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RESEARCH ARTICLE

Efficacy of Ocimum Sanctum on total antioxidant capacity levels in saliva of children with caries - A clinical study.

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Abstract

Despite great achievements in oral health of populations globally, problem still remain in many communities all over world, particularly among under privileged groups in the developing countries and dental caries is one of that. Saliva has many functions in oral cavity and is the first line of defense against dental caries. Oxidative stress may play an important role in the onset and development of several inflammatory oral pathologies and dental caries may also be included, suggesting that total antioxidant status may be of importance in determining the progression caries. Supplementation of antioxidants acts as scavengers of free radicals and prevents the progression and occurrence of disease. *Ocimum Sanctum* medically proven good source of antioxidant and it is abundantly available, easily accessible economically feasible.

Objectives: To evaluate the total antioxidant levels in saliva of caries active children before and after using *Ocimum Sanctum* leaves

Materials and Method Fifty caries active children aged between 8-14 year were selected for study. Base line saliva was collected and After 10 minutes the children were asked to chew *Ocimum Sanctum* leaves with 5ml of water in the mouth, rinse for 5-7minutes and expectorate .After 30 minutes saliva was collected. Each sample was analyzed for total antioxidant capacity using Spectro photometer.

Results: Total antioxidant capacity of saliva increased with caries active children. and greater drop in total antioxidant capacity of saliva after chewing *Ocimum Sanctum* leaves ($p < 0.001$).

Conclusion: Antioxidant properties of *Ocimum sanctum* can be considered as safe, effective and economic alternate option for preventing caries.

Introduction

Dental caries is still considered as the most important global oral health burden and 60%-90% of the children are affected, due to negligence, lack of prevention awareness, costly dental care services, scarce or no dental care services, especially in the rural areas[1, 2]

Saliva serves as a mirror of body's health with antioxidant system as one of the important defense mechanism, made up of various enzymes and small molecules[2, 3] Recently it has been claimed that the imbalance in levels of free radicals, reactive oxygen species and antioxidants in saliva plays an important role in the onset and development of caries[4] . However these three factors act in concert rather than the alone. Thus evaluation of total antioxidant capacity of saliva paves way in understanding the risk of
Individual to dental caries[5, 6]
It has been well accepted that supplementation of antioxidants help to slow down the excess oxidation process and protect the cells from free radicals and prevent the progression and occurrence of disease[2,7]
In recent years investigations on many plant based product revealed their usefulness as source of antioxidants and also received therapeutic significance[9]. *Ocimum Sanctum* which is regarded as the "Queen of herbs" is a medically proven good source of antioxidants, antimicrobial, antifungal, antidiabitic, analgesic, cardio-protective agent, which is non- toxic and widely available throughout India. [10, 11, 12]
Many studies have reported that flavonoids and phenol compounds like crisilineol, cirsimaritin isothymusin, apigenin and rosmarinic acid and eugenol present in fresh leaves of *Ocimum Sanctum* are responsible for its antioxidant activity. [13] So a need to explore, develop and promote the use of locally available and accessible methods of dental disease prevention is necessary. As studies related to antioxidant properties of *Ocimum Sanctum* in caries prevention is lacking in dentistry, research in this area is necessary to generate the required evidence.

Aims &Objectives:

Evaluation of total antioxidant levels in saliva of caries active children before and after using *Ocimum Sanctum* leaves.

Materials and Method:-

Study type and design:

In vivo study, experimental design

Methodology:-

Before the procedure, ethical clearance was obtained from the institutional ethical committee. Fifty children aged between 8 -14years reporting to the Department of Paedodontics and Preventive Dentistry, Yenepoya Dental College Mangalore were selected. And informed consent was obtained from parent / guardian of each child before starting the procedure and detailed case history was recorded.

Inclusion criteria:

Children aged between 8 -14years.
Free from systemic or local diseases, which affects salivary secretions.
Not physically or mentally compromised.

Exclusion criteria:

Children on long term medications.
Not wearing any intraoral appliances.

Clinical examination

Those children who fulfilled the above criteria were screened for caries status. All clinical examination was carried out by single examiner. Caries detection was based only on clinical caries, that observed with dental mirror and explorer and radiographic examination was not performed. Caries active group were selected within the subjects that had at least five clinical caries surface requiring restoration according to the WHO criteria. [20]

Saliva collection:-

On the day of saliva collection, participating children were instructed not to use any oral stimulation such as eating and drinking for 2hours prior to collection[21] .To control the circadian variation, samples were collected between 10am-11.30am. Children were made to sit on a dental chair with anterior head protrusion position. Saliva was allowed to accumulate in patient's mouth for 2minutes and was aspirated directly from the floor of the mouth with the help of disposable syringes and transferred to a sterile micro centrifuge tubes. The saliva sample were first weighed and reweighed again then immediately put on to ice and stored at 4 ^{0}C and transferred to the laboratory up to 20 minutes and kept at -80oC until the analysis. After 10 minutes of base line sample collection, the children were asked to chew five to six freshly collected and washed leaves of *Ocimum Sanctum* with 5ml of water in the mouth, rinse for 5-7min, and expectorate .After 30 minutes saliva was

collected by the same method used for collection of baseline saliva. And transferred aseptically to the laboratory at 4^0C. Each sample was estimated for total antioxidant capacity of saliva before and after chewing of *Ocimum Sanctum leaves*

Methodology:

Principle of the method:

Total antioxidant capacity of saliva by Phospho molybdenum method. [22]

This quantitative assay is based on the conversion of molybdenum (Mo V1) by reducing agents like antioxidants to molybdenum (Mo V) which reacts with phosphate under acidic pH resulting in the formation of a green colored complex the intensity of which can be read spectrophotometrically at 695nm.

Statistical analysis:

The obtained result was tabulated and then analyzed by using students paired t test

Result:

Paired Samples Statistics

		Mean	N	Std. Deviation	Std. Error Mean
Pair 1	Before	41.1600	50	7.20929	1.01955
	After	36.7200	50	7.29842	1.03215

Table 1

Paired Samples Test

	Paired Differences					t	df	P VALUE
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
Pair 1 Before – After	4.44000	1.66795	.23588	3.96598	4.91402	18.823	49	**<0.001**

Table 2

Students paired t test showed total antioxidant capacity of saliva in caries active children before chewing *Ocimum Sanctum* leaves mean was41.16 and after chewing *Ocimum Sanctum* leaves mean was 36.72 with the mean difference of 4.44 .The t value was 18.828 and P value was <0.001

There was a significant drop in total antioxidant capacity of saliva of caries active children after chewing *Ocimum Sanctum* leaves.(Figure1)

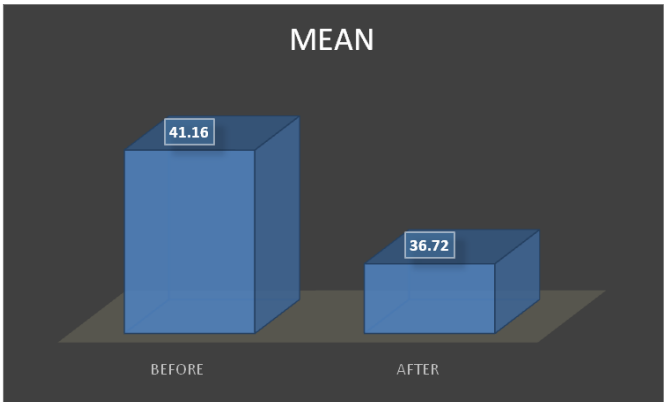

Figure 1

Discussion:-Dental caries is a common oral health problems and its prevention is one of the most important strategies in many countries. The first line of defense against dental caries is saliva. The composition and Physiology of saliva warrants thorough investigation because it clearly influences oral health[15]

Cells and biological fluid have an array of protective antioxidant mechanisms, both for preventing and production of free radicals and for repairing oxidative damage. Free radicals are produced during dental decay and the number appears to vary directly with caries activity. Many studies have been performed to correlate the total antioxidant capacity level in children with caries & reported that the total antioxidant capacity increases with increase in caries activity .[4,7, 23, 24, 25.] In the present study the total antioxidant capacity of saliva in caries active children was more before chewing the *Ocimum Sanctum* leaves. (Mean value 41.16). An important factor for the increase in total antioxidant capacity of saliva could also be triggered function of salivary peroxidase system which constitutes one of the major salivary antioxidant systems. Salivary peroxidase brings about the control of oral bacteria that form dental plaque, to imbalances in the ecology, and which lead to dental caries. [6]

In this study, we have evaluated the total antioxidant capacity of saliva as it is suggested that FR/ROS and antioxidant system appear to act in concert rather than alone. Investigation of individual antioxidant activity may be misleading, and the measurement of any individual antioxidant may be less representative of the whole anti oxidant status. Moreover the number of different antioxidants makes it difficult, and also expensive to measure each of them separately.[26]

Supplementation of antioxidants may prevent /delay oxidation and neutralize free radicals responsible of progression of diseases and plants are the natural source for antioxidants and therapies involving plants have been existed for thousands of years and some may be as old as human civilization itself. One such medicinal plant is *Ocimum Sanctum*, which is principle herb of Ayurveda, India's ancient holistic health system, which is medically proven antioxidant, antimicrobial, antifungal, antidiabitic, analgesic, cardio-protective agent, which is non- toxic and widely available throughout India. [10, 11, 12.] In the present study *Ocimum Sanctum* leaves were used and it was observed that greater drop in the total antioxidant capacity levels of saliva of caries active children 30 minutes after chewing. ($p<0.001$ highly significant.) The results of our study could not be compared with that of other studies as this is the first study of its kind.

Results of this study was encouraging which may **favor** the use of *Ocimum Sanctum* leaves in children as valuable health tool in preventing free radical-related disorders, as it is abundantly available, easily accessible, economically feasible and culturally acceptable and may possess minimal side effects. However to recommend confidently the antioxidant properties of *Ocimum sanctum* leaves, a long term study with larger sample is required and this study hopefully shall begin the journey.

Conclusion;

From the study we derived the following:

Total antioxidant capacity of saliva increased with caries active children. Greater drop in total antioxidant capacity of saliva after chewing *Ocimum Sanctum* leaves($p <0.001$).This is an encouraging result which may favour the promotion of antioxidant properties of *Ocimum Sanctum*, which is the possible source of economical dental health care for the low socioeconomic strata and can be effectively utilized to fight against common and prevalent oral health problems.

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