FPS-ZM1 Alleviates Circulating Indices of Liver Injury in Diet-Induced Type 2 Diabetic Mice

Somayeh Aslani[1], Saman Bahrambeigi[2], Davoud Sanajou[1]

[1] *Department of Biochemistry, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran*

[2] *Department of Basic Sciences, School of Veterinary Medicine, Urmia University, Urmia, Iran*

Received: 03 May 2021; Accepted: 21 Dec. 2021

Abstract- Despite dietary/lifestyle modifications as well as glycemic and lipid control, non-alcoholic fatty liver disease (NAFLD) imposes a considerable risk to the patients by advancing to non-alcoholic steatohepatitis (NASH). The present investigation aims to evaluate the protective potential of FPS-ZM1, a selective inhibitor for advanced glycation end products (RAGE), against circulating indices of liver injury in high fat diet-induced diabetic mice. FPS-ZM1 at 0.5. 1, and 2 mg/kg (orally) was administered for 2 months, starting 4 months after provision of the high-fat diet. Tests for glucose homeostasis, liver injury markers, and hepatic/plasma miR-21 expressions were performed. FPS-ZM1 attenuated diabetes-induced elevations in serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), glutamate dehydrogenase (GLD), and alpha glutathione-S-transferase (α-GST) as well as alkaline phosphatase (ALP) and gamma-glutamyl transpeptidase (GGT). It also decreased diabetes-associated elevations in serum ferritin and plasma cytokeratin 18 fragments. Additionally, FPS-ZM1 down-regulated elevated expressions of miR-21 in the liver and plasma of diabetic mice. These findings highlight the benefits of FPS-ZM in alleviating liver injury in mice evoked by high-fat diet-induced type 2 diabetes and suggest FPS-ZM1 as a new potential adjunct to the conventional diet/lifestyle modification and glycemic control in diabetics.

Acta Med Iran 2022;60(1):40-45.

Keywords: Diabetes; FPS-ZM1; Liver injury; Receptor for advanced glycation end products

Introduction

Diabetes mellitus is estimated to affect approximately 8.5% of the adult population universally, with type 2 diabetes constituting the majority (1). Diabetes is associated with several co-morbidities, including chronic liver, cardiovascular, and kidney diseases, as well as cancer. Non-alcoholic fatty liver disease (NAFLD) is the most frequent hepatic sequelae encountered in more than 70% of type 2 diabetic patients (2). Fat deposition in more than 5% of hepatic cells accompanied by trivial or no alcohol consumption is defined as NAFLD; indeed, it is termed non-alcoholic steatohepatitis (NASH) in more advanced forms, manifested by severe hepatocellular steatosis and liver inflammation, making the affected individuals prone to cirrhosis in subsequent years (3,4). While NAFLD is a benign condition, diagnosis and treatment of NASH are highly important as it will become the most frequent cause of liver transplantation in the near future (5).

To date, several randomized clinical trials (RCTs) have tested the efficacy of different treatment options on diabetes-associated NAFLD/NASH. In this respect, pioglitazone (a thiazolidinedione), vitamin E, liraglutide (a glucagon-like peptide-1 receptor agonist), and sitagliptin (a dipeptidyl peptidase-4 inhibitor) have been trialed (6-9). Additionally, essential phospholipids, fenugreek, and termis seeds have had alleviating effects on clinical and experimental cases of NAFLD (10,11). In spite of obtaining promising outcomes, a considerable proportion of patients continued to demonstrate active disease, which underlines the need for developing novel adjunctive therapies.

Increased production of advanced glycation end

Corresponding Author: D. Sanajou
Department of Biochemistry, School of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
Tel: +98 9144452530, Fax: +98 4133359680, E-mail address: davoodsanajoo@gmail.com

products (AGEs) under hyperglycemic conditions of diabetes exacerbates oxidative stress in hepatic tissues via stimulating its correspondent receptors, RAGEs (12). Considering increased expression and the crucial role of RAGE in the pathogenesis of liver diseases, including NAFLD/NASH (12,13), modulating these receptors seems to be a plausible therapeutic intervention in terms of alleviating hepatic injuries. FPS-ZM1 is the potent and specific inhibitor of the RAGE (14), capable of alleviating both glomerular and tubular injuries in experimental diabetic models of rats (15,16). This agent, moreover, have been shown to be effective against diabetic retinopathy and neuropathy, significantly improving the diabetes-associated lesions (17). On this basis, we aimed to investigate the potential protective effects of FPS-ZM1 against circulating indices of liver injury in type 2 diabetic mice from a biochemical perspective.

Materials and Methods

Animals and experimental protocol

30 male C57BL/6 mice (4 weeks old) were obtained from Pasteur Institute, Tehran, Iran. Standard housing conditions of 12 hours light, 12 hours dark, temperature of 24° C, and free access to water and food were observed. Study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences. All procedures were carried out according to the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health.

The animals were divided into 5 groups of six per each. Control group received the standard pelleted diet. The remaining groups were fed the high fat diet (HFD) containing 58% of fat, 26 % carbohydrate, and 16.4% of protein for a period of 6 month (18,19). After 4 months, HFD groups were arranged to receive vehicle (normal saline) and 0.5, 1, and 2 mg/kg/day of FPS-ZM1 by intra-gastric gavage and the administration continued for 2 months. At the ultimate day of the investigation, blood samples of the euthanized mice were collected by cardiac puncture and the livers were excised and freezed in liquid nitrogen.

Biochemical assays

Serum Glucose, total triglyceride (TG), total cholesterol (TC), high density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) as well as serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were measured via commercial kits (Man, Tehran, Iran) using an automated chemistry analyzer (Dirui, Shenzhen, China). Whole blood hemoglobin A1c (Hb A1c) was quantified by a micro-column chromatography assay kit (BioSystems, Barcelona, Spain).

Serum immunoassays

Serum c-peptide levels were tested via radioimmunoassay (RIA) kit (Izotop, Budapest, Hungary), followed by measuring the gamma emissions by using a gamma counter (RALS Gamma Counter GAMMA-10, Shin Jin Medics, Goyang, South Korea). An enzyme-linked immunosorbent assay (ELISA) kit was adopted to measure plasma cytokeratin 18 fragment levels (CK-18/M30 Fragment Asp 396, Pacific Biomarkers, Seattle, Washington, USA). Serum α-GST levels were assayed by using a chemiluminescent immunoassay (CLIA) kit (EIAab, Wuhan, China), and then a microplate luminometer (Lumex, Parsian Teb, Tehran, Iran) was implemented to perform final measurements. For quantifying serum ferritin levels, a fluoro-immunoassay (FIA) kit (BioMérieux, Marcy-l'Étoile, France) was utilized and the quantitative data were obtained by MINI VIDAS immuno-analyzer (BioMérieux, Marcy-l'Étoile, France).

RT-qPCR analysis of liver and plasma miR-21

Total RNA was extracted via miRNA Isolation Kit (Favorgen Biotech, Pingtung, Taiwan). miR-21 levels were quantified via TaqMan assay (Tm 00397, Applied Biosystems, Foster City, California, USA) according to the manufacturer's manual. snoRNA234 (Tm001234; Applied Biosystems) was used as the internal control for normalization. In the end, the $2^{-\Delta\Delta Ct}$ method was adopted to calculate mir-21 relative expressions.

Statistical analysis

Descriptive data were expressed as means±SD. Between-group comparisons were carried out using 1-way ANOVA followed by a post hoc Tukey test (SPSS Statistics software, version 18; SPSS Inc., USA). $P<0.05$ was considered significant.

Results

General characteristics of the study groups

Table 1 summarizes the general characteristics of the study groups. All diabetic mice had elevated serum glucose, blood Hb A1c, and serum c-peptide levels, and treatment with FPS-ZM1 had no effect on these indices. Similarly, elevated levels of serum TG, cholesterol, and

LDL-C along with decreased levels of serum HDL-C were observed in diabetic groups; and again, FPS-ZM1 had no impact on the indices of lipid profile (Table 1).

Table 1. General characteristics and lipid profile of the study groups

	Control	Diab	FPS (0.5)	FPS (1)	FPS (2)
Glucose (mg/dL)	80.75 ± 7.88	227.53 ± 19.21[a]	234.29 ± 20.66	221.38 ± 25.16	229.75 ± 23.51
Blood Hb A1c (%)	4.41 ± 0.86	8.95 ± 1.05[a]	9.07 ± 1.11	8.82 ± 1.16	9.12 ± 1.08
Serum c-peptide (ng/mL)	1.36 ± 0.22	4.77 ± 0.63[a]	4.51 ± 0.74	4.80 ± 0.59	4.68 ± 0.71
TG (mg/dL)	93.14 ± 8.55	113.30 ± 14.42[a]	118.25 ± 19.37	112.74 ± 15.10	110.59 ± 18.92
TC (mg/dL)	81.28 ± 13.72	366.72 ± 74.61[a]	379.29 ± 81.67	388.42 ± 70.35	358.84 ± 77.46
HDL-C (mg/dL)	58.51 ± 5.88	49.32 ± 3.91[a]	51.01 ± 5.07	50.29 ± 3.43	49.38 ± 4.67
LDL-C (mg/dL)	27.41 ± 2.79	76.92 ± 11.31[a]	80.17 ± 14.21	74.68 ± 10.88	79.26 ± 15.62

Control, healthy control mice; Diab, diet-induced diabetic control mice; FPS (0.5), diet-induced diabetic mice treated with 0.5 mg/kg FPS-ZM1; FPS (1), diet-induced diabetic mice treated with 1 mg/kg FPS-ZM1; FPS (2), diet-induced diabetic mice treated with 2 mg/kg FPS-ZM1. Data are presented as mean ± SD

Hb A1c, hemoglobin A1c; TG, total triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein-cholesterol; LDL-C, low-density lipoprotein-cholesterol

[a]$P < 0.01$ vs. Control

FPS-ZM1 dose-dependently attenuated serum levels of hepatic enzymes

Serum levels of ALT, AST, ALP, and α-GST are shown in (Table 2). Serum ALT, AST, and α-GST demonstrated significantly elevated concentrations in control diabetic mice. Indeed, serum ALP levels had slightly, albeit statistically significant, elevations in the control diabetic group. While FPS-ZM1 significantly reduced serum levels of these enzymes, the most pronounced reductions were observed for serum ALT, AST, and α-GST concentrations. It is worthy of underlining that all reductions followed a dose-dependent nature.

FPS-ZM1 dose-dependently decreased serum ferritin and plasma cytokeratin-18 fragment levels

As shown in (Table 2), control diabetic mice had remarkably raised levels of both cytokeratin-18 fragment and ferritin in their plasma and serum, respectively. 2-month FPS-ZM1 treatment in type 2 diabetic mice emerged to mitigate both plasma cytokeratin-18 fragments and serum ferritin levels dose-dependently (Table 2).

Table 2. Serum markers of liver injury in all study groups

	Control	Diab	FPS (0.5)	FPS (1)	FPS (2)
ALT (IU/L)	62.48 ± 8.31	275.62 ± 23.79[a]	231.58 ± 25.16[b]	208.37 ± 21.45[c]	189.63 ± 19.57[d]
AST (IU/L)	122.78 ± 15.29	419.36 ± 38.77[a]	382.83 ± 33.45[b]	327.23 ± 41.62[c]	290.93 ± 32.35[d]
ALP (IU/L)	105.72 ± 10.33	195.47 ± 18.49[a]	188.32 ± 19.15	173.56 ± 15.96[b]	154.27 ± 16.11[d]
α-GST (ng/mL)	6.73 ± 2.86	35.49 ± 4.23[a]	29.55 ± 3.77[b]	24.85 ± 3.91[c]	18.14 ± 4.29[d]
Ferritin (ng/mL)	166.81 ± 25.67	463.21 ± 42.75[a]	410.59 ± 39.68[b]	371.41 ± 43.74[c]	328.23 ± 34.49[d]
Cytokeratin 18 fragments (U/L)	107.39 ± 26.64	566.47 ± 91.32[a]	479.32 ± 71.51[b]	353.66 ± 82.35[c]	305.11 ± 70.88[d]

Control, healthy control mice; Diab, diet-induced diabetic control mice; FPS (0.5), diet-induced diabetic mice treated with 0.5 mg/kg FPS-ZM1; FPS (1), diet-induced diabetic mice treated with 1 mg/kg FPS-ZM1; FPS (2), diet-induced diabetic mice treated with 2 mg/kg FPS-ZM1. Data are presented as mean±SD

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; GGT, gamma-glutamyl trasnpeptidase; GLD, glutamate dehydrogenase; α-GST, alpha glutathione-S-transferase

[a]$P < 0.01$ vs. Control. [b]$P < 0.01$ vs. Diab. [c]$P < 0.01$ vs. FPS (0.5). [d]$P < 0.01$ vs. FPS (1)

FPS-ZM1 down-regulated liver and plasma expressions of miR-21

As illustrated in Figure 1, miR-21 showed notably elevated expressions in mice of the control diabetic group. These diabetes-induced elevations in liver and plasma miR-21 expressions were efficiently decremented by FPS-ZM; once more, the reductions turned out to be dose-dependent (Figure 1).

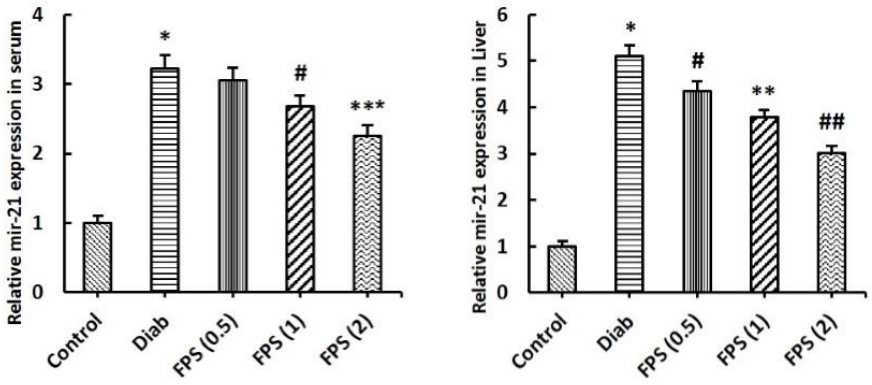

Figure 1. Effects of FPS-ZM1 on liver and plasma expressions of miR-21 in high fat diet-induced diabetic mice. Control, healthy control mice; Diab, diet-induced diabetic control mice; FPS (0.5), diet-induced diabetic mice treated with 0.5 mg/kg FPS-ZM1; FPS (1), diet-induced diabetic mice treated with 1 mg/kg FPS-ZM1; FPS (2), diet-induced diabetic mice treated with 2 mg/kg FPS-ZM1. **P*<0.01 vs. control; #*P*<0.01 vs. Diab; ***P*<0.01 vs. FPS (0.5); ****P*<0.01 vs. FPS (1); ##*P*<0.01 vs. FPS (1)

Discussion

In the present study, we showed that FPS-ZM1 attenuated serum markers of hepatobiliary injury, including ALT, AST, GLD, α-GST, ALP, and GGT in high-fat diet-induced type 2 diabetic mice. It further decreased diabetes-induced elevations in plasma cytokeratin-18 fragment and serum ferritin. Additionally, up-regulated liver and plasma expressions of miR-21 were obviated after FPS-ZM1 treatment for two months.

AGEs, final products of Amadori reactions formed by glycation of proteins, are increasingly produced under diabetic conditions due to persistent hyperglycemia (20). These compounds are termed glucotoxins as their interaction with their correspondent receptors (RAGEs) triggers a myriad of intracellular signaling pathways, including NF-κB, that leads to tissue dysfunction by initiating oxidative stress and inflammation (20). AGE-associated RAGE activation has been identified as the triggering factor for the NAFLD transit to NASH (21). Indeed, it has been demonstrated that antibody-mediated RAGE inhibition could alleviate lipopolysaccharide-induced hepatic oxidative stress along with its associated damages (22).

FPS-ZM1, the specific chemical inhibitor of RAGEs (14), has proven protective effects against diabetic nephropathy, lung emphysema, and neuroinflammation by down-regulating the levels of nuclear phosphorylated NF-κB p65 subunits and mitigating inflammatory cytokines and chemokines including, but not limited to, tumor necrosis factor alpha (TNF-α), interleukin-6 (IL-6), monocyte chemoattractant protein 1 (MCP-1), and transforming growth factor beta (TGF-β) in the affected tissues (23-25).

In the present investigation we first confirmed the development of high fat diet-induced type 2 diabetes mellitus by demonstrating high serum levels of glucose, whole blood hemoglobin A1c, and raised levels of serum c-peptide (26,27). Moreover, control diabetic mice had elevated serum levels of liver injury markers including ALT, AST, GLD, and α-GST. Also, Serum indices of biliary tract involvement i.e. ALP and GGT turned out to be mildly increased. GLD is principally located in the mitochondria of hepatic cells and therefore, its elevations denote the severeness of liver involvement (28). α-GST is a cytosolic enzyme in hepatic cells like ALT and AST; however, it is evenly distributed across the cells in hepatic lobules, which makes it a more sensitive marker of liver injury than ALT and AST (29-31). In addition to being an intracellular iron-storing protein, ferritin independently predicts histopathologic severity and tissue fibrosis in NAFLD patients (32). More importantly, cytokeratin 18, the structural protein abundant in hepatocytes, is fragmented by caspase-3 in apoptotic cells, and accordingly, its elevated levels in plasma is a reliable distinguishing marker for NAFLD and NASH (33). In the present study, FPS-ZM1 not only decreased serum levels of general hepatic injury markers; but also dose-dependently reduced serum and plasma levels of ferritin and plasma cytokeratin 18 fragments.

Accumulating evidence, obtained both by animal and human studies, signifies increased hepatic and plasma miR-21 levels as the fibrosis indicator in NASH (34,35). Mechanistically, in vitro studies on hepatic cell lines have documented the crucial role of miR-21 in promoting lipid accumulation by targeting 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) (36). In addition to enhancing lipid accumulation, miR-21 promotes the progression of

hepatocellular carcinomas through acting upon the HBP1-p53-Srebp1c pathway (37). The protective potential of FPS-ZM1 against hepatic injury was also revealed by demonstrating decreased miR-21 expressions, both in the liver and plasma, in the current investigation.

In conclusion, our findings uncovered that specific RAGE inhibition by FPS-ZM1 reduced circulating levels of liver injury indices in a dose-dependent manner; it also down-regulated hepatic and plasma levels of liver fibrosis indicator, miR-21 in high fat diet-induced type 2 diabetic mice. It should, however, be underlined that without liver histological assessments, FPS-ZM1's protective impact, specifically on NAFLD/NASH could not be claimed. Furthermore, the investigated enzymes and soluble proteins possess, more or less, wide tissue distribution from the individual perspective; in spite of that, their upraise in collection, could be regarded as the indicator of hepatic injury in diabetic mice.

Acknowledgements

This work was supported by grants from Students' Research Center, Tabriz University of Medical Science, Tabriz, Iran (IR.TBZMED.REC.1395.595).

References

1. Ahmad SNS, Nourollahi S, Nakhjavani M, Khojastehfard M, Mostafazadeh M, Hajipour H, et al. Preptin and myostatin independently increase in pre-diabetics and patients of type 2 diabetes mellitus Acta Med Iran 2019;57:160-6.
2. Loomba R, Abraham M, Unalp A, Wilson L, Lavine J, Doo E, et al. Association between diabetes, family history of diabetes, and risk of nonalcoholic steatohepatitis and fibrosis. Hepatology 2012;56:943-51.
3. Cobbina E, Akhlaghi F. Non-alcoholic fatty liver disease (NAFLD) - pathogenesis, classification, and effect on drug metabolizing enzymes and transporters. Drug Metab Rev 2017;49:197-211.
4. Alkassabany YM, Farghaly AG, El-Ghitany EM. Prevalence, risk factors, and predictors of nonalcoholic fatty liver disease among schoolchildren: a hospital-based study in Alexandria, Egypt. Arab J Gastroenterol 2014;15:76-81.
5. Wree A, Broderick L, Canbay A, Hoffman HM, Feldstein AE. From NAFLD to NASH to cirrhosis-new insights into disease mechanisms. Nat Rev Gastroenterol Hepatol 2013;10:627-6.
6. Belfort R, Harrison SA, Brown K, Darland C, Finch J, Hardies J, et al. A placebo-controlled trial of pioglitazone in subjects with nonalcoholic steatohepatitis. N Engl J Med 2006;355:2297-307.
7. Sanyal AJ, Chalasani N, Kowdley KV, McCullough A, Diehl AM, Bass NM, et al. Pioglitazone, vitamin E, or placebo for nonalcoholic steatohepatitis. N Engl J Med 2010;362:1675-85.
8. Armstrong MJ, Gaunt P, Aithal GP, Barton D, Hull D, Parker R, et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. Lancet 2016;387:679-90.
9. Cui J, Philo L, Nguyen P, Hofflich H, Hernandez C, Bettencourt R, et al. Sitagliptin vs. placebo for non-alcoholic fatty liver disease: A randomized controlled trial. J Hepatol 2016;65:369-76.
10. Dajani AI, Abu Hammour AM, Zakaria MA, Al Jaberi MR, Nounou MA, Semrin AI. Essential phospholipids as a supportive adjunct to the management of patients with primary NAFLD and NAFLD associated with type 2 diabetes mellitus or hyperlipidaemia. Hepatol Int 2013;7:748-54.
11. Mohamed WS, Mostafa AM, Mohamed JM, Serwah AH. Effects of fenugreek, Nigella, and termis seeds in nonalcoholic fatty liver in obese diabetic albino rats. Arab J Gastroenterol 2015;16:1-9.
12. Takeuchi M, Takino JI, Sakasai-Sakai A, Takata T, Ueda T, Tsutsumi M, et al. Involvement of the TAGE-RAGE system in non-alcoholic steatohepatitis: Novel treatment strategies. World J Hepatol 2014;6:880-93.
13. Leung C, Herath BC, Jia Z, Andrikopoulos S, Brown BE, Davies MJ, et al. Dietary advanced glycation end-products aggravate non-alcoholic fatty liver disease. World J Gastroenterol 2016;22:8026-40.
14. Deane R, Singh I, Sagare AP, Bell RD, Ross NT, LaRue B, et al. A multimodal RAGE-specific inhibitor reduces amyloid β–mediated brain disorder in a mouse model of Alzheimer disease. J Clin Invest 2012;122:1377-92.
15. Sanajou D, Haghjo AG, Argani H, Roshangar L, Ahmad SNS, Jigheh ZA, et al. FPS-ZM1 and valsartan combination protects better against glomerular filtration barrier damage in streptozotocin-induced diabetic rats. J Physiol Biochem 2018;74:467-78.
16. Sanajou D, Haghjo AG, Argani H, Roshangar L, Rashtchizadeh N, Ahmad SNS, et al., Reduction of renal tubular injury with a RAGE inhibitor FPS-ZM1, valsartan and their combination in streptozotocin-induced diabetes in the rat. Eur J Pharmacol 2019;842:40-8.
17. Sanajou D, Haghjo AG, Argani H, Aslani S. AGE-RAGE axis blockade in diabetic nephropathy: Current status and future directions. Eur J Pharmacol 2018;833:158-64.

18. Surwit RS, Kuhn CM, Cochrane C, McCubbin JA, Feinglos MN. Diet-induced type II diabetes in C57BL/6J mice. Diabetes 1988;37:1163-7.
19. Winzell MS, Ahrén B. The high-fat diet–fed mouse: a model for studying mechanisms and treatment of impaired glucose tolerance and type 2 diabetes. Diabetes 2004;53:215-9.
20. Singh VP, Bali A, Singh N, Jaggi AS. Advanced glycation end products and diabetic complications. Korean J Physiol Pharmacol 2014;18:1-14.
21. Patel R, Baker SS, Liu W, Desai S, Alkhouri R, Kozielski R, et al. Effect of dietary advanced glycation end products on mouse liver. PLoS One 2012;7:e35143.
22. Ribeiro CT, Gasparotto J, Teixeira AA, Portela LVC, Flores VNL, Moreira JCF, et al. Immune neutralization of the receptor for advanced glycation endproducts reduce liver oxidative damage induced by an acute systemic injection of lipopolysaccharide. J Biochem 2018;163:515-23.
23. Sharma I, Tupe RS, Wallner AK, Kanwar YS. Contribution of myo-inositol oxygenase in AGE:RAGE-mediated renal tubulointerstitial injury in the context of diabetic nephropathy. Am J Physiol Renal Physiol 2018;314:F107-21.
24. Lee H, Park JR, Kim WJ, Sundar IK, Rahman I, Park SM, et al. Blockade of RAGE ameliorates elastase-induced emphysema development and progression via RAGE-DAMP signalling. FASEB J 2017;31:2076-89.
25. Shen C, Ma Y, Zeng Z, Yin Q, Hong Y, Hou X, et al. RAGE-specific inhibitor FPS-ZM1 attenuates AGEs-induced neuroinflammation and oxidative stress in rat primary microglia. Neurochem Res 2017;42:2902-11.
26. Sandu O, Song K, Cai W, Zheng F, Uribarri J, Vlassara H. Insulin resistance and type 2 diabetes in high-fat–fed mice are linked to high glycotoxin intake. Diabetes 2005;54:2314-9.
27. Jones A, Hattersley A. The clinical utility of C- peptide measurement in the care of patients with diabetes. Diabetic Med 2013;30:803-17.
28. McGill MR, Sharpe MR, Williams CD, Taha M, Curry SC, Jaeschke H. The mechanism underlying acetaminophen-induced hepatotoxicity in humans and mice involves mitochondrial damage and nuclear DNA fragmentation. J Clin Invest 2012;122:1574-83.
29. Vaubourdolle M, Chazouillères O, Briaud I, Legendre C, Serfaty L, Poupon R, et al. Plasma alpha-glutathione S-transferase assessed as a marker of liver damage in patients with chronic hepatitis C. Clin Chem 1995;41:1716-19.
30. Matsumoto R, Watanabe S, Beppu T, Futagawa S. Serum alpha-glutathione S-transferase: a new marker of hepatocellular damage associated with hepatectomy. Hepatol Res 2000;18:10-8.
31. Federico A, Tuccillo C, Crafa E, Loguercio C. The significance of alpha-glutathione S-transferase determination in patients with chronic liver diseases. Minerva Gastroenterol Dietol 1999;45:181-5.
32. Kowdley KV, Belt P, Wilson LA, Yeh MM, Neuschwander- Tetri BA, Chalasani N, et al. Serum ferritin is an independent predictor of histologic severity and advanced fibrosis in patients with nonalcoholic fatty liver disease. Hepatology 2012;55:77-85.
33. Feldstein AE, Wieckowska A, Lopez AR, Liu YC, Zein NN, McCullough AJ. Cytokeratin- 18 fragment levels as noninvasive biomarkers for nonalcoholic steatohepatitis: a multicenter validation study. Hepatology 2009;50:1072-78.
34. Takeuchi-Yorimoto A, Yamaura Y, Kanki M, Ide T, Nakata A, Noto T, et al. MicroRNA-21 is associated with fibrosis in a rat model of nonalcoholic steatohepatitis and serves as a plasma biomarker for fibrotic liver disease. Toxicol Lett 2016;258:159-67.
35. Benhamouche-Trouillet S, Postic C. Emerging role of miR-21 in non-alcoholic fatty liver disease. Gut 2016;65:1781-3.
36. Park D, Jo IG, Jang JY, Kwak TH, Yoo SK, Jeon JH, et al. A Dunnione Compound MB12662 Improves Cisplatin-Induced Tissue Injury and Emesis. Biomol Ther(Seoul) 2015;23:449-57.
37. Wu H, Ng R, Chen X, Steer CJ, Song G. MicroRNA-21 is a potential link between non-alcoholic fatty liver disease and hepatocellular carcinoma via modulation of the HBP1-p53-Srebp1c pathway. Gut 2016;65:1850-60.