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Protective Effect of Exendin-4 Treatment On Oxidative Status Of Liver In Rats Exposed To Chronic Methylglyoxal

Running Title: Exendin-4 treatment in rats

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ABSTRACT

The protective effects of Glucagon-Like Peptide-1 (GLP-1) agonists against oxidative stress-induced cellular injury have been well established by previous experimental and clinical studies. Male Wistar rats (200-250 g weight, n=24) were used in this study. First group of rats were not treated with Methylglyoxal (MGO) and served as control group (C group). Second group of rats (MGO group) received MGO (75 mg/kg/day in drinking water) for 12 weeks. Third group of rats (MGO+Ex-4) received Exendin-4 (Ex-4) (1 μg/kg twice daily subcutaneously) concomitant with MGO for 12 weeks. At the end of the 12th week, total oxidant status (TOS), total antioxidant capacity (TAC), sulfhydryl groups (SH), myeloperoxidase (MPO), and advanced oxidation protein products (AOPP) in the liver tissues of all groups were measured spectrophotometrically. In MGO-administered rats, TOS, MPO and AOPP levels were significantly increased. Treatment with Ex-4 for 12 weeks caused a significant decline in the levels of these markers in rats exposed to MGO. Also, levels of TAC and SH were decreased significantly after the 12 weeks of MGO administration. 12

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weeks treatment with Ex-4 also increased the levels of TAC and SH in liver tissues of MGO-administered rats. Ex-4 treatment improves oxidative parameters of liver tissue in MGO-administered rats by improving oxidant-antioxidant balance.

Keywords: Exendin-4, Methylglyoxal, Oxidative stress, Live

INTRODUCTION

Methylglyoxal (MGO), as a dicarbonyl metabolite of glucose, is an intermediate product formed during glycation of proteins by glucose and its formation involves many pathways consisting of enzymatic and non-enzymatic reactions in all mammalian cells (Lo et al., 2006; L. Wu, 2005; Yim, Kang, Hah, Chock, & Yim, 1995). Increase in the plasma level of MGO has been reported in various metabolic diseases, including diabetes, obesity and fatty liver (Hanssen et al., 2017; Kong et al., 2014; Lapolla et al., 2003; Rabbani & Thornalley, 2011; Tappy & Lê, 2012). Excess production and/or decreased degradation of MGO give way to its high levels, generating cellular toxicity (Rabbani & Thornalley, 2015). Although it is critical to maintain normal liver function under metabolic stress, metabolic disorders, including diabetes and obesity, are associated with hepatic dysfunction because of the high levels of nutrients and metabolites (Marceau et al., 1999; Rabbani & Thornalley, 2011). Several studies have indicated the pathophysiological roles of MGO in the liver (Cheng, Cheng, Chiou, & Chang, 2012; Choudhary, Chandra, & Kale, 1997)glutathione (GSH. MGO and advanced glycation end products (AGEs) produced through MGO contribute to the pathophysiology of liver toxicity (Seo, Ki, & Shin, 2014; Yılmaz et al., 2018). Besides, in an early-stage liver damage model, the levels of MGO and its metabolite d-lactate were elevated suggesting that d-lactate could be useful as a reference marker for the early stage of hepatitis (W.-C. Wang, Chou, Chuang, Li, & Lee, 2018).

The cellular injury induced by MGO is provoked through the production of oxidative stress (Rabbani & Thornalley, 2015). Reactive oxygen species (ROS) that are generated and accumulated during the metabolism of excess MGO aggravate the oxidative stress (Desai et al., 2010; Rabbani & Thornalley, 2015). The role of MGO as activating and increasing oxidative stress, mitochondrial dysfunction and apoptosis have been shown in some previous *in vitro* studies (Maruf, Lip, Wong, & O'Brien, 2015; Seo et al., 2014). MGO has also been shown to induce mitochondrial dysfunction and cell death in liver by production of ROS (Seo et al., 2014). Moreover, exposure of mice to MGO induced significant changes in redox-homeostasis in the liver (Choudhary et al., 1997). MGO has been shown to decrease the glutathione (GSH) content and increase the lipid peroxidation. It could be concluded that the activities of the enzymes involved in the pro-

tective mechanism as well as GSH levels were altered in the liver of mice by administration of MGO which in turn may disturb the antioxidant status in the animals. Enhancement of lipid peroxidation in liver indicated the possibility of involvement of free radicals in the toxic effect of MGO. All of these studies suggest that oxidative stress is involved in liver toxicity induced by MGO. Thus, usage of antioxidant agents could be an important option for the prevention of liver toxicity induced by MGO. Exendin-4 (Ex-4), a long acting Glucagon-Like Peptide-1 (GLP-1) receptor agonist, was approved as a treatment, called "exenatide", for type 2 diabetes (Buse et al., 2004)"ISSN":"0149-5992","PMID":"15504997","abstract":"OBJECTIVE This study evaluated the ability of the incretin mimetic exenatide (exendin-4 by inducing pancreatic β-cell proliferation and inhibiting glucagon (Baggio & Drucker, 2007). In addition, studies in animal models have demonstrated that Ex-4 displays antioxidant properties in both *in vitro* and *in vivo* conditions (Oeseburg et al., 2010; Z. Wang, Hou, Huang, Guo, & Zhou, 2017; Zeng et al., 2016). Therefore, the antioxidant properties Ex-4 could potentially be of value in the treatment of liver toxicity induced by MGO.

To our knowledge, the in vivo role of Ex-4 treatment on hepatic oxidative stress induced by chronic MGO administration has not been presented in the literature. The goal of the present study was to determine if Ex-4 had an antioxidant activity in the liver tissues of rats exposed to chronic MGO. In light of the aforementioned studies, in the present study, we aimed to investigate the oxidative changes that occur in liver of rats that were chronically treated with MGO along with Ex-4.

METHODOLOGY

Experimental procedures

All animal experiments were carried out with the approval of the Animal Ethics Committee of Akdeniz University Medical Faculty, Antalya, Turkey (Document no: 65-2013.09.10). Totally 24 male rats were randomly assigned into three groups at the beginning of study. For each group, 8 animals were used. First group of rats were treated with neither MGO nor Ex-4 and served as control group (C group). Second group of rats (MGO group) received MGO (75 mg/kg/day in drinking water) for 12 weeks. Third group of rats (MGO+Ex-4) received Ex-4 (1 μg/kg twice daily subcutaneously) concomitant with MGO for 12 weeks. The dosage of Ex-4 was chosen according to our previous study showing that Ex-4 at a dose of 1 μg/kg twice daily did not alter blood glucose levels (Dalakklioglu et al., 2018).

Measurement of oxidative parameters in liver tissue

At the end of the 12th week, all rats were weighed and blood samples obtained from the abdominal vein were collected into test tubes following anesthesia with a cocktail of intramuscular ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) before sacrifice of each rat. Thereafter, serum was separated by centrifugation at 4000x g for 10 min at 4°C. HbA1c levels were measured using commercial kits from Roche Diagnostics according to the manufacturer's specifications. The analyzer was calibrated using Roche calibrators, and quality control sera from the manufacturer were tested alongside the serum samples. To determine the oxidative stress condition in the liver tissues, total oxidant status (TOS), total antioxidant capacity (TAC), sulfhydryl groups (SH), myeloperoxidase (MPO), and advanced oxidation protein products (AOPP) were measured spectrophotometrically.

TOS assay was performed according to the principle that oxidants present in the sample oxidize the ferrous ion–o-dianisidine complex to ferric ion. The oxidation reaction is enhanced by glycerol molecules, which are abundantly present in the reaction medium. The ferric ion makes a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is related to the total amount of oxidant molecules present in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed in terms of micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 equivalent/l). Intra and interassay CVs were 1.4% and 1.6%, respectively (Erel, 2005).

TAC was determined using a novel automated measurement method, developed by Erel (Erel, 2004)more stable, colored 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid radical cation (ABTS(*+. Briefly, the reduced 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid (ABTS) molecule is oxidized to $ABTS^+$ using hydrogen peroxide alone in acidic medium (the acetate buffer 30 mmol/l pH 3.6). In the acetate buffer solution, the concentrate (deep green) $ABTS^+$ molecules stay more stable for a long time. While it is diluted with a more concentrated acetate buffer solution at high pH values (the acetate buffer 0.4 mol/l pH 5.8), the color is spontaneously and slowly bleached. Antioxidants present in the sample accelerate the bleaching rate to a degree proportional to their concentrations. This reaction can be monitored spectrophotometrically, and the bleaching rate is inversely related with the TAC of the sample. The reaction rate is calibrated with Trolox, which is widely used as a traditional standard for TAC measurement assays, and the assay results are expressed in mmol Trolox equivalent/l. Intra and interassay CV were 2.6% and 2.9%, respectively.

SH levels were measured spectrophotometrically, using Ellman's reagent, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), with the thiol-disulfide interchange reaction between DTNB and thiol providing the basis of the spectrophotometric assay (Koster, Biemond, & Swaak, 1986).

The liver samples taken were washed in saline in an ice bath and homogenized in the ratio 1:10 (w:v) with ice-cold 150 mM KCl for MPO and protein determination. The rest of the homogenates were stored at -70°C until tissue MPO and protein levels of homogenates assays were performed. The samples were centrifuged at 12,000 g at 4 °C for 20 min. Liver extract MPO levels were estimated by a spectrophotometric method using O–Dianisidine Dihydrochloride as a substrate. MPO was assayed as follows: 0.1 ml of the liver extract supernatant was mixed with 2.9 ml of 50 mM phosphate buffer, pH 6.0, and containing 0.167 mg/ml O-dianisidine dihydrochloride and 1% hydrogen peroxide (Bradley, Priebat, Christensen, & Rothstein, 1982)a plentiful constituent of neutrophils, might serve as a marker for tissue neutrophil content. To completely extract MPO from either neutrophils or skin, hexadecyltrimethylammonium bromide (HTAB. The change in absorbance was measured at 450 nm using a spectrophotometer. One unit of MPO activity was defined as that degrading one μmole of peroxidase per minute at 25°C (*Worthington Enzyme Manual*, 1972). The total protein content of the homogenates was determined by the method of Lowry (LOWRY, ROSEBROUGH, FARR, & RANDALL, 1951).

AOPP levels were measured for only the supernatant fraction, using a spectrophotometric method (Witko-Sarsat et al., 1996). The values are expressed as μmol/g of protein in liver tissue.

Materials

MGO and Ex-4 were purchased from Sigma Chemical (St. Louis, MO, USA). All drugs were prepared fresh daily during experiments and were dissolved in distilled water before use.

Statistical analysis

All values were expressed as mean ± SEM. Statistical analysis of the results were performed by one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. A p value lower than 0.05 was considered significant.

RESULTS AND DISCUSSION

Rats treated with MGO showed significant increases in TOS and AOPP compared to control group rats (Figs.1 and 2). The treatment with Ex-4 for 12 weeks resulted in significant decreases in the levels of TOS and AOPP (Figs.1 and 2).

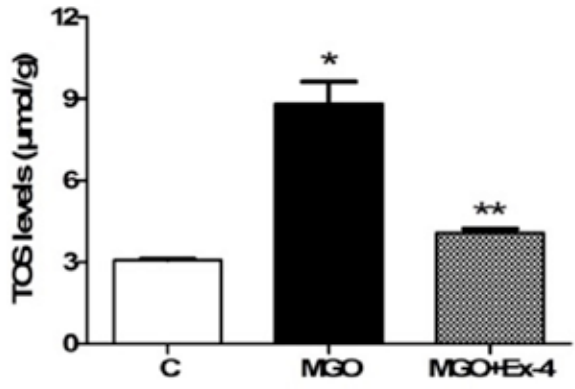

Figure 1: Levels of total oxidant status (TOS) in liver tissues obtained from all groups. C: Control, MGO: Methylglyoxal, Ex-4: Exendin-4. All values are expressed as mean ± SEM. *P<0.05 as compared with control rats; **P<0.05 as compared with MGO group rats.

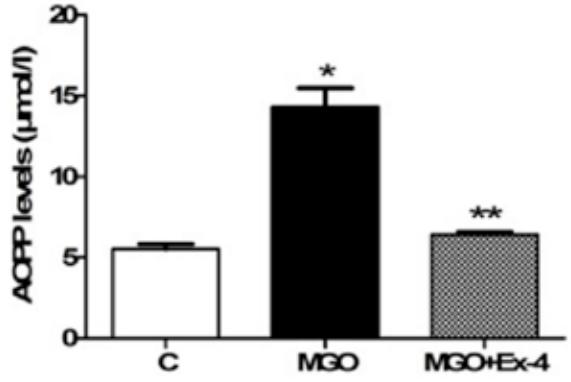

Figure 2: Levels of advanced oxidation protein products (AOPP) in liver tissues obtained from all groups. C: Control, MGO: Methylglyoxal, Ex-4: Exendin-4. All values are expressed as mean ± SEM. *P<0.05 as compared with control rats; **P<0.05 as compared with MGO group rats.

MGO-treatment also caused a significant reduction in hepatic TAC levels (Fig.3). Decreased hepatic TAC levels in MGO-administered rats were significantly increased with chronic Ex-4 treatment (Fig.3).

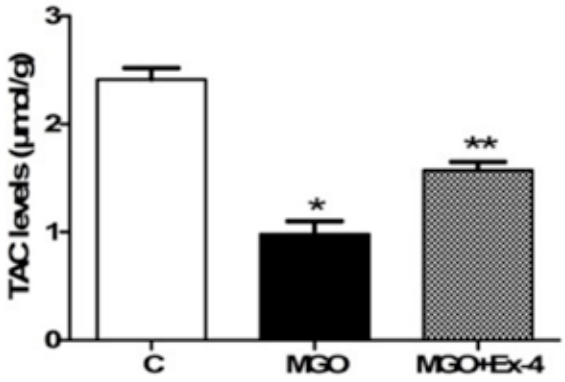

Figure 3: Levels of total antioxidant capacity (TAC) in liver tissues obtained from all groups. C: Control, MGO: Methylglyoxal, Ex-4: Exendin-4. All values are expressed as mean ± SEM. *P<0.05 as compared with control rats; **P<0.05 as compared with MGO group rats.

Fig.4 shows the levels of sulfhydryl groups (SH) in liver tissues obtained from all groups. After the chronic MGO administration, SH levels were decreased significantly (Fig.4). The treatment with Ex-4 for 12 weeks resulted in a significant increase in SH levels (Fig.4).

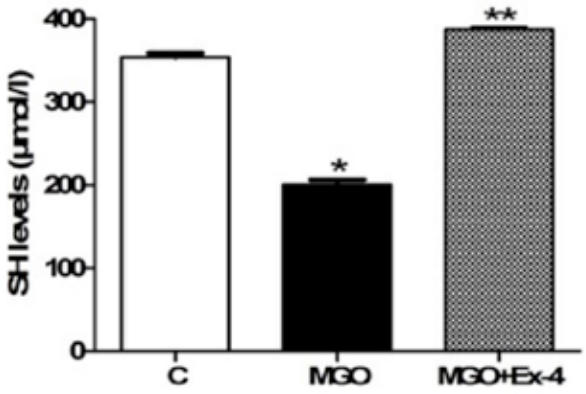

Figure 4: Levels of sulfhydryl groups (SH) in liver tissues obtained from all groups. C: Control, MGO: Methylglyoxal, Ex-4: Exendin-4. All values are expressed as mean ± SEM. *P<0.05 as compared with control rats; **P<0.05 as compared with MGO group rats.

As seen in Fig.5, MPO levels in liver tissues were higher in MGO-administered rats compared to control rats (Fig.5). Ex-4 treatment also caused a significant reduction in MPO levels in MGO-administered rats (Fig.5).

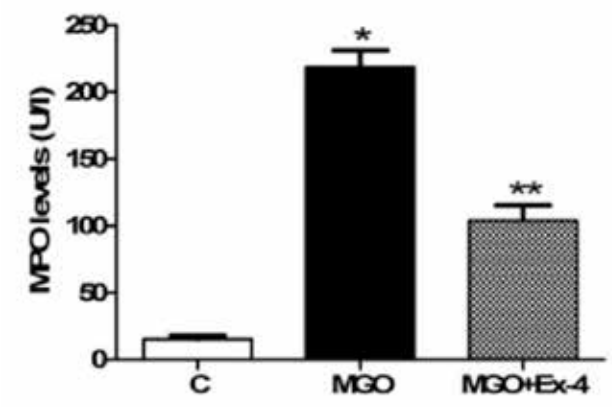

Figure 5: Levels of myeloperoxidase (MPO) in liver tissues obtained from all groups. C: Control, MGO: Methylglyoxal, Ex-4: Exendin-4. All values are expressed as mean ± SEM. *P<0.05 as compared with control rats; **P<0.05 as compared with MGO group rats.

In addition, percentage of HbA1c were not significantly different between percentage of HbA1c (Fig.6).

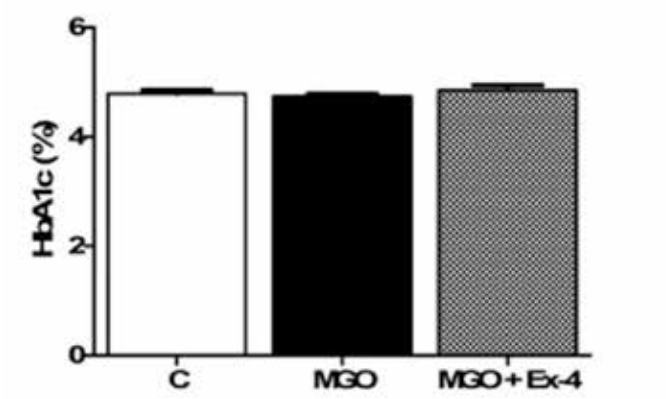

Figure 6: Comparison of HbA1c levels in all group rats. C: Control, MGO: Methylglyoxal, Ex-4: Exendin-4. All values are expressed as mean ± SEM.

The present study is the first to describe a protective effect of chronic Ex-4 treatment on MGO-induced oxidative stress in liver tissue of rats, suggesting a novel role for Ex-4 in protecting against MGO-related liver damage. The results of the present study indicate that chronic Ex-4 treatment ameliorates MGO-induced hepatic oxidative stress as confirmed by biochemical assays.

The liver is among the primary organs susceptible to oxidative stress because it is involved in metabolism and detoxifying processes in the body, which may lead to liver tissue injury (Palsamy, Sivakumar, & Subramanian, 2010). However, the liver is also equipped with cellular antioxidant defense such as SH and MPO as not only to neutralize free radicals but also to protect the liver cells from oxidative damage. TOS and TAC are usually measured to determine the toxicity level in damaged tissues (Oguz et al., 2015). The results of Seo et al. showed that MGO increases cell death and induces liver toxicity, which results from ROS-mediated mitochondrial dysfunction and oxidative stress (Seo et al., 2014). The decrease in total antioxidant defense system and the increase in oxidative parameters in liver tissue were also reported in MGO-administered rats (Choudhary et al., 1997). In agreement, our results showed that levels of TOS in the liver was increased in MGO group of rats. Otherwise, protein oxidation is also often studied alongside oxidative stress status (Kalousová, Skrha, & Zima, 2002). Oxidant-mediated protein damage can be determined by the level

of AOPP. In the present study, significantly increased AOPP levels in the liver tissues were found after MGO administration. Moreover, one of the principal molecules released after recruitment and activation of phagocytes is MPO, an important enzyme involved in the generation ROS (Klebanoff, 2005)the phagocytosis and destruction of microorganisms. When coated with opsonins (generally complement and/or antibody. The measurement of MPO may serve as a reliable marker to estimate the degree of oxidative stress (C.-C. Wu et al., 2005)little is known of how different dialysis membranes contribute to the oxidative stress induced by the dialysis procedure per se. We therefore studied the influence of two different dialysis membranes on oxidative stress during HD. METHODS Eight patients undergoing HD three times per week were enrolled in this cross-controlled study. Patients sequentially received HD using polysulphone (PS. Importantly, liver MPO levels also significantly increased in MGO-administered rats as compared with controls. All these findings clearly indicated to an increased oxidative stress in the liver tissues of rats exposed to chronic MGO.

MGO administration can also reduce the hepatic antioxidant defense leading to accumulation of free radicals in hepatocytes. MGO may inhibit several antioxidant enzymes and thereby, increased oxidative stress may be due to reduction in the activities of antioxidant enzymes. One of them, thiol groups are important members of the antioxidant team and have been shown to destroy ROS and other free radicals by enzymatic and non-enzymatic mechanisms (Jones et al., 2000)the redox potential of the GSSG/2GSH pool (-137 +/- 9 mV. Total thiol groups of proteins are mainly responsible for their antioxidant response, and they can serve as a sensitive indicator of oxidative stress (Halliwell & Gutteridge, 1990; Soszyński & Bartosz, 1997). MGO exposure was reported to decrease protein-SH and reduce GSH levels in different cell types and in the liver (Leoncini, Maresca, & Buzzi, 1989; Ray & Ray, 1984)methylglyoxal was found to be the best substrate. The pH optimum of the enzyme was found to be 6.5, and Km for methylglyoxal was 0.4 mM. The molecular weight of the enzyme was found to be 89000 by gel filtration on a Sephadex G-200 column. Electrophoresis on sodium dodecyl sulfate-polyacrylamide gel revealed that the enzyme is composed of two subunits. The enzyme is highly sensitive to sulfhydryl group reagents. The inactivation by p-chloromercuribenzoate could be substantially protected by methylglyoxal in combination with NADH, indicating a possible involvement of one or more sulfhydryl group(s. In the present study, we observed decreased levels of SH groups in liver tissues of MGO-administered rats which further supported the involvement of SH in MGO-induced liver damage. Our results also showed that levels of TAC in liver tissues were significantly de-

creased in MGO group rats compared to controls. Hence, it might be suggested that in addition to increased TOS levels due to excessive release of free radicals, reduction in TAC and SH groups in hepatic tissue may also contribute to the MGO-induced hepatotoxicity in rats.

In a previous study, we have demonstrated that Ex-4 attenuated MGO-induced erectile dysfunction through inhibition of oxidative stress (Dalaklioglu et al., 2018). In the present study, we have showed that MGO-induced oxidative stress in liver was also significantly improved by Ex-4 treatment for 12 weeks. The increased oxidative stress in livers from MGO-treated rats as indicated by TOS, AOPP and MPO levels was significantly reversed by Ex-4 treatment. Antioxidant therapy is a potential future therapeutic strategy; increasing antioxidant levels in patients with diabetes mellitus-induced liver damage may hopefully counter the effects of oxidative stress, thereby reducing the severity of diabetic complications. Besides its ability to scavenge free radicals, Ex-4 may also have indirect antioxidant actions. Ex-4 has been shown to enhance several antioxidant enzymes (Ahangarpour, Oroojan, & Badavi, 2018). In accordance with the previous studies, the results of the current study also demonstrated that treatment of rats with Ex-4 for 12 weeks slightly increased the activities of SH and TAC in the liver tissue. Therefore, the finding of increased activities of antioxidant enzymes in the liver tissues of rats treated with Ex-4 alone suggest that Ex-4 not only exhibits a direct scavenging effect on free radicals but also partly stimulates intracellular antioxidant defense mechanisms.

Importantly, it is also to be mentioned that preventive effect of Ex-4 occur by a mechanism independent from the glucose-lowering effects of this drug. Importantly, in agreement with previous results (Cardoso et al., 2014; Dalaklioglu et al., 2018), when MGO (75 mg/kg b.w./day; in drinking water) was given to animals for a period of 12 weeks, no significant change in serum HbA1c levels was found as compared to controls. Moreover, in the present study, we demonstrated that levels of HbA1c did not change with chronic Ex-4 treatment at a dose of 1 μg/kg in MGO-administered rats. Thus, the protective effect of Ex-4 against MGO-induced oxidative stress in liver does not seem to be associated with well-known glucose lowering effect of this drug.

In conclusion, the results of present study provide first evidence for the combined effect of decreased oxidative stress and increased antioxidant defense mechanisms contributing to therapeutic effect of Ex-4 against MGO-induced oxidative stress in liver tissue. Based on the results of the present study, it is possible to suggest that Ex-4 treatment may offer a novel therapeutic approach for the prevention of hepatotoxicity induced by MGO, especially in diabetic adults.

ACKNOWLEDGEMENTS

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Figure Legends

Figure 1. Levels of total oxidant status (TOS) in liver tissues obtained from all groups. C: Control, MGO: Methylglyoxal, Ex-4: Exendin-4. All values are expressed as mean ± SEM. *P<0.05 as compared with control rats; **P<0.05 as compared with MGO group rats.

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Figure 6. Comparison of HbA1c levels in all group rats. C: Control, MGO: Methylglyoxal, Ex-4: Exendin-4. All values are expressed as mean ± SEM.

REFERENCES

1. Ahangarpour, A., Oroojan, A. A., & Badavi, M. (2018). Exendin-4 protects mice from D-

galactose-induced hepatic and pancreatic dysfunction. *Pathobiology of Aging & Age Related Diseases*, *8*(1), 1418593. https://doi.org/10.1080/20010001.2017.1418593

2. Baggio, L. L., & Drucker, D. J. (2007). Biology of incretins: GLP-1 and GIP. *Gastroenterology*, *132*(6), 2131–2157. https://doi.org/10.1053/j.gastro.2007.03.054

3. Bradley, P. P., Priebat, D. A., Christensen, R. D., & Rothstein, G. (1982). Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. *The Journal of Investigative Dermatology*, *78*(3), 206–209. https://doi.org/10.1111/1523-1747.ep12506462

4. Buse, J. B., Henry, R. R., Han, J., Kim, D. D., Fineman, M. S., Baron, A. D., & Exenatide-113 Clinical Study Group. (2004). Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care*, *27*(11), 2628–2635. https://doi.org/10.2337/diacare.27.11.2628

5. Cardoso, S., Carvalho, C., Marinho, R., Simões, A., Sena, C. M., Matafome, P., … Moreira, P. I. (2014). Effects of methylglyoxal and pyridoxamine in rat brain mitochondria bioenergetics and oxidative status. *Journal of Bioenergetics and Biomembranes*, *46*(5), 347–355. https://doi.org/10.1007/s10863-014-9551-2

6. Cheng, A.-S., Cheng, Y.-H., Chiou, C.-H., & Chang, T.-L. (2012). Resveratrol upregulates Nrf2 expression to attenuate methylglyoxal-induced insulin resistance in Hep G2 cells. *Journal of Agricultural and Food Chemistry*, *60*(36), 9180–9187. https://doi.org/10.1021/jf302831d

7. Choudhary, D., Chandra, D., & Kale, R. K. (1997). Influence of methylglyoxal on antioxidant enzymes and oxidative damage. *Toxicology Letters*, *93*(2–3), 141–152. https://doi.org/10.1016/s0378-4274(97)00087-8

8. Dalaklioglu, S., Tasatargil, A., Kuscu, N., Celik, S., Celik-Ozenci, C., Ozdem, S., … Kucukcetin, I. (2018). Protective effect of exendin-4 treatment on erectile dysfunction induced by chronic methylglyoxal administration in rats. *Peptides*, *106*, 1–8. https://doi.org/10.1016/j.peptides.2018.05.005

9. Desai, K. M., Chang, T., Wang, H., Banigesh, A., Dhar, A., Liu, J., … Wu, L. (2010). Oxidative stress and aging: is methylglyoxal the hidden enemy? *Canadian Journal of Physiology and Pharmacology*, *88*(3), 273–284. https://doi.org/10.1139/Y10-001

10. Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clinical Biochemistry*, *37*(4), 277–285. https://doi.org/10.1016/j.clinbiochem.2003.11.015

11. Erel, O. (2005). A new automated colorimetric method for measuring total oxidant status. *Clinical Biochemistry*, *38*(12), 1103–1111. https://doi.org/10.1016/j.clinbiochem.2005.08.008

12. Halliwell, B., & Gutteridge, J. M. (1990). The antioxidants of human extracellular fluids. *Archives of Biochemistry and Biophysics*, *280*(1), 1–8. https://doi.org/10.1016/0003-9861(90)90510-6

13. Hanssen, N. M. J., Scheijen, J. L. J. M., Jorsal, A., Parving, H.-H., Tarnow, L., Rossing, P., … Schalkwijk, C. G. (2017). Higher Plasma Methylglyoxal Levels Are Associated With Incident Cardiovascular Disease in Individuals With Type 1 Diabetes: A 12-Year Follow-up Study. *Diabetes*, *66*(8), 2278–2283. https://doi.org/10.2337/db16-1578

14. Jones, D. P., Carlson, J. L., Mody, V. C., Cai, J., Lynn, M. J., & Sternberg, P. (2000). Redox state of glutathione in human plasma. *Free Radical Biology & Medicine*, *28*(4), 625–635.

https://doi.org/10.1016/s0891-5849(99)00275-0

15. Kalousová, M., Skrha, J., & Zima, T. (2002). Advanced glycation end-products and advanced oxidation protein products in patients with diabetes mellitus. *Physiological Research*, *51*(6), 597–604. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12511184

16. Klebanoff, S. J. (2005). Myeloperoxidase: friend and foe. *Journal of Leukocyte Biology*, *77*(5), 598–625. https://doi.org/10.1189/jlb.1204697

17. Kong, X., Ma, M., Huang, K., Qin, L., Zhang, H., Yang, Z., … Su, Q. (2014). Increased plasma levels of the methylglyoxal in patients with newly diagnosed type 2 diabetes 2. *Journal of Diabetes*, *6*(6), 535–540. https://doi.org/10.1111/1753-0407.12160

18. Koster, J. F., Biemond, P., & Swaak, A. J. (1986). Intracellular and extracellular sulphydryl levels in rheumatoid arthritis. *Annals of the Rheumatic Diseases*, *45*(1), 44–46. https://doi.org/10.1136/ard.45.1.44

19. Lapolla, A., Flamini, R., Dalla Vedova, A., Senesi, A., Reitano, R., Fedele, D., … Traldi, P. (2003). Glyoxal and methylglyoxal levels in diabetic patients: quantitative determination by a new GC/MS method. *Clinical Chemistry and Laboratory Medicine*, *41*(9), 1166–1173. https://doi.org/10.1515/CCLM.2003.180

20. Leoncini, G., Maresca, M., & Buzzi, E. (1989). Inhibition of the glycolytic pathway by methylglyoxal in human platelets. *Cell Biochemistry and Function*, *7*(1), 65–70. https://doi.org/10.1002/cbf.290070111

21. Lo, C.-Y., Li, S., Tan, D., Pan, M.-H., Sang, S., & Ho, C.-T. (2006). Trapping reactions of reactive carbonyl species with tea polyphenols in simulated physiological conditions. *Molecular Nutrition & Food Research*, *50*(12), 1118–1128. https://doi.org/10.1002/mnfr.200600094

22. LOWRY, O. H., ROSEBROUGH, N. J., FARR, A. L., & RANDALL, R. J. (1951). Protein measurement with the Folin phenol reagent. *The Journal of Biological Chemistry*, *193*(1), 265–275. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/14907713

23. Marceau, P., Biron, S., Hould, F. S., Marceau, S., Simard, S., Thung, S. N., & Kral, J. G. (1999). Liver pathology and the metabolic syndrome X in severe obesity. *The Journal of Clinical Endocrinology and Metabolism*, *84*(5), 1513–1517. https://doi.org/10.1210/jcem.84.5.5661

24. Maruf, A. Al, Lip, H., Wong, H., & O'Brien, P. J. (2015). Protective effects of ferulic acid and related polyphenols against glyoxal- or methylglyoxal-induced cytotoxicity and oxidative stress in isolated rat hepatocytes. *Chemico-Biological Interactions*, *234*, 96–104. https://doi.org/10.1016/j.cbi.2014.11.007

25. Oeseburg, H., de Boer, R. A., Buikema, H., van der Harst, P., van Gilst, W. H., & Silljé, H. H. W. (2010). Glucagon-like peptide 1 prevents reactive oxygen species-induced endothelial cell senescence through the activation of protein kinase A. *Arteriosclerosis, Thrombosis, and Vascular Biology*, *30*(7), 1407–1414. https://doi.org/10.1161/ATVBAHA.110.206425

26. Oguz, A., Kapan, M., Kaplan, I., Alabalik, U., Ulger, B. V., Uslukaya, O., … Polat, Y. (2015). The effects of sulforaphane on the liver and remote organ damage in hepatic ischemia-reperfusion model formed with pringle maneuver in rats. *International Journal of Surgery (London, England)*, *18*, 163–168. https://doi.org/10.1016/j.ijsu.2015.04.049

27. Palsamy, P., Sivakumar, S., & Subramanian, S. (2010). Resveratrol attenuates hyperglycemia-mediated oxidative stress, proinflammatory cytokines and protects hepatocytes ultrastructure in streptozotocin-nicotinamide-induced experimental diabetic rats. *Chemico-Biological Interactions*, *186*(2), 200–210. https://doi.org/10.1016/j.cbi.2010.03.028

28. Rabbani, N., & Thornalley, P. J. (2011). Glyoxalase in diabetes, obesity and related disorders. *Seminars in Cell & Developmental Biology*, *22*(3), 309–317. https://doi.org/10.1016/j.semcdb.2011.02.015

29. Rabbani, N., & Thornalley, P. J. (2015). Dicarbonyl stress in cell and tissue dysfunction contributing to ageing and disease. *Biochemical and Biophysical Research Communications*, *458*(2), 221–226. https://doi.org/10.1016/j.bbrc.2015.01.140

30. Ray, M., & Ray, S. (1984). Purification and partial characterization of a methylglyoxal reductase from goat liver. *Biochimica et Biophysica Acta*, *802*(1), 119–127. https://doi.org/10.1016/0304-4165(84)90041-2

31. Seo, K., Ki, S. H., & Shin, S. M. (2014). Methylglyoxal induces mitochondrial dysfunction and cell death in liver. *Toxicological Research*, *30*(3), 193–198. https://doi.org/10.5487/TR.2014.30.3.193

32. Soszyński, M., & Bartosz, G. (1997). Decrease in accessible thiols as an index of oxidative damage to membrane proteins. *Free Radical Biology & Medicine*, *23*(3), 463–469. https://doi.org/10.1016/s0891-5849(97)00117-2

33. Tappy, L., & Lê, K.-A. (2012). Does fructose consumption contribute to non-alcoholic fatty liver disease? *Clinics and Research in Hepatology and Gastroenterology*, *36*(6), 554–560. https://doi.org/10.1016/j.clinre.2012.06.005

34. Wang, W.-C., Chou, C.-K., Chuang, M.-C., Li, Y.-C., & Lee, J.-A. (2018). Elevated levels of liver methylglyoxal and d-lactate in early-stage hepatitis in rats. *Biomedical Chromatography : BMC*, *32*(2). https://doi.org/10.1002/bmc.4039

35. Wang, Z., Hou, L., Huang, L., Guo, J., & Zhou, X. (2017). Exenatide improves liver mitochondrial dysfunction and insulin resistance by reducing oxidative stress in high fat diet-induced obese mice. *Biochemical and Biophysical Research Communications*, *486*(1), 116–123. https://doi.org/10.1016/j.bbrc.2017.03.010

36. Witko-Sarsat, V., Friedlander, M., Capeillère-Blandin, C., Nguyen-Khoa, T., Nguyen, A. T., Zingraff, J., … Descamps-Latscha, B. (1996). Advanced oxidation protein products as a novel marker of oxidative stress in uremia. *Kidney International*, *49*(5), 1304–1313. https://doi.org/10.1038/ki.1996.186

37. Worthington Enzyme Manual. (1972). Worthington Biochemical Corparation.

38. Wu, C.-C., Chen, J.-S., Wu, W.-M., Liao, T.-N., Chu, P., Lin, S.-H., … Lin, Y.-F. (2005). Myeloperoxidase serves as a marker of oxidative stress during single haemodialysis session using two different biocompatible dialysis membranes. *Nephrology, Dialysis, Transplantation : Official Publication of the European Dialysis and Transplant Association - European Renal Association*, *20*(6), 1134–1139. https://doi.org/10.1093/ndt/gfh764

39. Wu, L. (2005). The pro-oxidant role of methylglyoxal in mesenteric artery smooth muscle cells. *Canadian Journal of Physiology and Pharmacology*, *83*(1), 63–68. https://doi.org/10.1139/y04-112

40. Yim, H. S., Kang, S. O., Hah, Y. C., Chock, P. B., & Yim, M. B. (1995). Free radicals generated during the glycation reaction of amino acids by methylglyoxal. A model study of protein-cross-linked free radicals. *The Journal of Biological Chemistry*, *270*(47), 28228–28233. https://doi.org/10.1074/jbc.270.47.28228

41. Yılmaz, Z., Kalaz, E. B., Aydın, A. F., Olgaç, V., Doğru-Abbasoğlu, S., Uysal, M., & Koçak-Toker, N. (2018). The effect of resveratrol on glycation and oxidation products in plasma and liver of chronic methylglyoxal-treated rats. *Pharmacological Reports : PR*, *70*(3), 584–590.

https://doi.org/10.1016/j.pharep.2017.12.005

42. Zeng, Y., Yang, K., Wang, F., Zhou, L., Hu, Y., Tang, M., … Xu, G.-T. (2016). The glucagon like peptide 1 analogue, exendin-4, attenuates oxidative stress-induced retinal cell death in early diabetic rats through promoting Sirt1 and Sirt3 expression. *Experimental Eye Research*, *151*, 203–211. https://doi.org/10.1016/j.exer.2016.05.002