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Title: THEOBROMINE: THE NEXT FRONTIER IN REMINERALISATION OF DENTAL CARIES

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Key words: Theobromine, remineralisation, dental caries, initial lesions

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THEOBROMINE: THE NEXT FRONTIER IN REMINERALISATION OF DENTAL CARIES

ABSTRACT

Remineralization is the process of returning mineral ions into a hydroxyapatite structure characterized by mineral deposition on the enamel surface. The use of fluorine as remineralization agent with side effects such as fluorosis. Cocoa bean extract contains theobromine that can be used as an alternative remineralization ingredient. Cocoa (Theobroma cacao) as natural materials can be utilized in the field of dentistry with the extract containing theobromine, which is expected to be an alternative to fluoride. Some research on Theobromine (3,7-dimethylxantine) contained in the cocoa fruit extracts, found that theobromine can be used as a natural ingredient to prevent dental caries. This paper reviews the remineralisation potential in dental caries and the possibility of being an adjunct to fluoride.

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INTRODUCTION

The non-invasive treatment of early caries lesion by remineralisation has the potential to be a major advance in the management of dental caries(1). Remineralisation occurs by two modes, either at the surface of the lesion causing the arrest of the lesion or it extends into the subsurface leading to complete remineralisation(2).

Dental caries in its early stages of formation (non cavitated) can be remineralised and this remineralisation can be facilitated by Fluoride(3). The ability of Fluoride to promote remineralisation and inhibit caries formation in the oral environment is limited by the availability of Calcium and Phosphate in saliva and plaque formation(4).

Over the years, Fluoride containing dentifrices have been the usual choice of prevention and remineralisation of dental caries. However, Fluoride has been undergoing scrutiny and researches from the US Environment Protection Agency’s National Health and Environmental effects research laboratory have categorised Fluoride as a chemical having substantial evidence of developmental neurotoxicity(5). Scientists are still anticipating real breakthrough in using more effective agents for caries prevention. Thus, a constant need for the development of newer biomaterials, which can act as an adjunct to the existing fluorides or can act individually for promoting safer remineralisation is advocated(6). Currently, modern medicine is shifting to add newer, safer and natural ingredients since it is believed that drugs derived from natural materials are relatively safer and inexpensive. A plethora of remineralisation agents are available in the market. Some of these are CPP-ACP, Tri Calcium Phosphate, Amine Fluoride, Ozone, Xylitol, Calcium Sodium Phosphosilicate, Galla Chinesis, Theobromine.
Theobromine, previously known as Xantheose(7) was first discovered by the Russian chemist, Alexander Voskresensky in 1841 and first synthesized from xanthine by Herman Emil Fischer. It is a natural dimethyl xanthine alkaloid of plant origin, viz, chocolate, leaves of tea plant and kola nut. Its chemical formula is \( \text{C}_7\text{H}_8\text{N}_4\text{O}_2 \). The name theobromine is derived from Theo (God) and Broma (Food) meaning “Food of the Gods”.

Cocoa is the natural source of theobromine found at a concentration of 1.5-3.0% in cacao bean (Theobroma cacao), reaped from the husk of the bean. The Mayans of Central America are credited for being the first people to consume cocoa. The Mayan historians refer cacao to as Mayan gold. The Mayan elites would often pulverize and grind the cacao pods with spices, corn and hot water to form hot frothy beverages. The hot water served as a method by which Theobromine was physically extracted. The consumption of copious amounts of theobromine has been the hallmark of Mayan dental health and had a direct correlation to enamel growth and thickness.

Cocoa contains antioxidants like flavonoids and polyphenols aid in reducing the inflammatory activity of cytokines(9). Cocoa also contains Oleic acids and linoleic acids have antibacterial action against S mutans. Alkaloids present in cocoa seeds and bark have enamel remineralisation ability and increase the crystal size, creating a much harder and acid dissolution resistant enamel(10–12).

**PROPERTIES OF THEOBRONINE**

Theobromine is a purine alkaloid derived from xanthosine, a nucleoside. Cleavage of the ribose and N-methylation yields 1-methylxanthosine. It is the precursor to theobromine, which in turn is a precursor to caffeine.

It is a white crystalline powder with a sublimation point of 290-295 C. The plasma half-life of theobromine is 50% on consumption in 6hours. It has a boiling point of 357 C and a molecular weight of 180.17. It is soluble in water at a concentration of 1g/1.5 L and soluble in ethanol at 1g/2.2L. It is bitter in taste

Its levels are higher in dark chocolates (approx. 10 g/kg) than in milk chocolates (1-5 g/kg). The mean Theobromine content of cocoa beans is approximately 20.3 mg/g.
STUDIES ON THEOBROMINE

Theobromine may possess the unique property of preventing dental caries by causing an increase of the hydroxyapatite crystal size which inhibits dissolution of apatite of the enamel surface. Theobromine has been observed to be much better than fluoride at increasing the hardness of the enamel surface which supports the idea that chocolate may be less cariogenic.

A series of studies by Stralfors suggest that cocoa powder and chocolate may contain a caries inhibitory substance. He demonstrated that cocoa powder washed with water possessed considerably less anti-cariogenic effect than unwashed cocoa powder. Nevertheless, he still observed a considerable anti-caries effect in the washed cocoa powder group, "indicating an existence of a non-water soluble cariostatic factor," and alluded to the existence of "two caries-inhibitory substances in cocoa: one water-soluble and another which is sparingly soluble in water". He also speculated that the tannin in cocoa could be a caries inhibitive constituent and that other caries-inhibitive constituents may be present. In another study, Whole cocoa powder inhibited caries by 84%, 75%, 60%, and 42%, when the cocoa content of the diet was 20%, 10%, 5%, and 2%, respectively. However, cocoa butter incorporated into a diet in an amount of 15% increased dental caries. He concluded that the cariostatic factors are found in the non-fat part of cocoa.

He also tested the effect of Calcium phosphate mixed into chocolate and observed that there was some anti-caries factor in chocolate. These reports suggest that cocoa extract, the main component of chocolate could contain an agent with anticaries potential(13,14).

Besic FC, Bayard M, Weimann MR (1975) supplemented the maternal diet of rats with theobromine at a concentration of 1 mg/100g of the dams’ weight. During the lactating period, the suckling rat pups received theobromine through the milk. On post-natal day 22, the maxillary and mandibular molars were extracted and the acid solubility of the enamel surface was studied. The data showed that the first molars were formed with bigger HAP crystals resistant to acid dissolution(15).

Falster et al 1993 while researching the effect of caffeine on the mineralisation of teeth, showed that caffeine decreases the crystal size on enamel when exposed early at the critical period of growth(14).
During the caffeine study, it was discovered that Theobromine which is the same Xanthine family, showed that Theobromine enhances crystallinity\(^{(15)}\).

Nakamoto et al (1999, 2001) showed that new born rats exposed to theobromine while growing up showed comparatively lesser dissolution of Ca, P and Magnesium from the enamel apatite compared to the control rats when the teeth were exposed to weak acid solution. In view of the above animal studies, Nakamoto demonstrated that theobromine causes a lesser dissolution of minerals in an acidic medium\(^{(16)}\).

In an extensive study on theobromine by Sadeghpour and Nakamoto in 2011 subjecting the remineralised enamel surfaces to further acidic challenge observed that theobromine treated teeth had lost 8% less Calcium than Fluoride treated teeth\(^{(17)}\).

Kargul et al (2012) conducted studies using human teeth to investigate the effect of theobromine exposure on enamel surface in vitro. The results showed that the microhardness of the enamel surface of the theobromine group was greatly enhanced when compared to fluoride. They also concluded that less Theobromine than fluoride is required to obtain harder enamel surfaces\(^{(18)}\).

Grace Synfira et al (2012) found that increased enamel microhardness after treatment with four different concentrations of theobromine. A statistically significant increase of enamel microhardness was shown with increase in theobromine concentration\(^{(19)}\).

Amaechi BT et al. (2013) demonstrated that theobromine in a apatite forming medium can enhance the remineralisation potential in comparison to a standard Sodium fluoride dentifrice. It was demonstrated that theobromine at a molar level 71 times less than that of fluoride has an enamel remineralisation effect comparable to that of fluoride\(^{(17)}\).

Abdillah et al (2014) concluded that application of theobromine was able to equally increase the hardness of the enamel surface similar to fluoride\(^{(20)}\).

Tetsuo Nakamoto et al (2016) stated that theobromine causes the formation of larger HAP crystallites in vitro. An SEM demonstrated qualititaive repair of the enamel surface by theobromine making the process
of enamel remineralisation produces smooth enamel surface. Hence, they concluded that theobromine maybe a better alternative than fluoride (16).

An increase in micro hardness of enamel was observed after the application of Theobromine gel as reported by Irawan (2017), Suryana (2018) (21)(22).

Taneja et al (2019) has reported remineralisation of smooth surface lesions with Theobromine (23).

In another study by Fabricio Gonzalez et al (2019), they added 1 %Theobromine to GIC and some properties of GIC was modified and it enhanced the microhardness and reduced the amount of biofilm deposition over the material surface and it did not interfere with the sorption, solubility, colour or fluoride release to saliva (24).

Additionally, Theobromine can be incorporated to mouth rinses and dentifrices and showed remineralisation (20).

MECHANISM OF ANTICARIES ACTIVITY OF THEOBROMINE

The Theobromine molecule (C₇H₈N₄O₂) binds with the Hydroxyapatite crystal of the enamel. The highly electronegative Nitrogen and Oxygen of the Theobromine attract Calcium and Phosphate ions with lower electronegativity. Additionally, the H ions of the Hydroxyapatite dissolve easily in the relative lower acidic pH and are replaced by Calcium and Phosphate ions. This leads to the emergence of a more stable Theobromine HAP [Ca₁₀(PO₄)₆(OHC₇H₈N₄O₂)] which is 4 times the size of hydroxyapatite crystal which is less acid soluble. The larger crystalline size is caused by dental crystallinity bond through the apatite forming system. The Theobromine apatite crystal thus formed has an increased crystal hardness and density and thus stronger and less vulnerable to acid attacks. Larger crystals provide less surface for reaction with acid in comparison with smaller crystals (25,26).

Theobromine increases the hardness of tooth enamel through interstitial reaction as a substitution for the loss of hydroxyl apatite crystals. Theobromine crystals are smaller than HAP crystals and this makes it easier to penetrate into the enamel micro channels and replace ions in the composition of the apatite (27).
Gundogar et al (2019) observed that Theobromine at 500 mg/L aids in Calcium and Phosphate accumulation on the enamel in a manner similar to that of 1450 ppm Fluoride thereby increasing the surface hardness.

Furthermore, Theobromine negatively interacts with Streptococci mutans. The bacteriostatic activity of Theobromine can be attributed to several factors:

1. Hindering bacterial adhesion to the tooth surface(24)
2. Retarding biofilm progression and acid production by S mutans (28)
3. Supressing the activity of Streptococcus mutans (29,30)
4. inhibitory activity in glucosyltransferase(7,29)
5. Inhibiting bacterial amylase activity causing alteration in carbohydrate metabolism which furthermore reduces the rate of acid production by S mutans(24)
6. Triggering irreversible damage to the cytoplasmic membrane of bacteria(24)

DENTIN HYPERSENSITIVITY

When added to dentifrices, Theobromine aids in dentin tubule obliteration which might aid in reducing sensitivity(11,24)

CONCLUSION

Theobromine can be considered as a natural and biologically active supplementary choice to fluoride compounds, providing greater anti-bacterial, remineralisation and desensitization efficacy. Also, this innovative material can be readily absorbed and metabolised by the human body making it free from unpropitious effects of fluorides, specifically, fluoride toxicity, gastric irritation etc. Theobromine has a promising result and can be acclaimed as an additional strategy for caries prevention.
REFERENCES


