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Efficacy of a cocoa-based non-fluoridated remineralizing agent (Theobromine): A systematic review

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***Abstract*---**Background and Objectives: This systematic review aimed in investigating the effectiveness of theobromine as a remineralizing agent on early enamel lesions. Materials and Methods: An electronic search was performed in the three databases (Google scholar, Pubmed and Medline) using keywords. Articles published in the English language and without any time limit were and 6038 articles were collected. Among the collected articles, considering the inclusion and exclusion criteria, a total of eight articles were selected for this systematic review. Results: All the selected articles assessed and compared the remineralization potential of theobromine containing agents (solution, dentifrices, gel) with fluoridated remineralizing agents. Majority of the selected studies indicated that theobromine is an effective remineralizing agent. Conclusion: The novel theobromine has remineralizing potential equivalent to fluoridated agents and it can be considered as an alternative for fluoridated remineralizing agents.

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***Keywords*---**Theobromine, Remineralization, Fluoride, Enamel caries, Incipient caries.

Introduction

Alterations in the remineralization and demineralization cycle in the oral cavity led to the formation of dental caries, a common oral disease. The early enamel caries can be reversed if it is diagnosed in an earlier stage. As prevention of oral disease is the foremost goal of modern dentistry, it is focused to explore an ideal remineralizing agent to treat early enamel caries [1]. Numerous remineralizing agents were introduced, broadly classified into fluoridated and non-fluoridated remineralizing agents. Fluoride is an extensively used remineralizing agent either systemic intake or topical application. However, the major limitation of fluoride is that excessive intake results in local and systemic adverse effects such as skeletal and dental fluorosis or even death. which led to a search for an alternative non-fluoridated remineralizing agent [2].

Theobromine (3,7 dimethylxanthine) is one such non fluoridated remineralizing agent gaining interest in recent years. Theobromine is a bitter, alkaloid, organic compound, derived from cocoa tree, that enhances the crystalline growth in enamel. Its chemical formula is C7H8N4O2. The word theobromine was derived from the genus name of cocoa tree and suffix -ine was given to nitrogen-containing compound [3]. Chocolates were considered as caries-promoting food due to the presence of caffeine. But theobromine a cocoa product present abundance in chocolates is found to provide a beneficial effect on dentition by promoting enamel remineralization. The waste material disposed from the chocolate factory named cocoa bean husk contains approximately 1 - 4 % of natural theobromine [3].

Theobromine was first discovered by Russian chemist Alexander Woskresensky in 1841 [4]. Nakamoto [5] did research on methylated xanthines and discovered the potential benefits of theobromine on teeth. Sadeghpour [6] expanded Nakamoto's research by measuring the microhardness of tooth enamel treated with artificial saliva with theobromine. Theobromine attracts the minerals (calcium and phosphorus) from saliva and stimulates the growth of larger unit hydroxyapatite crystals (2 mu mm). These crystals influence the enamel surface hardness thereby increasing the resistance of tooth enamel to acid attack. Sadeghpour and Nakamoto study paved a way for further research on a newer remineralizing agent, theobromine.

It is one of the excellent non fluoridated remineralizing agent, holding a promising future of preventive dentistry. It is found in chocolate (higher concentration in dark chocolates) and cocoa-containing foods, as well as in the leaves of tea plants, and the cola nut. The anticaries potential of theobromine is proved in many studies [3]. Thus, the present systematic review is focused to evaluate the remineralization potential of theobromine.

Methodology

An extensive literature search was performed in Google Scholar, Pubmed, Medline and Dentistry and Oral Sciences databases using the keywords, “theobromine” AND “remineralization”, “theobromine” AND “enamel”, “cocoa” AND “enamel remineralization”, “theobromine” AND “dental caries”, “theobromine” AND “dentistry”. An author independently collected all relevant articles without any limit in the time period.

PICO

The formulated research question was
‘Is theobromine a superior remineralizing agent when compared to other remineralizing agents?’
P-enamel; I-theobromine; C-other remineralizing agents; O- remineralizing effectiveness of theobromine.

Eligibility criteria

The articles evaluating the effectiveness of theobromine in enamel remineralization were selected based on the following inclusion and exclusion criteria: (a) Studies in English language were included; (b) Studies assessing the remineralization potential of theobromine were included; (c) Review articles were excluded; (d) Animal studies were excluded; (e) Studies evaluating the potential of theobromine on reducing plaque formation, dentin tubule occlusion (dentin sensitivity), and other criteria were excluded; (f) Comparison study (consisting of a control group) was included.

Data extraction

All the relevant articles (6038 articles - before screening) were collected by an individual author. Initial screening was performed by two authors after reading all the titles and abstracts of the articles. If the information in the abstract was insufficient to make a decision, full text was read. Final decision was taken after a discussion between the two authors. Disagreements between the authors were resolved by discussion until a consensus had been reached.

Risk of bias and quality assessment

The quality of the selected articles was assessed using the modified CONSORT checklist. Studies with four or five factors are considered acceptable quality; studies with two or three factors are moderate quality and; only one factor or not provided the assessed data was considered low-quality studies. (Table 2)

Results

In this study, a total of 6038 articles were retrieved through electronic search from the databases. 61 articles extracted for full-text after applying limits (review of literature, studies evaluating dentin tubule occlusion, anti-bacterial effect, effect of toothbrushing, bovine incisors). Though an attempt was made to collect

clinical and laboratory studies, we ended up collecting only laboratory studies, as there was a lack of clinical studies on this topic. For homogeneity of the systemic review, only those laboratory studies assessing remineralization potential of theobromine in pH cycling (replicating oral environment) were included for the review. 8 articles addressing the remineralization potential of theobromine on human teeth were collected and evaluated in this study. Figure 1 shows the flow chart of the included articles.

Also, we found that all the retrieved articles were comparing theobromine with fluoride. There was no article comparing the remineralization potential of theobromine with other fluoridated remineralizing agents such as silver diamine fluoride or non-fluoridated remineralizing agent such as ACP-CCP. The selected teeth were premolars in six studies, permanent molars in one study and both premolars and molars in one study. The summary of the articles included in this systematic review is described in table 1. The risk of bias and quality assessment of the selected articles were shown in table 2.

Discussion

In recent decades, researchers are searching for a novel non-fluoridated remineralizing agent with minimum or no potential adverse effects on the host even on the administration of the highest quantity. Theobromine has evolved as one such remineralizing agent with the anti-cariostatic property. Theobromine also has systemic effects on humans. It is a myocardial stimulator and vasodilator. It increases the heartbeat and also dilates the blood vessels. It acts as a smooth muscle relaxant [7,8]. In dentistry, theobromine can be used for remineralization of white spot lesions and for sensitivity (dentin tubule occlusion).
Though we included both clinical and laboratory studies, till now no clinical trials were conducted to evaluate the effectiveness of theobromine as a remineralizing agent. We had only choice to include laboratory studies. For the homogeneity of the study as well as to minimize bias we limited our review to only those in-vitro studies where the specimens were subjected to pH cycling (demineralization/ remineralization). pH cycling involves sequential exposure of specimens to demineralization and remineralization that mimics the oral environment.

Nakamoto et al [9,10] from his research concluded that 1.1 mmol/l concentration of theobromine increases the surface hardness of the enamel more effectively. Later in 2012, Kargul [11] et al evaluated different concentrations of theobromine (100 mg/l and 200 mg/l). They demonstrated the required minimal concentration of theobromine is 200 mg/ l to exhibit superior enamel surface hardness.
Amaenchi B et al evaluated 1.1mmol/l theobromine concentration [12]. Four studies [13,14,15,16], except two studies on dentifrices [17,18] and one study on novel varnish[19].

Pushpalatha C et al invented a novel varnish with theobromine as an active ingredient and compared it with the commercial 5% sodium fluoride varnish and demonstrated the superior hardness of enamel in the theobromine group [19]. They reported much harder and more resistant enamel surface to dissolution on theobromine application than fluoride thus increasing remineralization.

Theobromine varnish enhances calcium deposition on the demineralized enamel surface resulting in an increased mineral gain and surface microhardness. Pushpalatha C et al stated that the novel varnish will provide a promising alternative for fluoride in enamel remineralization [19].

Toothpaste containing theobromine as an active ingredient is available commercially in the trade name Theodent [fig 2]. It is more favorable and safer for children and has its advantages as it does not produce harmful side effects on ingestion when compared to fluoride. The composition of Theodent is given in table 3. Premanth JP et al [17] and Shawky R et al provided comparable results on theodent toothpaste. Insignificant differences were found between theobromine and fluoride dentifrices [18]. In spite of the result, the studies concluded that theobromine is an effective agent for tooth remineralization.

Five studies assessed the surface characteristics of the enamel using Scanning Electron Microscope (SEM), [14,15,18] Energy Dispersive X-ray analysis (EDXA), [12,14,15,16] and confocal laser scanning microscopy (CLSM) [17]. SEM is one of the most sensitive methods to assess the demineralization and remineralization process under laboratory circumstances. Contrast results were obtained on SEM analysis. Hussein S I et al [14] demonstrated smooth enamel surface in theobromine solution with fewer rods exposed in some regions with circular concavities. A rough enamel surface with wide irregular deep depressions was observed on theobromine gel group. Shawky R et al [18] reported smoother and nearly normal enamel surface treated with theobromine toothpaste. Whereas Elsherbini MS et al [15] demonstrated enhanced enamel surface characteristics in theobromine gel group. The cervical and middle third of the tooth presented relatively smooth enamel with occasional depressions. Occlusal surface alone showed frequent defects with noticeable circular shallow depressions in different sizes and shapes. The size of the enamel crystals is one of the factors that control acid dissolution. Theobromine leads to the formation of large apatite crystals that have limited surface area for chemical reactivity. Hence, its dissolution is slower than the small crystals which could be beneficial in theobromine group.

EDXA is a surface analytical technique in which the chemical characterization of the sample is quantitatively analyzed. Hussein SI et al showed the highest mean values of calcium and phosphorus in theobromine solution group (1.98 ± 0.42) and the least in theobromine gel group (1.56 ± 0.25) but the results were insignificant [14]. Elsherbini MS et al showed highest Calcium/ phosphate ratio in theobromine gel (1.89 ± 0.11) than in fluoride gel (1.73 ± 0.13) but the results were insignificant [15]. Farhad F et al showed similar results. Higher Calcium/ phosphate deposition was found in theobromine solution (3.82 ± 1.83) [16].

Amaenchi B et al reported higher calcium deposition in theobromine and NaF treatments but no statistically significant results were found [12]. In theobromine molecule ($C_7H_8N_4O_2$) the oxygen and nitrogen are presented in higher electronegativity that attracts the low electronegativity ions (calcium and phosphate) resulting in the formation of new crystals of theobromine apatite with higher calcium deposition.

Premanth P et al utilized CLSM to assess the lesion depth [17]. Maximum lesion depth was evident in theobromine (375.30 ± 15.37) and minimum lesion depth in tricalcium phosphate dentifrice (240.68 ± 26.68). Pushpalatha C et al recorded the mineral content of the tooth using DIAGNOdent pen [19]. Significant difference was found in comparing theobromine varnish and sodium fluoride (NaF) varnish with superior remineralization in theobromine varnish (5.27 ± 0.46). Amaenchi B et al evaluated the mineral loss and lesion depth using transverse microradiography and found theobromine toothpaste group exhibited significantly higher mineral gain (remineralization) [12].

Three studies investigated the surface microhardness using a microhardness tester (Vicker's microhardness test,[12,16,19] and Wilson instrument [12]). Pushpalatha C et al suggested that theobromine varnish (280.48 ± 7.47) is slightly better than NaF varnish (280.48 ± 3.91) but no significant difference was found [19]. The surface microhardness in theobromine group was harder and resistant to dissolution than fluoride. The larger crystals in the theobromine treated teeth improve the resistance of the tooth surface to acid attack. In a study conducted by Farhad F et al [16] all the treated groups resulted in a significant increase in microhardness than control group. They concluded that teeth treated with theobromine (36.56 ± 4.95) resulted in maximum lesion rehardening than sodium fluoride (23.25 ± 3.92) which was statistically significant. Lipert F [13] found no statistically significant difference in theobromine treated teeth alone or in combination with strontium and fluoride.

The quality of all the included studies were interpreted as moderate to high bias. Therefore, the result of this review should be carefully interpreted. Although seven of the eight evaluated studies stated that theobromine is an effective and alternative remineralizing agent to novel fluoride, Lippert F [13] observed least enamel rehardening using theobromine and concluded that theobromine does not provide anti-caries benefit. However, studies have to be conducted comparing theobromine with other non-fluoridated remineralizing agents. Majority of the studies reported theobromine as an alternative to fluoride in remineralizing the artificially induced enamel lesions. The limitation of this systemic review is all the studies included in this review were laboratory studies. In pH cycle, the specimens will be subjected to vigorous remineralization and demineralization than the usual acid attacks in the oral cavity. So, the pH cycling does not mimic the oral environment accurately. Therefore, well-designed clinical trials should be conducted to provide more information on theobromine.

Conclusion

Within the limitations of this systemic review, we could conclude that theobromine is a viable and safe replacement for fluoride in enamel remineralization. Further clinical studies are required to evaluate the effect of this novel theobromine containing mouthwash, varnish, floss, solution, and gel.

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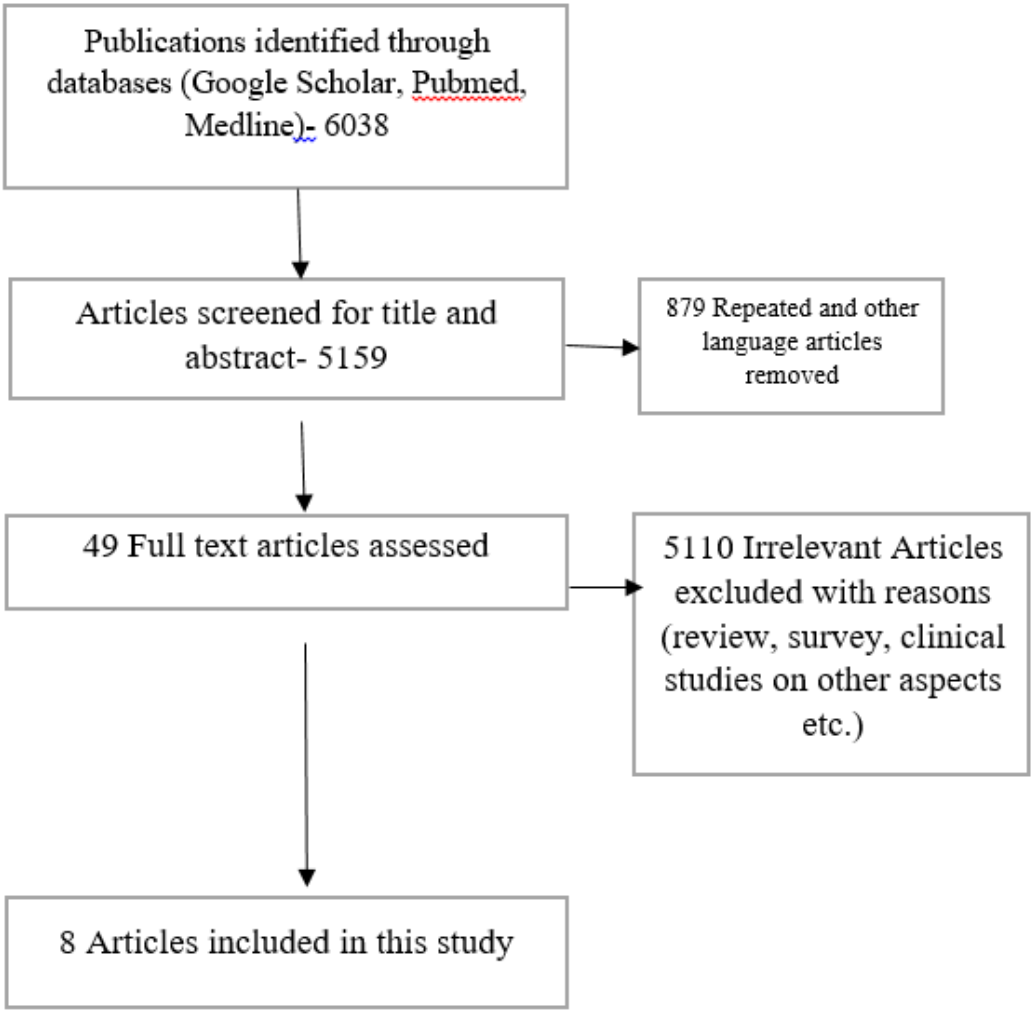

Fig 1: Prisma flow diagram of included studies

Table 1: Summary of the included studies

Sn no	Author name & year	Test group	Tested against	No of Specimens	Study conclusion
1	B.T.Amaechi et al. (2012)	G2- artificial saliva with theobromine (0.0011 mol/l), and	G1- artificial saliva; G3- NaF toothpaste slurry (0.0789 mol/l F)	30 Molars	Theobromine can enhance the remineralization potential of the medium.
2	Frank Lipert (2017)	G5- 1.1mM F theobromine, G6- Sr+theobromine, G7-F+Sr, G8- F+theobromine, G9- F+Sr+theobromine	G1-Placebo, G2-11.9mM sodium fluoride (F), G3-23.8mM sodium fluoride (2×F), G4-1.1mM strontium chloride hexahydrate (Sr),	16 per group (Molars and premolars)	Theobromine does not offer anti-caries benefit
3	Safaa Ismail Hussein et al. (2018)	G3- theobromine solution G4- theobromine gel	G1- control G2- Demineralization group G5- fluoride solution G6- fluoride gel	30 (Premolars)	Theobromine solution and fluoride gel are more effective remineralizing agents. Fluoride solution and theobromine gel have has less remineralizing potential.

4	Premnath et al. (2019)	G3- theodent,	G1- clinpro (0.21% NaF dentifrice with Functionalized tricalcium phosphate), G2- Amflor (1450-ppm amine fluoride-containing dentifrice)	27 (Maxillary Premolars)	Theobromine toothpaste toothpaste is effective in remineralizing enamel carious lesions
5	Elsherbini MS. (2020)	G4- Theobromine	G1- control group G2- Demineralization group G3- sodium fluoride (2000 mg /l)	40 (Premolars)	Theobromine gel had more effective remineralizing potential than fluoride gel
6	C.Pushpalatha et al. (2020)	G2- Novel varnish (1.8% theobromine + 0.005% catechin + 0.005% epicatechin + 0.3% agarose + 2 % benzoin powder+ 2% ethanol)	G1- 5% NaF varnish	45 (Premolars)	Novel varnish containing cocoa compounds has almost similar remineralizing effect in comparison to commercially available fluoride varnish.
7	Farnaz Farhad et al (2021)	G2- theobromine	G1- artificial saliva G3- sodium fluoride (0.05%)	90 (Premolars)	Theobromine is an effective cariostatic agent, can be considered as an alternative for fluoride
8	Rania Mostafa Shawky et al. (2021)	G1- Theodent-classic®	G2- Sensodyne f® (fluoride-containing) and G3- Sensodyne Original® (negative control)	15 (Premolars)	Theobromine could be a suitable alternative for fluoride in terms of safety and efficiency.

Table 1 continuation…

S n. n o	Duration of demineralization to produce enamel lesion	pH cycle	Theobromine concentration	Duration of application (remineralizing agent) pH cycling model	Mode of measurement/ outcome analysis	Study outcome
1	7 days	28 days	1.1 mmol /l	1 hour acid challenge 2 minutes remineralizing agent	Electron Dispersive Sperctroscopy (EDS), Transverse Micro Radiography (TMR), Vickers hardness test.	Theobromine can enhance the remineralization of the tooth.
2	24 hours	5 days	200ppm	3×1min-treatment; 2×60min-demineralization; 4×60min & overnight-artificial saliva	Microhardness tester (Wilson instruments)	In the absence of fluoride, strontium, theobromine and their combination were not effective. Theobromine and fluoride, either in presence or absence of strontium, did not enhance lesion fluoride content.
3	4 days	5 days	200mg/ l	3 minutes-remineralizing agent 3 hours-demineralizing solution	Scanning Electron Microscope (SEM) and Energy-Dispersive X-ray Analysis (EDXA).	Theobromine solution and fluoride gel groups showed improvement of the enamel surface.
4	96 hours	7 days	NA	3 hours-demineralizing solution 2 hours-remineralizing solution	Confocal laser scanning microscopy	No significant difference was found between the groups
5	3 days	5 days	200mg/l	3 minutes-remineralizing agent 3 hours-demineralizing solution	Scanning Electron Microscope (SEM) and Energy Dispersive X ray Analysis (EDXA).	No significant difference was found between two groups
6	96 hours	28 days	1.8%	1-hour acid challenge 2 minutes remineralizing	Vickers hardness test, DIAGNOdent	DIAGNOdent values showed that theobromine has superior

				agent		remineralizing potential than NaF varnish. No significant difference was found on Vickers microhardness test values.
7	Not mentioned	7 days	200 mg/l	1-minute remineralizing solution, 6-hour demineralizing solution, 1-minute remineralizing solution, 18 hour	Vickers hardness tester, Energy-dispersive X-ray Spectroscopy	Surface microhardness was significantly higher in theobromine than in the sodium fluoride group.
8	4 days	3 days	NA	2 minutes toothpaste treatment 6 hours acid challenge	Scanning Electron Microscope	Theobromine reported higher remineralization than fluoride but, the result was statistically insignificant.

Table 2: Risk of bias and quality assessment

Sn.no	Author name & year	Sample size estimation	Allocation concealment	Mechanism used for random allocation of sample	Blinding the evaluators	Risk of bias
1.	B.T.Amaechi et al. (2012)	NR	NR	NR	NR	High bias
2.	Frank Lipert (2017)	NR	NR	NR	NR	High bias
3.	Safaa Ismail Hussein et al. (2018)	NR	NR	NR	NR	High bias
4.	Premnath et al. (2019)	YES	YES	YES	NR	Moderate bias
5.	Elsherbini MS. (2020)	NR	NR	NR	NR	High bias
6.	C.Pushpalatha et al. (2020)	NR	NR	NR	NR	High bias
7.	Farnaz Farhad et al. (2021)	NR	NR	NR	NR	High bias
8.	Rania Mostafa	YES	NR	NR	NR	High

	Shawky et al. (2021)					bias

*NR- Not Reported

Table 3: Composition of Theobromine

	Active Ingredients	Inactive Ingredients
Theodent	Rennou (theobromine, calcium acetate, sodium hydrogen phosphate)	Purified water, hydrated silica, sorbitol, xylitol, glycerin, titanium dioxide, citric acid, sodium benzoate, stervia extract, sodium bicarbonate, suger free chocolate extract, suger free vanilla extract.

Fig 2: Theobromine containing toothpaste