

The effect of intracrevicular injection of fucose on serum interleukine -1beta and tumor necrosis factor alpha

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ABSTRACT

Background: α -L-Fucose is a methyl pentose sugar, had the ability to kill bacteria, controlling infection and normalize immune function. The objective of this study is to determine the effect of sulcular injection of fucose on rabbits periodontium, throughout measuring the level of some proinflammatory cytokine; interleukine 1beta (IL-1beta) and tumor necrosis factor (TNF-alpha) in sera of rabbits before fucose injection and at 3 days after fucose injection.

Materials and Methods: The study was carried on using (20) male rabbits of the same species weighted (1-1.5 kg), the blood samples were collected from hearts of 20 rabbits before fucose injection and consider as (non injected group), and after 3 days of fucose injection and consider as (fucose injected group) and analyzed for determination of the concentration of IL-1 β and TNF- α .

Results: The results showed a highly significant decrease in the mean concentration of TNF- α in sera of fucose injected group (19.05 ± 1.166) pg/ml when compared to its mean concentration in non injected group (27.25 ± 7.371) pg/ml, also a highly significant decrease in the mean concentration of IL-1 β in sera of fucose injected group (34.19 ± 3.1) pg/ml as compared to its mean concentration in sera of non injected group (38.86 ± 4.565) pg/ml.

Conclusion: both proinflammatory cytokines were influenced and inhibited by local fucose injection

Key words: α -L-fucose, interleukine 1beta (IL-1 β), tumor necrosis factor (TNF- α). (J Bagh Coll Dentistry 2013; 25(2):125-129).

INTRODUCTION

Fucose (6-deoxy-L-galactose) is a monosaccharide that is found on glycoprotein and glycolipids in vertebrates, invertebrates, plants, bacteria¹, and is present in low concentration in normal serum but is increased in diabetes, cancer, and inflammatory diseases².

A study found that fucose containing glycans modulated a wide range of physiological processes, such as; cell migration, proliferation, embryogenesis, fetal development, neuron-transmission, leukocytes adhesion, signal transduction and apoptosis³. Fucose is a powerful immune modulator. It is distributed in macrophages, (which are largest white blood cells in the body) which are critically important to immune function, its necessity in immune function, especially that of an overactive immune system, the cause of autoimmune disorders, so fucose is well-documented as playing a vital role in immune function².

Inflammation is one of the first responses of the immune system to an infection, irritation or tissue injury, and the local response to an infection or tissue injury involves production of cytokine, which are released to site of inflammation⁴.

Inflammation is stimulated by chemical factors released by injured cells and serves to establish a physical barrier against the spread of infection, and to promote healing of any damaged tissue following the clearance of pathogens⁵. These chemical factors produced during inflammation (histamine, bradykinin, serotonin, leukotrienes also prostaglandins) sensitize pain receptors, cause vasodilation of the blood vessels at the scene, and attract phagocytes, especially neutrophils⁵. Neutrophils then trigger other parts of the immune system by releasing factors that summon other leukocytes and lymphocytes. Cytokines produced by macrophages and other cells of the innate immune system mediate the inflammatory response. These cytokines include TNF and IL-1⁶. Researchers found that alpha-L-fucose has been demonstrated to inhibit lymphokine activity in vitro are effective in suppressing in vivo manifestation of cellular immunity⁷, other study suggested that alpha-L-fucose suppresses contact allergy by locally inhibiting the efferent phase of the cellular immune response⁸.

A fucose-containing glycoprotein fraction which stimulates spleen cell proliferation and cytokine expression has been identified from the water-soluble extract of *Ganoderma luicedum*, further studies on the activities of this glycoprotein fraction through selective proteolysis and glycosidic cleavage indicate that a fucose containing polysaccharide fraction is

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responsible for stimulating the expression of cytokines, especially IL-1, IL-2 and INF-gamma⁹.

Another study, described the isolation of Reishi polysaccharides for the study of their effect on cytokine expression in mouse splenotypes, a fraction(F3) has been shown to activate the expression of IL-1,IL-6,IL-12,IFN-gama, and TNF-alpha, and from this three subfractions have been prepared where F3G1 activates IL-1,IL-12,and TNF-alpha , F3G2 activates all the cytokines as F3 does , and F3G3 activates only IL-1 and TNF-alpha¹⁰.

MATERIALS AND METHODS

1- Setting of the study:

The present study was carried out in Erbil city, Hawler Medical university, College of Dentistry, Department of Basic Science, and Medical Research center.

2- Experimental animal:-

Twenty rabbits were used, these 20 rabbits were used as a non injected group (group A) that not receive any injection of fucose; and the same 20 rabbits were received intra crevicular injection of a single dose of fucose solution of 50 µl fucose / kg rabbit weight and of a concentration of 150 mM fucose in normal saline 0.9% NaCl, into mid-labial area of lower right central incisor and consider them as a fucose injected group, the blood samples were collected from 20 rabbits heart (cardiac puncture)¹¹ before fucose injection and after 3 days of intracrevicular injection of fucose.

3-Sulcular injection technique and administration of L-fucose

Sulcular injection techniques are standard techniques which are used most often to achieve nerve block and infiltration anesthesia in dentistry. In this technique the needle is inserted gently approximately 5 mm into the gingival tissue at the bottom of the gingival sulcus of lower right central incisor and a small amount of solution is injected slowly, and the amount of solution was delivered to each site over a period of approximately ten seconds¹². Fucose was infiltrated through the labial gingival tissue of the lower right central incisor using disposable insulin syringe (0.33×13mm)¹³.

4-Sampling

Blood sample had been collected from rabbits heart (Cardiac puncture)¹¹ of 20 rabbits by using disposable syringes of (5 cc syringes), the animals were anesthetized and restrained in dorsal recumbency then the needle (21 gauge needle) was inserted under the xyphoid cartilage slightly to the left of midline. The needle is advanced at a

20 to 30 degree angle from the horizontal axis to the sternum to enter the heart. The blood should be withdrawn slowly, four ml of blood were collected from each rabbit before fucose injection , then the samples were transferred into sterilized glass tubes(gel tubes), quitting for 30 minutes for clotting and centrifuged at 3000 rpm for 5 minutes to obtain clear supernatant, the serum was separated and transferred into sterile screw capped labeled tubes and stored at -20 C for subsequent analysis of proinflammatory cytokine IL-1 beta , TNF- alpha , and the same procedure of blood collection was done for the same 20 rabbits after 3 days of fucose injection.

5-Methods

A- Determination of serum TNF-alpha using indirect ELISA Technique.

1-All reagents and serum samples were brought to room temperature.

2-Standered was diluted to 1µg/ml with distilled water. Then serial dilution of the standard (1000, 500,250, 125, 62.5, 31.25, 15.625, 7.8125 pg/ml) were prepared from the original standard.

3- 200 µl of assay buffer was pipette into the appropriate microtiter wells. The wells were aspirated to remove liquid, and then each plate was washed 4 times by using 300 µl of Washing Solution. After the last wash, the plate was inverted to remove residual solution and blotted on paper towel.

4- 100 µl of standard, sample, and control groups were pipetted into the wells, and then covered with Plate Sealer, incubate at room temperature for 2 hours.

5- After incubation, the plates were aspirated to remove liquid, then washed 4 times with diluted Wash Buffer. The wells were inverted and taped dry on paper towel.

6- 100 µl of the diluted Detected Antibody (diluted streptavidin-HRP conjugate 0.5µg/ml) were added to each well, then covered with Plate Sealer , and incubated at room temperature for 2 hours.

7- The plates were aspirated to remove liquid, and then washed 4 times with diluted Wash Buffer. The wells were inverted and taped dry on paper towel.

8- 100 µl of the diluted color development Enzyme(ready- to-use-TMB substrate solution) were added to each well, and then covered with Plate Sealer and incubated at room temperature for 30 minutes.

7 - The plates were aspirated to remove liquid, and then washed 4 times with diluted Wash Buffer. 9- 100 µl of color development solution was added to each well, covered, and then incubated at room temperature for a proper color

development for 4minutes. 100 µl of the stop solution was added to each well in order to stop the color reaction.

9- The absorbance of the well contents were read at 450 nm , by using a microliter plate reader. 10- The TNF-alpha concentration of unknown samples and control groups were calculated from the following equation.

$$\text{Concentration of TNF-alpha} = \frac{\text{OD test}}{\text{OD standard}} \times \text{concentration of standard}$$

B- Determination of serum IL-1 beta using indirect ELISA Technique .

1-All reagents and serum samples were brought to room temperature

2-Standard was diluted to 0.1µg/ml with distilled water .Then serial dilution of the standard (125, 62.5, 31.25, 15.625, 7.8125 pg/ml) were prepared consecutively from the original standard.

3- 200 µl of Washing Solution (PBST) was pipette into the appropriate microtiter wells. The wells were aspirated to remove liquid, and then the plate washed 3 times with 300 µl of Washing Solution .After the last wash, the plate was inverted to remove residual solution and blotted on paper towel.

4- 100 µl of standred, sample, and control groups were pipette into the wells, then covered the plate with Plate Sealer, and incubated at room temperature for 2 hours.

5-The plate was aspirated and washed 4 times with diluted Wash Buffer.

6-100 µl of the diluted Detected Antibody (0.5µg/ml) was added to each well, then covered with Plate Sealer, and incubated at room temperature for 2 hours.

7- At the end of incubation time, the plate was aspirated and washed 4 times with diluted Wash Buffer.

8- 100 µl of the diluted color development Enzyme was added to each well, then the plate was covered with Plate Sealer and incubated at room temperature for 30 minutes, at the end of incubation time, the plate was aspirated and washed 4 times.

9- 100 µl of color development solution was added to each well .Covered, then incubated at room temperature for a proper color development

for 4 minutes, 100 µl of stop solution was added to each well to stop the color reaction.

10- The absorbance of the well contents were read at 450 nm, by using a microtiter plate reader.

11- The IL-1 beta concentration of unknown samples and control groups were obtained from the following equation:

$$\text{Concentration of IL-1 beta} = \frac{\text{OD test}}{\text{OD standard}} \times \text{concentration of standard}$$

RESULTS

Table 1 and figure 1 showed that there was a high significant decrease in the mean concentration of TNF-α in sera of rabbits after fucose injection (19.05±1.166 pg /ml) when compared with its mean concentration in sera of same rabbits before fucose injection (27.25±7.371 pg/ ml). Also a high significant decrease in the mean concentration of IL-1beta in sera of rabbits after fucose injection (34.19±3.1pg/ ml) when compared to its mean concentration in sera of same rabbits before fucose injection (38.86±4.565pg/ ml), as shown in table 2 and figure 2.

Table1: The mean concentration of TNF-α (pg/ml) in sera of non injected and fucose injected groups.

Study groups	Number	Range of conc.	Mean ± SD	±SE	Sig.
Non injected	20	19.8-49.4	27.25±7.371	1.648	HS
Fucose injected	20	15 - 20.4	19.05±1.166	0.261	

Table 2: The mean concentration of Il-1 beta (pg/ml) in sera of non injected and fucose injected groups.

Study groups	Number	Range of conc.	Mean ± SD	±SE	Sig.
Non injected	20	32.5-46.1	38.86±4.56	1.021	HS
Fucose injected	20	30.3-39.3	34.19± 3.1	0.693	

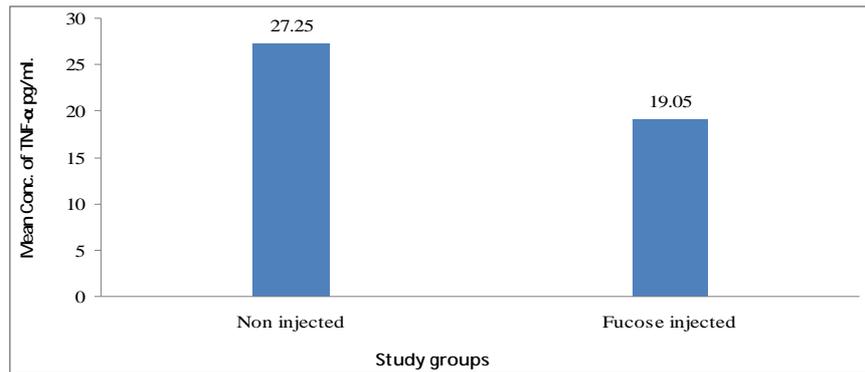


Figure 1: The mean concentration of TNF- α (pg/ml) in sera of non injected and fucose injected groups.

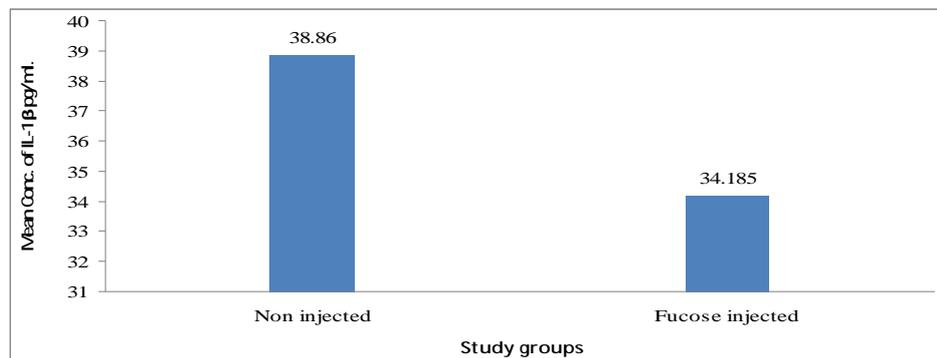


Figure 2: The mean concentration of IL-1 β (pg/ml) in sera of non injected and fucose injected groups.

DISCUSSION

1- Serum IL-1 β

The present study demonstrated that local injection of fucose was significantly associated with serum level of IL-1 beta, and a highly statistically significant decrease in serum concentration of IL-1 beta were observed in fucose solution injected group compared to the non injected group. This result was in agreement with that found by other researchers^{14,15, 16,7,8}, Rocklin¹⁴, found that L-fucose capable of inhibiting lymphokine activity in vitro, and found that human macrophage inhibition factor (MIF) activity on blood monocyte was significantly reduced by L-fucose, also Remold¹⁵, strongly suggested that alpha-L-fucose comprises an essential part of the macrophage membrane receptor to migration inhibition factor, and he showed that alpha-L-fucose abolishes the activity of guinea pig migration inhibitory factor (MIF) on the macrophages, so macrophages no longer responded to MIF. Also Baba *et al*⁷ studied the effect of α -L-fucose on cell mediated immunity in vivo, and found that L-fucose injected intravenously could inhibit the ability of lymphokine containing supernatants to induce skin reaction or cause reduction in the

macrophage content of peritoneal exudates, moreover, L-fucose can inhibit the cutaneous delayed hypersensitivity reaction and the peritoneal macrophage disappearance reaction (MDR) induced by antigen in actively immunized guinea pigs, the result demonstrated that monosaccharides capable of inhibiting lymphokine activity in vitro are effective in suppressing in vivo manifestation of cell mediated immunity. Knop and Reichman⁸ suggested that local application of α -L-fucose on the ear before elicitation of contact allergy to dinitrofluorobenzene (DNFB) in mice results in a suppression of the contact allergic response by locally inhibiting the efferent phase of the cellular immune response.

Clark *et al*¹⁶, studied cytokine in animal model (vivo and vitro studies), in vivo model, they measured the amount of rabbit IL-1 α and IL-1 beta protein present in brain, kidney, liver, lung, muscle, and spleen at various times after the injection of endotoxine, and found that IL-1 present all tissues studied but largely in spleen; and found that IL-1 levels were transient, reaching peak levels by 4 h after injection and rapidly decreased to low levels by 24h, and in vitro studies IL-1 β was maximal at 24h then decreased. In contrast this result was in

disagreement with that found by other researchers⁹, Wang et al⁹, found that a fucose containing polysaccharide fraction is responsible for stimulating the expression of cytokines, especially IL-1.

2- Serum TNF- α

Regarding TNF-alpha, the present study demonstrated a high statistically significant differences was found between fucose solution injected group and non injected group, and the mean concentration of TNF-alpha in sera of non injected group was 27.25 and decrease to 19.05 in fucose injected group, this result was in agreement with that found by other researches^{7, 8, 17}, baba et al⁷, found that L-fucose capable of inhibiting lymphokine activity in vitro and was effective in suppressing in vivo manifestation of cellular immunity, and Knop and Riechman⁸, suggested that local application of α -L-fucose suppresses contact allergy by locally inhibiting the efferent phase of the cellular immune response, also Stankova¹⁷, found that fucose induced significantly higher secretion of TNF-alpha by both lymphocytes and monocytes, and found that fucose induced the accumulation of TNF-alpha accumulation in a time – dependent manner with a peak concentration at 8 hours and returned to baseline values at 20 hours after stimulation, in contrast to our finding, Chen et al¹⁰, reported that Ganoderma Luicedum (Reishi) polysaccharide (F3) and there sub fractions (F1,F2,and F3) all activated the expression of TNF-alpha.

9. Wang YY, Khoo KH, Chen ST et al. Studies on the immuno-modulating and antitumor activities of Ganoderma Luciden (Reishi) polysaccharides. functional and proteomic analysis of a fucose-containing glycoprotein fraction responsible for the activities. *Bioorg Med Chem* 2002;10(4): 1057-62.
10. Chen HS, Tsai YF, Lin CC et al. Studies on the immuno- modulating and anti-tumor activities of Ganoderma lucidum (Reishi) polysaccharides. *Bioorg Med Chem* 2004; 1;12(21):5595-601.
11. Standardized operating procedure for rabbit immunization and blood collection 2011. University at Buffalo. Buffalo.
12. Peterson JE, Matsson L, Nation W. Cementum and epithelial attachment response to the sulcular and periodontal ligament injection techniques. *Ped Dentis American Acadey of Periodn* 1983; 5(4).
13. Taka S. The local effects of S2-complex on periodontium. Master thesis, College of Dentistry, Baghdad University. 2001.
14. Rocklin RE. Role of monosaccharides in the interaction of 2 lymphocytes mediators with their target cells. *J Immunol* 1976;116(3):816-20.
15. Remold HG. Requirement for α -l-Fucose on the Macrophage membrane receptor for Mif. *J Exp Med* 1973;38(5):1065-76.
16. Clark BD, Bedrosian I, Schindler R et al. Detection of interleukin 1 alpha and 1 beta in rabbit tissues during endotoxemia using sensitive radioimmunoassays. *J Appl Physi* 1991; 71(6)2412-8.
17. Stanková J. Fucose-activated killer cells. I. Enhanced TNF-alpha mRNA accumulation and protein production. *J Leukoc Biol* 1992; 52(2):188-96.

REFERENCES

1. Moriwaki K, Miyoshi E. Fucosylation and gastrointestinal cancer. *Word J Heptaol* 2010; 27; 2 (4):151-61.
2. American International Association of Nutritional Education. Fucose. *J Nutr Edu for the world* 2006; 23(5):67-69.
3. Orczyk-Pawilowicz M. The role of fucosylation of glyconjugatas in health and disease. *Postepy Hig Med Dosw* 2007; 61:240-252.
4. Pedersen BK. Special feature for the Olympics: Effects of Exercise on the Immune System. *Immunol and cell bio* 2000; 78:532-5.
5. Stvrtinova V, Jan J, Hulin I. Inflammation and Fever from Pathophysiology: Principles of Disease. Computing Centre, Slovak Academy of Sciences 1995.
6. Lotze MT, and Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Natu rev Immunol* 2005; 5(4):331-42.
7. Baba T, Yoshida T, Cohen S. Suppression of Cell-Mediated Immune Reactions by Monosaccharides. *J Immunol* 1979; 122(3):838-41.
8. Knop J, Reichmann R. Suppression of the elicitation phase of contact allergy by epicutaneous application of alpha –L-fucose 1983; 247(1-2) 155-8.