Genetic Study on the Effect of the Antidiabetic Drug (Sitagliptin) on DNA and Chromosomes of Human Lymphocyte Culture

Original Article

Soad Shebl Shebl[1], Rania Ibrahim Yassien[1], Shaimaa Abou-Ghanima[2], Sobhy E. Hassab El-Nabi[3] and Eman S. El-Roghy[1]

[1]*Department of Histology, Faculty of Medicine, Menoufia University, Egypt.*

[2]*Department of Biochemistry, King Khalid University, Abha, KSA.*

[3]*Department of Zoology Department, Faculty of Science, Menoufia University, Egypt.*

ABSTRACT

Background: Diabetes mellitus is a worsening worldwide health problem. It constitutes a major global health concern. Sitagliptin selectively inhibits dipeptidyl peptidase-4 (DPP-4) to manage type 2 diabetes mellitus (T2DM). It regulates the blood glucose level without risk of hypoglycemia or increase in body weight. In our study we investigated the effects of Sitagliptin on DNA and chromosomes in cultured human lymphocytes.

Aim: To assess the genotoxic and cytotoxic effects of different concentrations of Sitagliptin on cultured human lymphocytes.

Material and Methods: Cultures were divided into 6 groups: control, positive control (Cisplatin) at concentration of 10 µg/mL and 4 different concentrations of Sitagliptin (125,250,500,1000 µg/mL). Sitagliptin genotoxicity and cytotoxicity were determined by using chromosomal aberrations (CAs), mitotic index (MI), comet assay and nucleic acids electrophoresis.

Results: There was high significant increase in total chromosomal aberrations (TCAs) at 500, 1000 µg/mL of Sitagliptin compared to control. Other studied concentrations of Sitagliptin exhibited an increase in TCAs without significant relation. Compared to control, there was a significant increase in mitotic index at 125 µg/mL of Sitagliptin but non-significant increase at 250 µg/mL of Sitagliptin. However, at 500, 1000 µg/mL of Sitagliptin, there was a significant decrease in MI. Regarding comet assay, there was significant and high significant increase in total DNA damage at 500,1000 µg/mL of Sitagliptin respectively. Nucleic acids electrophoresis not digested with RNase showed that optical density value of RNA was maximum at 125 µg/mL then gradually decreased till reach the minimum level at 1000 µg/mL of Sitagliptin indicating its toxicity. Genomic DNA fragmentation results indicated that Sitagliptin caused a slight damage of DNA in the form of necrosis in a concentration dependent manner.

Conclusion: Sitagliptin induces significant genotoxic and cytotoxic effects on the cultured human lymphocytes at concentrations of (500, 1000 µg/mL).

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Key Words: CAs, comet assay, Genomic DNA fragmentation, MI, sitagliptin.

Corresponding Author: Soad Shebl Shebl, Department of Histology, Faculty of Medicine, Menoufia University, Egypt, **Tel.**: +201 067589364, **E-mail:** soadshebl18@gmail.com

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INTRODUCTION

Diabetes mellitus is a metabolic disease in which the blood glucose level is increased. It constitutes a major health problem. The International Diabetes Federation's 2019 data has demonstrated that approximately 463 million adults are living with diabetes. Diabetes caused about 4.2 million deaths[1].

Hyperglycemia is related to decreased life expectancy and quality due to different vascular complications. The aim of treatment strategies in type II diabetes is to restore the normal blood glucose level thus decreasing the risk of complications. Sitagliptin is widely used with other medications as it decreases hemoglobin A1C, without risk of hypoglycemia or weight gain[2].

Sitagliptin is involved in the degradation of two endogenous hormones; glucagon-like peptide 1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) so improving the level of these hormones and enhances function of islet of Langerhans leading to glycemic control[3]. Sitagliptin was accepted by FDA "Food and Drug Administration" as a combination treatment and also as a monotherapy. It is used in type II diabetic patients to control blood glucose levels if exercise and diet alone are not adequate[4].

Recently, the relationship between cancer and diabetes is greater than expectations[5]. Different studies had demonstrated that patients with diabetes who needed surgery or chemotherapy were exposed to higher rate of death[6]. Most diabetic patients receive medical treatment for the rest of their lives. Sitagliptin is a promising anti-diabetic drug so it should be well evaluated regarding its genotoxic and cytotoxic effects for better evaluation of management plan[7].

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Genotoxicity indicates the ability of the substance to impair different cellular components as spindle fibers, DNA polymerases, DNA repair system. As well as cytotoxicity means a potential cell death[8].

Chromosomal aberration and comet assay are usually used in *vivo* and in *vitro* to examine the genotoxicity and mutagenic features of various substances[9,10]. Genotoxicity studies about Sitagliptin are very limited. European Medicines Agency (EMEA) reported contradictory results about Sitagliptin genotoxicity and cytotoxicity[11].

Until now, the reports about Sitagliptin are controversial. Therefore, we assessed the in *vitro* genotoxicity and cytotoxicity of Sitagliptin at different concentrations via various tests; chromosomal aberrations, mitotic index, comet assay and nucleic acids electrophoresis.

MATERIALS AND METHODS

Ethics

This study was carried out in compliance with guidelines accepted by the Ethical Committee of the faculty of medicine, menoufia university. We took signed consent from all contributors before the start of the study.

Chemicals

Sitagliptin was purchased from Sigma-Aldrich Corp (Cas no. 654671-78-0, USA).

Cisplatin

Was obtained from Sigma-Aldrich (EC number 239-733-8, St. Louis, MO, USA).

Donors and blood samples collection

Blood samples were obtained from three healthy male donors aged 20, 24 and 29 years. They were non-smoker, non-alcoholic and didn't receive any medications. Peripheral blood samples were collected into heparinized tubes. The cell cultures were performed at the same day.

Lymphocyte cultures and cell harvesting

The culture medium consisted of 0.5 mL whole blood sample, 5 mL RPMI (Roswell Park Memorial Institute) 1640 medium, 20% fetal calf serum, 6 µg/mL PHA-L (Phytohemagglutinin-L), 0.5 mL L-glutamine and antibiotics. It was incubated for 72 h at 37 °C[12].

In our study, cultures were divided into 6 groups with addition of treatment after 48 hours of incubation time:

Group I: Control (without treatment).

Group II: Positive control (Cisplatin): treated with 10 µg of Cisplatin/ mL of culture medium[13].

We use four different concentrations of Sitagliptin according to Yuzbasioglu *et al*.[14]. Sitagliptin was dissolved in distilled water.

Group III: (Sitagliptin 125 µg/mL): treated with 125 µg of Sitagliptin /mL of culture medium.

Group IV: (Sitagliptin 250 µg/mL): treated with 250 µg of Sitagliptin /mL of culture medium.

Group V: (Sitagliptin 500 µg/mL): treated with 500 µg of Sitagliptin /mL of culture medium.

Group VI: (Sitagliptin 1000 µg/mL): treated with 1000 µg of Sitagliptin /mL of culture medium.

Methods

I. Chromosomal aberrations (CAs) assay

The method of Evans[15] was followed for the assessment of CAs. We add 0.06 µg/mL of colchicine two hours before harvesting. Centrifugation and addition of hypotonic solution were done to harvest the cells. We centrifuged the cells and used a mixture of cold methanol and acetic acid (3:1) as a fixative. Finally, leukocytic cells were re-suspended and dropped onto slides. Giemsa used to stain the slides of chromosomes. For each group, 100 metaphases were scored for chromosomal aberrations. Only cells with well spread chromosomes were selected. Slides were examined at (×1000) magnification by light microscope. Using the digital camera, representative images were captured.

II. Mitotic index

The same slides of chromosomal aberrations were used to evaluate the mitotic index. It is important to determine the percentage of proliferating cells. 100 cells for each group were assessed at (×200) magnification by light microscope. Cells were classified according to their division to non-dividing cells, prophase and metaphase. Mitotic index was evaluated according to: Mitotic index (%) = (number of metaphases) ×100 / ((number of non-dividing cells + prophase number + metaphase number).

III. Comet assay

It is used to assess the DNA single strand breaks according to Singh *et al*.[16]. 5 ul blood was added to 120 ul low melting agarose. They were placed on a microscopic slide pre-coated with normal melting agarose and protected by a cover slip. At 4°C, the agarose was gelled. We then pulled the cover. Slides were subsequently embedded in the lysis buffer then in the electrophoresis buffer. The slides were neutralized then washed by PBS (phosphate buffered saline). Ethidium bromide was used to stain them. Finally, slides were examined using fluorescence microscope. Interpretation: breaks identified as fluorescent tails in damaged cells that extend from the center to the anode. The length of tail indicated the amount of DNA breakage in the cell. The DNA damaged spots were further categorized into damaged and strongly damaged according to the length of the migrated fragments[17].

IV. Nucleic acids electrophoresis

We followed the simple salting our technique which was explained by Aljanabi & Martinez[18] and modified by El-Garawani and Hassab El-Nabi[19]. Peripheral white

blood cells were collected by centrifugation and lysed by using lysing buffer. The suspension of cells was lightly shaken and kept overnight at 37℃. DNA was obtained by adding saturated NaCl and centrifugation at 1000 rpm for 10 min. DNA was precipitated by using cold isopropanol followed by centrifugation. The sediment was cleaned by ethyl alcohol for 7 min at 1000 rpm. The supernatant was removed and the remaining pellet was lightly mixed with TE buffer. Optical density value of RNA was determined before digestion with RNase. To digest RNA, a suitable amount of RNase was added and incubated at 37℃ for 1 h. DNA samples were added to the loading buffer and examined on an ethidium bromide stained agarose gel. Gel was photographed using digital camera. Image analyzer software (Image J 1.47v national institute of health, USA) was used to analyze these photos.

Statistical analysis

The data was statistically analyzed by SPSS (Statistical Package for the Social Sciences) program, version 20 for Windows (SPSS Inc., Chicago, Illinois, USA). Data was expressed as Mean ± S.D (standard deviation). The mean of each group was compared to that of the control using one-way analysis of variance (ANOVA) then "Tukey" post hoc test. Results were considered significant if *P values* < 0.05 and highly significant if *P values* < 0.001[20].

RESULTS

I. Chromosomal aberrations (CAs) in cultured human lymphocyte

In our study, Sitagliptin increased the frequency of CAs in a concentration dependent manner. The most noticeable structural aberrations were fragment, gap and deletion. There was an increase in the total chromosomal aberrations (TCAs) in all groups treated with Sitagliptin compared to control. But highly significant (P<0.001) only at 500, 1000 µg/mL. There was no significant difference of TCAs caused by 1000 µg/mL Sitagliptin vs Cisplatin. There was a significant decrease (P <0.05) of TCAs at 500 µg/mL Sitagliptin vs Cisplatin. (Figure 1, Table 1, Histogram 1).

II. Mitotic index

There was a significant increase (P <0.05) in mitotic index at 125µg/mL Sitagliptin compared to control. At 250µg/mL, there was an increase in mitotic index but not significant compared to control. While at 500,1000 µg/mL, there were a significant decrease of mitotic index compared to control. Also, there was no significant difference of MI at 1000 µg/mL Sitagliptin compared to Cisplatin. There was a significant increase of MI at 500 µg/mL Sitagliptin compared to Cisplatin. (Table 2, Histogram 2).

III. Comet assay (Alkaline single cell gel electrophoresis)

Sitagliptin increased total DNA damage in a concentration dependent manner. There was no significant difference of total DNA damage caused by 125, 250 µg/mL Sitagliptin compared to control. While there was a significant increase (P<0.05) of total DNA damage caused by 500 µg/mL Sitagliptin vs control. There was high significant increase (P <0.001) of total DNA damage caused by 1000 µg/mL Sitagliptin vs control. There was no significant difference of total DNA damage caused by 1000 µg/mL Sitagliptin vs Cisplatin. There was high significant decrease of total DNA damage caused by 500 µg/mL Sitagliptin vs Cisplatin (Figure 2, Table 3, Histogram 3).

IV. Nucleic acids electrophoresis

a. Nucleic acids electrophoresis without digestion with RNase

The optical density value of RNA of lymphocytes was assessed. It was maximum at 125 µg/mL Sitagliptin. It decreased gradually till reach the minimum level at 1000 µg/mL Sitagliptin. There was high significant increase (P<0.001) of optical density value of RNA at 125, 250 µg/mL compared to control. There was a significant increase (P<0.05) of optical density value of RNA at 500µg/mL Sitagliptin compared to control. While it was significantly decreased at 1000µg/mL compared to control. There was no significant difference of optical density value of RNA at 1000 µg/mL Sitagliptin vs Cisplatin. While it was highly significantly increased at 500 µg/mL Sitagliptin vs Cisplatin. (Figure 3, Table 4, Histogram 4).

b. Genomic DNA fragmentation

Sitagliptin caused a slight damage of DNA in the form of necrosis in a concentration dependent manner. Necrotic DNA fragments appeared as a smear shape. The optical density value of fragmented DNA was highly significantly increased (P<0.001) at 125, 250, 500, 1000 µg/mL Sitagliptin vs control. There was no significant difference of optical density value of fragmented DNA at 500,1000 µg/mL Sitagliptin compared to Cisplatin. (Figure 4, Table 5, Histogram 5).

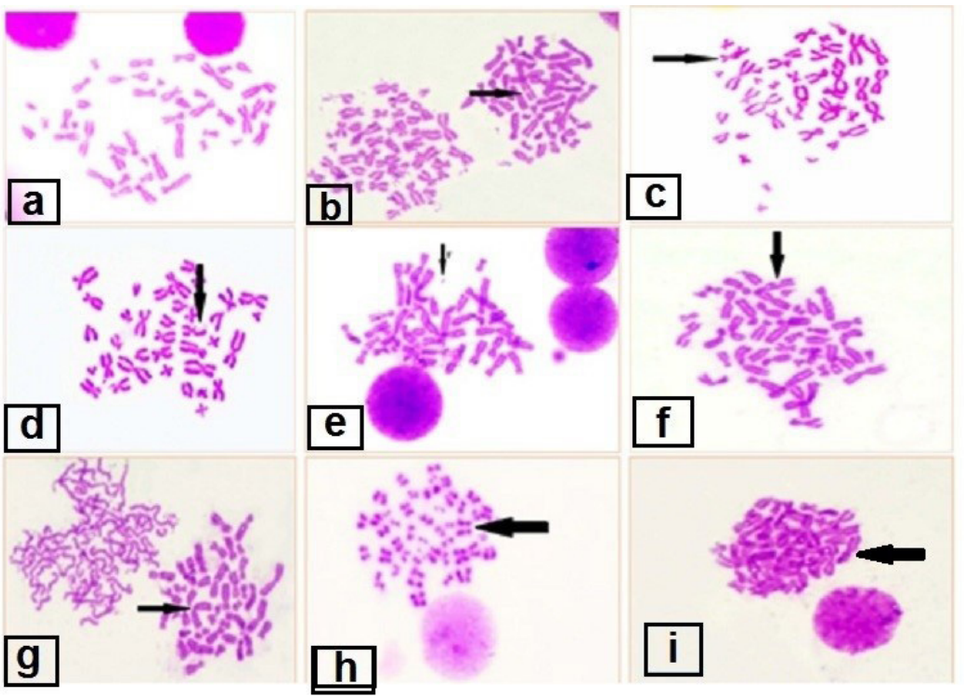


Fig. 1: Showing photographs of chromosomes of cultured human lymphocytes with spread metaphase of normal chromosomes in photograph (a) and different chromosomal aberrations (arrows): gap (b), end to end (c), deletion (d), fragment (e), break (f), centric fusion (g), centric attenuation (h), stickness (i). (x1000 Giemsa stain)

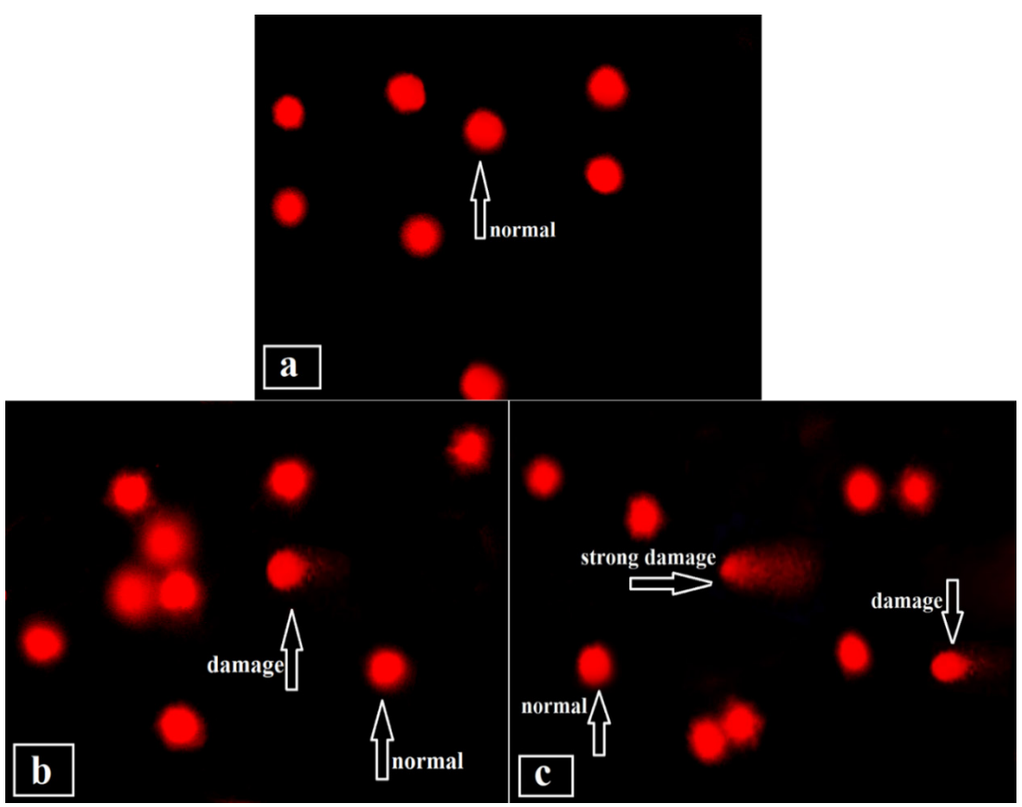


Fig. 2: Showing representative photographs for the effect of different concentrations of sitagliptin on single cell gel electrophoresis (comet assay) of cultured human lymphocytes. Photograph (a) representing DNA at 125, 250 µg/mL of sitagliptin which is more or less similar to control. Photograph (b) representing damage caused by 500 µg/mL of sitagliptin. While photograph (c) representing damage caused by 1000 µg/mL of sitagliptin which is more or less similar to that caused by cisplatin.

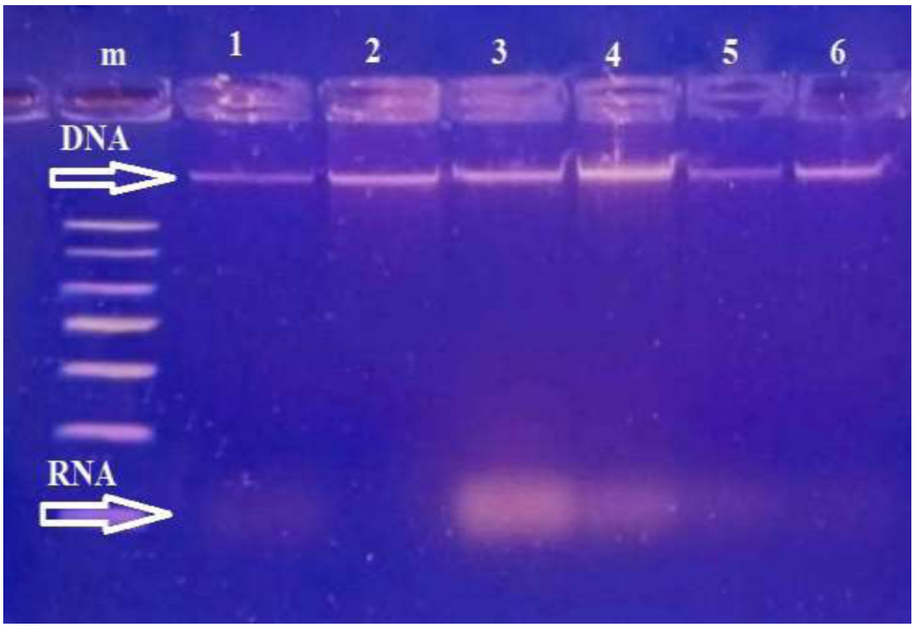


Fig. 3: Showing digital photograph of nucleic acids electrophoresis of cultured human lymphocytes not digested with RNase showing DNA, RNA of all studied groups. Lane1(control), lane2(cisplatin), lane3(125 µg/mL sitagliptin), lane4(250 µg/mL sitagliptin), lane5 (500 µg/mL sitagliptin), lane6 (1000 µg/mL sitagliptin), m (DNA marker).

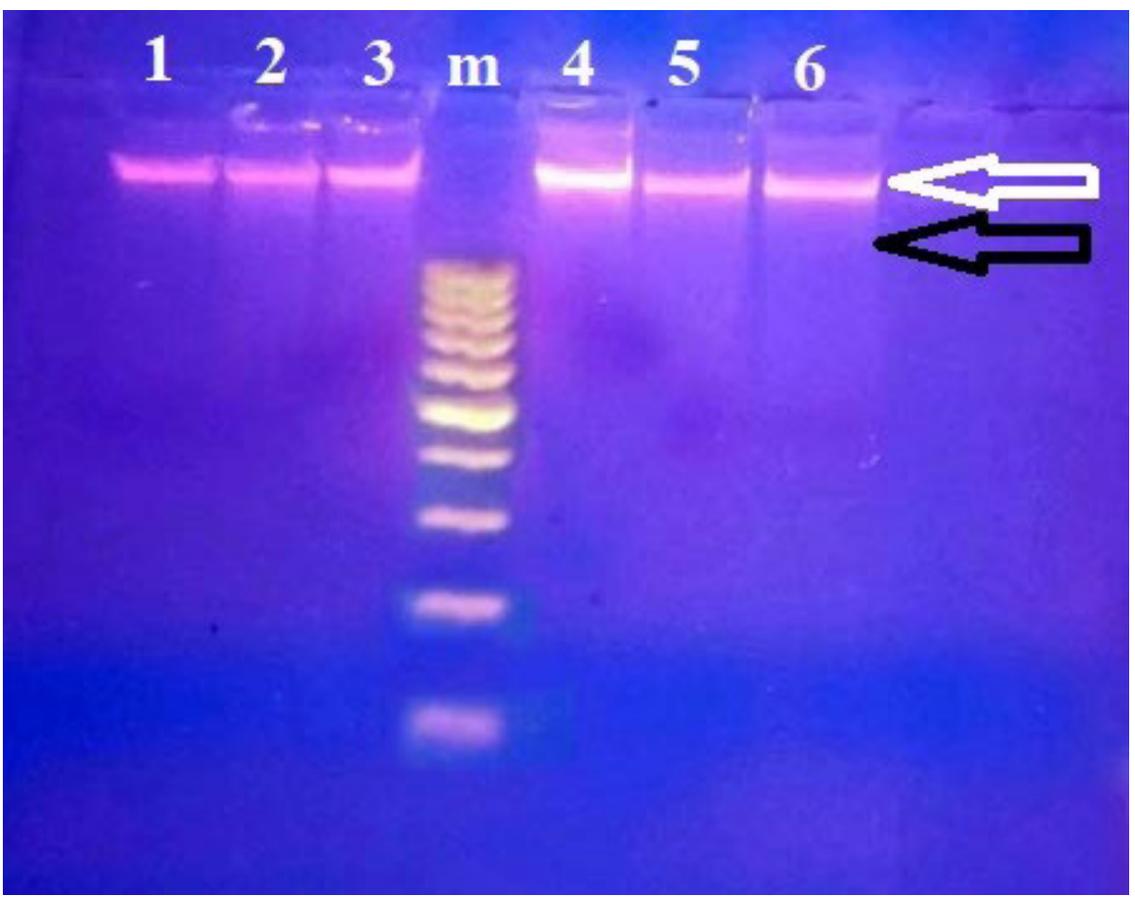


Fig. 4: Showing digital photograph of DNA electrophoresis of cultured human lymphocytes showing DNA of all studied groups. Lane1(control), lane2(cisplatin), lane3(125 µg/mL sitagliptin), lane4(250 µg/mL sitagliptin), lane5 (500 µg/mL sitagliptin), lane6 (1000 µg/mL sitagliptin), m (DNA marker). White arrow: intact DNA, black arrow: fragmented DNA.

Table1: Statistical means of each CA and total CAs of all studied groups.

Groups	CHROMOSOMAL ABERRATIONS (CAs) (Mean ± SD)								*P value* of TCAs
	Deletion	Fragment	Break	Gap	End to end	Centric Fusion	Centric attenuation	Stickiness	Ring	TCAs	
Group I: (Control)	1.33±0.58	1 ± 1	2 ± 1	1.33±0.58	2 ± 1	1 ± 1	1.33 ± 1.15	1 ± 1	-	11±1.73	
Group II: (Cisplatin)	7.67 ± 1.15	9.33 ± 1.53	8.33 ± 3.06	12 ± 2	11.67± 3.06	5± 1.73	8.67 ±2.52	7.33±2.52	0.33± 0.58	70±5.57	0.00003**
Group III: (Sitagliptin 125 µg/mL)	2 ± 1	1.67± 1.15	1.67± 0.58	1.67±0.58	2.67 ± 0.58	1.67 ± 0.58	2 ± 1	1.67± 0.58	-	15±4	0.09
Group IV: (Sitagliptin 250 µg/mL)	2.67± 2.08	2.67 ± 0.58	2.33 ± 0.58	2.33±1.53	2.33 ± 1.15	1.67± 0.58	2± 1	1.67± 0.58	-	17.67± 5.69	0.06
Group V: (Sitagliptin 500 µg/mL)	7.33± 2.08	8± 1	7.67± 2.52	10.67±1.53	8 ± 2.65	5± 1	6.67 ± 2.08	4.67± 1.53	0.67± 0.58	56.67±2.08	0.0000** 0.007#
Group VI: (Sitagliptin 1000µg/mL)	9 ± 2.52	9.33± 2.52	7.67± 2.08	11.33 ± 1.53	11± 3.61	4.33 ± 1.53	8.33 ± 1.53	7 ± 2	0.67 ± 0.58	68.67±2.52	0.0000** 0.3

* Significant vs control; ** high significant vs control; # significant vs cisplatin.

Table 2: Statistical means of mitotic index of all studied groups

Groups	Mitotic Index (Mean ± SD	*P value*
Group I:(Control)	8± 1	
Group II: (Cisplatin)	2.67 ± 1.53	0.004*
Group III: (Sitagliptin 125 µg/mL)	11 ± 2	0.04*
Group IV: (Sitagliptin 250 µg/mL)	9± 1.73	0.2
Group V: (Sitagliptin 500 µg/mL)	5.67 ± 1.15	0.03* 0.03#
Group VI: (Sitagliptin 1000 µg/mL)	3.67 ± 1.53	0.01* 0.2

* Significant vs control; # significant vs cisplatin

Table 3: Statistical means of total DNA damage of all studied groups

Groups	Total DNA damage (Mean ± SD	*P value*
Group I:(Control)	5± 1	
Group II: (Cisplatin)	21 ± 1.73	0.00008**
Group III: (Sitagliptin 125 µg/mL)	5.33 ± 1.15	0.4
Group IV: (Sitagliptin 250 µg/mL)	6 ± 1	0.1
Group V: (Sitagliptin 500 µg/mL)	10.33 ± 1.53	0.004* 0.0007##
Group VI: (Sitagliptin 1000 µg/mL)	19.67 ± 2.08	0.0002** 0.2

* Significant vs control; ** high significant vs control; ## high significant vs cisplatin

Table 4: Statistical means of optical density values of RNA of all studied groups

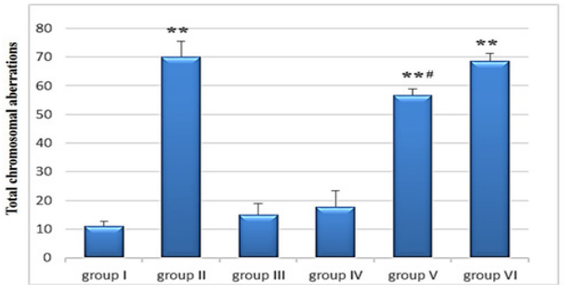
Groups	Optical density of RNA (Mean ± SD	*P value*
Group I:(Control)	132.8 ± 1.61	
Group II: (Cisplatin)	128.2 ± 2.02	0.02*
Group III: (Sitagliptin 125 µg/mL)	175.3 ±1.53	0.0000**
Group IV: (Sitagliptin 250 µg/mL)	159.7 ± 1.53	0.00002**
Group V: (Sitagliptin 500 µg/mL)	143 ± 2	0.002* 0.0004##
Group VI: (Sitagliptin 1000 µg/mL)	127 ± 1.53	0.06* 0.2

*Significant vs control; **high significant vs control; ## high significant vs cisplatin

Table 5: Statistical means of optical density values of fragmented DNA of all studied groups

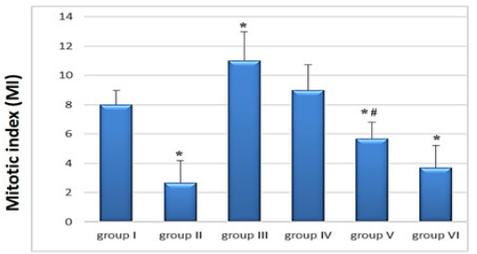
Groups	Optical density of fragmented DNA (Mean ± SD	*P value*
Group I:(Control)	58.67 ± 1.53	
Group II: (Cisplatin)	86.67 ± 2.08	0.00002**
Group III: (Sitagliptin 125 µg/mL)	81 ±1	0.00002**
Group IV: (Sitagliptin 250 µg/mL)	82.67 ± 1.53	0.00002**
Group V: (Sitagliptin 500 µg/mL)	85 ± 1	0.0000** 0.1
Group VI: (Sitagliptin 1000 µg/mL)	87.67 ± 2.08	0.00002** 0.3

**high significant vs control



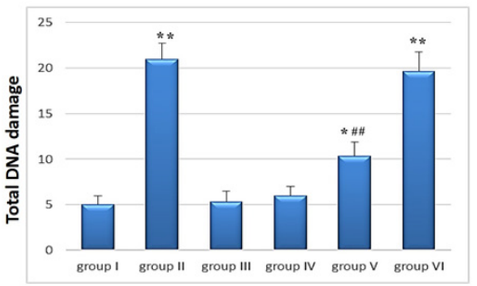
** high significant vs control; # significant vs cisplatin

Histogram 1: Statistical means of total chromosomal aberrations of all studied group.



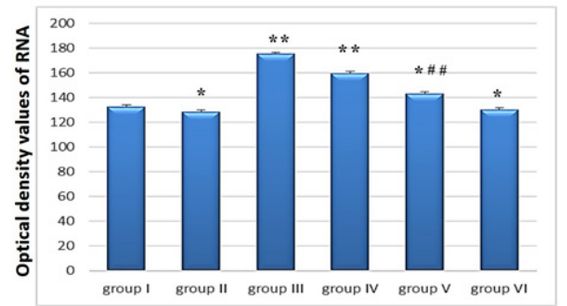
* Significant vs control; # significant vs cisplatin

Histogram 2: Statistical means of mitotic index of all studied groups.



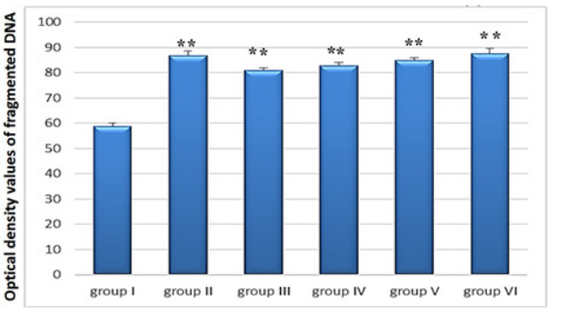
* Significant vs control; ** high significant vs control; ## high significant vs cisplatin.

Histogram 3: Statistical means of total DNA damage of all studied groups.



*Significant vs control; **high significant vs control; ## high significant vs cisplatin

Histogram4: Statistical means of optical density values of RNA of all studied groups.



** High significant vs control

Histogram5: Statistical means of optical density values of fragmented DNA of all studied groups.

DISCUSSION

Diabetes mellitus type II considers the most common type of diabetes. It is caused due to improper response of body cells to insulin. In the last three decades, there was an epidemic increase in the number of T2DM patients[21].

Many researches indicated a relation between diabetes and cancer. There is increased rate of certain cancers in diabetic patients as uterine, colorectal, liver, pancreatic and breast cancers[22]. The antidiabetic drugs are used for long time or forever. So it is necessary to evaluate these drugs regarding their genotoxicity and cytotoxicity. They should be selected carefully especially in case of cancer and pregnancy[23].

Sitagliptin is an oral antidiabetic drug used in the treatment of type II diabetic patients. It has the ability to regulate the level of blood glucose[24]. The aim of the current study was to assess the genotoxic and cytotoxic effects of Sitagliptin on the cultured human lymphocytes. CAs, mitotic index and comet assay have been commonly used to estimate genotoxic and cytotoxic effects of carcinogens[25].

In this study, Sitagliptin induced structural chromosomal aberrations (CAs) in a concentration dependent manner. Where, Total CAs were highly significantly increased at 500, 1000 µg/mL of Sitagliptin compared to control. These findings could be correlated to Yuzbasioglu *et al*.[14] who reported a significant increase of CAs frequency at 1000 µg/mL. CAs may induce oncogenic transformation by inhibition of a tumor suppressor gene. Additionally, by the stimulation of an oncogene by proteins that initiate carcinogenesis[26].

On the other hand, Kasurka *et al*.,[27] found that Sitagliptin didn't increase chromosomal aberrations compared to control. European Medicines Agency reported that Sitagliptin was not mutagenic. However, this report did not give complete information. Also, the genotoxicity studies related to Sitagliptin were so limited[14].

MI is commonly used to assess replication of DNA, cell division and cell death. So, it is used to determine the cytotoxicity of various physical and chemical agents. Any decrease in the MI refers to cytotoxicity of this agent[28].

In our study, Sitagliptin significantly decreased the MI at 500, 1000 µg/mL indicating its in*vitro* cytotoxic effects. This result has been documented in previous studies[14,27]. They stated that Sitagliptin decreased the MI of cultured lymphocytes at 1000 µg/mL. Oz Gul *et al*[29] documented that Sitagliptin has in*vivo* genotoxic and cytotoxic effects. They found that Sitagliptin inhibit three indices of cell-division NDI (nuclear division index), PI (proliferation index), and MI (mitotic index) in treated patients compared to those received medical nutrition therapy.

As regard to Pinheiro *et al*.[30], 50 µg/mL plus the higher Sitagliptin concentrations decrease cell proliferation in cultured lymphocytes. Palus *et al*.[31] reported that the irreparable chromosomal aberrations might cause cell death even before cell division. This is in harmony with our results about decreased MI by the same concentrations of Sitagliptin that caused increased TCAs.

The most common types of DNA damage are double-strand breaks, single-strand breaks and base lesions. The specific repair pathway is activated by either of the above forms[32]. In our study, we evaluated single stranded and double stranded DNA damage by using comet assay and genomic DNA fragmentation respectively.

Our comet assay results, indicated that total DNA damage increased significantly at 500 µg/mL and highly significantly at 1000 µg/mL Sitagliptin respectively. This is in agreement with Yuzbasioglu *et al*.[14] and Kasurka *et al*.[27] who reported that Sitagliptin increased the intensity of comet tail at 1000 µg/mL significantly. In contrast, Giordani *et al*.[11] documented that neither Sitagliptin nor vildagliptin elicited in *vitro* DNA breaks at the tested concentrations (10, 100, and 1000 µM).

In our study, during nucleic acids electrophoresis, before digestion with RNase, we found that the optical density value of RNA was maximum at 125 µg/mL Sitagliptin indicating increased cell activity. This is the same concentration that caused significant increase in MI compared to control. The optical density value of RNA gradually decreased till reach the minimum level at 1000 µg/mL Sitagliptin indicating its toxicity. This may be explained by McKay and Bruce[33] who stated that changes in gene expression and transcriptional regulation may occurred after DNA damage.

Regarding genomic DNA fragmentation, we observed that Sitagliptin caused slight DNA damage in the form of necrosis in a concentration dependent manner. This slight damage in our study is in partial agreement with the study of Najam *et al*.[34] who found massive DNA damage but at higher concentrations (1520 and 3040 µg/mL). Cells that do not correctly repair DNA damage experience cell death. While, inaccurate repaired DNA damage may induce genomic instability. This is associated with tumorigenesis and human disorders[35].

Pfeiffer *et al*.[36] demonstrated that DNA double-strand breaks are critical primary lesions in chromosomal aberrations formation. This may explain our findings regarding increased chromosomal aberrations by the same concentrations that cause increase in DNA damage.

Recently, several studies have documented a cross-link between the mitotic machineries and the DNA damage response. There are check points at all cell cycle phases that enable the cell cycle to stop and activate the mechanisms of repair. This is important to avoid propagation of any genetic changes to the next cell generations[37]. This arrest in cell cycle could explain our findings regarding decreased MI by the same concentrations of Sitagliptin which caused DNA damage.

CONCLUSION

Sitagliptin has significant genotoxic and cytotoxic effects at 500 ,1000 µg/mL on cultured human lymphocytes. But it has no genotoxic or cytotoxic effects at 125 ,250 µg/mL in in*vitro*. We recommend further studies on different concentrations of Sitagliptin to accurately determine the safe Sitagliptin dose for diabetic patients. Also, to explain the mechanism of genotoxicity, cytotoxicity and DNA damage of Sitagliptin.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة وراثية عن تأثير العقار المضاد لمرض السكري (سيتاجليبتين) على الحمض النووي والكروموسومات في مزرعة الخلايا الليمفاوية البشرية

سعاد شبل شبل[١]، رانيا ابراهيم ياسين[١]، شيماء ابو غنيمة[٢]، صبحي السيد حسب النبي[٣]، ايمان شحاته الرغي[١]

[١]قسم الهستولوجي، كلية الطب_ جامعة المنوفية

[٢]قسم الكيمياء الحيوية، جامعة الملك خالد- السعودية

[٣]قسم الوراثة والبيولوجية الجزيئية، كلية العلوم – جامعة المنوفية

المقدمة: يعد مرض السكري مشكلة صحية عالمية تزداد سوءًا ويمثل مصدر قلق كبير للصحة العامة. السيتاجليبتين يثبط بشكل انتقائي ٤-dipeptidyl peptidase (٤-DPP) لعلاج داء السكري من النوع الثاني. وهو ينظم مستوى السكر في الدم دون التعرض لخطر الإصابة بنقص السكر في الدم أو زيادة وزن الجسم. في هذه الدراسة ، قمنا بدراسة آثار السيتاجليبتين على الحمض النووي والكروموسومات في مزرعةالخلايا الليمفاوية البشرية.

الهدف من الدراسة : تقييم التأثير السمي لتركيزات مختلفة من السيتاجليبتين علي الجينات و الخلايا في مزرعة الخلايا الليمفاوية البشرية

المواد المستخدمة وطريقة البحث: تم تقسيم المجموعات الي ٦ مجموعات . المجموعة الاولي (المجموعة الظابطة)، المجموعة الثانية(مجموعة السيسبلاتين) تم استخدام السيسبلاتين كضابط إيجابي بتركيز ١٠ ميكروغرام / مل. لقد تم استخدام اربعة تركيزات مختلفة من السيتاجليبتين (١٢٥ ، ٢٥٠ ، ٥٠٠، ١٠٠٠ ميكروغرام السيتاجليبتين / مل). لقد حددناالسمية الجينية والسمية الخلوية للسيتاجليبتين باستخدام الانحرافات الكروموسومية (CAs) ، ومؤشر الانقسام (MI) ، وفحص المذنب والرحلان الكهربي للأحماض النووية.

النتائج: كانت هناك زيادة كبيرة في إجمالي الانحرافات ا لكروموسومية عند ٥٠٠، ١٠٠٠ ميكروغرام من السيتاجليبتين / مل) مقارنة بالمجموعة الضابطة . أظهرت التركيزات الأخرى المستخدمة من السيتاجليبتين زيادة في إجمالي الانحرافات ا لكروموسومية دون علاقة ذات مغزي احصائي بالمقارنة مع الضابطة ، كما كانت هناك زيادة كبيرة في مؤشر الانقسام (MI) عند ١٢٥ ميكروغرام / مل من السيتاجليبتين . ولكنها كانت غير ملحوظة عند ٢٥٠ ميكروغرام / مل من الستاجليبتين. بينما عند ٥٠٠، ١٠٠٠ ميكروغرام / مل من السيتاجليبتين ، كان هناك انخفاض كبير في مؤشر الانقسام . فيما يتعلق بمقايسة المذنب ، كانت هناك زيادة كبيرة وذات دلالة كبيرة في تلف الحمض النووي الكلي عند ٥٠٠، ١٠٠٠ ميكروغرام / مل من السيتاجليبتين على التوالي. أظهر الرحلان الكهربي للأحماض النووية التي لم يتم هضمها باستخدام RNase أن قيمة الكثافة الضوئية للحمض النووي الريبي RNAكانت بحد أقصى ١٢٥ ميكروغرام / مل ثم انخفضت تدريجياً حتى وصلت إلى المستوى الأدنى عند ١٠٠٠ميكروغرام / مل من السيتاجليبتين مما يشير إلى سميتها. أشارت نتائج تجزئة الحمض النووي الجينومي إلى أن السيتاجليبتين تسبب في تلف طفيف للحمض النووي في شكل تنخر بطريقة تعتمد على التركيز.

الاستنتاج : يسبب الستاجليبتين في حدوث تأثيرات جينية وخلوية سامة ذات مغزي للجينات والخلايا عند تركيزات ٥٠٠، ١٠٠٠ ميكروغرام / مل في مزرعة الخلايا الليمفاوية البشرية