The effect of Alloxan induced diabetes on baseline comet test in male laboratory mice fed on different feeding systems

Mina N. Hassan* **Ferial F. Hussein** **Hazem I. Al-Ahmad**[1]

Affiliation: Food sciences / College of Agriculture / Tikrit University
[1]Biotechnology Research Center / Al-Nahrain University

*Correspondence: **Mina.n.hasan@st.tu.edu.iq**

Received: 25/5/2022

Accepted: 15/8/2022

Published:27/11/2022

Abstract:

Background: The comet analysis (electromagnetic relay analysis of a single cell) is a highly sensitive method for determining DNA damage because to exposure to carcinogens and other substances that consequently affect fertility.

Objective: the aim of this study is to Evaluation the broken DNA in male diabetic mice. Created by alloxan and knowledge of the effect of some nutritional systems on the treatment and repair of genetic material due to diabetes.

Materials and methods: In this study were used, 42 male Albino mice labs at the age of 2-3 months and weight (25-30) g then 6 animals were isolated to represent the control group, The remaining animals were injected between the thighs by alloxan 150 mg / kg and left the next day to make sure hit by diabetes and randomly distributed to six groups in addition to a set of Not affected control transactions.

Results and conclusion: It was found from the results of the statistical analysis shown in figure(1) showed the presence of the highest percentage of injuries in the genetic material that was in the group of animals with diabetes and untreated for the duration of the experiment, as it was clear from the photos of this group that the tail length resulting from the migration of the genetic material DNA to outside the nucleus because to its damage, as well as the results showed that the group of animals affected by diabetes and the treatment by adding the Salvia officinalis plant to both plant and animal nutrition had a significant decrease (<0.05> P) in the proportion of genetic material injuries where the proportion of high (long guilt) infections was low in these two The two groups compared to the group of animals with diabetes and untreated for the duration of the experiment.

Key words: Diabetes, the basal comet test, Salvia officinalis, nutritional systems.

Introduction:

During the past ten years, comet examination has been used as a fast and sensitive technique to detect DNA damage, and this technique has gained widespread acceptance to test for genetic toxicity, and this technology has spread to its ease, cheapness, simplicity, and flexibility, in addition to its high sensitivity in detecting DNA damage. Also, this test has the ability to detect DNA damage in individual cells (1,2,3). The examination was recently used in different laboratory studies on Organism for the purpose of monitoring exposure to mutagens and carcinogens That perform injury different DNA damage (4,5).

In this study, diabetes was concentration and its effect on genetic material as it is considered one of the common chronic organic diseases., as it affects a large number of people of both sexes either because of acute insulin deficiency or a defect in the sensitivity of cell reception to it, which occurs due to different factors such as heredity and the environment (6). Also, in this study, types of plants were used to treat diabetes and reduce its harmful effects. Which affects the body because the plants contain various effective compounds are considered valuable treasures that rid humans of many incurable diseases such as cancer, polio, etc. (7).

Materials and methods:

The experience was conducted at the Center for Biotechnology Research, Al-Nahrain University/ Baghdad. Were used at (42) male Albino mice age of (2-3) months and weight (25-30) g. The animals were randomly divided into seven groups. Each group includes (6) mice; one uninfected group was isolated, then the remaining 6 groups where infected with alloxan at a concentration of (150) mg / kg for the purpose of diabetes and the groups were divided according to the transactions are as follows:

N	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	nutrition + salvia Vegetarian	(23.8%) vegetarian diet + (4.76%) Salvia
6	Infected	Animal protein + salvia	(13.8%) plant diet + (41.66) animal protein + (2.77%) Salvia
7	Infected	nutrition + vit Vegetarian (D3+ B12)	(250) g of vegetable diet + (250) micrograms of vit D3 + (500) micrograms of vit B12

the electric grinding was used to grind and mix the components of the diet in the proportions specified for each group then feeding for (30) days the animals were fasted for 12 hours, after which the blood was drawn from the animal (0.5 - 1) ml by cardiac puncture with the insulin syringes (1 ml) coated from the inside with heparin to prevent blood clotting. The blood was placed in dedicated abandroves and inserted into the centrifuge to isolate the serum from the blood and was stored in the freezer until use.

Alkaline Comet test: The ready-made kit was used by the US-based Trevigen company, as shown in the following steps (8, 9):

1- Prepare and dissolve the dissolution solution at (4 °C) for at least (20) minutes before using it.
2- Dissolution the agarose in a cup of boiling water, leave it for (5) minutes, put the cap and put it in a (37m) م water bath for at least (20) minutes.
3- Grouping the cells with molten agarose 37 ° C at 1:10 (V / V) and immediately pull 50 microliters of the mixture and spread it evenly onto the special comet slice.
4- The slides are placed after completion of publication in a dark place at (4 °C) (in the refrigerator) for 10 minutes. After this period, a clear ring will form on the edge of the comet slice.

5- The slides are immersed in the dissolve solution for a period of 30-60 minutes at a temperature of (4 °C), and to ensure decomposition, they are placed in the incubator at a temperature of (4 °C) for the next day.

6- Immerse the slides with an alkaline disassembly solution at pH 13 while keeping the slices immersed in the solution for (20) minutes at room temperature or at (4 °C) for an hour in a dark place (in the refrigerator) for the purpose of removing the neutralized solution in excess of the need from the slices with the need to deal with caution and wear gloves.

7- Performing the electrical relocation of the comet by placing 850 ml of the device's alkaline electrical solution at a temperature of (4 °C) and quietly placing the slides at the bottom of the device as the end of the slide is placed near the positive electrode (black cathode) and the slide cover is placed on it and then put the device cover and power delivery and set it to 21 Volts for half an hour.

8- Dispose of the excess electrolyte by immersing the slides in DH_2O two times five minutes at a time and then placing it in 70% ethanol for another five minutes.

9- Dry the samples at 37 °C for 10-15 minutes. This is for the purpose of attracting and grouping cells in one flat surface for easy observation and examination, and then keeping samples at room temperature.

10- Place 100 microliters of the SYBR Green dye diluted on each clear ring formed on the slide for 30 minutes and put it in a dark place at room temperature and then remove the excess dye by gently moving the slide and washing it with water for a short time and then dry the slides completely at a temperature of 73 °C.

11- Scan the slides with a radiological microscope and have the highest clarity on the power of 496-522 nm.

50 cells were randomly selected for each sample to determine the comet cells, then calculate the result from the comet ratio to determine the indicators of the comet (CL), as the rate is recorded from 1.2 - 2 are considered low injuries (LD) and from 2-3 - 3 are medium injuries (MD) and higher than 3 if damage DNA is largely HD (10,11).

Then the comet's measurements were calculated using the analysis program. Various changes were calculated for all comets, as there are 3 variables that indicate DNA migration, namely: (12,13).

1- The length of the tail (the distance from the center of the head to the end of the tail).

2- The proportion of DNA in the tail.

3- The density of DNA cells.

Results:

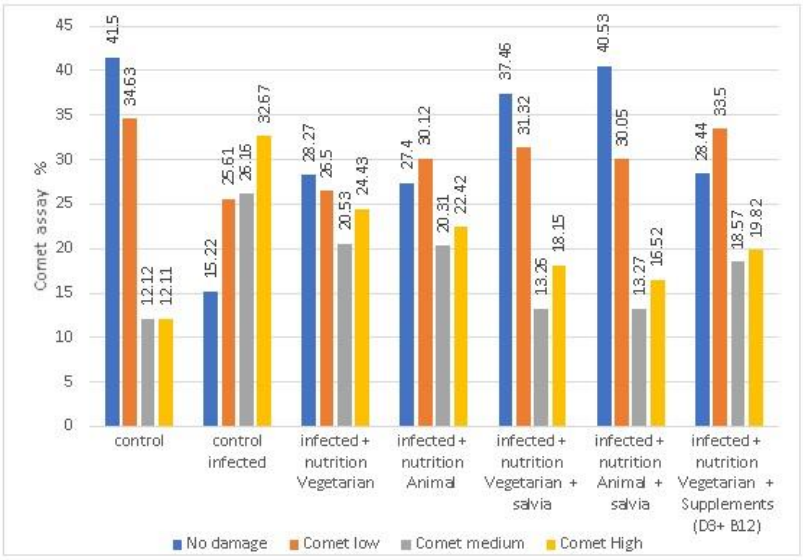

Figure (1): the effect of the mentioned treatments on the comet examination in the serum of male mice with diabetes induced by alloxan

The results of this study showed, which are illustrated in figure (1), that the highest incidence of DNA was in the diabetic and untreated group for the duration of the experiment, as it turns out from the photos of this group that the tail length increased the result is that the genetic material migrates DNA out of the nucleus due to its damage (14). As for the group of infected animals treated with the use of the Salvia officinalis add it to both plant and animal diet, the results showed a significant decrease in the proportion of DNA infections, as the high rate of (long guilt) was low in these two groups. The results showed that animals with diabetes and treated with plant diet, animal diet, and nutritional supplements, clarified a marked improvement in DNA compared to the group of untreated infected animals.

Discussion

The reason for the high injuries in the group of infected and untreated animals may be due to the length of the experiment, as shown in the following figure (2) Comparison with the group of uninfected animals' figure (3) to oxidative stress and free radical formation due to disease attacking the pancreas and damage to pancreatic beta cells.

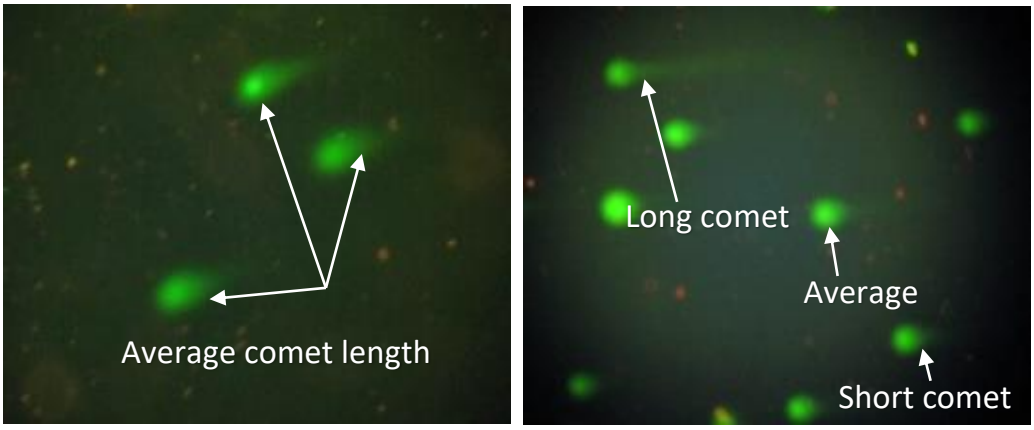

Figure (2): shows the shapes of the cell nucleus with a long, medium, and short comet in the male group of diabetic animals without treatment, as the tail shape resulting from the migration of the destroyed genetic material from the cells appears.

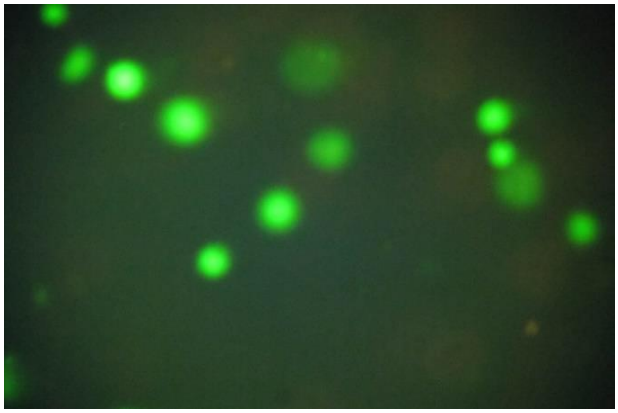

Figure (3): The normal nucleus cell form without a comet in the male group of healthy control animals

This result was consistent with the results of (15), which proved in its results that the DNA is caused by a high rate of damage due to oxidative stress, as indicated by (16) The DNA in a person's healthy cells remains intact inside the nucleus without any harm, but when the cells are subjected to oxidative stress or unsuitable environmental conditions, the single strands of DNA lose its structural shape and it goes outside the nucleus. When photographing the electrophoresis, these pieces appear guilty. (Comet) With heads distinct contained on the DNA and proper tail containing pieces of DNA damaged (17).

As for the group of infected animals treated with the use of the Salvia officinalis add it to both plant and animal diet, the results showed a significant decrease in the proportion of DNA infections, as the high rate of (long guilt) was low in these two groups as shown in the following figure (4).

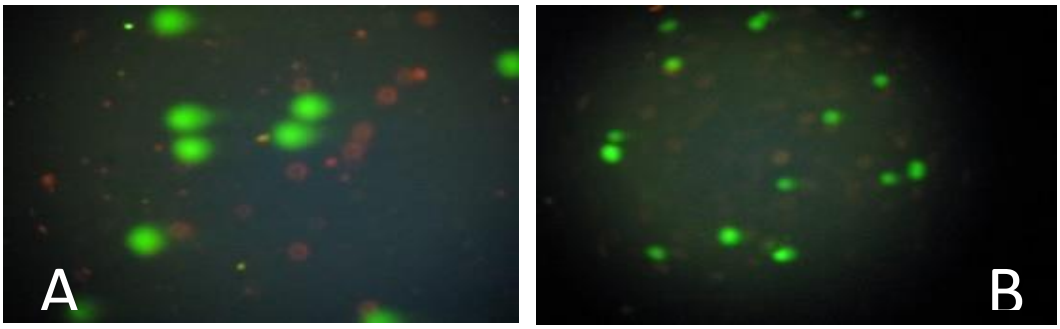

Figure (4): shows the forms of cell nuclei in the two groups of male mice with diabetes and treatment by adding Salvia to both plant (A) and animal (B), as it is clear from the two forms a significant improvement in the ratio of migration of the genetic material to DNA outside the nucleus.

The reason may be due to the inclusion of Salvia officinalis on the components of flavones, which work on reducing the free radicals formed by diabetes, which cause these roots to cause significant damage and damage to DNA (18). In addition to containing vitamin C and E and their role as antioxidants, they have the ability to reduce oxidative stress and reduce the high damage to DNA that was measured by comet test (19). The presence of antioxidants in the body helps control the damage to DNA (20). The results showed that animals with diabetes and treated with plant diet, animal diet, and nutritional supplements, clarified a marked improvement in DNA compared to the group of untreated infected animals as shown in the following figures:

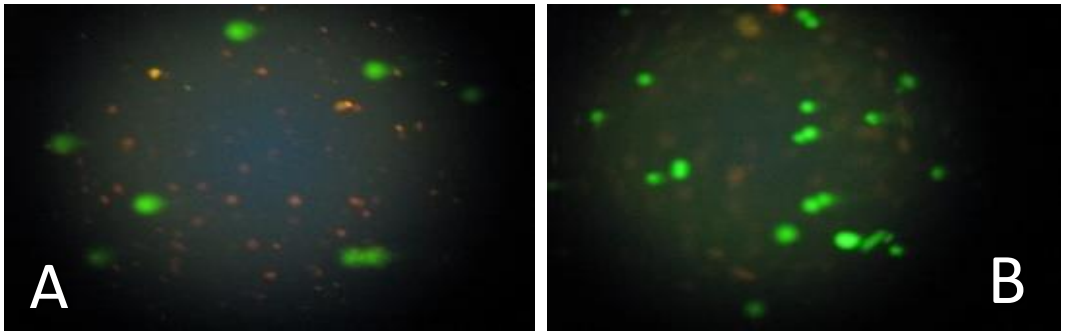

Figure (5): shows the shape of the cell nucleus in the two groups of male diabetic mice treated with plant-based feeding (A) and animal feeding (B). As the two figures show, an improvement in the migration rate of genetic material from cells.

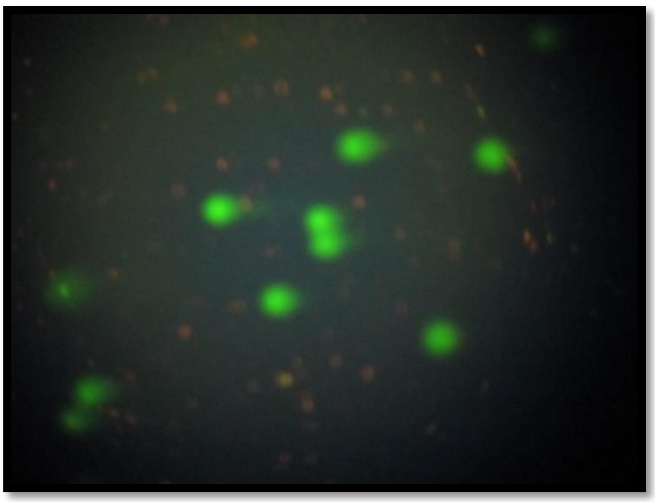

Figure (6): shows the nucleus shapes in the male group of diabetic mice and treated by adding nutritional supplements (B12 + D3) to plant nutrition, as the figure shows a decrease in the damage of DNA cells compared to the group of infected and untreated animals throughout the experiment period.

References:

1. McKenna DJ, McKeown SR, McKelvey-Martin VJ. Potential Use of the Comet Assay in the Clinical Management of Cancer. Mutagenesis, (2008); 23: 183-190.
2. Collins AR. The Comet Assay for DNA Damage and Repair: Principles, Applications, and Limitations. Mol. Biotechnol, (2008); 26: 249-261.
3. Tice. RR, Agurell E, Anderson D, Burlinson B, Hartmann A, Kobayashi H, Miyamae Y, Rojas E, Ryu JC, Sasaki YF. Single cell gel/ comet assay: guidelines for in vitro and in vivo genetic toxicology testing. Environ Mol Mutagen, (2008); 35: 206-221.
4. Gillian R, w.; Valerie J, McKelvey-Martin, C. Stephen Downes .The Use of the Comet Assay in the Study of Human Nutrition and Cancer Mutagenesis. (2008); 23(3): 153–162.
5. Piperakis SM, Visvardis EE, Tassiou AM. Comet Assay for Nuclear DNA Damage. Methods Enzym, (1999); 300: 184–194.
6. WHO. "World Health Organization" (2000). Diabetes Mellitus. Regional office for the Eastern.
7. Galleto R, V. Siqueira, E. Ferreira, A. Olivera, R. Bazotti .Absence of antidiabetic and hypolipidemic effect of Gymnema Sylvester in non-diabetic and alloxan-diabetic rats. Brazilian Archives of biology and Technology, (2004); 47(4): 545-551.
8. Olive PL, Wlodek D, Banath JB. DNA double- strand breaks measured in individual cells subjected to gel electrophoresis. Cancer Res. (1991); 51: 4671- 4676.
9. De Boeck M, Touil N, De Visscher G, Vande PA, Kirsch–Volders M. Validation and implementation of an internal standard in comet assay. Mutat.Res. (2000); 469: 181-197.
10. Collins AR, Harrington V, Drew J, Melvin R. Nutritional modulation of DNA repairs in a human intervention study. Carcinogenesis. (2003); 24: 511-515.
11. Al-Jewari HSJ. In vitro Cytotoxic Activity of the L-asparaginase Extracted and Purified from Pathogenic Escherichia coliagainst four Leukemic cell lines. PhD Thesis, Genetic Engineering Biotechnology for Post Graduate Studies, (2010). University of Baghdad, Iraq.
12. Azqueta A, Shaposhnikov A, Collins A. Mutation research genetic toxicology and environmental mutagenesis. Mutation Research, (2009); 674: 101-108.
13. Gontijio A, Elias F, Salvadori D, Oliveira M, Correa L, Goldberg J. Single cell gel comet assay detects primary DNA damage in nonneoplastic urothelial cells of smokers and Ex-smokers. Cancer Epidemiology biomarkers and prevention, (2001); 10: 987-993.
14. Lopez- Fernandez CB, Perez-Llano P, García-Casado R, Sala A, Gosalbez F, Arroyo J, L. Fernandez, Gosálvez J. Sperm DNA fragmentation in a random sample of the Spanish boar livestock. Animal Reprod., (2008); 103: 87-98.

15. Abbas RN. Cytogenetic and Hematological study for some Iraqi Women suffer from Abortion, College of Science / University of Al-Mustanririya, (2016): 62p.
16. Kisby GE, Muniz JF, Scherer J, Lasarev MR, Koshy M, Kow YW, McCauley L. Oxidative stress and DNA damage in agricultural Workers. Journal of aeromedicine, (2009);14(2): 206-214.
17. Mahood R. Abdul-Hussein. Biochemical and Cytogenetic Study of Recurrent Spontaneous Abortion in a Sample of Women from Baghdad Governorate. PhD thesis, College of Science / University of Baghdad, (2015): 86p.
18. Kaya Gİ, Somer NÜ, Konyalıoğlu S. Antioxidant and antibacterial activities of Ranunculus marginatus var. trachycarpusand R. sprunerianus. Turk J Biol. (2010); 34: 139-146.
19. Gloria NF, Soares N. Lycopene and Beta Carotene induce cell –cycle arrest and apoptosis in human breast cancer cell line.; (2014); Mar. 34, 3: 1377- 1386.
20. Tudek B, Winezura A, Jaik J, Siomek A, Foksinski M, Olinski R. Involvement of Oxidatively damaged DNA and repair in cancer development and aging. American journal of translational Research, (2010); 2(3): 254-284.

تأثير داء السكري المستحدث بالآلوكسان على فحص المذنب القاعدي في ذكور الفئران المختبرية المغذاة على أنظمة تغذوية مختلفة

مينه نصري حسن* **فريال فاروق حسين** **حازم إسماعيل الأحمد[1]**

علوم الاغذية / كلية الزراعة / جامعة تكريت
[1] مركز بحوث التقنيات الاحيائية / جامعة النهرين
*Correspondence: **Mina.n.hasan@st.tu.edu.iq**

الملخص:

خلفيةعن الموضوع: يعد تحليل المذنب (تحليل الترحيل الكهربائي لخلية منفردة طريقة شديدة الحساسية لتحديد تضرر الحامض النووي الرايبوزي منقوص الأوكسجين (DNA) بسبب التعرض للمواد المسرطنة والمواد الأخرى والتي تؤثر بالتالي على الخصوبة.
الهدف من البحث: ان الهدف من هذه الدراسة هو لتقييم الدنا المتكسر في ذكور الفئران المصابة بالسكري المستحدث بالآلوكسان ومعرفة تأثير بعض الأنظمة التغذوية في علاج وإصلاح المادة الوراثية بسبب الإصابة بالسكري، المواد وطرق العمل تم في هذه الدراسة استعمال 42 ذكر من الفئران المختبرية 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 تم توزيعها عشوائياً على ستة مجاميع بالإضافة لمجموعة السيطرة السليمة.
النتائج والمناقشة: لقد أظهرت نتائج التحليل الإحصائي والموضحة في الشكل (1) وجود اعلى نسبة إصابات في المادة الوراثية كانت في مجموعة الحيوانات المصابة بالسكري والغير معالجة طوال مدة التجربة حيث اتضح من صور هذه المجموعة زيادة طول الذيل (Tail length) الناتج من هجرة المادة الوراثية الدنا الى خارج النواة بسبب تضررها وكذلك فقد أوضحت النتائج ان مجموعة الحيوانات المصابة بالسكري والمعاملة بإضافة عشبة نبات الميرامية الى كل من التغذية النباتية والتغذية الحيوانية حصل فيها انخفاض معنوي ($P<0.05$) في نسبة إصابات المادة الوراثية حيث كانت نسبة الإصابات العالية (طويلة الذنب) منخفضة في هاتين المجموعتين مقارنةً مع الحيوانات المصابة بالسكري والغير معالجة طوال التجربة.

الكلمات المفتاحية: داء السكري، اختبار المذنب، عشبة الميرامية، أنظمة تغذوية.