptional activation of cell defense genes[29], Expression of endogenous antioxidants is regulated by Nrf2 system. Exogenous antioxidants is supposed to activate that system as; omega 3, curcumin, allicin (in garlic), sulforaphane (in broccoli), pterostilbene (in grapes and blue berries) and green tea[30-33]. So the direct role of folic acid is by anti-oxidative action as above examples.

Recently, it was confirmed that folic acid can efficiently scavenge such free radicals like •OH, CCl3O2•, SO4•-, N3•, Br2•-, and O•-.[34] in the circumstances of extreme oxidative stress, the exhaustion of folates may occur. It was suggested that the main antioxidant activity of the fully reduced and active form of folate (5-MTHF) resides in its pterin core and an electrondonating effect[35] .The radical-scavenging activities of folates are strongly depend on pH. Folic acid is a superior free radical scavenger at acidic and basic pH than at neutral pH. In the normal state, the pH is kept up near neutrality by saliva. Bacterial plaque get calcium compounds and use minerals to defend them from the high pH which is necessary for plaque growth proposing the alkaline pH of the saliva obtained from the subjects with generalized chronic gingivitis.

While pH of periodontitis is acidic, so we proposed that folic acid is useful in gingivitis and periodontitis[36-38].

Oral hygiene and gingival index scores of treatment group at the entire period of the study have little but non-significant difference as shown in table 4, this is supposed to be due to insufficient dose of folic acid also due to patient incompliance with their oral hygiene. We chose the dose of folic acid according to institution of medicine (IOM) recommendation that suggested the tolerable upper intake dose (TUL) must be 1 mg/day for any period without inducing any side effects nor development of cancer. Side effects appear due to unmetabolized folic acid which accumulates in circulation as masking B12 deficiency and while cancer is induced in people with tumor foci by the effect of accumulated folic acid[39-41].

Vogel et al at 1976 have been used folic acid in their study on gingivitis, results indicate that folic acid treatment leads to a reduction in inflammation[23]. Another study was conducted in 1984 by Pack A., treatment group used mouth wash (MW) which contained 5 mg folate per 5 ml, twice daily for 4 weeks, rinsing for 1 min before expectoration. The control group used a placebo MW. She has found that folate MW appears to have an effect on health of gingiva through gingival color and bleeding index improvement[42]. During comparisons of means of salivary TP levels in treatment with folic during our study period, there is a slight decrease (Table 10). That supposed to be due to anti inflammatory effect of folic acid[23-27].

During the chronic gingivitis condition there is are up-regulation of pro-inflammatory mediators such as IL-6 and Tumor necrosis factor alpha in response to the chronic infection by oral micro-organisms. Pro-inflammatory cytokines have been shown to increase genes expression of oral host defense system proteins in gingival tissues[43,44]. In general, there are moderate to strong correlations between oral inflammatory cytokines production and levels of several salivary cytoprotective proteins in inflammation and resolution of gingivitis.[42]

CONCLUSIONS

Sub-acute treatment of chronic gingivitis with 1 mg/day of oral folic acid supplements can improve gingivitis by increasing salivary total antioxidant capacity and slightly decreasing in salivary total protein . We recommend to study the effect of another doses of folic acid on patients with chronic gingivitis also to measure their effects on specific proteins in their saliva.

Acknowledgment

Authors are greatly appreciative to the University of Mosul / College of Dentistry for their kind assessment to reach best quality of this research.

REFERENCES

1- Almerich-silla JM, Montiel-Company JM, Pastor S, Serrano F, Puig-Silla, M, Dasí F. Oxidative stress parameters in saliva and its association with periodontal disease and types of bacteria. *Disease markers*,2015,2015.ArticleID653537,http://dx.doi.org/10.1155/2015/653537.

2- Kornman, K.S. Mapping the pathogenesis of periodontitis: A new look. *J. Periodontol*. 2008, 79 (Suppl. 8),1560–1568.

3- Alkan, Duygu, et al. Folate-receptor 1 level in periodontal disease: a pilot study. *BMC oral health*, 2019, 19.1: 1-5.

4- D'Aiuto, F.; Nibali, L.; Parkar, M.; Patel, K.; Suvan, J.; Donos, N. Oxidative stress, systemic inflammation, and severe periodontitis. *J. Dent. Res.* 2010, 89, 1241–1246.

5- Ray, Paul D.; Huang, Bo-Wen; Tsuji, Yoshiaki. Reactive oxygen species (ROS) homeostasis and redox regulation in cellular signaling. *Cellular signalling*, 2012, 24.5: 981-990.

6- Sakallioğlu, Umur, et al. Reactive oxygen species scavenging activity during periodontal mucoperiosteal healing: an experimental study in dogs. *Archives of Oral Biology*, 2005, 50.12: 1040-1046.

7- Dalai, Camelia, et al. Correlation between histopathological aspects of periodontitis and biochemical changes of oxidative stress. *Romanian Journal of Morphology and Embryology*, 2013, 54.3: 817-822

8- Miricescu, Daniela, et al. Salivary biomarkers: relationship between oxidative stress and alveolar bone loss in chronic periodontitis. *Acta Odontologica Scandinavica*, 2014, 72.1: 42-47.

9- Genco, Robert J. Current view of risk factors for periodontal diseases. *Journal of periodontology*, 1996, 67: 1041-1049.

10- Kamodyová, Natália, et al. Salivary markers of oxidative stress and antioxidant status: influence of external factors. *Disease Markers*, 2013, 34.5: 313-321.

11- Borges JR, Ivan, et al. Proinflammatory and oxidative stress markers in patients with periodontal disease. *Mediators of inflammation*, 2007, 2007.

12- Nieuw Amerongen AV, Veerman ECI: Saliva – the defender of the oral cavity. *Oral Dis* 2002;8:12–22.

13- Kumar, J. Sarvesh; GAYATHRI, R.; PRIYA, V. Vishnu. Evaluation of salivary total proteins, albumin, globulin, and albumin/globulin ratio among healthy individuals and patients with chronic gingivitis. *Drug Invention Today*, 2018, 10.6.

14- Kirwan JP, O'Gorman D, Evans WJ. A moderate glycemic meal before endurance exercise can enhance performance. *J Appl Physiol (1985).* 1998;84(1):53–59.

15- Joshi R, Adhikari S, Patro BS, Chattopadhyay S, Mukherjee T. Free radical scavenging behavior of folic acid: evidence for possible antioxidant activity. *Free Radic Biol Med*. 2001;30:1390–1399.

16- Racek J, Rusnáková H, Trefil L, Siala KK. The influence of folate and antioxidants on homocysteine levels and oxidative stress in patients with hyperlipidemia and hyperhomocysteinemia. *Physiol Rev*. 2005;54:87–95.

17- Bayés B, Pastor MC, Bonal J, Juncà J, Romero R. Homocysteine and lipid peroxidation in hemodialysis: role of folinic acid and vitamin E. *Nephrol Dial Transplant*. 2001;16:2172–2175

18- Żukowski, Piotr; Maciejczyk, Mateusz; Waszkiel, Danuta. Sources of free radicals and oxidative stress in the oral cavity. *Archives of Oral Biology*, 2018, 92: 8-17.

19- Celec, Peter, et al. Oxidative stress and antioxidants in the diagnosis and therapy of periodontitis. *Frontiers in physiology*, 2017, 8: 1055.

20- AmbatI, Manasa, et al. Evaluation of oxidative stress in chronic periodontitis patients following systemic antioxidant supplementation: A clinical and biochemical study. *Journal of natural science, biology, and medicine*, 2017, 8.1: 99.

21- Da silva, Júlia Christ, et al. The effect of periodontal therapy on oxidative stress biomarkers: A systematic review. *Journal of clinical periodontology*, 2018, 45.10: 1222-1237

22- Albandar, J.M.; Brown, L.J.; Brunelle, J.A.; Loe, H. Gingival state and dental calculus in early-onset periodontitis. *J. Periodontol.* 1996, 67, 953–959.

23- Vogel, Richard I., et al. The effect of folic acid on gingival health. *Journal of periodontology*, 1976, 47.11: 667-668.

24- Esaki, M., et al. Relationship between folic acid intake and gingival health in non-smoking adults in Japan. *Oral diseases*, 2010, 16.1: 96-101.

25- Chen, Hui, et al. Folic acid supplementation mitigates Alzheimer's disease by reducing inflammation: a randomized controlled trial. *Mediators of inflammation*, 2016, 2016.

26- Paniz, Clovis, et al. A daily dose of 5 mg folic acid for 90 days is associated with increased serum unmetabolized folic acid and reduced natural killer cell cytotoxicity in healthy Brazilian adults. *The Journal of nutrition*, 2017, 147.9: 1677-1685.

27- Makino, Emi, et al. Subacute oral administration of folic acid elicits anti-inflammatory response in a mouse model of allergic dermatitis. *The Journal of nutritional biochemistry*, 2019, 67: 14-19.

28- Matsuoka, Yuichiro, et al. IL-6 controls resistance to radiation by suppressing oxidative stress via the Nrf2-antioxidant pathway in oral squamous cell carcinoma. *British journal of cancer*, 2016, 115.10: 1234-1244.

29- Taguchi, Keiko; MOTOHASHI, Hozumi; Yamamoto, Masayuki. Molecular mechanisms of the Keap1–Nrf2 pathway in stress response and cancer evolution. *Genes to cells*, 2011, 16.2: 123-140.

30- Zgórzy ´ nska, E.; Dziedzic, B.; Gorzkiewicz, A.; Stulczewski, D.; Bielawska, K.; Su, K.-P.; Walczewska, A. Omega-3 polyunsaturated fatty acids improve the antioxidative defense in rat astrocytes via an Nrf2-dependent mechanism. *Pharmacol. Rep.* 2017, 69, 935–942.

31- Majkova, Z.; Layne, J.; Sunkara, M.; Morris, A.J.; Toborek, M.; Hennig, B. Omega-3 fatty acid oxidation products prevent vascular endothelial cell activation by coplanar polychlorinated biphenyls. *Toxicol. Appl. Pharmacol*. 2011, 251, 41–49.

32- Gao, L.; Wang, J.; Sekhar, K.R.; Yin, H.; Yared, N.F.; Schneider, S.N.; Sasi, S.; Dalton, T.P.; Anderson, M.E.; Chan, J.Y.; et al. Novel n-3 fatty acid oxidation products activate Nrf2 by destabilizing the association between keap1 and cullin3. J. *Biol. Chem.* 2007, 282, 2529–2537.

33- Bryan, H.K.; Olayanju, A.; Goldring, C.E.; Park, B.K. The Nrf2 cell defence pathway: Keap1-dependent and independent mechanisms of regulation. *Biochem. Pharmacol*. 2013, 85, 705–717.

34- Joshi, R.; Adhikari, S.; Patro, B. S.; Chattopadhyay, S.; Mukherjee, T. Free radical scavenging behavior of folic acid: Evidence for possible antioxidant activity. *Free Radical Biol. Med.* 2001, 30, 1390–1399.

35- Rezk, B. M.; Haenen, G. R. M. M.; van der Vijgh, W. J. F.; Bast, A. Tetrahydrofolate and 5-methyltetrahydrofolate are folates with high antioxidant activity. Identification of the antioxidant pharmacophore. a\a*FEBS Lett.* 2003, *555*, 601–605.

36- Gliszczyńska-świgło, Anna; Muzolf, Małgorzata. pH-Dependent radical scavenging activity of folates. *Journal of agricultural and food chemistry*, 2007, 55.20: 8237-8242.

37- Baliga, Sharmila; Muglikar, Sangeeta; Kale, Rahul. Salivary pH: A diagnostic

biomarker. *Journal of Indian Society of Periodontology*, 2013, 17.4: 461.

38- Verhaar, Marianne C., et al. 5-methyltetrahydrofolate, the active form of folic acid, restores endothelial function in familial hypercholesterolemia. *Circulation*, 1998, 97.3: 237-241.

39- Dolin, Cara D.; Deierlein, Andrea L.; Evans, Mark I. Folic acid supplementation to prevent recurrent neural tube defects: 4 milligrams is too much. *Fetal diagnosis and therapy*, 2018, 44.3: 161-165

40- Saldanha, Leila G., et al. Perspective: Time to Resolve Confusion on Folate Amounts, Units, and Forms in Prenatal Supplements. *Advances in Nutrition*, 2020.

41- Mason, Joel B. Folate, cancer risk, and the Greek god, Proteus: a tale of two chameleons. *Nutrition reviews*, 2009, 67.4: 206-212.

42- Pack, Angela RC. Folate mouthwash: effects on established gingivitis in periodontal patients. *Journal of Clinical Periodontology*, 1984, 11.9: 619-628.

43- Mcauley, Julie L., et al. MUC1 cell surface mucin is a critical element of the mucosal barrier to infection. *The Journal of clinical investigation*, 2007, 117.8: 2313-2324.

44- Aboodi, Guy M., et al. Salivary cytoprotective proteins in inflammation and resolution during experimental gingivitis—a pilot study. *Frontiers in cellular and infection microbiology*, 2016, 5: 92.