



EFFECT OF APPLE ON TOTAL ANTIOXIDANT CAPACITY OF SALIVA (AN IN VIVO STUDY)

¹Dr. Nikhitha Aswath. P*, ² Prof. Dr. Vabitha Shetty and ³Prof Dr. Amitha M. Hegde

¹B.D.S, M.D.S (Final Year Post Graduate Student)

Department of Pedodontics and Preventive dentistry, A B Shetty Memorial Institute of Dental Sciences, Mangalore.

²B.D.S, M.D.S Professor Department of Pedodontics and Preventive dentistry, A B Shetty Memorial Institute of Dental Sciences, Mangalore.

³B.D.S, M.D.S Professor and Head of the Department of Pedodontics and Preventive dentistry Department of Pedodontics and Preventive dentistry, A B Shetty Memorial Institute of Dental Sciences, Mangalore.

*Corresponding Author: Dr. Nikhitha Aswath. P

B.D.S, M.D.S (Final Year Post Graduate Student) Department of Pedodontics and Preventive dentistry, A B Shetty Memorial Institute of Dental Sciences, Mangalore.

Article Received on 09/02/2016

Article Revised on 02/03/2017

Article Accepted on 22/03/2017

ABSTRACT

Aims and Objective: Oxidative stress may play an important role in the onset and the development of several inflammatory oral pathologies and dental caries may also be included. Apples contain very high content of phytochemicals accounting to their high antioxidative property. **Methodology:** 50 children aged between 6-11 were selected to participate in the study. Total Antioxidant Capacity (TAC) of saliva before and after the consumption of Kashmiri apple was determined using spectrophotometer. **Results:** In our study the TAC of saliva significantly increased from a baseline value of 0.934685 $\mu\text{M/L}$ to 1.460478 $\mu\text{M/L}$ 20min after consumption of apple. **Conclusion:** Natural substitutes like apple increases the antioxidant capacity of saliva and could help prevent or reduce the oxidative stress present due to an already established pathology.

KEYWORDS: Saliva, Total Atioxidant Capacity, Apple.

INTRODUCTION

Dental caries and periodontal disease are the most common pathologies amongst the various oral diseases in humans.^[1]

Oxidants and free radicals are produced as a part of various metabolic activities carried out in human body. However, when there is excess production of these free radicals, damage of the larger bio molecules like DNA, lipids, and proteins can occur. This situation is called as oxidative stress.

In the recent past researchers are claiming that oxidative stress may play an important role in the onset and progression of several inflammatory oral pathologies and dental caries may also be included.^[2] Studies have shown that there is a positive co-relation between total antioxidant capacity of saliva and dental caries^[3]

Humans have complex antioxidant defence systems to combat oxidative stress. However, they are not perfect enough to fight against all the possible oxidative damage.^[2] Hence external supply of antioxidants maybe needed to assist the body to combat this oxidative stress.

Phytochemicals, the non nutrient plant compounds such as the carotenoids, flavonoids, isoflavonoids, and phenolic acids, are the major contributor for most of protective effect produced by fruits and vegetables. A major function of the phytochemicals is protecting us from highly oxidative environment.^[4,5]

Apples contain very high content of phytochemicals accounting to their high antioxidative property, they not only contain their own antioxidants, but cause the body to make more of one of its own internal antioxidants; it can be further stated that this internal antioxidant impacts health beyond what can be done by external antioxidants from foods.^[2]

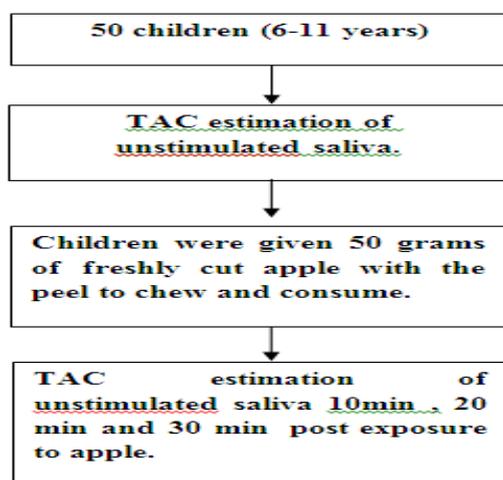
Since the economic burden associated with dental diseases can be extremely high, need for care is high among the poor and under privileged population.^[6] Hence bolstering the body's natural defence mechanism could reduce the risk of developing oral diseases. Apples are ranked the second for total concentration of phenolic compounds, and perhaps more importantly, apples had the highest portion of free phenolics when compared to other fruits.^[2] However, little is known about their impact on dental health.^[7] and there is paucity

of data about the effects of antioxidants in apple on dental health, hence the need of the study.

AIM OF THE STUDY

To assess the effect of apple on the total antioxidant capacity (TAC) of saliva.

MATERIALS AND METHOD



Study was approved by the ethical committee of the institution. The children and their parents were given verbal and written information, and informed consent was taken. Children were selected according to inclusion and exclusion criteria.

SOURCE OF DATA

50 children aged 6 to 11 years reporting to the Department of Pedodontics and Preventive dentistry, A.B. Memorial Institute of Dental Sciences.

INCLUSION CRITERIA

- Children with DMFT more than 3 (active carious lesions)
- Children whose parents/guardians are willing to participate in the study.

EXCLUSION CRITERIA-

Children

- using vitamin substitutes
- with underlying systemic disorders
- using orthodontic and prosthodontic appliances.

APPLE SAMPLE

Kashmiri apples (sourced from local market) were used.

Method Of Obtaining baseline TAC of Saliva.

2ml of unstimulated salivary sample was collected.^[8] in the morning between 10-10.30a.m. two hours after the consumption of their breakfast and stored at 4°C in plastic or glass vials. The collected saliva was subjected to antioxidant analysis using Spectrophotometer. This quantitative assay is based on the conversion of

OBJECTIVES OF THE STUDY

To estimate the total antioxidant content of saliva before and after administration of whole apple fruit.

molybdenum (Mo VI) by reducing agents like antioxidants to molybdenum (Mo.V), which further reacts with phosphate under acidic pH resulting in the formation of a green coloured complex, the intensity of which can be read spectrophotometrically at 695nm.^[9]

Chemicals required

- Conc. Sulphuric acid (H₂SO₄)
- Sodium di hydrogen phosphate (Na H₂PO₄)
- Ammonium heptamolybdate (NH₄)₆Mo₇O₂₄.4H₂O)
- Tri Chloro acetic acid (TCA)
- Ascorbic acid (Vit.C)

Estimation of total antioxidant capacity of the sample:

- 100µL of the sample (saliva) was pipetted out into a clean test tube and 100µL of 5% TCA is added to it to precipitate out the proteins in the sample, the mixture was then allowed to stand for about 5 minutes and centrifuged.
- 100µL of the clear supernatant was transferred into a clean test tube and 1 mL of TAC reagent [0.6Mm Conc. Sulphuric acid (H₂SO₄) + 28Mm Sodium di hydrogen phosphate (Na H₂PO₄) + 4mM Ammonium heptamolybdate (NH₄)₆Mo₇O₂₄.4H₂O) + Ascorbic acid (Vit.C)]was added to it. The mixture was then incubated in water bath at 90°C for 90 minutes.
- A blank was also maintained simultaneously by substituting 100µL of water instead of sample in the reaction mixture.
- Following the incubation the reaction mixture was cooled and the optical density of the greenish to bluish colour formed was read at 695nm against blank.

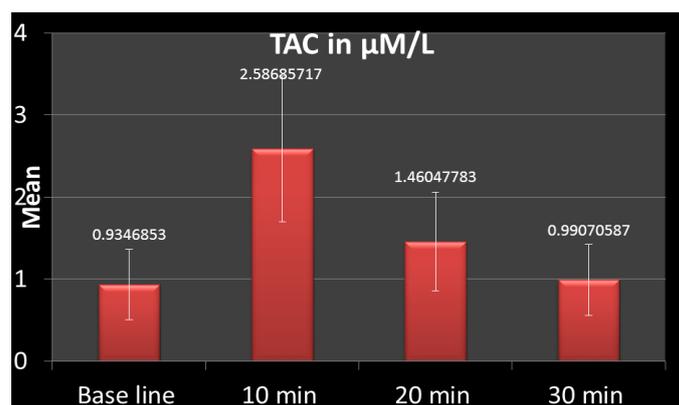
Kashmiri apple sourced from the local market were washed thoroughly using sterile water. The apples were then cut to weigh 50gm. Each child was asked to consume 50gm of freshly cut apple with the peel. 2ml of unstimulated salivary sample was collected 10 min, 20 min and 30 min after the consumption of apple and was subjected to antioxidant analysis using Spectrophotometer.^[9] as mentioned above.

STATISTICAL METHOD OF ANALYSIS

The concentration of the total antioxidants in the saliva was obtained by plotting the absorbance of the test against the standard graph, and the concentration is expressed as $\mu\text{g/ml}$. Results were subjected to statistical analysis namely

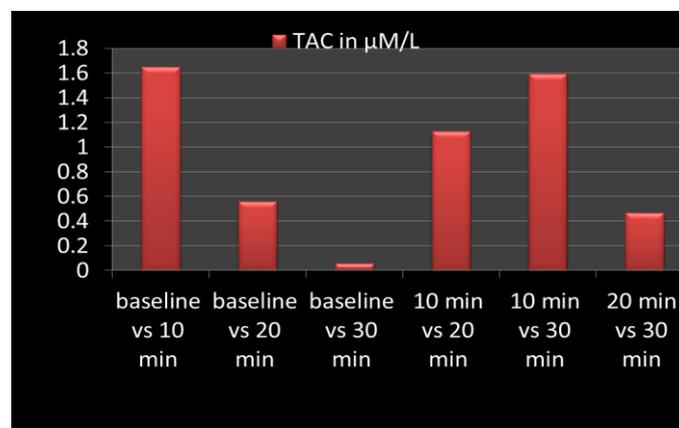
- Repeated measures ANOVA was used to compare the difference in TAC of saliva at different time intervals.
- Bonferroni adjustment was used for analysing the difference in the TAC of saliva between the various time intervals.

RESULTS



Graph 1: Salivary TAC levels pre and post exposure to apple

Post consumption of apple, a statistically significant ($p < 0.001$) rise in the TAC level of saliva was observed from baseline value of $0.934685 \mu\text{M/L}$ to $1.460478 \mu\text{M/L}$ at the end of 20 min.



Graph 2: Difference in the TAC of saliva between the various time intervals.

When the mean TAC values of saliva obtained at baseline time (Pre exposure), 10 and 20 min were compared with each other, a statistically significant ($p < 0.001$) raise in TAC levels was observed at all time intervals. We however found no significant difference between the TAC values compared at base line and at 30min.

DISCUSSION

Free Radicals and/or Reactive Oxygen Species (ROS) are high energy molecules released as a metabolic end

product during the reduction of molecular oxygen to water.^[10]

Oxidative stress, which occurs as a result of an imbalance between free radicals/reactive oxygen species and antioxidant system has been implicated as one of the important contributory etiologic factors in many of the oral inflammatory pathologies and dental caries is no exception.^[11]

"An antioxidant is any substance that when present at low concentrations compared to those of an

oxidisable substrate significantly delays or prevents oxidation of that substrate".^[10] Reports by Diab-Ladki R *et al.*^[12] and Sculley DV *et al.*^[13] have shown that decreased antioxidant activities of crevicular fluid and saliva are associated with the development of periodontitis and oral diseases.

Antioxidant micronutrients are important not only for limiting such oxidative and tissue damage, but also in preventing increased cytokine production, which is a result of prolonged activation of the immune response^[14]

Free radical/reactive oxygen species and antioxidant system appear to act in concert rather than alone, and measurement of any individual antioxidant may be less representative of whole antioxidant status.^[15] Few studies which evaluated individual antioxidants in caries free and caries active individuals showed no statistical difference between the groups and have suggested the evaluation of TAC.^[16] Hence, In our study, we have evaluated total antioxidant capacity .

Saliva may contribute a first line of defence against free radical-mediated oxidative stress, since the process of mastication promotes a variety of such reactions including lipid peroxidation.^[16] Although blood is the gold standard for doing many medical tests, it's not particularly convenient. Expecterated saliva serves as a nice alternative. Healthy individuals produce about a litre and a quarter of saliva per day. Nearly all the analytes that are in blood are also present within saliva^[17]. The unstimulated saliva samples has been preferred in determination of antioxidant defence parameters to stimulated saliva and it is claimed that TAC is higher in unstimulated saliva.^[17,18] Thus in our study we decided to use unstimulated saliva as a diagnostic tool.

Sometimes the inherent antioxidant system of the body might not be able to combat the oxidative stress. Need for external source of antioxidants at this point becomes essential.

Reports in recent years both in the popular and scientific press have stressed the value and advantages of natural ingredients as food preservatives. There is an implied assumption of safety for compounds that occur naturally in foods and that have been consumed for many centuries. It is preferable, however, to use substances that do not pose problems of proof of safety.^[19]

Fruits are a major natural resource of anti oxidants. Polyphenols found in fruits may contribute to increase the antioxidant activity of oral fluids.^[20]

Apple fruit is not only most commonly consumed fruit by the population but also ranked the second for total concentration of phenolic compounds, its pulp contains catechin, procyanidin, caffeic acid and chlorogenic acid among other components. The skin contains all the aforementioned substances as well as flavonoids, not

present in pulp, such as quercetin glycosides and cyanidin glycosides.^[21]

Most importantly, apples have the highest portion of free phenolics when compared to other fruits. This means that these compounds are not bound to other compounds in the fruit, and may be more available for eventual absorption hence increasing the beneficial effect of polyphenols compared to other fruits.^[22] In our present study we chose Kashmiri apple which are readily available in our Indian market and are a good source of antioxidants and can be incorporated into a child's daily diet, to improve general as well as oral health .

In our study the TAC of saliva significantly increased from a baseline value of 0.934685 $\mu\text{M/L}$ to a value of 1.460478 $\mu\text{M/L}$ 20min after consumption of apple. A maximum value of 2.58685717 $\mu\text{M/L}$ was recorded 10 min post consumption of apple. This observation was in accordance with the study performed by Shi Zhao, B.A.^[3] wherein he compared the TAC of saliva between whole apple fruit, apple with apple products and apple extract. Salivary total antioxidant capacity test significantly increased in the apple group, but not the other groups. However we observed no significant differences in the TAC of saliva measured at baseline and at the end of 30 min. Salivary clearance could be one of the reasons for drop in the salivary antioxidant capacity at the end of 30 min.

CONCLUSION

Within the limitations of the study we conclude that, regular consumption of whole apple fruit could aid the body in combating oxidative stress caused due to an established pathology in the oral cavity or could prevent a situation of oxidative stress by enhancing the endogenous antioxidant system by increasing the local availability of antioxidants through a rise in the TAC levels of saliva.

LIST OF REFERENCES

1. McDougall HA. studies on dental plaque. Aust Dent J 1963; 8: 261-265.
2. Zhao S. *Antioxidant Effects of Apples and Apple Products in Diet* (Doctoral dissertation, The Ohio State University); Ohio(US); 2011.
3. Hegde AM, Rai K, Padmanabhan V. Total antioxidant capacity of saliva and its relation with early childhood caries and rampant caries. J Clin Pediatr Dent. 2009 Spring; 33(3): 231-4.
4. Hollman P, Katan M: Absorption, metabolism and health effects of dietary flavonoids in man. Biomed Pharmacother 1997; 51: 305-10
5. Liu RH: Health benefits of fruit and vegetables are from additive and synergistic combination of phytochemicals. Am J Clin Nutr 2003; 78(3 Suppl): 517S-520S.
6. Peterson PE, Bourgeois D, Ogawa H, Estupinan-DS, Ndiaye C. The global burden of oral diseases and risk to oral health. Bull WHO 2005; 3: 661-669.

7. Slack, G. L.; Martin, W. J. Apple and Dental Health. *Br. Dent.J.* 1958; 18: 366-71
8. Sreebny L.M., Banoczy J, Baum BJ, Edgar WM, Epstein JB, Fox PC et al. Saliva its role in health and disease. *International Dental Journal*, 1992; 42; 291-304.
9. Prieto P, Pineda M, Aguilar M. Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem.* 1999 May 1; 269(2): 337-41.
10. Halliwell B. Antioxidants: the basics- what they are and how to evaluate them. In: *Antioxidants in disease mechanisms and therapy. Advances in pharmacology.* San Diego: Academic Press, 1997; 38: 3-20
11. Battino M, Bullon S, Wilson M, Newman H. Oxidative injury and inflammatory periodontal diseases: The challenge of antioxidants to free radicals. *Crit Rev Oral Biol Med* 1999; 10: 458-76.
12. Diab-Ladki R, Pellat B, Chahine R. Decrease in the total antioxidant activity of saliva in patients with periodontal disease. *Clin Oral Investig* 2003; 7: 103-7.
13. Sculley DV, Langley-Evans SC. Periodontal disease is associated with lower antioxidant capacity in whole saliva and evidence of increased protein oxidation. *Clin Sci* 2003; 105: 167-72.
14. S. Carnelio¹, S. A. Khan² and G. Rodrigues³ Definite, probable or dubious: antioxidants trilogy in clinical dentistry *British Dental Journal* 2008; 204: 29-32.
15. Prior RL, Cao G. In vivo total antioxidant capacity: Comparison of different analytical methods. *Free Radic Biol Med* 1999; 27: 1173-81.
16. Terao J, Nagao A. Antioxidative effect of human saliva on lipid peroxidation. *Agric Biol Chem* 1991; 55: 869-72.
17. Edgar WM. Saliva: its secretion, composition and functions. *BDJ.* 1992; 122: 305-312.
18. US Department of Health and Human Services: *Oral Health in America: a report of the surgeon general*, Rockville, MD, 2000, National Institutes of Health.
19. Pratt DE, Hudson BJ. Natural antioxidants not exploited commercially. In *Food antioxidants* Springer, Amsterdam, Netherlands 1990; 171-191.
20. Kumar D, Pandey RK, Agrawal D, Agrawal D. An estimation and evaluation of total antioxidant capacity of saliva in children with severe early childhood caries. *Int J Paediatr Dent.* 2011 Nov; 21(6): 459-64.
21. Koo H, Jeon JG. Naturally occurring molecules as alternative therapeutic agents against cariogenic biofilms. *Adv Dental Research.* 2009 Aug 1; 21(1): 63-8.
22. Boyer J, Liu RH. Apple phytochemicals and their health benefits. *Nutr J.* 2004 May.