Effect of Some Nutrient on Some Fertility Characteristics of Male Laboratory Mice Infected with Diabetes

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ABSTRACT

Key words: nutrient, Fertility, diabetic, male mice.

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Accepted:11/2/2018

This study was conducted in the Biotechnology Research Center at Nahrain University, Baghdad, aimed to investigate the effect of different nutritional formula to the diabetic rats, which were developed by Alloxan on some The properties fertility such as total number of sperm, percentage of sperm, live and distorted and movement activity, as well as the level of Testosterone hormone. In this study were used 42 male mice type Albino mice at 2-3 months aged and weight at 25-30 g. Six mice were isolated as a control group. The remaining animals were injected with 150 mg/kg alloxan between the thighs and after ascertaining the incidence of diabetes was randomly distributed to six groups in addition to the Non-infected control group, each group has a specific treatment as follows:

1- Control group. 2- Diabetes group. 3- Diabetes group and treatment with plant nutrition. 4- Diabetes group and treatment with animal protein. 5- Diabetes group and treatment with Salvia officinalis added to plant nutrition. 6- Diabetes group and treatment with Salvia officinalis added to animal protein. 7- Diabetes group and treatment with Food Supplements $(D_3 + B_{12})$ added to plant nutrition.

The results of the statistical analysis showed that the animals with diabetes developed with alloxan had a significant decrease (P<0.05) in the preparation of sperm, the percentage of live sperm and the increase in the percentage of Distorted sperm also showed a significant decrease in the concentration of testosterone hormone compared to the control group. As for the group of animals infected with diabetes and treatment using plant nutrition and animal nutrition, the results showed a significant increase in the preparation and movement of live sperm and decrease in the percentage of mutilated semen, or testosterone hormone the results showed no significant differences compared with the group of infected animals without treatment.

The results showed that the group of infected animals and the treatment using the salvia officinalis added to the plant nutrition and animal nutrition had a significant increase (P<0.05) in the preparation and movement of live healthy sperm and decrease in the proportion of mutilated and dead sperm and a significant increase in the concentration of testosterone hormone.

It was also found that the group of infected animals and the treatment with supplements had a significant increase in the preparation and movement of live sperm, as well as in the Testosterone hormone and a decrease in the numbers of mutilated sperm.

تأثير بعض المغذيات على بعض صفات الخصوبة لدى ذكور الفئران المصابة بالسكري

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الخلاصة

الكلمات المفتاحية: تم اجراء الدراسة في مركز بحوث التقنيات الأحيائية في جامعة النهرين / بغداد. هدفت هذه الدراسة التغذية النباتية والحيوانية والميرامية الى معرفة تأثير الأنظمة التغذوية المختلفة في الفئران المصابة بالسكري المستحدث بالآلوكسان على بعض والمكملات الغذائية والخصوبة وداء صفات الخصوبة كالعدد الكلي للنطف والنسبة المئوية للنطف الحية والمشوهة ونشاط حركتها وكذلك على السكري.

¹ This article is a part of MSc. Thesis for the first author.

مستوى هورمون الشحمون الخصوي. استعمل في هذه الدراسة 42 ذكر من الفئران نوع Albino mice بعمر 2-3 شهر ووزن 25-30 غم ثم عزلت ستة فئران باعتبارها مجموعة السيطرة السليمة وتم حقن الحيوانات المتبقية بين الفخذين بالآلوكسان 150 ملغم/ كغم وتركت الى اليوم التالي للتأكد من اصابتها بالسكري ثم وزعت عشوائياً إلى ستة مجاميع إضافة الى مجموعة السيطرة السليمة ولكل مجموعة معاملة محددة وكالآتي: للمراسلة:

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الاستلام: 2017/9/13

القبول: 2018/2/11

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1- مجموعة السيطرة السليمة 2- مجموعة السيطرة المصابة 3- مجموعة الحيوانات المصابة والمعاملة بالتغذية النباتية 4- مجموعة الحيوانات المصابة والمعاملة بالبروتينات الحيوانية 5- مجموعة الحيوانات المصابة والمعاملة بالتغذية النباتية 6- مجموعة الحيوانات المصابة والمعاملة بإضافة الميرامية الى التغذية النباتية 6- مجموعة الحيوانات المصابة والمعاملة بإضافة الميرامية الى التغذية النباتية 6- مجموعة الحيوانات المصابة والمعاملة بالتذية النباتية 4- مجموعة الحيوانات المصابة والمعاملة بالبروتينات الحيوانية 5- مجموعة الحيوانات المصابة والمعاملة بالتغذية النباتية 6- مجموعة الحيوانات المصابة والمعاملة الحيوانات المصابة والمعاملة الميرامية الميرامية الى التغذية النباتية 6- مجموعة الحيوانات المصابة والمعاملة بإضافة الميرامية الميرامية الميرامية الميرامية المعاملة محموعة الحيوانات المصابة والمعاملة الميرامية المعاملة بإضافة الميرامية الميرامية الى التغذية النباتية 6- مجموعة الحيوانات المصابة والمعاملة بإضافة الميرامية الى التغذية النباتية 6- مجموعة الحيوانات المصابة والمعاملة بإضافة الميرامية الى التغذية النباتية 6- مجموعة الحيوانات المصابة والمعاملة بإضافة الميرامية الى التغذية النباتية 6- مجموعة الحيوانات المصابة والمعاملة بإضافة الميرامية الى التغذية 10- مجموعة الحيوانات المصابة والمعاملة بإضافة الميرامية الى المعاملة التغذية النباتية.

لقد اشارت نتائج التحليل الاحصائي ان الحيوانات المصابة بالسكري المستحدث بالألوكسان قد حصل لها انخفاض معنوي (P<0.05) في اعداد النطف والنسبة المئوية للنطف الحية وارتفاع في نسبة النطف المشوهة وكذلك اتضح حصول انخفاض معنوي في تركيز هورمون الشحمون الخصوي مقارنة مع مجموعة السيطرة. اما مجموعة الحيوانات المصابة بالسكري والمعالجة باستعمال التغذية النباتية والتغذية الحيوانية فقد أوضحت النتائج حصول ارتفاع معنوي في اعداد النطف الحية وحركتها وانخفاض في نسبة النطف المشوهة ما هورمون الشحمون الخصوي في معنوي عن عداد النطف الحية وحركتها وانخفاض في نسبة النطف المشوهة اما هورمون الشحمون الخصوي فأوضحت النتائج عدم حدوث فروق معنوية مقارنة مع

مجموعة الحيوانات المصابة دون معالجة. واوضحت النتائج ايضا ان مجموعة الحيوانات المصابة والمعاملة باستعمال عشبة نبات الميرامية المضافة لكل من التغذية النباتية والتغذية الحيوانية فقد حصل فيها ارتفاع معنوي (P<0.05) في اعداد النطف السليمة الحية وحركتها وانخفاض في نسبة النطف المشوهة والميتة وكذلك ارتفاع معنوي في تركيز هورمون الشحمون الخصوي. وكذلك اتضح من النتائج ان مجموعة الحيوانات المصابة والمعاملة بالمكملات الغذائية حصل فيها ارتفاع معنوي في اعداد النطف الحيوانية وحركتها وكذلك في هورمون الشحمون الخصوي وانخفاض في اعداد النطف المداد النطف المشوهة.

Introduction:

Nutrition factor is one of the most important factors affecting the health, physical and mental state of people (Sadiq, 2011). The use of medicinal plants has been common as a source of treatment for many diseases that have been affecting humans for a long time. The interest in these plants has returned to the fact that they contain effective compounds with a physiological medical effect (Yakuba *et al.*, 2007). Salvia officinalis plants are effective in treating many diseases Such as Kidneys and intestines, as well as to reduce blood sugar and reduce cholesterol. It also has an anti-free radical effect of oxidative stress that destroys protein bonds and breaks down oxygen-depleted DNA (Tsao *et al.*, 2004; Megan, 2016; Velickovic *et al.*, 2007).

The human body, in addition to carbohydrates, fats and proteins, requires mineral elements and vitamins in small quantities necessary for its various activities (Al-Madany, 2014). Therefore, people tend to use Food Supplements to supply the body with energy, which is the purpose of supplementing the individual's diet with nutrients such as vitamins, minerals and fiber and fatty acids that may not feed the body's need for them (janji, 2008). The most people Consumption supplementation are vegetarians, older people and pregnant women (Al-Salhi, 2014).

Diabetes is one of the most common diseases, where the number of infected in Iraq is estimated to be around 2 million if a large number of people of all ages suffer from severe insulin deficiency or a lack of sensitivity to cell uptake due to several factors such as heredity, the environment and others (Al-Saidaly, 2000 : WHO, 2000).

Aims of the study:

- 1- Study the effect of diabetes on some properties fertility by calculating the accounts of live sperm and its activity, abnormality sperm and calculating the concentration of the hormone testosterone.
- 2- Recognition of the effect of some food treatments such as plant proteins, animal proteins, salvia officinalis, and food supplements on some of the damage to sperm and testosterone hormone caused by diabetes.

Materials and Methods:

The study was conducted at the Biotechnology Research Center, Nahrain University, Baghdad. Used 42 albino mice males, aged between 2-3 months and 25-30 g. they randomly divided into seven groups, each ones consisted of 6 mice. The first group was the control group and the other six groups were injected with alloxan at 150 mg /kg for the injury of diabetes.

Ν	Group	Treatment	Feed components
1	Non-Infected control	Standard	The usual diet
2	Infected control	Standard	The usual diet
3	Infected	Vegetarian nutrition	25%For (soybeans, corn meal, wheat, barley)
4	Infected	Animal protein	15.5% of the plant diet + 38% animal protein
5	Infected	Vegetarian nutrition + salvia	23.8% of the vegetarian diet + 4.76% Salvia
6	Infected	Animal protein + salvia	13.8% of the plant diet + 41.66 animal protein + 2.77% Salvia
7	Infected	Vegetarian nutrition + Supplements (D ₃ + B ₁₂)	250 g of vegetable diet + 250 micrograms of vitamin D ₃ + 500 micrograms of vitamin B ₁₂

After the grinding of the components and mixing with the ratios mentioned for each group then feeding for 30 days, blood was withdrawn from the animal to assay the blood and physiological parameters then dissection of animals Using sterile anatomy tools and isolate the testicle and put in a dedicated bottles containing formalin diluted 10% and reduced to use, and then isolate the epididymis and cut it into small pieces and place it with the Phosphate solution for the purpose of obtaining sperm and testing:-

First: sperm characteristics tests

• **Sperm accounts:** Using the counting slice, counting the number of sperm after crushing the tail of the epididymis with a sharp scalpel in a dish containing the formaldehyde 10% by 9.8 ml and adding two drops of the tincture Eocene and took one drop and put it on the slide and left for five minutes to settle the sperm on four squares and square in the center Using the optical microscope Strongly enlarge 40X, the total number of sperm was calculated as follows (Sakamoto & Hashimato, 1996).

Total sperm number = $(n / 80) \times 400 \times 1000 \times 10 \times 10$

• Sperm activity: 200 sperm were taken for each smear at least and calculated as follows: -

(Live sperm ratio% = number of live sperm / total number of sperm $\times 100$). and then calculate the distorted sperm ratios of the equation (Zeneveld & Polakski, 1977)

(Percentage of distorted sperm% = number of mutilated sperm / total number of sperm \times 100).

Second: Testosterone concentration: - The concentration of the testosterone hormone test using the Kit ready with Elisa technique according to the company Monobind American origin (Tietz, 1995), where reagents use the antibody, the enzyme - the associated antigen, the original antigen. After mixing and the interactions that end in a blue-colored complex, it is measured along a 450-nm wavelength by the optical spectrometer. The intensity of the color is proportional to the amount of the enzyme and is inversely associated with the amount of the testosterone hormone.

Results and discussion:

Fertility tests: The results shown in Figure 1 that the group of animals infected with diabetes by alloxane had a significant decrease (P < 0.05) in live sperm and motive and significant increase in mutilated sperm compared with the control group. This result was agreed with Hussein *et al.*, (2013) and kate'a (2015). The reason is to the formation of free radicals as a result of oxidative stress due to diabetes, which affects the tissues and cells of the body and cause many diseases, including testicular cells and other sexual glands, as well as affect the functions of the testis and inhibit the formation of sperm or Impaired sperm maturation (Ishihara *et al.*, 2000 : Krishnamoorthy *et al.*, 2007).

The results showed that the group of animals infected with diabetes and treatment with the addition of Salvia officinalis to both plant nutrition and animal nutrition obtained a significant increase (P <0.05) in live sperm and its movement and decreased the percentage of distorted sperm. The reason is that it contains the necessary elements of sexual health, especially zinc, which has an important role in increasing the number of sperm through its effect on sperm cells lining the spermatozoa (Agrawal *et al.*, 2012) or may be due to the containment of Salvia Vitamin C, which has a role in stimulating the growth of sperm and division of sperm and also necessary for the differentiation of sperm cells and spermatozoa (Al-Hamedawi, 2006).





The result in the figure above showed a significant increase in the accounts and movement of live sperm and a decrease in the percentage of sperm abnormalities in the plant nutrition and animal protein groups. The reason in animal proteins are rich in essential amino acids and most of the elements and minerals needed by the organism as well as for plant nutrition (Soybean) It contains a high percentage of essential amino acids to the extent that they match animal proteins as well as contain antioxidants, especially arginine, which is known to enhance sexual ability and improve the number of sperm and increase (Sinclair and Lac, 2000).

As for as the group of animals infected with diabetes and treatment using food supplements ($D_3 + B_{12}$), the results showed a significant increase in the preparation and movement of live sperm, but less than the above mentioned factors due to the improvement of sperm to the role of vitamin B_{12} in improving fertility and maintain the safety and activity of sperm, Vitamin D_3 has the potential to Increase sperm movement and activity by freeing calcium from these cells and pushing them forward faster (Papadopoulou *et al*, 2013).

The following is a set of pictures illustrating some forms of sperm in each treatment of treatments mentioned: -

1- Not infected control group



Picture (1) show the shape of the natural spermatozoa in the control group is not infected **2- infected control group**



Picture (2) show the shape of the abnormality sperm in the infected control group

3- Two groups of animals infected with diabetes and treatment with both vegetarian nutrition and animal nutrition.



Picture (3) show one form of sperm abnormalities such as the existence of cytoplasmic droplet in the group of infested animals and treatment with vegetarian nutrition (A) and natural sperm in the group of infested animals and treated with animal nutrition.

4- Two groups of animals infected with diabetes and treatment with the addition of salvia officinalis for both vegetarian nutrition and animal nutrition



Picture (4) show the natural sperm forms in the two groups of infected animals and treatment with the addition of salvia officinalis to both vegetarian nutrition and animal nutrition.

5- Group of animals infected and The Supplements (D₃+B₁₂) is added to vegetarian nutrition treatment



Picture (5) show the natural and abnormality sperm in the infected animals group and treatment by adding food supplements (D_3+B_{12}) to vegetarian nutrition.

Second: The Testosterone hormone: - The result in Figure (2) show a significant decrease in the concentration of testosterone in the group of diabetic and non-treated animals during the duration of the experiment. This result was Agreed with (Al- Aredhi & Al- Ahmad, 2015) The level of fat and insulin, such as glucose intolerance, which leads to endocrine changes, it produces a decrease in testosterone. This result was agreed with (Chnang *et al.*, 2015 : Al- Bayati *et al.*, 2010) who indicated

that type 2 diabetes was associated with low levels of testosterone hormone.

The result showed a significant increase (P < 0.05) in the testosterone hormone in the group of animals with diabetes, which was developed by alloxane and treated with the herb of Salvia officinalis plant added to both plant nutrition and animal nutrition. And the presence of an abnormal endoplasmic reticulum, it is the center of making testosterone hormone (Hesketh, 1982), In addition to the containment of the Salvia plant on phenolic compounds and flavonoids, which increases the concentration of testosterone hormone (Presnell & Schreibman, 1997).



Figure 2: Levels of Testosterone hormone

The result showed no significant differences in the group of infected animals and treatment using plant nutrition Compared with infected animal control group. The reason is that soy proteins contain phytoestrogen, which works similar to estrogen and compete with the male hormone. There is a loss of balance between testosterone and estrogen. This negatively affects the Ledge testicular cells (Davis *et al*, 1999).

This result showed a significant increase (P <0.05) in the concentration of Testosterone hormone in the group of animals Infected diabetes and treatment with $B_{12} + D_3$ supplementation. The role of B_{12} and D_3 was to improve sexual capacity and increase the concentration of testosterone (Sinclair and Lac, 2000).

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