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Effect of coumarin and some derivatives on glycosyltransferase that purified from *Streptococcus mutans*

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Abstract

Background: Dental caries, a persistent infectious disease, caused by *Streptococcus mutans*, was settled on the exterior part of dentil further outcome that vandalize robust teeth. Glycosyltransferase enzyme produced by S. *mutans that* fermentable sucrose and fructose present in dental caries. **Aims**: This study estimates effects of coumarins and their derivatives on purified Glycosyltransferase. **Methods:** S. mutans were cultured in an aerobically for 48 hours on mitis salivaris bacitracin-agar medium to produce glycosyltransferase, which was purified by Debois method, and different concentration of synthetic coumarin was applied on the enzyme**. Results**: The results clarified the 7-ethyl-4-methyl coumarin at the concentration of 400 µg /ml decreased the enzyme activity from (104.32 U/ml to 19.619 U/ml) compared with other inhibitors**. Conclusion:** the enzyme activity affected by coumarin with different concentrations but to extend effects of coumarin on the enzyme activity, add the substitute on different sites of the strong heterocyclic parts.

Keywords: Coumarin, coumarin derivatives, Glycosyltransferases, Streptococcus *mutans.*

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Introduction

Streptococcus mutans is a normal inhabitant of the human oral fissure represented as one of the major etiologic agents of caries. Main settlement of *Streptococcus mutans* on oropharyngeal cavity. (1). preliminary inhabitant *Streptococcus mutans* in oral fissure by means of glutinous particle of saccharide (2). The acidogenic of *S. mutans* is capable of composing extracellular polysaccharide in the existence of sucrose, fructose, and glucose. The extracellular polysaccharides are lengthy, concatenation and giant molecular throng polymers (3).

Glycosyltransferases are enzymes that represent an extensive ancestor particular are imperative in nature for the biogenesis of oligo- and polysaccharides along with compound glycans. Glycosyltransferases are catalyzing the deportation of glycosyl ensemble get going posterior of the segregate of sucrose to expanding alpha glucose concatenation. (4).

Coumarins molecules found in higher plants where they arise from the general phenylpropanoid pathway (5). Coumarins are

derived from 1,2-benzopyrones, these are essential class of composites of both natural and synthetic origin. The benefit of pharmaceutical and biological activities of coumarin moiety exhibit when the substituents occur on the bear in the parent benzopyran moiety. (6)

Materials and Methods

Streptococcus mutans was cultured on the mitis salivaris bacitracin -agar medium for 48 hours in candle jar then aerobically for 24 hours. *S.mutans* isolates were identified by Biochemical tests were also performed by fermentation of glucose, inulin, manitol, sucrose, sorbitol according the method of Guthof (1970)(7)**.**

Modification method described by Al-Hebshi (8) was estimated the enzyme activity. Fraction of Glycosyltransferase describes the quantity of enzyme stimulates the integration of one micromole of glucose taken away sucrose under the circumstances of experiment. Glycosyltransferase activity was throughout the guess quantity of glucan that was manufacture by the action of the enzyme, in accordance with the phenol-sulfuric acid method .(9) .

Assessment the activity of enzyme after adding inhibitors to purified enzyme:

This activity estimate according the Debois, (9) ,then complete the activity by method Al-Hebshi *et al* (2005).

Results

The samples of study has taken place at the seven months, it encompass thirty six clinical swab oral cavity specimens, and saliva.

Table (1) exhibit biochemical tests, in addition to the phenotypic features focus attention on belonging of these isolates to genus *Streptococcus mutans*. That is gram positive, α hemolytic qualified to grow on blood agar under 5% CO2, catalase negative, and dextran production.

Table (1): Biochemical test

Isolate	Gram stain	Hemolytic	Dextran production	Catalase
S.mutans	+	**A**	+	-

Table (2) clarified the result of carbohydrate fermentation , the bacteria have the ability to convert the red color of media has manitol ,inulin , sucrose ,sorbitol and glucose to yellow.

Table (2): Carbohydrate fermentation

Isolate	Mannitol	inulin	sorbitol	Sucrose	glucose
S.mutans	+	+	+	+	+

(+) means fermentation and change the color from red to yellow

The effect of coumarin and its derivatives on GTF activity:

Different concentration of seven-ethyl-four-methyl coumarin , four, seve dimethyl-six-nitro coumarin, and seven-hydroxy-four-methyl coumarin when applied on purified glycosyltransferase appeared in Figure (1) and table (3) .

Table (3) The enzyme activity with different concentration of inhibitors

	Enzyme activity (U/ml)				
	Enzyme untreated	104.32			
	concentration / Inhibitors	100µg/ml	200 µg/ml	300 µg/ml	400 µg/ml
1	7-ethyl-4-methyl coumarin	74.142	42.571	29.39	19.619
2	Coumarin	68.190	51.238	30.120	28.523
3	4,7dimethyl-6-nitro coumarin	93.661	56.285	42.642	29.571
4	7-hydroxy-4-methyl coumarin	84.619	52.144	31.912	36.834

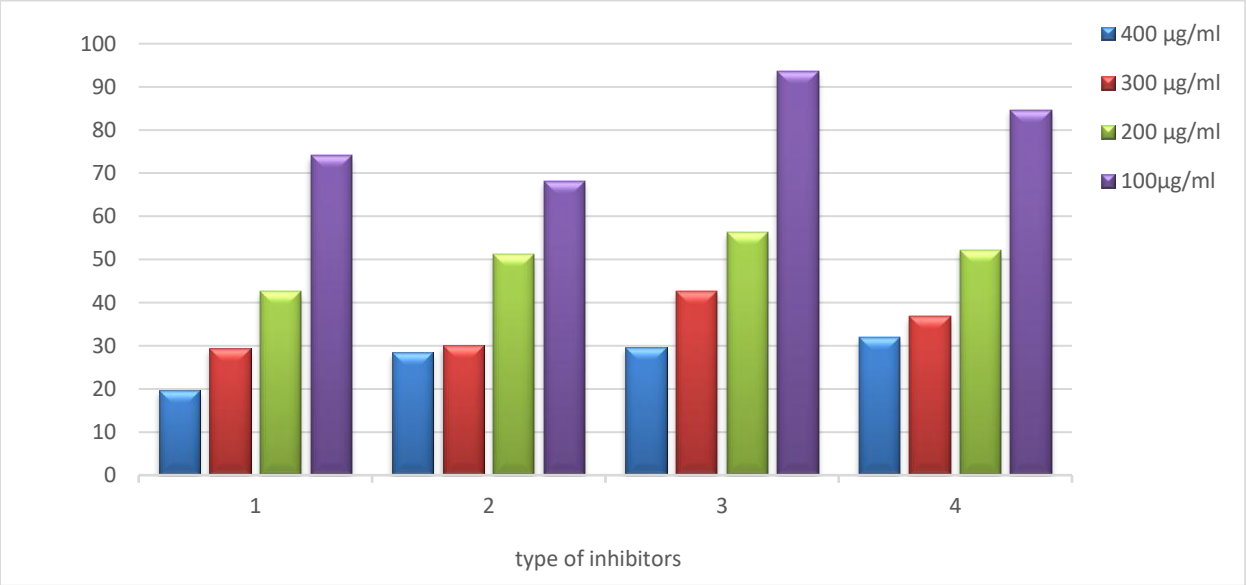

Figure (1): The enzyme activity with different concentrations of inhibitors

Discussion

carbohydrate fermentable and further act a source for the make whole of extracellular polysaccharides and intracellular polysaccharides in dental superficial (10). figured out sucrose act decline the concentration of calcium, inorganic phosphorus and fluoride in the dental biofilm . Main virulence factor in the dental carie due to the production of glucan and the massive quantity of lactic acid as a result of fermentation of carbohydrates. decline the concentration of calcium, inorganic phosphorus and fluoride in the dental biofilm (11).

From the results show that 7-ethyl-4-methyl coumarin at the concentration 400 µg /ml was decrease the enzyme activity from (104.32 U/ml to 19.619 U/ml). the inhibition activity due to change at coumarin molecules raise the repressor possibility of those components, also oxygen, methyl ,ethyl and hydroxyl groups those a raise of linkage with link site due to effects on enzyme activity. (12) While the other derivatives have different effect on the enzyme activity but lower compare with 7-ethyl-4-methyl coumarin (16).

coumarins and its derivatives like acyl coumarins, four-hydroxyl, 7-hydroxycoumarins and coumaric amide dimers and were examine aganist to *Bacillus subtilis*, *Staphylococcus aureus, Escherichia coli* , and *Pseudomonas*. (13). Mashelkar and Audi, (2006) (14) manufacture some uncommon four-substituted coumarins to examine against Gram positive *Staphylococcus aureus* and Gram negative *Salmonella typhi*.The active sit of enzyme has been hydrogen bonds and fill up with van der Waals distance that thereby served to anchor the complex throughout catalysis. hydroxyl group on C-6 of the Glucose participate by hydrogen bonds that anchored of this portion in the active side (15).

Conclusion: Coumarin has an effect on the enzyme but increases the activity by adding the substitute on different sites of the strong heterocyclic parts. Clinical applications of coumarin, such as its antimicrobial and antioxidant effects and enzyme inhibitory actions with different concentration, made us conclude that purification of coumarin from a natural product is beneficial for application in the line of drugs because of less side effect compared with chemicals.

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