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A novel biological rote of α-L-fucose in mutans group streptococci

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Abstract:
This study includes (50) samples were collected from patients with dental diseases (30) swabs taken from dental carries and (20) swabs from periodontal cases, these patients were of both sexes, their ages ranged from 10-65 years. There results of bacterial culture were positive in (20) patients of mutans group streptococci. Versus (30) patients revealed other types of and negative cultures.it was found that (10) isolates (50%) were identified as *St. mutans*, where (8) isolates (40%) identified as *St. salivaris,* and (2) isolates (10%) identified as *St. oralis*. The inhibition effect of fucose in different concentration of bacterial growth were studied, the results showed that there is a great inhibition growth on all studied bacterial isolated form oral cavity (mutans group streptococci) and the best inhibition concentration of fucose on bacterial growth found to be (80mM).
Key words: Mutans group streptococci**,** Fucose

1. Introduction:

Mutans group streptococci is a wide spread pathogen and a major cause of dental carries and periodontal cases (Toder, 2008). Established antibiotic treatments of mutans group streptococci have become less effective due to the emergence of drug- resistant isolates (Tapiainen *et al*., 2001). Fucose is one of the eight essential sugars that body required for optimal function of cell-to-cell communication (chan *et al.,* 2003). It is a hexose deoxy suger with the chemical formula $C_6H_{12}O_5$. It is found on N-liked glucans on the mammalian, insect and plant cell surface. α-(1——3) linked core fucose is suspected carbohydrate antigens for IgE-mediated allergy (Backer and Lawe, 2003), and it is claimed to have application in cosmetics, pharmaceuticals and dietary supplements (Dang *et al*., 2010).fucose is a powerful immune modulator, distributed in macrophages, which are critically important to immune function especially that of an overeractive immune system, that cause of autoimmune disorders (Abbas, 2004). It is showing promis in its ability to normalized immune function. It is particularly active in inflammatory diseases and has the ability to suppress such allergic skin reactions as contact dermatitis (Ruiz-Palacios *et al*., 2003). Fucose is monosaccharide that is considered to be one of the essential sugars, or polysaccharides that the body needs to function properly. It is relatively new phenomenon that being studied in order to help such disease as Al-Zheimers-Tajiri et al., 2008). It is located in the nerves of the body, the kidneys, the testes, and outer layer of the skin. Recently studies showing that fucose can be administrated to the body wherever a deficiency resides (Omer, 2010). New studies reavel that, since bacteria have lectins on their surfaces that stick to the hosts saccharide receptors, supply the body with these essential sugars can help deflect host-binding so that an infection can either be foiled or lessened (Baldoma and Aguilar, 2008). In addition to that, fucose has the ability to kill bacteria and help the body strengthen itself against infection. Fucose helps the cells in the body deflect bacteria so that infection can be fought off more efficiently (Liu, 2009). Fucose can help inhibit growth of many types of pathogenic bacteria and can as well as kill cancer cells in the body (Sawke and Sawke, 2010).

1.1 Aim of study: The aim of this study is to fucose wether fucose prevent growth of mutans group streptococci and to evaluate the inhibition effect.

2. Material and methods:

2.1Bacterial isolates: Twenty isolates of mutans group streptococci were isolated from dental disease, periodontal cases. All swabs and samples were collected from each patient plated on to blood agar, nutrient agar and incubated aerobically at 37^{o}C overnight. Isolates were identified to the species level based on the standards biochemical and microbiological methods (Macfaddin, 2000).

2.2 Bacterial count: The microorganisms were counted using hematocytometer to give an actual and precise number of organisms that were used throughout the assessment of fucose activity which were 1×10^8 cell/ml. methylene blue dye was used to differentiate viable cells from dead cells under light microscope prior to cells counting. Viable cells appeared bright color and ring shaped whereas dead cells were stained dark. Concentration of bacteria was calculated according to the following formula:
Bacterial concentration (cell/ml) = Total viable cells counted in four squares × Dilution factor × 10000.
Bacterial counts were done in different times (1 hr, 2 hrs, 4 hrs, 12 hrs and 24 hrs) (Al-Bayaty *et al*., 2011).

2.3 Preparation of different concentration of L-fucose: different concentration of L-fucose were prepared in deionized water DDW (10, 20, 30, 50, 60, 80, 100 and 120) µm all these gradient concentration were tested against the bacterial growth to clarify the minimum inhibitory concentration after filtrated through 0.2µm pore size filter (special communication prof. Dr. Mufeed Ewadh).

2.4 Minimal inhibitory concentration (MIC) test: A minimum inhibitory concentration test was carried out to determine the lowest concentration of L-fucose needed to inhibit visable (99%) bacterial growth of fixed concentration of experimental microorganism after an overnight incubation. The MIC value was confirmed based on the inhibition and growth observed on the agar plate which had been spot inoculated. The test was carried out in triplicate and the mean value of MIC was calculated (Al-Bayaty *et al*., 2011).

2.5 McFarland tube standard (0.5): A barium sulfate turbidity standard solution equivalent to a 0.5 McFarland standard was prepared as described by (CLSI, 2010).

2.6 Detection of bacterial growth by optical density: The Optical density of each tube was measured at a wavelength of 750nm against the slandered medium, and the measurement being performed every 1 to 2 hrs. during the logarithmic phase to growth. The OD results were collected as the means of three measurements. To confirm the relationship between the OD and the total number of viable bacterial cells, viability counts were made by the standard dilution method on sheep blood agar plate at the beginning. At the end of experiment (24 hrs) the samples were cultured on sheep blood plate and in the basic medium to ensure the presence of visable tested bacteria.

2.7 Screening of fucose effect in bacterial growth: L-fucose effect in different concentration was analyzed for inhibition activities against indicator bacteria (*St. mutans*, *St. salivaris, St. oralis*) by agar-well diffusion assay (Barefoot and Kalaenhammmer, 1989). Muller-Hinton agar seeded with bacterial isolates. The inoculums to be used in this test were prepared by adding 5 isolated colonies grown on blood agar plate to 5ml of nutrient broth and incubated at 37^{o}C for 18 hrs. and compared with (0.5) McFarland standard tube. A sterile swabs was used to obtain an inoculums was streaked on a Muller-Hinton agar plate and left to dry. Wells 5mm were hollowed out in agar using a sterile cork borer, a volume of 50µl of tested fucose were dropped separately in each well, and incubated at 37^{o}C for 24 hrs, and inhibition zone around the wells were measured and recorded in millimeter after subtraction 5mm (well diameter).

3. Results:

A total of (50) samples from patients with dental disease were collected in this study, 30 (60%) swabs taken from dental carries and 20 (40%) swabs taken from periodontal cases, only 20 patients with bacteria identified as mutans group streptococci verses 30 patients revealed other types of bacteria and negative culture. These results were show in Figure (1).

Twenty isolates of mutans group streptococci were identified to the species level based on the standard biochemical and microbiological methods in *Streptococcus mutans St. salivaris* and *St. oralis*. This results show in Table (1).

Bacterial isolates were subjected to study the effect of fucose in different concentration on their growth these results showed in Table (2)

It was found that this sugar have the ability to inhibit the growth of bacteria isolated from oral cavity *Streptococcus mutans, St. salivaris* and *St. oralis*. And the best inhibitory concentration was determined as 80mM as showed in Table (3).

A maximam antibacterial activity of fucose was shown by *Streptococcus mutans* its exhibited a larger inhibition zone with 26mm diameter, while the isolate of *Streptococcus salivaris*, 22mm diameter and *Streptococcus oralis* 20mm diameter as shown in Figure (2).

Also, the viable count of bacteria was determined by using hematocytometer in different times of incubation as shown in Figure (3).

The mean optical density for the mutans group streptococci at the beginning of the culture where fucose was

used (0.28-0.32), while the mean OD after 24 hrs. for the some group arranged between (0.15-0.18). These results as showed in Figure (4).

4. Discussion:

In this study, out of (50) samples only (20) isolates of mutans group streptococci were isolates. (10)(50%) isolates of *Streptococcus mutans*, (8)(40%) of *Streptococcus salivaris* and(2)(10%) isolated were identified as *Streptococcus oralis*. All_*Streptococcus mutans* were isolated from dental caries lesion and other bacteria were taken from dental plaque. *Streptococcus mutans* represent the main causative agent of dental disease especially dental caries and to lesser degree the periodontal disease, these results similar to those obtained by (Yoo *et al*., 2007, Shimotoydome *et al.,* 2007). Results also show that *Streptococcus mutans* isolated from dental caries (50%) was more than those isolated from dental plaque and saliva. These results were attributed to that *Streptococcus mutans* bacteria play an important role in pathogenesis of dental caries this finding was also compatible with the results obtained by (Toder, 2008). The antimicrobial activity of selected concentration of L-fucose against the experimental organisms is shown in Table (3). It illustrates the bacterial growth seen on agar plates after overnight incubation in the confirmation step (spot inoculation). The lowest fucose concentration that inhibits the growth of these organisms is regarded as the minimum inhibitory concentration (MIC) value. This study showed minimum activity with a MIC value 80mM which the concentration is recommended for inhibition of mutans group streptococci. The results obtained by revealed the fact that fucose has the ability to causes marked inhibition of mutans group streptococci (*Streptococcus mutans*, *Streptococcus salivaris* and *Streptococcus oralis*). The other suggested that the mechanism of growth inhibition in streptococci is mediated by a system regulated by fucose. The phosphotransferase system of oral streptococci is flexible mechanism capable of taking different sugars depending on the current sugar enviromant (Vadeboncoeur and Pelletier, 1997). So that, it could be the first step of L-fucose metabolism in mutans group streptococci is entry into the bacterial cell via the ficokinase phosphate system than fucose metabolized by this bio functional enzyme with both h-fucokinase activity and L-fucose-L-phosphate (L-fuc-1-P) guanayl tranferase activity. Under catalysis of fucokinase phosphate FKP free L-fucose-1-P by the L-fucokinase activity, L-fucose-1-P than utilized as the immediate substrate for L-fucose-1-P guanayl tranferase activity to form GDP-L-fucose. This activated form of L-fucose it can be used as an L-fucosyldonor for fucosyle transferases (Yoshidi *ei al*., 2012). The fucose-1-P, which mutans group streptococci cannot utilize further and which may even be toxic to bacteria it must therefore be expelled from the cell. From sugar inhibition studies showed that agglutinin mediated aggregation of mutans group streptococci was most potently inhibited by fucose and lactose this inhibition lead to lysis of bacterial cells by different steps of fucose metabolism (Demuth *et al*., 1990). from those for cultures grown on the control medium, the mutans group streptococci in the medium containing fucose remained viable until the end of the test. The autolysis of bacterial isolates was seen in BHI containing 80mM fucose which is a typical concentration for bacterial inhibition growth. Growth of these bacteria was detected after transfer to fresh basic medium.

5. Conclusions and Recommendation:

This study showed the possibility of noval rote for α-L-fucose in inhibiting the growth of mutans group streptococci. The underlying mechanism of fucose-induced inhibition of growth of streptococci may be mediated by fucokinase transferases system. This study showed that 80mM α–L-fucose can be used as antibacterial for plaque control and could be recommended as anti-dental plaque agent.

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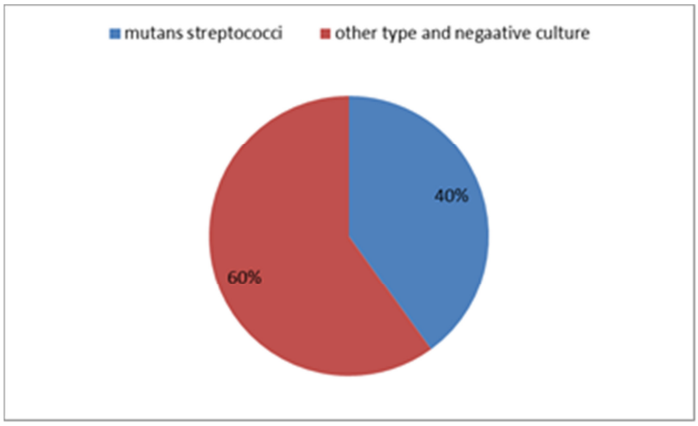

Figure (1): Percentage of bacterial culture for patients with dental disease

Table (1): Mutans group streptococci isolated from dental disease

Bacterial isolates	Number of isolates	Percentage
Streptococcus mutans	**10**	**50%**
Streptococcus salivaris	**8**	**40%**
Streptococcus oralis.	**2**	**10%**
Total	**20**	**100%**

Table (2): Growth media used in the experiments in all asolates

Control medium	**Test medium**
BHI only	BHI+10mM fucose
BHI only	BHI+20mM fucose
BHI only	BHI+30mM fucose
BHI only	BHI+50mM fucose
BHI only	BHI+60mM fucose
BHI only	BHI+70mM fucose
BHI only	BHI+80mM fucose

- BHI: Brain Heart Infusion

Table (3): Effect of different concentration of fucose on mutans group streptococci isolated from oral cavity

Type of bacteria	**Fucose concentration mM**					
	10	**20**	**30**	**50**	**60**	**80**
Streptococcus mutans (10)	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**
Streptococcus salivaris (8)	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**
Streptococcus oralis (2)	**-ve**	**-ve**	**-ve**	**-ve**	**-ve**	**+ve**

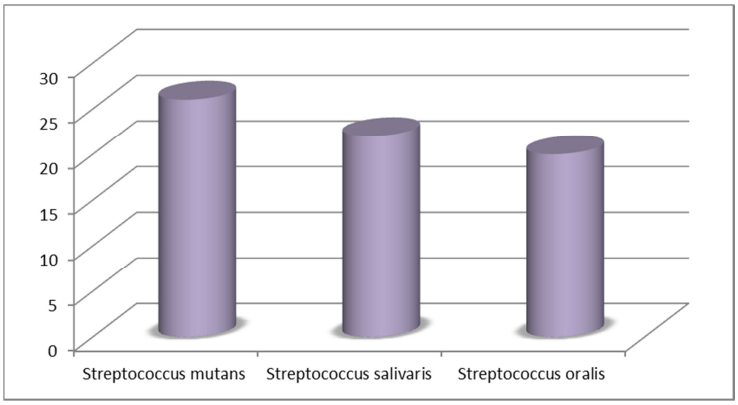

Figure (2): Inhibition zone of oral cavity bacteria (inhibited by 80mM fucose)

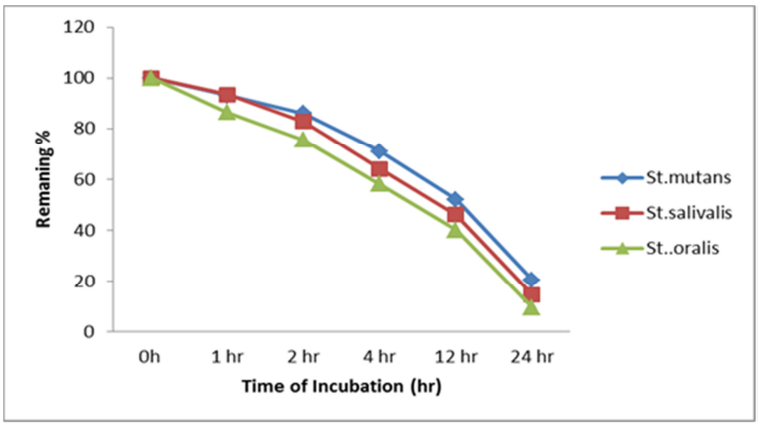

Figure (3): Effect of fucose (80 mM) on bacterial growth in different times

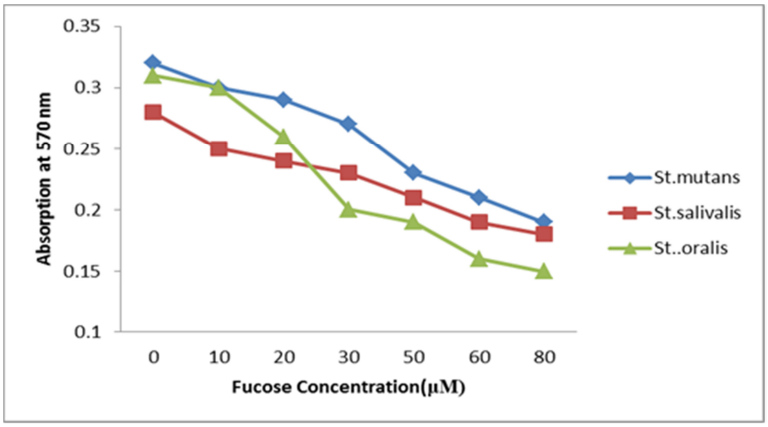

Figure (4): The optical density in different concentration of fucose

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