# Evaluation the antioxidant effect of α-L- Fucose injection into rabbit periodontium

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## ABSTRACT

Background: α-L-Fucose is a methyl pentose sugar similar to L-galactose except for the loss of alcohol group on carbon number 6. The objective of this study is to evaluate the biochemical and antioxidant effect of intracrevicular injection of fucose into rabbits periodontium, throughout measuring the levels of total protein (TP), total fucose (TF), protein bound fucose PBF) , Malondialdehyde (MDA) , and vitamin C in sera of fucose injected rabbit groups.(

Materials and Methods: The existing study was carried out on 55 male rabbits and were divided randomly into three groups ; first group was injected with 50µl of 150mM fucose solution into gingival sulcus ; second group was injected with 50 µl of normal saline ; while the third group was not received any injection ( normal group) .Blood samples were collected from injected groups at time intervals of 1, 3 ,24 ,72, and 168 hours after injection, for measuring of serum TP, TF, , PBF , MDA, and vitamin C and compared with normal group.

Results: The results showed a significant increase in the mean concentration of TF and PBF reaching its maximum value 3hrs after injection, then it decline until reached its normal value 168 hours after injection, whereas serum total protein increased significantly only 3 hours after injection. Also serum MDA level did not change after injection, while serum vitamin C increased immediately after fucose injection, even 72 hours after injection.

Conclusion: Intracrevicular injection of α-L- Fucose has an observable effect on TF and PBF this may give an indication about its effect on periodontal tissue and has a role in the body defence against oxidative stress, throughout increasing the production of vitamin C.

Key words: Total protein, Total fucose , Protein bound fucose , Malondialdehyde ,Vitamin C. (J Bagh Coll Dentistry 2013; 25(2):119-124).

## INTRODUCTION

α-L-Fucose is a six carbon deoxy–hexose (6-deoxy-L-galactose) with a general formula of C6H12O5 [1], and is important component of glycoproyein and glycolipid. In mammals, fucose-containing glycans have important roles in blood transfusion reactions, selecting-mediated leukocyte endothelial adhesion, host -microbe interactions and numerous oncogenic events, including signaling events by the notch receptor family [2]. Fucose glycoconjugates (glycoproteins and glycolipids) are an essential part of eliminating or reversing such disease processes as cancer, inflammation, and immunity [3].

Studies showed the importance of serum, saliva and gingival fluid fucose and its related parameters in the detection of oral disease, such as; gingivitis, periodontitis and oral cancers [4,5]. A study suggested that serum glycoproteins components (fucose, sialic acid, hexose and hexosamine) were a useful index of inflammation [6].

Other study revealed that α-L-Fucose could be used as therapeutic agent for many diseases, throughout oral administration or intravenous injection. This might be due to the inhibiting and reversing effect of L-Fucose on the disease process[7] .Studies also report that fucose had the ability to kill bacteria, controlling infection and modulate immune system and normalize immune function [8,9].

## MATERIALS AND METHODS

The study was carried out on 55 male white rabbits of the same species and nearly the same age (10-12 months) that weighed 1-1.5 kg. They were divided randomly into three main groups, first group was called fucose injected group which consisted of 25 rabbits and had been subdivided randomly into 5 subgroups ; B1,B2,B3,B4, and B5 . Each subgroup included five rabbit that received intracrevicular injection of a single dose of 50µl ⁄ kg of 150mM fucose into the mid –labial of the gingival sulcus of the lower right central incisor. Then blood samples were collected by cardiac puncture[10], after a specific time intervals of 1hrs (B1group), 3 hrs (B2group), 24hrs (B3group), 72 hrs (B4group), 168 hrs (B5 group) after fucose injection. The second group called normal saline injected group, consisted of 25rabbits, this group also subdivided randomly into 5 subgroups; C1, C2, C3, C4, and

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C5. Each subgroup included 5 rabbit that received local injection of 50µl ⁄ kg of normal saline into the same area as fucose group at time injected group. Blood samples were collected from the hearts at the same intervals of 1 hrs (C1group), 3 hrs (C2 group), 24hrs (C3 group), 72hrs (C4group), 168 hrs (C5group) after saline injection. The third group called non-injected group (group A), which consisted of 5 rabbits, the rabbits of this group were not received any injection .Blood samples were collected in the same way as in fucose and saline injected groups. The study was carried for:-

**A. Estimation of serum total protein (TP):**

Serum total proteins for the rabbit groups were estimated, using biuret method [11] .

**B- Estimation of serum total fucose ( TF):-**

Serum TF was estimated according to the method of Disch and Shettels [12].The principle depends on the direct formation of a chromogen after addition of concentrated $H_2SO_4$ and cysteine reagent into the tube containing the sample, and the color product measured at (390 and 430 nm).The difference in absorbance was directly proportional to α-L-Fucose content of the sample.

**C- Estimation of serum protein bound fucose (PBF):-** Serum PBF was also determined according to the method of Disch and Shettels[12]. The protein was precipitated by ethanol. The precipitate was resuspended in NaOH to resolubilize the protein. A color product was formed when fucose, in strong acid medium, combined with cysteine hydrochloride. The color intensity was measured at 390nm and 430nm.

**D-Determination of serum Malondialdehyde (MDA)**:- Serum MDA was estimated by **NWK-MDA01** assay**,** The NWK-MDA01 assay was based on the reaction of MDA with thiobarbituric acid ((TBA);forming an MDA-TBA2 , a product that absorbed stronglyat532nm[13].

**E -Determination of serum vitamin C:-** Serum vitamin C was determined using Stanely method [14]. The principle depends on ascorbic acid oxidased to form dehydroascorbic acid and Diketogluonic acid ,which react with 2, 4-phenylhydrazine to form a derivative of 2,4-dinitrophenylhydrazine, then this compound with concentrated H2SO4 , gets rearrangement and forms a product that gives absorbance at520nm.

# RESULTS

1. **Serum Total Protein (TP):-**

Table (1) shows that, there was an increase in the mean of TP after fucose injection, but this increase was significant only, 3 hours after injection ( B2 group) (P<0.05) . Table (2) showed the TP values in serum of non injected group (group A) and normal saline injected subgroups; C1, C2, C3, C4, and C5 after time intervals of injection with saline solution. No significant changes in TP values were observed, indicating that intracrevicular injection of saline solution had no significant effect on serum TP levels (P>0.05).

**2- Serum Total Fucose (TF)**

Table (3) shows that, serum TF levels increased significantly after fucose injection, reaching optimum value, 3 hours after injection (group B2) as compared to the non-injected group, then it decline but still significantly high after 24, and 72hours of injection (group B3, and B4), then it returned to normal base line value, 168 hours (7days) after fucose injection (group B5) (P>0.05).

Table (4) shows the TF values in sera of non-injected group (A) and normal saline injected groups (C1, C2, C3, C4 and C5) after time intervals of local injection with normal saline solution. No significant changes in TF values were observed in sera of saline injected subgroups comparing with non-injected group(P>0.05).

**3- Serum PBF**

Table 5 shows that, PBF level increased significantly after injection reached optimum value, 3 hours after injection (groupB2), then it declined step by step, but still more than its value in non- injected group, finally it returned back to normal base line value, 168 hours after fucose injection (group B5). Table (6) shows the PBF values in sera of non-injected group (group A) and normal saline subgroups after time intervals of intracrevicular injection with saline solution. No significant changes in PBF values were observed in sera of saline injected subgroups comparing with non-injected group ( P>0.05) .

**4.Serum Malondialdehyde (MDA):-**

Table (7and 8) shows that there were non significant differences in the mean level of MDA between both fucose injected groups and normal saline injected subgroups as compared to non-injected group(P>0.05).

**5. Serum Vitamin C:-**

Table (9) shows the mean, and standard deviation of serum vitamin C levels in non-injected group (group A) and fucose injected groups after time intervals of fucose injection. The results indicated that the mean serum levels of vitamin C in fucose injected groups increased highly after 1hour of injection, then it declined, but still higher than that of non-injected group. Finally it returned to normal value , 168hours after injection ( P > 0.05) . Table (10) shows non significant differences in the mean serum levels

of vitamin C between normal saline injected subgroups and non-injected group (P>0.05).

**Table 1: The mean, and standard deviation (SD)of serum total protein in rabbits of non-injected group and groups after time intervals of intracrevicular injection with 50µl/kg of 150mM fucose in normal saline solution (P>0.05).**

| Rabbit Groups | N. of Rabbits | Time intervals (Hours) | Fucose & Control ( TP gr/dl) | | | |
|---|---|---|---|---|---|---|
| | | | mean | ±SD | P-Value | Sig. |
| A | 5 | Control | 5.29 | 1.42 | | |
| B1 | 5 | 1hr | 6.50 | 1.39 | 0.21 | NS |
| B2 | 5 | 3hrs | 6.93 | 0.53 | 0.04 | S |
| B3 | 5 | 24hrs | 6.56 | 1.42 | 0.19 | NS |
| B4 | 5 | 72hrs | 5.91 | 0.86 | 0.42 | NS |
| B5 | 5 | 168 hrs | 5.88 | 1.24 | 0.55 | NS |

**Table 2: The mean, and standard deviation of serum total protein levels in rabbits of non-injected group and groups after time intervals of intracrevicular injection with 50µl/kg of normal saline solution( P>0.05).**

| Rabbit Groups | N. of Rabbits | Time intervals (Hours) | NS & Control ( TP gr/dl) | | | |
|---|---|---|---|---|---|---|
| | | | mean | ±SD | P-Value | Sig. |
| A | 5 | Control | 5.29 | 1.42 | | |
| C1 | 5 | 1hr | 6.42 | 0.94 | 0.18 | NS |
| C2 | 5 | 3hrs | 5.74 | 2.66 | 0.74 | NS |
| C3 | 5 | 24hrs | 4.91 | 1.03 | 0.64 | NS |
| C4 | 5 | 72hrs | 5.72 | 0.51 | 0.54 | NS |
| C5 | 5 | 168 hrs | 5.47 | 0.43 | 0.79 | NS |

**Table 3:The mean, and standard deviation of serum total fucose in rabbits of non-injected group (A)and groups after time intervals of intracrevicular injection with 50 µl/kg of 150 mM fucose in normal saline solution(P<0.05).**

| Rabbit groups | N. of Rabbits | Time intervals (Hours) | Fucose&Control( TFmg/dl) | | | |
|---|---|---|---|---|---|---|
| | | | mean | ±SD | P-Value | Sig. |
| A | 5 | Control | 10.39 | 0.16 | | |
| B1 | 5 | 1hr | 13.85 | 2.32 | 0.01 | S |
| B2 | 5 | 3hrs | 15.41 | 1.38 | 0.000 | HS |
| B3 | 5 | 24hrs | 14.07 | 0.07 | 0.000 | HS |
| B4 | 5 | 72hrs | 13.63 | 2.22 | 0.01 | S |
| B5 | 5 | 168 hrs | 11.13 | 0.86 | 0.09 | NS |

**Table 4:The mean , and standard deviation of serum total fucose in non-injected group (A)and groups after time intervals of sulcular injection of 50 µl/kg saline (P>0.05).**

| Rabbit groups | N. of Rabbits | Time intervals (Hours) | NS &Control(TFmg/dl) | | | |
|---|---|---|---|---|---|---|
| | | | mean | ±SD | P-Value | Sig. |
| A | 5 | Control | 10.39 | 0.16 | | |
| C1 | 5 | 1hr | 10.85 | 0.60 | 0.14 | NS |
| C2 | 5 | 3hrs | 10.75 | 1.34 | 0.57 | NS |
| C3 | 5 | 24hrs | 10.59 | 2.70 | 0.87 | NS |
| C4 | 5 | 72hrs | 10.56 | 3.30 | 0.91 | NS |
| C5 | 5 | 168 hrs | 10.07 | 1.47 | 0.63 | NS |

**Table 5: The mean, and standard deviation of serum protein bound fucose in non-injected group and groups after time intervals of sulcular injection with 50µl/kg of 150mM fucose in normal saline solution (P>0.05).**

| Rabbit groups | N. of Rabbits | Time intervals (Hours) | Fucose & Control ( PBF mg/dl) | | | |
|---|---|---|---|---|---|---|
| | | | mean | ±SD | P-Value | Sig. |
| A1 | 5 | Control | 2.79 | 0.82 | | |
| B1 | 5 | 1hr | 6.16 | 2.43 | 0.01 | S |
| B2 | 5 | 3hrs | 6.83 | 1.41 | 0.000 | HS |
| B3 | 5 | 24hrs | 6.63 | 0.90 | 0.000 | HS |
| B4 | 5 | 72hrs | 6.27 | 0.43 | 0.000 | HS |
| B4 | 5 | 168hrs | 2.48 | 0.34 | 0.46 | NS |

**Table 6: The mean , and standard deviation of serum PBF in rabbits of non-injected group (A) and groups after time intervals of intracrevicular injection with 50µl/kg of normal saline solution.( P>0.05).**

| Rabbit Groups | N. of Rabbits | Time intervals (Hours) | NS & Control (PBF mg/dl) | | | |
|---|---|---|---|---|---|---|
| | | | mean | ±SD | P-Value | Sig. |
| A | 5 | Control | 2.79 | 0.82 | | |
| C1 | 5 | 1hr | 2.78 | 0.54 | 0.98 | NS |
| C2 | 5 | 3hrs | 2.76 | 0.30 | 0.93 | NS |
| C3 | 5 | 24hrs | 2.75 | 0.31 | 0.92 | NS |
| C4 | 5 | 72hrs | 2.75 | 0.61 | 0.93 | NS |
| C5 | 5 | 168 hrs | 2.74 | 0.69 | 0.93 | NS |

**Table 7: The mean, and standard deviation of serum malondialdehyde levels in rabbits of non-injected group and groups after time intervals of intracrevicular injection with 50µl/kg of 150 mM of fucose solution( P>0.05).**

| Rabbit Groups | N. of Rabbits | Time intervals (Hours) | Fucose & Control (MDAnmol/L) | | | |
|---|---|---|---|---|---|---|
| | | | mean | ±SD | P-Value | Sig. |
| A | 5 | Control | 4.220 | 1.087 | | |
| B1 | 5 | 1hr | 4.560 | 1.328 | 0.669 | NS |
| B2 | 5 | 3hrs | 4.340 | 1.571 | 0.892 | NS |
| B3 | 5 | 24hrs | 4.160 | 0.929 | 0.928 | NS |
| B4 | 5 | 72hrs | 4.340 | 0.723 | 0.842 | NS |
| B5 | 5 | 168 hrs | 4.260 | 0.498 | 0.943 | NS |

**Table 8: The mean, and standard deviation (SD)of serum malondialdehyde levels in rabbits of non-injected group and groups after time intervals of intracrevicular injection with 50µl/kg of normal saline solution( P>0.05).**

| Rabbit Groups | N. of Rabbits | Time intervals (Hours) | NS & Control (MDAnmol/L) | | | |
|---|---|---|---|---|---|---|
| | | | mean | ±SD | P-Value | Sig. |
| A | 5 | Control | 4.220 | 1.087 | | |
| C1 | 5 | 1hr | 4.160 | 1.324 | 0.94 | NS |
| C2 | 5 | 3hrs | 4.140 | 1.534 | 0.927 | NS |
| C3 | 5 | 24hrs | 4.280 | 1.730 | 0.949 | NS |
| C4 | 5 | 72hrs | 4.320 | 1.264 | 0.897 | NS |
| C5 | 5 | 168 hrs | 4.240 | 1.108 | 0.978 | NS |

**Table 9: The mean, and standard deviation of serum vitamin C levels in non-injected group and groups after time intervals of intracrevicular injection with 50µl/kg of 150 mM fucose solution (P<0.05) .**

| Rabbit Groups | N. of Rabbits | Time intervals (Hours) | Fucose & Control (Vitamin C mg/100 ml) | | | |
|---|---|---|---|---|---|---|
| | | | Mean | ±SD | P-Value | Sig. |
| A | 5 | Control | 0.192 | 0.022 | | |
| B1 | 5 | 1hr | 0.442 | 0.097 | 0.000 | HS |
| B2 | 5 | 3hrs | 0.387 | 0.048 | 0.000 | HS |
| B3 | 5 | 24hrs | 0.246 | 0.019 | 0.004 | HS |
| B4 | 5 | 72hrs | 0.237 | 0.035 | 0.043 | S |
| B5 | 5 | 168 hrs | 0.202 | 0.003 | 0.057 | NS |

**Table 10: The mean, and standard deviation (SD) of serum vitamin C levels in rabbits of non-injected group and groups after time intervals of intracrevicular injection with 50µl/kg of normal saline solution (P>0.05) .**

| Rabbit Groups | N. of Rabbits | Time intervals (Hours) | Fucose & Control ( Vitamin C mg/100 ml) | | | |
|---|---|---|---|---|---|---|
| | | | mean | ±SD | P-Value | Sig. |
| A | 5 | Control | 0.192 | 0.022 | | |
| C1 | 5 | 1hr | 0.218 | 0.016 | 0.076 | NS |
| C2 | 5 | 3hrs | 0.217 | 0.047 | 0.314 | NS |
| C3 | 5 | 24hrs | 0.206 | 0.002 | 0.210 | NS |
| C4 | 5 | 72hrs | 0.203 | 0.001 | 0.319 | NS |
| C5 | 5 | 168 hrs | 0.201 | 0.000 | 0.426 | NS |

# DISCUSSION

**1 - Serum total protein (TP) :**

Serum TP level significantly increased after 3 hours of fucose injection (B2 group), then the mean value declined with non significant changes and nearly returned to the normal value after 168 hours of injection .The result may be due to break down of tissue protein and glycoprotein which may be occured as a result of the inflammation induced by injection of foreign material into gingival tissue as well as the injury processes that induced by the introduce of needle [15].

**2-Serum total fucose (TF):**

In this study, the serum content of total fucose increased after intracrevicular injection of fucose solution into gingival sulcus. This increase may be due to the time that needed for fucose solution to be transferred from gingival sulcus into gingival connective tissue through the epithelial lining of sulcus , then it passes into the serum through blood vessels plexus that exist in gingival tissue. Some amount of injected fucose can enter to the gingival tissue and can incorporate into glyconjugates ,throughout its conversion to the main substrate ( GDP-fucose) [16] , as indicated in the following reactions:- Fucose→fucose-1-P→GDP-fucose→glycoprotein It was published that ,if exogenous fucose is injected into animals, it is first conjugated intracellularly to form fucose -1-phosphate followed by conversion into GDP- L-fucose ,and finally, this nucleotide –sugar functions as a sugar donor in glycoprotein synthesis [17.] It was reported that exogenous fucose administrated to animals is unlike the other monosaccharides, it is not converted into other sugars or substances of another nature but amounts can enter to the circulation and recovered from the urine[17,18].

**3- Serum protein bound fucose (PBF):**

From the results of serum PBF , one can conclude that intracrevicular injection of fucose can accelerate the biosynthesis and secretion of serum glycoprotein from gingival tissue . Fucose injection

also can induce fucosylation of newly synthesized glyconjugates in the tissue .So the maximum induction of fucosylation of newly synthesized glyconjugate and its secretion into extra-cellular fluid (serum glycoprotein) can reach maximum rate, 3 hours after fucose injection, then it declines step by step until reaches to its normal value ,168 hrs after injection. The results of fucose injection and its effect on serum PBF level in this study are in line with the results obtained by [16], who injected a single dose of L-fucose-1-$^{14}$ C in 0.9% sodium chloride solution intraperitoneally .They found that the incorporation of fucose- into the proteins of serum and tissues was time dependent. Their result showed that the value in serum reached highest activity , 3 hours after injection then declined step by step. They concluded that liver, small intestine, and serum were the most highly labeled (contained high labeled protein bound fucose) than the other tissue. Although the liver is the major site of synthesis of serum glycoproteins, the appearance of protein-bound fucose in the serum of hepatectomized rats indicated that extrahepatic tissues contributed to the circulating glycoproteins. Researchers studied the metabolic fate of L-fucose-1-$^{14}$C and serum glycoprotein labeled with $^{14}$C-fucose in rats after daily periods of parenteal or oral administration of these compounds. They found that the time of maximum serum protein bound fucose was 3 hours after injection[19]. So our results indicated that intracrevicular injection of fucose solution caused an increased in serum PBF levels for a long duration that reached 72 hours after injection.

**4.Effect of fucose injection on serum MDA and vitamin C.**

Since it's the first study to evaluate the antioxidant effect of local injection of fucose solution on healthy periodontium, throughout measuring serum vitamin C and MDA. The results showed a non significant difference in serum MDA between fucose injected groups and non-injected group (groupA ) ,this results indicated that oxidative stress parameter (MDA) was not affected by fucose injection and remained normal ,while serum content of vitamin C was found to be affected by fucose injection and increased. Thus fucose may has an indirect antioxidant effect, throughout increasing the blood level of vitamin C for a long duration, reached 72 hrs after injection. This increase may be due to the enhancement effect of fucose injection on the endogenous secretion of vitamin C which is a potent antioxidant in the body. Whereas no change was observed in the basal levels of lipid peroxidation markers (MDA) in both injected groups. For normal saline, the result showed that the endogenous secretion of both MDA and vitamin C. was not affected by normal saline and remained normal .

Since the profound oxidative stress that occurs following injury results in significant depletion of many endogenous antioxidants (vitamin C, E, selenium). Evidence suggested that antioxidant supplementation reduce infectious complications and organ dysfunction following injury and hemorrhagic [20].

Vitamin C is a preferred antioxidant, denotes two electrons and the species formed after the loss of one electron is a free radical,semihydroascorbic acid or ascorbyl radical , but is relatively stable with a half life of $10^{-5}$ seconds and is fairly unreactive .In simple terms, a reactive and possibly harmful free radical can interact with ascorbate , then reactive free radical is reduced ,and the ascorbyl radical formed in its place is less reactive. Then upon loss of second electron, the compound formed is dehydroascorbic acid. Once formed ascorbyl radical and dehydroascorbic acid can be returned back into ascorbic acid by at least three enzyme pathways as well as by reducing compound in biological system such as glutathione .The action of L-fucose and a fucose-rich oligosaccharide (FROP-3) on skin explant cultures and fibroblast cell cultures, alone or together with three vitamins (A, C, E, often used in topical preparations) was studied. Both L-fucose and FROP-3 modulated the action of the two above-mentioned vitamins in most experimental conditions used. The combined action of the three vitamins (all-trans retinol, ascorbate, alpha-tocopherol) with L-fucose and even more so with FROP-3 can be considered as favorable for the modulation of the biosynthetic activity of fibroblasts [2]

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