THE *IN VITRO* CYTOTOXICITY, GENOTOXICITY AND OXIDATIVE DAMAGE POTENTIAL OF THE ORAL DIPEPTIDYL PEPTIDASE-4 INHIBITOR, LINAGLIPTIN, ON CULTURED HUMAN MONONUCLEAR BLOOD CELLS

K. Çadirci^{1,*}, H. Türkez², Ö. Özdemir²

¹Health Science University, Erzurum Regional Training and Research Hospital - Department of Internal Medicine, ²Erzurum Technical University, Department of Molecular Biology and Genetics, Erzurum, Turkey

Abstract

Background. Linagliptin (LNG) is a selective dipeptidyl peptidase-4 (DPP-4) inhibitor that ameliorates blood glucose control of patients with type 2 diabetes, without developing hypoglycemic risk and weight gain with a good clinical and biological tolerance profile. To the best of our knowledge, its cytotoxic, genotoxic and oxidative effects have never been studied on any cell line.

Aim. To evaluate the *in vitro* cytotoxic, genotoxic damage potential and antioxidant/oxidant activity of LNG in cultured peripheral blood mononuclear cells (PBMC).

Material and methods. After exposure to different doses (from 0.5 to 500 mg/L) of LNG, cell viability was measured by the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) and lactate dehydrogenase (LDH) leakage tests. The antioxidant activity was assessed by the total antioxidant capacity (TAC) and total oxidative stress (TOS) assays. To evaluate the genotoxic damage potential, chromosomal aberration (CA) frequencies and 8-oxo-2'-deoxyguanosine (8-oxo-dG) levels were determined.

Results. Treatment with LNG did not cause statistically significant decreases of cell viability at lower concentrations than 100 mg/L as compared to untreated cultures. However, LNG exhibited cytotoxic action at 250 and 500 mg/L. Also, IC₂₀ and IC₅₀ values of LNG were determined as 8.827 and 70.307 mg/L, respectively. In addition, the oxidative analysis revealed that LNG supported antioxidant capacity at concentrations of 2.5, 5, 10, 25, 50 and 100 mg/L without generating oxidative stress. Besides, the results of CA and 8-oxo-dG assays showed *in vitro* non-genotoxic feature of LNG. As a conclusion, our findings clearly revealed that LNG had no cytotoxic and genotoxic actions, but exhibited antioxidative activity. In conclusion, therefore it is suggested that LNG use in diabetic patients is safe and provides protection against diabetic vascular and oxidative complications.

Key words: DPP-4 inhibitor, linagliptin, cytotoxicity, oxidative stress, genotoxicity.

INTRODUCTION

Diabetes mellitus, a metabolic disorder characterized by chronic hyperglycemia, influences approximately 285 million adults worldwide and leads to nearly four million deaths each year (1, 2). Hyperglycemia is related to decreased life expectancy and quality due to both micro-vascular and macro-vascular complications. The aim of treatment strategies in type 2 diabetes is maintaining glycemic control to decrease the risk of complications. Dipeptidyl peptidase-4 (DPP-4) inhibitors are one of the current drug options for the treatment of type 2 diabetes (3-5). DPP-4 inhibitors are involved in the degradation of two endogenous incretin hormones glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) and enhance active levels of these hormones and, in doing so, improve islet function as well as glycemic control in T2DM. Collectively, the therapeutic importance of DPP-4 inhibitors in T2DM is rapidly evolving as their potential strengths and is thought that this significance will become better defined via further investigations (6-11).

Linagliptin (LNG), as a selective and competitive dipeptidyl peptidase-4 (DPP-4) inhibitor is being used for the treatment of type 2 diabetes since 2011 (12). LNG leads to decreases of glucagon secretion and increases of insulin secretion in a glucose-dependent manner. Thus LNG provides an overall amelioration in the glucose homeostasis (13). In addition, this promising agent inhibits DPP-4 activity with an IC₅₀ of ~1 nM and has a long duration of action (>80% DPP-4 inhibition at 24-h post dose). It is known that LNG is well tolerated at relatively higher doses than 100-fold in excess of the therapeutic dose of 5 mg, thus exhibits a large safety line (14-16).

From the literature scanning, it was propounded

*Correspondence to: Kenan Çadirci MD, Health Science University, Regional Training and Research Hospital, Department of Internal Medicine, Erzurum, 25050, Turkey, E-mail: doktorcadirci@hotmail.com

that the cytotoxic, genotoxic and oxidative effects of LNG had not been investigated on human PBMC cultures yet. Therefore, in this present study, we aimed to assess *in vitro* biological activities consisting cytotoxic (by the MTT and LDH tests), oxidative (by measuring TAC and TOS levels) and genotoxic (by the CA and 8-oxo-dG assays) damage potentials by LNG in cultured human PBMCs for the first time.

MATERIALS AND METHODS

Chemicals and Reagents

Trajenta® tablets labeled to contain 5 mg of LNG were obtained from Boehringer Ingelheim Pharmaceuticals. Tablets were dissolved in methanol and diluted in cell culture medium.

Mononuclear cell isolation

Human PBMCs were obtained from five healthy non-smoking volunteers between 22 and 25 years of age, with no history of exposure to any genotoxic agent. All participants provided their signed informed consent forms before participating in this research. The blood samples were diluted with the equal volume of phosphate-buffered saline (PBS), layered on Ficoll-Hypaque-Plus (GE Healthcare BioSciences Corp., Piscataway, NJ, USA) and centrifuged (400 g for 30 min at 18-24°C). PBMCs were carefully removed and transferred into RPMI-1640 media (GIBCO, Grand Island, NY, USA) supplemented with 10% FBS and 1% penicillin/streptomycin (Sigma, USA). The PBMC cells were counted using a haemocytometer. The cultures without LNG were used as negative control group. Mitomycin C (10 mg/L, Sigma-Aldrich, USA) was used as the positive control group in CA and 8-oxo-dG assays. Ascorbic acid (10 mg/L, Sigma-Aldrich, USA) and hydrogen peroxide (25 mg/L, Sigma-Aldrich, USA) were also used as positive controls in TAC and TOS analysis, respectively.

MTT Assay

The PBMC cells were inoculated into each well of 48-well plates containing the growth medium. The cells were incubated in the absence or presence of LNG (0.5, 1, 2.5, 5, 10, 25, 50, 100, 250 and 500 mg/L) for 72 h. After incubation period, 10 µL of MTT solution (5 mg/mL MTT in PBS) was added to each well and incubated for 4 h. Then, 100 µL DMSO was added to dissolve any formazan crystals formed and then optical density was determined at 570 nm using a micro plate reader (Synergy-HT; BioTek Winooski, VT, USA). Cell

viability was expressed in percentage of viable cells.

LDH Release Assay

LDH assay was performed using LDH Cytotoxicity Assay kit (CytoSelect, USA) according to the manufacturer's guide. Briefly, the PBMC cells were placed in 48-well plates and incubated at 37 °C in a humidified 5% CO₂/95% air mixture and treated with LNG at different concentrations (0.5 to 500 mg/L) for 72 h. At the end of incubation, 90 µL of the supernatant from each well (including positive control, negative control and test groups) was transferred to a new plate. Afterwards, 10 µL of LDH assay reagent was added into each well and incubated at 37°C for 30 min. The absorbance was measured at 450 nm using a micro-plate reader (Synergy-HT; BioTek Winooski, VT, USA).

Total antioxidant capacity (TAC) and total oxidant status (TOS) assays

The PBMC cells seeded in a 48-well plate were treated with various concentrations of LNG and incubated for 72 h at 37°C with 5% CO₂ cultures. At the end of incubation, PBMC cells were homogenized and centrifuged at 600 g for 10 min at 4°C, and then the supernatants were used to examine total antioxidant capacity (TAC) assay and the total oxidant status (TOS) assay using commercial kits (Rel Assay Diagnostics®, Turkey) according to the manufacturer's protocol.

Genotoxicity testing

CA Assay

Human lymphocytes were treated with LNG at different concentrations and cultured for 72h. Two hours prior to harvesting, 0.02 µg/mL of colchicine was added to the culture. After performing hypotonic treatment (0.075 M KCl/37.4 °C) and fixation (methanol plus acetic acid) three times, cells were harvested by centrifugation. The slides were prepared from each fixed-cell suspension and air-dried. Then, the slides were stained with Giemsa in phosphate buffer (pH 6.8). For each treatment, 30 well-spread metaphases were analyzed for chromosome aberration scoring. All the aberrations (chromatid or chromosome gap and chromatid or chromosome break) were classified according to the criteria of Environmental Health Criteria 46 for environmental monitoring of human populations.

8-oxo-dG level

8-hydroxy-2-deoxyguanosine assay kits were obtained from Cayman Chemical (USA) for assessing

8- oxo-dG levels in the cultures. All procedures were performed due to the provider's guide.

Statistical analysis

Statistical analysis was performed using SPSS software (version 20.0, SPSS, Chicago, IL, USA). The Duncan's test was used for the statistical analysis of all experimental values. P values of <0.05 were used for determining significant differences between means.

RESULTS

In order to determine toxicity of LNG, lymphocytes were treated with increasing concentrations of the agent for 72h and the MTT cell proliferation assay was performed. The assay results showed that there were not statistically significant ($p>0.05$) decreases in cell proliferation rates after treatment with LNG concentrations (except for 250 and 500 mg/L) when compared to untreated controls (Fig. 1). From the results of MTT analysis, it was concluded

that LNG at 250 and 500 mg/L showed cytotoxic action. 250 and 500 mg/L concentrations of LNG were 63.61 and 127.22 times higher than the approved therapeutic concentration (3.93 mg/L). We also investigated the toxic effect of LNG on the proliferation of lymphocytes cells by LDH release assay. As shown in Figure 2, LNG (at 100, 250 and 500 mg/L) caused to slight ($p>0.05$, $p<0.1$) increases of LDH activity as compared to control sample. IC20 and IC50 values of LNG were determined according to the MTT assay and calculated as 8.827 and 70.307 mg/L, respectively.

The oxidative effects of LNG on human whole blood cultures were evaluated via using TAC and TOS analysis. According to the results shown in Figure 3, 0.5 and 1 mg/L concentrations of LNG exposure did not lead to significant ($p>0.05$) alterations in TAC levels, while 2.5, 5, 10, 25, 50 and 100 mg/L concentrations of LNG treatment caused significant ($p<0.05$) increases of TAC levels without leading any alterations in TOS levels.

Potential genotoxic effect of LNG was assayed on human lymphocytes cells using CA assay. The

Figure 1. Viability of human blood cells after 72h of exposure to LNG. Control (-): negative control; Control (+): positive control. * symbol presents statistical difference from the control(-) group at level of $p<0.05$.

Figure 2. LDH levels in cultured human blood cells treated with different concentrations (0-500 mg/L) of LNG for 72 h. * and # symbols present statistical difference from the control(-) group at level of $p<0.05$ and $p<0.1$, respectively.

Figure 3. TAC levels in cultured human peripheral blood cells exposed to LNG for 72h. * symbol presents statistical difference from the control(-) group at level of $p<0.05$.

Figure 4. TOS levels in cultured human peripheral blood cells exposed to LNG for 72h. * symbol presents statistical difference from the control(-) group at level of $p<0.05$.

Figure 5. Representative images of chromosomal aberrations observed in cultured human peripheral blood cells exposed to different concentrations of LNG (a) Control (-): normal undamaged chromosomes are seen; (b) treatment with IC₂₀ concentration of LNG: chromosomes seem normal like control; (c) treatment with IC₅₀ concentration of LNG: chromosomes seem normal like control.

results are shown in Figure 5. *In vitro* exposure of LNG at tested concentrations did not cause to increases of CA rates as compared to control group ($p>0.05$). Likewise, LNG at all tested concentrations did not show an increase ($p>0.05$) in the levels of 8-oxo-dG on treated cultures when compared with the control group (Table 1).

DISCUSSION

Recent researches have focused on the impact and safety profile of LNG on human. These studies showed that LNG was an effective anti-diabetic drug on human and safe according to profile of side effect (17,18). To our best knowledge, the cytotoxic, genotoxic and oxidative effects of LNG have not been investigated on human blood cell cultures yet. Therefore, we aimed to evaluate *in vitro* biological activities consist of cytotoxic, genotoxic and oxidative effects of different doses of LNG in cultured human blood cells for the first time.

The MTT assay is a colorimetric method widely used to determine cell viability and proliferation.

LDH assay is also a technique to detect cell death via measuring the release of a stable cytosolic enzyme, LDH, upon membrane damage in necrotic cells (19-22). In the present study, MTT and LDH cytotoxicity assays on human lymphocyte cells gave similar results and showed that LNG has a weak inhibition effect on cell viability and low LDH activity. These results were in accordance with the previous clinical studies, which have reported that treatment with LNG was overall safe and well tolerated (23, 24). In addition, treatment of LNG with doses almost 400-fold higher than the human-equivalent clinical dose for 2 years was found not associated with side effects in rodents (25). Similar findings have been observed in long-term preclinical studies of other DPP-4 inhibitors such as saxagliptin, sitagliptin and vildagliptin (26-28).

The reduction in β -cell mass results from increased apoptosis, most probably caused by the combined action of cytokines and increased plasma glucose and free fatty acid levels. The gluco-incretin hormones glucagon-like peptide (GLP)-1 and gastric inhibitory peptide (GIP) protect β -cells against apoptosis induced by cytokines or glucose and free

Table 1. The genotoxic effects of LNG treatments on cultured human blood cells

Groups	CAs/cell	8-oxo-dG level (p mol / μ g DNA)
Control (-)	0.20 ± 0.02	0.81 ± 0.08
Control (+)	$2.59 \pm 0.18^*$	$4.22 \pm 0.35^*$
0.5 mg/L	0.22 ± 0.03	0.70 ± 0.05
1 mg/L	0.24 ± 0.02	0.72 ± 0.07
2.5 mg/L	0.18 ± 0.01	0.68 ± 0.06
5 mg/L	0.22 ± 0.03	0.73 ± 0.08
10 mg/L	0.24 ± 0.02	0.75 ± 0.11
25 mg/L	0.26 ± 0.04	0.72 ± 0.07
50 mg/L	0.28 ± 0.03	0.77 ± 0.09
100 mg/L	0.26 ± 0.03	0.80 ± 0.10

* symbol presents significant statistical difference from the control (-) group at level of $p<0.05$.

fatty acids (29). At the same time, Exendin-4 (Ex4), a long acting glucagon-like peptide 1 receptor agonist, has similar effects upon β cells in rodents and humans, and it stimulates β cell growth (30).

In a study by Taskinen *et al.* (23) LNG was reported to reduce the risk of hypoglycemia and an increase in the risk of weight gain on the reduced HbA1C, fasting plasma glucose (FPG), HBA1c and 2 hour post-prandial glycemia. LNG enhanced β -cell function consistent with the increased availability of endogenous GLP-1, which stimulates the proliferation and differentiation of pancreatic β cells. Beta cell dysfunction and insulin resistance are accents of type 2 diabetes. Pancreatic beta cells are vulnerable to oxidative stress due to the relatively low expression of antioxidant enzymes such as catalase and glutathione peroxidase. Oxidative stress generation and certain inflammatory cytokine productions cause destructions of beta cells. In diabetic patients, oxidative stress is caused by hyperglycemia and hyperglycemia reduces the expression and secretion of insulin (31). Excessive oxidative stress caused oxidative damage to proteins, lipids, and other biological macromolecules, which finally leads to organ injury (32).

To detect the antioxidant/oxidant effects of LNG, TAC and TOS assays were used in the study. Results obtained from the present study demonstrate that LNG led to increase in the TAC level and without altering in the TOS level. In agreement with our findings, Salheen *et al.* (33) investigated the potential of various DPP-4 inhibitors including LNG, sitagliptin, and vildagliptin on the reduction of vascular superoxide levels and found that only LNG showed a significant reduction of superoxide production. Likewise, Kröller-Schön *et al.* (34) indicated that sitagliptin, vildagliptin, alogliptin and saxagliptin did not inhibit the oxidative burst in human leucocytes. However, LNG exhibited strong antioxidant and anti-inflammatory effects. In diabetic patients, hyperglycemia increases insulin resistance by increasing oxidative stress and contributes to the progression of the disease. Oxidative stress and hyperglycemia play an important role in the process of endothelial dysfunction, which causes complications by affecting all organs.

In diabetic patients, advanced glycation end products (AGEs) and their receptor (RAGE) contribute to vascular damage by forming endothelial damage. AGE implements this via increasing reactive oxygen species and reducing endothelial nitric oxide synthase. A previous study was performed in order to demonstrate the protective effect of sitagliptin, a DPP-

4 inhibitor, on AGE-RAGE-stimulated endothelial cells. Finally, it was revealed that sitagliptin completely inhibited AGE-induced elevations in RAGE mRNA in umbilical vein endothelial cells. This action appeared to be a consequence of the antioxidative property. In this study, the observed elevations in TAC levels after treatment with LNG indicates that LNG may interact with AGEs, hence it provides antioxidative protection (35).

Epidemiologic evidence suggests that cancer incidence is associated with diabetes as well as certain diabetes risk factors and diabetes treatments (36-37). The oxidative DNA damage was associated with different tissue injuries in experimental animals (38), antioxidative agents could provide protection (39, 40). At this point, the *in vitro* genotoxic effects of LNG have not been investigated. In this investigation, we assessed the genotoxicity of LNG on human lymphocyte cultures using chromosomal aberration (CA) assay. This assay showed that LNG was non-genotoxic *in vitro*. Similar to these findings, it was reported that DPP-4 inhibitors such as saxagliptin, vildagliptin and sitagliptin had no genotoxic potentials (41, 42). The assessment of genotoxicity and cytotoxicity of anti-diabetic medications deserve special consideration due to an increasing number of patients with T2DM who require long-lasting therapeutic interventions. In fact, Gul *et al.* (43) investigated, genotoxic (sister chromatid exchange assay, micronucleus assay and CA) and cytotoxic damage potentials of pioglitazone, rosiglitazone and sitagliptin as compared with medical nutritional therapy in T2DM. They found that the genotoxic damage scores were higher in the group treated with sitagliptin in comparison to medical nutritional therapy. However, the observed damage scores were lower than that occurring in subjects who received thiazolidinedione (TZD).

In conclusion, it is suggested that linagliptin has no cytotoxic, pro-oxidative and genotoxic effects on cultured human lymphocytes. Also, the results of the present investigation support the antioxidant properties of linagliptin reported in the previous studies. To summarize, linagliptin is a well-tolerated DPP-4 inhibitor and exhibits a large-scale of safety profile.

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. *Diabetes Res Clin Pract* 2010; 87: 4-14.
- Roglic G, Unwin N. Mortality attributable to diabetes: estimates for the year 2010. *Diabetes Res Clin Pract* 2010; 87: 15-19.
- Mori K, Emoto M, Numaguchi R, Yamazaki Y, Urata H, Motoyama K, Morioka T, Shoji T, Inaba M. Potential advantage of repaglinide monotherapy in glycemic control in patients with type 2 diabetes and severe renal impairment. *Acta Endocrinologica-Bucharest* 2017; 13(2): 133-137.
- American Diabetes Association. Standards of medical care in diabetes-2009. *Diabetes Care* 2009; 32(Suppl. 1): 13-61.
- Nathan DM, Buse JB, Davidson MB, Ferrannini E, Holman RR, Sherwin R. Medical management of hyperglycemia in type 2 diabetes: a consensus algorithm for the initiation and adjustment of therapy: a consensus statement of the American Diabetes Association and the European Association for the Study of Diabetes. *Diabetes Care* 2009; 32(1): 193-203.
- Duez H, Cariou B, Staels B. DPP-4 inhibitors in the treatment of type 2 diabetes. *Biochem Pharmacol* 2012; 83(7): 823-832.
- Scheen AJ. DPP-4 inhibitors in the management of type 2 diabetes: a critical review of head-to-head trials. *Diabetes Metab* 2012; 38(2): 89-101.
- Cahn A, Cernea S, Raz I. An update on DPP-4 inhibitors in the management of type 2 diabetes. *Expert Opin Emerg Drugs* 2016; 21(4): 409-419.
- St Onge EL, Miller SA, Taylor JR. Novel approaches to the treatment of type 2 diabetes. *J Pharm Pract* 2009; 22: 320-332.
- Richter B, Bandeira-Echtler E, Bergerhoff K, Lerch CL. Dipeptidyl peptidase-4 (DPP-4) inhibitors for type 2 diabetes mellitus. *Cochrane Database Syst Rev* 2008; CD006739.
- Bode BW. Defining the importance of daily glycemic control and implications for type 2 diabetes management. *Postgrad Med* 2009; 121: 82-93.
- Fuchs H, Runge F, Held HD. Excretion of the dipeptidyl peptidase-4 inhibitor linagliptin in rats is primarily by biliary excretion and P-gp mediated efflux. *European Journal of Pharmaceutical Sciences: Official Journal of the European Federation for Pharmaceutical Sciences* 2012; 45(5): 533-538.
- Del Prato S, Barnett AH, Huisman H, Neubacher D, Woerle HJ, Dugi KA. Effect of linagliptin monotherapy on glycaemic control and markers of B-cell function in patients with inadequately controlled type 2 diabetes: a randomised controlled trial. *Diabetes Obes Metab* 2011; 13: 193-287.
- Thomas L, Eckhardt M, Langkopf E, Tadayyon M, Himmelsbach F, Mark M. (R)-8-(2-Amino-piperidin-1-yl)-7-but-2-ynyl-3-methyl-1-(4-methyl-quinazolin-2-ylmethyl)-3,7-dihydro-purine-2,6-dione (BI 1356), a novel xanthine-based dipeptidyl peptidase 4 inhibitor, has a superior potency and longer duration of action compared with other dipeptidyl peptidase-4 inhibitors. *J Pharmacol Exp Ther* 2008; 325: 175-182.
- Hüttner S, Graefe-Mody EU, Withopf B, Ring A, Dugi KA. Safety, tolerability, pharmacokinetics and pharmacodynamics of single oral doses of BI 1356, an inhibitor of dipeptidyl peptidase 4 in healthy male volunteers. *J Clin Pharmacol* 2008; 48: 1171-1178.
- Heise T, Graefe-Mody EU, Hüttner S, Ring A, Trommeshauser DA, Dugi KA. Pharmacokinetics, pharmacodynamics and tolerability of multiple oral doses of linagliptin, a dipeptidyl peptidase-4 inhibitor in male type 2 diabetes patients. *Diabetes Obes Metab* 2009; 11: 786-794.
- Wu D, Li L, Liu C. Efficacy and safety of dipeptidyl peptidase-4 inhibitors and metformin as initial combination therapy and as monotherapy in patients with type 2 diabetes mellitus: a meta-analysis. *Diabetes Obes Metab*. 2014; 16: 30-37.
- Rehman MB, Tudrej BV, Soustre J, Buisson M, Archambault P, Pouchain D, Vaillant-Roussel H, Gueyffier F, Faillic JL, Perauld-Pochat MC, Cornu C, Boussageon R. Efficacy and safety of DPP-4 inhibitors in patients with type 2 diabetes: meta-analysis of placebo-controlled randomized clinical trials. *Diabetes Metab*, 2017; 43:48-58.
- Kornelius E, Lin C, Chang H, Li H, Huang W, Yang Y, Lu Y, Peng C, Huang C. DPP-4 inhibitor Linagliptin attenuates A β -induced cytotoxicity through activation of AMPK in neuronal cells. *CNS Neuroscience & Therapeutics* 2015; 21: 549-557.
- Furuhashi M, Hiramitsu S, Mita T, Fuseya T, Ishimura S, Omori A, Matsumoto M, Watanabe Y, Hoshina K, Tanaka M, Moniwa N, Yoshida H, Ishii J, Miura T. Reduction of serum FABP4 level by sitagliptin, a DPP-4 inhibitor, in patients with type 2 diabetes mellitus. *Journal of Lipid Research* 2015; 56: 2372-2380.
- Turkez H, Yousef MI, Sönmez E, Togar B, Bakan F, Sozio P, Stefano AD. Evaluation of cytotoxic, oxidative stress and genotoxic responses of hydroxyapatite nanoparticles on human blood cells. *J Appl Toxicol* 2014; 34(4): 373-339.
- Patrino A, Fornasari E, Di Stefano A, Cerasa LS, Marinelli L, Baldassarre L, Sozio P, Turkez H, Franceschelli S, Ferrone A, Di Giacomo V, Speranza L, Felaco M, Cacciatore I. Synthesis of a novel cyclic prodrug of S-allyl-glutathione able to attenuate LPS-induced ROS production through the inhibition of MAPK pathways in U937 cells. *Mol Pharm* 2015; 12(1): 66-74.
- Taskinen MR, Rosenstock J, Tamminen I, Kubiak R, Patel S, Dugi KA, Woerle HJ. Safety and efficacy of linagliptin as add-on therapy to metformin in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled study. *Diabetes Obes Metab* 2011; 13(1): 65-74.
- Scherthaner G, Barnett AH, Emser A, Patel S, Troost J, Woerle HJ, von Eynatten M. Safety and tolerability of linagliptin: a pooled analysis of data from randomized controlled trials in 3572 patients with type 2 diabetes mellitus. *Diabetes Obes Metab*. 2012; 14(5): 470-478.
- Boehringer Ingelheim Pharmaceuticals, Inc. Tradjenta™ (linagliptin) tablets. US Prescribing information, 2011.
- Bristol-Myers Squibb Company. ONGLYZA™ (saxagliptin) tablets. US prescribing information, 2011.
- Merck Sharp & Dohme Corp. JANUVIA® (sitagliptin) tablets. US prescribing information, 2011.
- Novartis Pharmaceuticals UK Ltd. Galvus 50 mg tablets. Summary of product characteristics. Electronic Medicines Compendium (eMC), 2011.
- Cornu M, Yang JY, Jaccard E, Poussin C, Widmann C, Thorens B. Glucagon-like peptide-1 protects beta-cells against apoptosis by increasing the activity of an IGF-2/IGF-1 receptor autocrine loop. *Diabetes*. 2009; 58(8): 1816-1825.
- Park S, Dong X, Fisher TL, Dunn S, Omer AK, Weir G, White MF. Exendin-4 uses Irs2 signaling to mediate pancreatic beta cell growth and function. *J Biol Chem* 2006; 281: 1159-1168.
- Rochette L, Zeller M, Cottin Y, Vergely C. Diabetes, oxidative stress and therapeutic strategies. *Biochim Biophys Acta*. 2014; 1840 (9): 2709-2729.
- Çolak S, Geyikoğlu F, Bakır TÖ, Türkez H, Aslan A. Evaluating the toxic and beneficial effects of lichen extracts in normal and diabetic rats. *Toxicol Ind Health*. 2016; 32 (8): 1495-1504.
- Salheen SM, Panchapakesan U, Pollock CA, Woodman OL. The DPP-4 inhibitor linagliptin and the GLP-1 receptor agonist exendin-4 improve endothelium-dependent relaxation of rat mesenteric arteries in the presence of high glucose. *Pharmacological Research* 2015; 94: 26-33.
- Kröller-Schön S, Knorr M, Hausding M, Oelze M, Schuff A, Schell R, Sudowe S, Scholz A, Daub S, Karbach S, Kossmann S, Gori T, Wenzel P, Schulz E, Grabbe S, Klein T, Münzel T, Daiber A. Glucose-independent improvement of vascular dysfunction in experimental sepsis by dipeptidyl-peptidase 4 inhibition. *Cardiovasc Res* 2012; 96:140-149.
- Ishibashi Y, Matsui T, Takeuchi M, Yamagishi S. Sitagliptin

augments protective effects of GLP-1 against advanced glycation end product receptor axis in endothelial cells. *Horm Metab Res.* 2011; 43(10): 731-734.

36. Giovannucci E, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report, *Diabetes Care* 2010; 33: 1674-1685.

37. Bala C, Craciun AE, Hancu N. Updating the concept of metabolically healthy obesity. *Acta Endocrinologica-Bucharest* 2016; 12: 197-205.

38. Türkez H, Geyikoglu F, Yousef MI. Ameliorative effect of docosahexaenoic acid on 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced histological changes, oxidative stress, and DNA damage in rat liver. *Toxicol Ind Health* 2012; 28(8): 687-696.

39. Friedrich A, Olejniczak K. Evaluation of carcinogenicity studies of medicinal products for human use authorised via the European centralised procedure (1995–2009). *Regulatory Toxicology and Pharmacology* 2011; 60: 225-248.

40. Uboh F, Udosen E, Ebong P. Protective effect of vitamin C against gasoline vapours - induced reproductive toxicity in male wistar rats. *Acta Endocrinologica-Bucharest* 2010; 6: 305-314.

41. Mathieu C, Kozlovski P, Paldanius PM, Foley JE, Modgill V, Evans M, Serban C. Clinical safety and tolerability of vildagliptin-insights from randomized trials, observational studies and post-marketing surveillance. *Eur Endocrinol* 2017;13:68-72.

42. National Institutes of Health (NIH), DailyMed. Current medication information for onglyza (saxagliptin) tablet, film coated (Revised: May 2013).

43. Gul ÖO, Cinkilic N, Gul CB, Candr S, Vatan Ö, Ersoy C, Yılmaz D, Tuncel E. Comparative genotoxic and cytotoxic effects of the oral antidiabetic drugs sitagliptin, rosiglitazone, and pioglitazone in patients with type-2 diabetes: A cross-sectional, observational pilot study. *Mutation Research* 2013; 757: 31-35.