Original Article

Epimedii folium Polysaccharide Ameliorated Glucose Metabolic Disorder in Type 2 Diabetic Mice by Regulating the SIRT1/PPARγ Signaling Pathway

Ruo-Fei Huang, Lin-Lin Xu, Tian-Xi Chen, Ke Li, Jing-Yao Xia, Hui-Shang Wang*

The First People's Hospital of Yongkang, Zhejiang, CHINA.

ABSTRACT

Background and Aim: *Epimedii folium* is a widely used herbal medicine with numerous medicinal effects. In this study, we aimed to investigate the effects of *Epimedii folium* polysaccharide (EFP) in db/db metabolic disorder mice model. **Materials and Methods:** Studies were carried out by the db/db mice with separately receiving EFP (100 mg/kg, 400 mg/kg) and metformin (300 mg/kg/day) for 8 weeks. **Results:** In the present study, we show that EFP and metformin ameliorated metabolic disorders and decreased the levels of glucose metabolism parameters in a dose-dependent manner. Besides, the hepatorenal function was also improved with EFP and metformin. In addition, EFP and metformin also reduced the lipid vacuoles accumulated in the hepatocytes and alleviated the hepatocyte's hypertrophy and nuclei shrunk. Moreover, EFP and metformin also reduced the activity level of MDA and increased the antioxidant enzymes levels, including CAT, SOD, and GSH-Px, as well as the mRNA and proteins expression of SIRT1 and PPARγ in the mice liver. **Conclusion:** Our data demonstrate that EFP ameliorated hyperglycemia mediated oxidative stress and promoted lipid metabolism in T2D mice, and was partially associated with activation of SIRT1/PPARγ pathway in db/db mice.

Key words: Type 2 diabetes, *Epimedii folium* polysaccharide, Hyperglycemia, Oxidative stress, Lipid metabolism.

INTRODUCTION

Type 2 diabetes (T2D) is a common and complex, chronic metabolic disease affecting the aging population and middle-aged people with unhealthy lifestyles.[1,2] The typical hallmark of T2D was hyperglycemia caused by insulin resistance, dyslipidemia, and induction of pro-inflammatory response.[3,4] These mutually influenced factors resulted in diabetic nephropathy, diabetic ophthalmopathy, coronary artery disease, obesity, and nonalcoholic fatty liver disease, etc.[4,5] The liver, one of the central metabolic organs, plays a pivotal role in the regulation of various biochemical reactions, thus keeping the metabolic homeostasis of whole-body glucose, fatty acids, and amino acids.[6] While mitochondria are key organelles in the regulation of liver energy metabolism. Hence, the hyperglycaemic status particularly threatened the mitochondria and induced the mitochondria oxidative stress and mitochondria injury, which contributes to ROS production, the reduction of antioxidant capacity, and changes in mitochondrial membrane potential.[7,8]

The disturbance of ROS production activated the stress kinases, such as c-Jun N-terminal kinase and protein kinase C, which phosphorylated insulin receptor-1 and induced insulin resistance.[9] Besides, the antioxidative defenses system was also damaged resulting in excessive free radical production.[10] These alterations accordingly contributed to dysregulated fatty acid metabolism with plasma non-esterified fatty acids (NEFA) accumulation, which leads to hepatic oxidative stress and lipotoxicity.[11,12] Hence, it's paramount to find efficient medicines to conquer these questions.

Submission Date: 28-10-2021;
Revision Date: 24-12-2021;
Accepted Date: 16-03-2022.

DOI: 10.5530/ijper.56.2s.98

Correspondence:
Dr. Hui-Shang Wang
The First People's Hospital of Yongkang, No. 599, Jinshan W. Road, Dongcheng Street, Yongkang City, Zhejiang-321300, CHINA.
E-mail: fly7250805@sina.com

www.ijper.org

Traditional Chinese Medicine displayed pharmacological effects with wide clinical applications in Asia. Besides, it was more and more popular worldwide in recent years. The *Epimedii folium* (Chinese name "YinYangHuo") is a proverbial herbal medicine that has been broadly used as a aphrodisiac, tonic, and antirheumatic.[13] Also, *Epimedii folium* polysaccharides (EFP) are one of the active ingredients in the *Epimedii folium* (Berberidaceae). In scientific researches, several reports had shown that EF exhibited varieties of biological effects, such as antitumor,[14] immunoregulatory,[15] antioxidant,[16,17] and anti-hyperlipidemic, cholesterol-lowering activities in obesity.[18] However, the anti-diabetic effect of EFP on the db/db mouse model and its potential mechanisms are still unclear. Therefore, there is great medicinal utilization potential of EFP in metabolic diseases.

The NAD+-dependent deacetylase, sirtuin 1 (SIRT1) was a key protein that played important role in mitochondrial oxidative activities.[19] And, it could deacetylate the peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) to modulate glucose homeostasis.[20] Additionally, the transcriptional factor peroxisome proliferator-activated receptor γ (PPARγ) engaged in the ability of lipid esterification and β-oxidation through the mTOR signaling pathway.[21] Hence, to our knowledge, we attempted to investigate the medicinal effect of EFP in modulating hyperglycemia-induced hepatic oxidative stress through the SITR1/PPARγ axis in the T2D mice model.

MATERIALS AND METHODS

Chemical reagents

Epimedii folium polysaccharides and metformin were purchased from Solarbio (Beijing, China. A high-fat diet was obtained from Nanjing jinyibo biotechnology co. LTD (D12450B, Nanjing, China). Alanine aminotransferase (ALT), alanine aminotransferase (AST), triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) assay kits, UA assay kits, Ucr assay kits, and U-ALB assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). ADVIA Centaur chemiluminescence apparatus, TRIZOL Kit, SYBR Green PCR Master Mix, and Prime Script RT Enzyme Mix were purchased from TaKaRa (Takara, Tokyo, Japan). RIPA buffer was purchased from Sigma. SDS-PAGE gel kits and Hematoxylin and eosin Staining Kit (C0105) were purchased from Beyotime (Shanghai, China). PVDF membranes were purchased from Millipore. Primary antibodies against SIRT1 and PPARγ were purchased from Cell Signaling Technology (Danvers, MA, USA, Cat no. 2028, 95128). Chemiluminescence detection reagents (ECL) were purchased from Thermo Fisher Scientific.

Animal and experimental design

Thirty diabetic mice (db/db) (aged six weeks with a body-weight of 35g ± 2.45g) and the other six age-matched non-diabetic mice (db/m) (18.5g ± 1.25g), were purchased from the Nanjing University Model Animal Research Institute, and maintained at animal experimental research center of Zhejiang Chinese Medical University (Zhejiang, China). The mice were housed in a standardized environment with the controlling conditions: temperature, 20-25°C; humidity, 40%-70%; circadian conditions; 12 hr light/dark cycle, and had free access to regular diet and water. After adaptation for 2 weeks, the db/db mice that met with the diagnostic criteria for T2D (random blood glucose ≥11.1 mmol/L) were randomly divided into the following four groups, $n = 6$ in each group, based on the blood glucose and body weight levels: T2D group, intragastrically treated with 0.3% CMC-Na; EFP groups, intragastrically administered with 100 mg/kg or 400 mg/kg EFP, respectively, and metformin (Met) group, intragastrically administered with 300 mg/kg/day. Meanwhile, the control mice have also received saline solution. At the end of the 8-week treatment period, the food intake, water intake, and urine volume in all the mice were evaluated, and record the body weight of the mice, then we collected the blood via the tail vein of the mice to evaluate the fasting blood glucose and haemoglobin A1c (HbA1c) levels. Afterward, all the mice were anesthetized with 2% isoflurane and sacrificed, then we collected the blood samples from all the mice aorta abdominalis immediately and all the mice liver tissue samples were snap-frozen in liquid nitrogen or immersed in formalin for experiments. All animal experimental procedures were approved by the Committee for Animal Research of Zhejiang Chinese Medical University (Zhejiang, China).

Biochemical parameters assays

The blood samples collected from the aorta abdominalis were centrifugated at 3500 r/min for 15 min at 4°C. Then, the fasting serum insulin (FINS) and glucagons levels were measured by radio-immunoassay (RIA) method with chemiluminescence apparatus. In addition, the levels of ALT, AST, TC, TG, HDL-C, LDL-C, Scr, and BUN in serum and UA, Ucr, and U-ALB in urine were separately quantified with the commercial assay kits following the manufacturer's protocols.

Histological analysis

After treatment for 8 weeks, mice were weighed, and the blood samples were collected from the aorta abdominalis under anesthesia. Then, mice were sacrificed. Next, the liver tissues were removed and fixed with 10 % neutral formaldehyde for 48 hr. After that, paraffin sections were prepared and stained with hematoxylin and eosin (H&E). Finally, Images were obtained with an Olympus BX41 microscope.

Hepatic oxidative parameters assays

The collected liver samples were homogenated in cold PBS and then centrifuged at 12000 r/min for 10 min at 4°C. Then, the supernatant of the homogenization medium was collected. The amount of malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) was analyzed with the commercial assay kits according to the manufacturer's instructions.

qRT-PCR analysis

Total RNA was extracted from the isolated liver tissues with TRIzol, then the RNA was reverse-transcribed into cDNA and amplified with Real-time RT-PCR by SYBR Green PCR Master Mix in MyCycler PCR apparatus. Afterward, we calculated the mRNA expression levels with the $2^{-\Delta\Delta Ct}$ method with GAPDH as the reference marker. The primers sequences of the genes utilized are shown as follows: PPARγ: forward, 5'-GGAGCCTAAGTTTGAGTTTGCTGTG-3', reverse,5'-TGCAGCAGGTTGTCTTGGATG-3';SIRT1: forward, 5'-GCGATTGGGTACCGAGATAAC-3', reverse,5'-GGCCTTGGAGTCCAGTCACTA-3';GAPDH: forward, 5'-TGCACCACCACCTGCTTAGC-3', reverse, 5'-GAGGGGCCATCCACAGTCTTC-3'.

Western blotting

The total proteins were extracted from the isolated liver tissues with RIPA buffer containing PMSF, then the proteins were denatured and evaluated the protein concentration with the BCA protein assay kit. Proteins (20 μg) were subsequently separated by 10% SDS-PAGE and then blotted onto polyvinylidene difluoride (PVDF) membranes. Next, the transferred membranes were blocked with 5% fat-free milk for one hour. Next, the membranes were incubated with antibodies against SIRT1 and PPARγ or β-actin and GAPDH at 4°C overnight. After washing, the membranes were incubated with the second anti-mouse IgG antibody for 1hr. After that, the PVDF membranes were incubated in an ECL reagent and the signals thus obtained were visualized by exposure to hyper-performance chemiluminescence film for 30 sec to 5 min. Finally, the proteins' gray intensity was measured with Image J Software.

Statistical analysis

All data are presented as mean ± standard deviation (SD). Statistical significance was determined with a one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test using the SPSS statistic 19.0 (IBM, USA) software. $P<0.05$ was considered statistically significant.

RESULTS

EFP improved the metabolic profiles of T2D mice

The effects of EFP on metabolic disorder were measured on T2D mice. As elucidated in Figure 1A, the body weight of the T2D group was higher than that of the control group. Also, EFP and metformin treatment slightly decreased the body weight in db/db mice. Also, in week 8, the levels of food intake, water intake, and urine volume in the EFP and metformin groups were significantly lower than that in the T2D group, respectively (Figure 1B-1D).

EFP improved glucose metabolism of T2D mice

The db/db mice had higher levels of fasting insulin, glucagon, fasting blood glucose, and HbA1c than those of the control mice (Figure 2A-2D). Eight-week-old male mice were administered EFP and metformin for 8 weeks. As expected, EFP and metformin significantly reduced fasting insulin, glucagon, fasting blood glucose, and HbA1c levels in db/db mice (Figure 2A-2D).

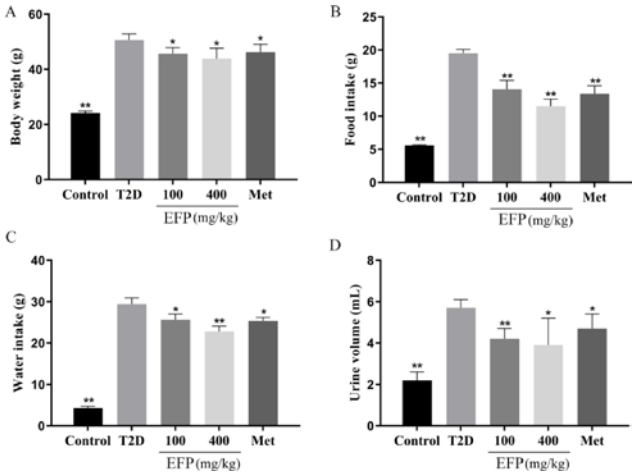


Figure 1: Effects of EFP on body weight and metabolic changes in db/db mice.

(A) Body weight (A), food intake (B), water intake (C), and urine volume changes in db/db mice and non-diabetic mice that were administered EFP for 8 weeks. All data were expressed as mean ± SD (n =6). *$P<0.05$, **$P<0.01$ *vs* T2D.

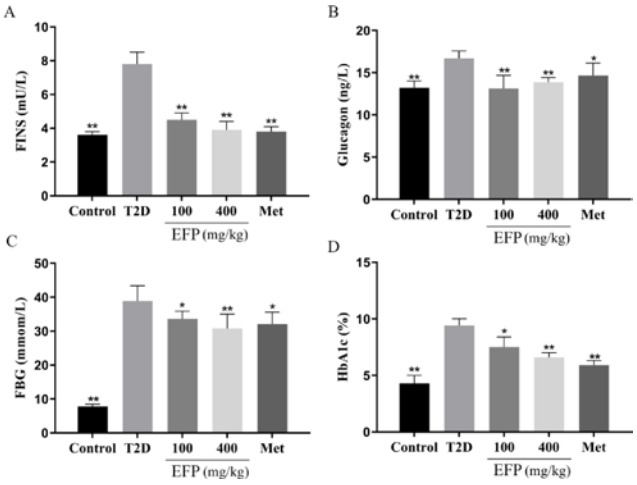


Figure 2: Effects of EFP on glucose metabolism levels in db/db mice.

At the end of the experiment, fasting insulin (A), glucagon (B), fasting blood glucose (FBG, C), and glycosylated hemoglobin (HbA1c, D) levels were measured. Data are mean ± SD. Data were analyzed by a one-way ANOVA test. *$P<0.05$, **$P<0.01$ *vs* T2D.

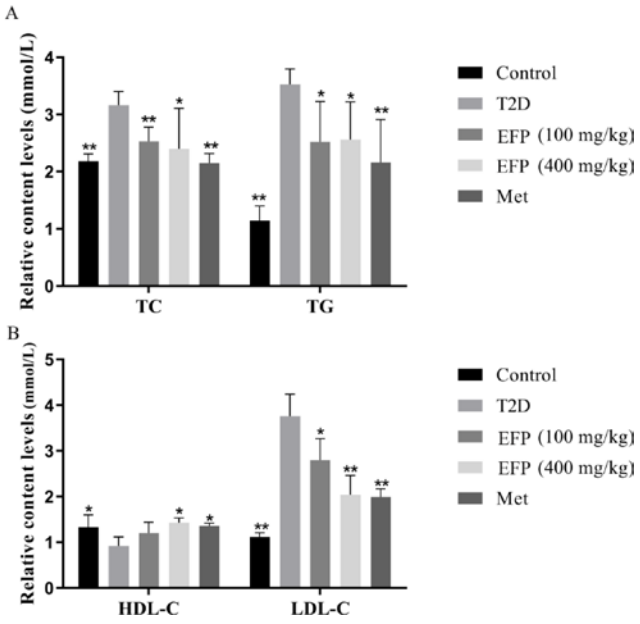


Figure 3: Effects of EFP on serum lipid profile in db/db mice and non-diabetic mice.

(A) Serum levels of TC and TG in db/db mice. (B) Levels of LDL-C and HDL-C in serum of db/db mice. Data are mean ± SD. *$P<0.05$, **$P<0.01$ *vs* T2D.

EFP reduced lipid accumulation in the serum in T2D mice

The effect of EFP on TC, TG, HDL-C, and LDL-C levels is illustrated in Figure 3A-3B. Compared with those in the control group, the TC, TG, and LDL-C levels were significantly higher in the T2D group. In contrast, the levels of HDL-C were significantly lower in the T2D group. Meanwhile, EFP and metformin-treated db/db mice demonstrated a lower TC, TG, and LDL-C levels in serum as compared with the db/db mice. And, we also found that a markedly increased HDL-C was detected in EFP (400 mg/kg) and metformin groups at the end of the experiment (Figure 3A-3B).

EFP ameliorated liver and renal function in T2D mice

Serum ALT, AST, creatinine, and BUN levels were higher in diabetic mice than in non-diabetic mice (Figure 4A-4B). Moreover, urine creatinine, UA, and U-ALB levels were also higher in db/db mice (Figure 4D-4F). After EFP and metformin administration, the levels of ALT, AST, Scr, BUN, Ucr, UA, and U-ALB were markedly reduced compared to the T2D group. Taken together, these results suggested that EFP and metformin could improve liver and renal function to protect T2D.

EFP decreases oxidative stress in db/db mice

In the liver of db/db mice, the level of MDA increased, and the activity of SOD, CAT, and GSH-PX was significantly reduced (Figure 5A-5D). EFP and metformin significantly improved the oxidative stress

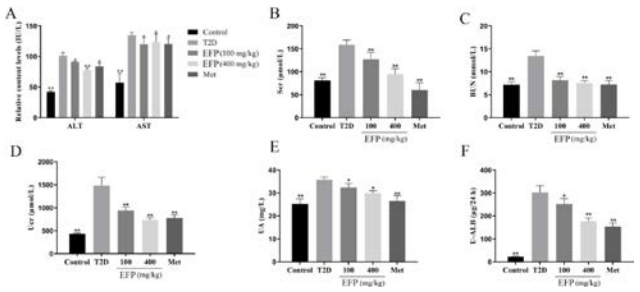


Figure 4: Effects of EFP on the liver and renal function in db/db mice and non-diabetic mice.

(A) Serum levels of ALT, AST, Scr, and BUN (B) in db/db mice. Urine levels of Ucr (D), UA (E), and U-ALB (F) in db/db mice. Data are mean ± SD. *$P<0.05$, **$P<0.01$ *vs* T2D.

when compared with the T2D group. These results showed that EFP could significantly inhibit the oxidative stress in db/db mice.

EFP ameliorated the histopathological changes and activated the SIRT1/PPARγ pathway in the liver of db/db mice

As shown in Figure 6A, in the control group, the hepatocytes exhibited regular arrangement. Additionally, the hepatocytes of the T2D group exhibited disordered arrangement, swelling, and steatosis. Compared with the T2D group, the swelling and steatosis of the hepatocytes were significantly alleviated in the EFP and met groups. In both q-PCR and western blot results, SIRT1 and PPARγ expression levels were significantly reduced

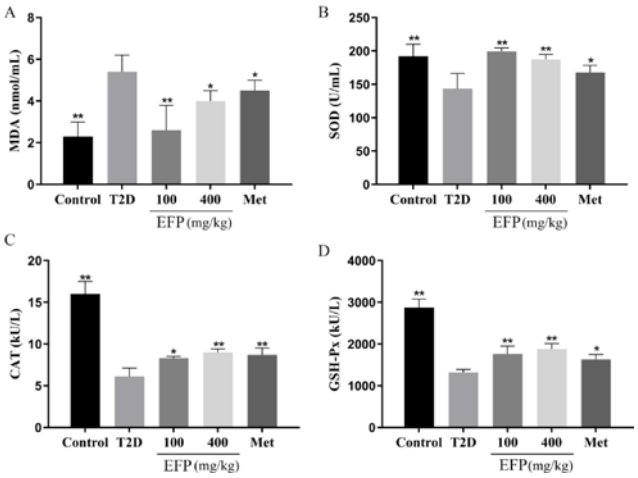


Figure 5: Effects of EFP on the hepatic oxidative stress in db/db mice and non-diabetic mice.

(A) liver levels of malondialdehyde (MDA), (B) superoxide dismutase (SOD), (C) catalase (CAT), and (D) glutathione peroxidase (GSH-Px) in db/db mice that were administered EFP for 8 weeks. Data are mean ± SD. **P<0.05*, ***P<0.01 vs* T2D.

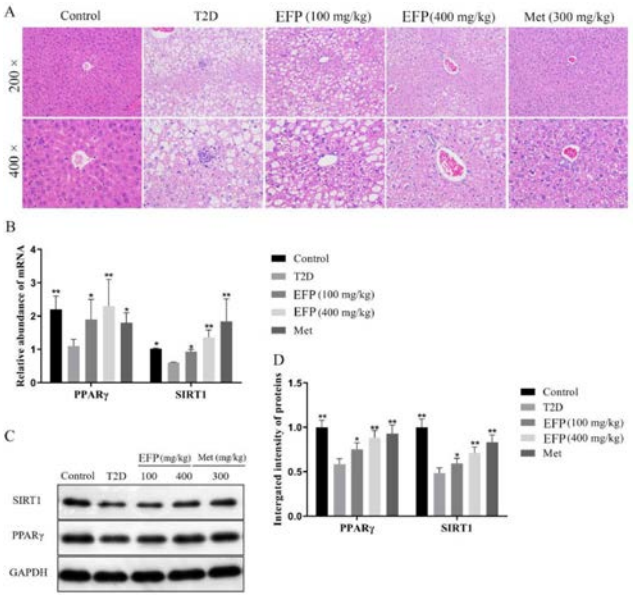


Figure 6: Effects of EFP on the pathological changes and SIRT1/PPARγ signaling pathway of the liver in db/db mice.

(A) The effect of EFP and Met on the pathological changes of liver in db/db mice. The liver was stained with hematoxylin and eosin (magnification: ×200 and ×400). (B, C) The mRNA and protein expression levels of SIRT1 and PPARγ were determined by qPCR and Western blotting analysis, respectively. (C) The representative images and band intensity analysis (D) are shown. Data are mean ± SD. Data were analyzed by a one-way ANOVA test. **P<0.05*, ***P<0.01 vs* T2D.

in the T2D group (Figure 6B-6D). EFP is effective in increasing the mRNA and protein expression levels of SIRT1 and PPARγ, particularly in the high dose group. Overall, activation of the SIRT1/PPARγ pathway via EFP treatment might ameliorate liver injury, which in turn alleviated the features of T2D.

DISCUSSION

Type 2 Diabetes mellitus (DM) is increasing in prevalence at a worrying rate with hyperglycemia and hyperlipidemia.[22] Hyperglycaemia and hyperlipidemia also resulted in hepatic oxidative stress, hepatocyte damage, macrovascular, microvascular endothelial cell injury,[23,24] and chronic inflammatory response.[25] Our results displayed EFP with an anti-diabetogenic therapeutic effect, which was through alleviating metabolic disorder, improving glucose metabolism, insulin sensitivity, and hepatorenal function. Besides, EFP ameliorated hepatic oxidative stress and facilitated hepatic lipid metabolism. Thus, resulting in the reduction of liver pathology lesions in this study.

In addition, hyperglycemia promoted hepatic oxidative stress, which resulted in the accumulation of lipid peroxidation product in T2D mice livers. Moreover, EFP enhanced the hepatic antioxidative function through up-regulating the expression of antioxidant enzymes, including CAT, SOD, GSH-Px in this study. Importantly, these medicinal effects of EFP coincided with Gyurko *et al.*[26] Studies of Pan *et al.*,[27] and Ferré *et al.*,[28] demonstrated that the protein PPARγ engaged in promoting fatty acid oxidation and inducing adiponectin secretion to enhance insulin sensitivity, and eventually, promoting glucose utilization. Hence, our further study can investigate the effects of EFP in adiponectin change of T2D mice. In addition, studies also confirmed SIRT1 to be a potential therapeutic target in T2D.[29,30] For SIRT1 and PPARγ played crucial roles in reducing inflammation and mitochondrial oxidative stress to ameliorate insulin resistance and protect pancreatic β cells. Therefore, the proteins of SIRT1 and PPARγ may be potential pharmacological therapeutic targets for treating T2D. As expected, EFP relieved liver oxidative stress by up-regulating the expression of SIRT1 and facilitating lipid esterification through activating the transcriptional factor PPARγ. However, a recent study also showed that EFP not only ameliorated lipid metabolic disorders but also regulated hepatic inflammation response.[31] Hence, it's also necessary to further investigate the therapeutic effects of EFP in mediating gut microbiota to shape T2D. Taken together, in this study, we found EFP ameliorated metabolic disorder in type 2 diabetic mice.

CONCLUSION

EFP might ameliorate metabolic disorder and hyperglycemia-mediated hepatic oxidative stress through activating the SIRT1/PPARγ pathway in the liver of type 2 diabetic mice.

Funding

This work was supported by grants from the Zhejiang medical and health science technology program (No. 2019RC305).

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ABBREVIATIONS

EFP: *Epimedii folium* polysaccharide; **T2D:** Type 2 diabetes; **NEFA:** non-esterified fatty acids; **SIRT1:** NAD+-dependent deacetylase, sirtuin 1; **PGC-1α:** peroxisome proliferator-activated receptor γ coactivator-1α; **PPARγ:** peroxisome proliferator-activated receptor γ; **ALT:** Alanine aminotransferase; **AST:** alanine aminotransferase; **TG:** triglyceride; **TC:** total cholesterol; **HDL-C:** high-density lipoprotein cholesterol; **LDL-C:** low-density lipoprotein cholesterol; **Met**: metformin; **HbA1c:** haemoglobin A1c; **FINS:** fasting serum insulin; **RIA:** radio-immunoassay; **HE:** hematoxylin and eosin; **MDA:** malondialdehyde; **SOD:** superoxide dismutase; **CAT:** catalase; **GSH-Px:** glutathione peroxidase; **PVDF:** polyvinylidene difluoride; **SD:** standard deviation; **ANOVA:** one-way analysis of variance; **DM:** Diabetes mellitus.

Ethical approval

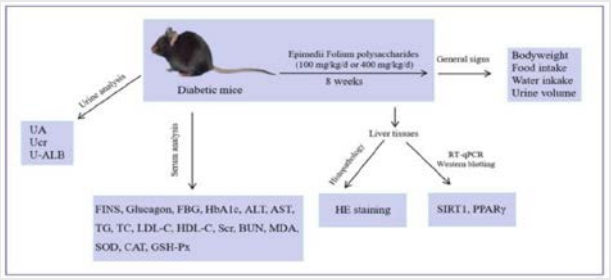
Animals' use and disposal had been approved by the animal ethics committee of Zhejiang Chinese Medicine University.

REFERENCES

1. Lovic D, Piperidou A, Zografou I, Grassos H, Pittaras A, Manolis A. The growing epidemic of diabetes mellitus. Curr Vasc Pharmacol. 2020;18(2):104-9. doi: 10.2174/1570161117666190405165911, PMID 30961501.
2. Lam DW, LeRoith D. The worldwide diabetes epidemic. Curr Opin Endocrinol Diabetes Obes. 2012;19(2):93-6. doi: 10.1097/MED.0b013e328350583a, PMID 22262000.
3. Imai Y, Cousins RS, Liu S, Phelps BM, Promes JA. Connecting pancreatic islet lipid metabolism with insulin secretion and the development of type 2 diabetes. Ann N Y Acad Sci. 2020;1461(1):53-72. doi: 10.1111/nyas.14037, PMID 30937918.
4. Sung KC, Lee MY, Kim YH, Huh JH, Kim JY, Wild SH, *et al*. Obesity and incidence of diabetes: Effect of absence of metabolic syndrome, insulin resistance, inflammation and fatty liver. Atherosclerosis. 2018;275:50-7. doi: 10.1016/j.atherosclerosis.2018.05.042, PMID 29860108.
5. Bardini G, Rotella CM, Giannini S. Dyslipidemia and diabetes: Reciprocal impact of impaired lipid metabolism and Beta-cell dysfunction on micro- and macrovascular complications. Rev Diabet Stud. 2012;9(2-3):82-93. doi: 10.1900/RDS.2012.9.82, PMID 23403704.
6. Li W, Zhang W, Chang M, Ren J, Xie W, Chen H, *et al*. Metabonomics reveals that triclocarban affects liver metabolism by affecting glucose metabolism, β-oxidation of fatty acids, and the TCA cycle in male mice. Toxicol Lett. 2018;299:76-85. doi: 10.1016/j.toxlet.2018.09.011, PMID 30261223.
7. Szendroedi J, Phielix E, Roden M. The role of mitochondria in insulin resistance and type 2 diabetes mellitus. Nat Rev Endocrinol. 2011;8(2):92-103. doi: 10.1038/nrendo.2011.138, PMID 21912398.
8. Rolo AP, Palmeira CM. Diabetes and mitochondrial function: Role of hyperglycemia and oxidative stress. Toxicol Appl Pharmacol. 2006;212(2):167-78. doi: 10.1016/j.taap.2006.01.003, PMID 16490224.
9. Aguirre V, Werner ED, Giraud J, Lee YH, Shoelson SE, White MF. Phosphorylation of Ser307 in insulin receptor substrate-1 blocks interactions with the insulin receptor and inhibits insulin action. J Biol Chem. 2002;277(2):1531-7. doi: 10.1074/jbc.M101521200, PMID 11606564.
10. Martínez-Cisuelo V, Gómez J, García-Junceda I, Naudí A, Cabré R, Mota-Martorell N, *et al*. Rapamycin reverses age-related increases in mitochondrial ROS production at complex I, oxidative stress, accumulation of mtDNA fragments inside nuclear DNA, and lipofuscin level, and increases autophagy, in the liver of middle-aged mice. Exp Gerontol. 2016;83:130-8. doi: 10.1016/j.exger.2016.08.002, PMID 27498120.
11. Pereira S, Park E, Mori Y, Haber CA, Han P, Uchida T, *et al*. FFA-induced hepatic insulin resistance *in vivo* is mediated by PKCδ, NADPH oxidase, and oxidative stress. Am J Physiol Endocrinol Metab. 2014;307(1):E34-46. doi: 10.1152/ajpendo.00436.2013, PMID 24824652.
12. Ament Z, West JA, Stanley E, Ashmore T, Roberts LD, Wright J, *et al*. PPAR-pan activation induces hepatic oxidative stress and lipidomic remodelling. Free Radic Biol Med. 2016;95:357-68. doi: 10.1016/j.freeradbiomed.2015.11.033, PMID 26654758.
13. Wang Z, Xu G, Wang H, Zhan X, Gao Y, Chen N, *et al*. Icariside II, a main compound in *Epimedii Folium*, induces idiosyncratic hepatotoxicity by enhancing NLRP3 inflammasome activation. Acta Pharm Sin B. 2020;10(9):1619-33. doi: 10.1016/j.apsb.2020.03.006, PMID 33088683.
14. Zhang ZB, Ip SP, Cho WC, Hu Z, Huang YF, Luo DD, *et al*. Evaluation of the effects of androgenic Chinese herbal medicines on androgen receptors and tumor growth in experimental prostate cancer models. J Ethnopharmacol. 2020;260:113058. doi: 10.1016/j.jep.2020.113058.
15. Zhang X, Han N, Li G, Yang H, Cao Y, Fan Z, *et al*. Local icariin application enhanced periodontal tissue regeneration and relieved local inflammation in a minipig model of periodontitis. Int J Oral Sci. 2018;10(2):19. doi: 10.1038/s41368-018-0020-3, PMID 29895944.
16. Li L, Chen B, An T, Zhang H, Xia B, Li R, *et al*. BaZiBuShen alleviates altered testicular morphology and spermatogenesis and modulates Sirt6/P53 and Sirt6/NF-κB pathways in aging mice induced by D-galactose and NaNO2. J Ethnopharmacol. 2021;271:113810. doi: 10.1016/j.jep.2021.113810.
17. Xie QF, Xie JH, Dong TT, Su JY, Cai DK, Chen JP, *et al*. Effect of a derived herbal recipe from an ancient Chinese formula, Danggui Buxue Tang, on ovariectomized rats. J Ethnopharmacol. 2012;144(3):567-75. doi: 10.1016/j.jep.2012.09.041, PMID 23036809.
18. Zhang D, Liu L, Jia Z, Yao X, Yang M. Flavonoids of Herba Epimedii stimulate osteogenic differentiation and suppress adipogenic differentiation of primary mesenchymal stem cells via estrogen receptor pathway. Pharm Biol. 2016;54(6):954-63. doi: 10.3109/13880209.2015.1079224, PMID 26453492.
19. Feige JN, Lagouge M, Canto C, Strehle A, Houten SM, Milne JC, *et al*. Specific SIRT1 activation mimics low energy levels and protects against diet-induced metabolic disorders by enhancing fat oxidation. Cell Metab. 2008;8(5):347-58. doi: 10.1016/j.cmet.2008.08.017, PMID 19046567.
20. Rodgers JT, Lerin C, Haas W, Gygi SP, Spiegelman BM, Puigserver P. Nutrient control of glucose homeostasis through a complex of PGC-1alpha and SIRT1. Nature. 2005;434(7029):113-8. doi: 10.1038/nature03354, PMID 15744310.
21. Li Z, Xu G, Qin Y, Zhang C, Tang H, Yin Y, *et al*. Ghrelin promotes hepatic lipogenesis by activation of mTOR-PPARγ signaling pathway. Proc Natl Acad Sci USA. 2014;111(36):13163-8. doi: 10.1073/pnas.1411571111, PMID 25157160.
22. Patel TP, Rawal K, Bagchi AK, Akolkar G, Bernardes N, Dias DDS, *et al*. Insulin resistance: An additional risk factor in the pathogenesis of cardiovascular disease in type 2 diabetes. Heart Fail Rev. 2016;21(1):11-23. doi: 10.1007/s10741-015-9515-6, PMID 26542377.
23. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, *et al*. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: Potential role of PGC1 and NRF1. Proc Natl

Acad Sci USA. 2003;100(14):8466-71. doi: 10.1073/pnas.1032913100, PMID 12832613.
24. Fowler MJ. Microvascular and macrovascular complications of diabetes. Clin Diabetes. 2008;26(2):77-82. doi: 10.2337/diaclin.26.2.77.
25. Gyurko R, Siqueira CC, Caldon N, Gao L, Kantarci A, Van Dyke TE. Chronic hyperglycemia predisposes to exaggerated inflammatory response and leukocyte dysfunction in Akita mice. J Immunol. 2006;177(10):7250-6. doi: 10.4049/jimmunol.177.10.7250, PMID 17082643.
26. Pan D, Zhang D, Wu J, Chen C, Xu Z, Yang H, *et al*. A novel proteoglycan from Ganoderma lucidum fruiting bodies protects kidney function and ameliorates diabetic nephropathy via its antioxidant activity in C57BL/6 db/db mice. Food Chem Toxicol. 2014;63:111-8. doi: 10.1016/j.fct.2013.10.046, PMID 24211521.
27. Ferré P. The biology of peroxisome proliferator-activated receptors: Relationship with lipid metabolism and insulin sensitivity. Diabetes. 2004;53;Suppl 1:S43-50. doi: 10.2337/diabetes.53.2007.s43, PMID 14749265.
28. Miles PD, Barak Y, He W, Evans RM, Olefsky JM. Improved insulin-sensitivity in mice heterozygous for PPAR-gamma deficiency. J Clin Invest. 2000;105(3):287-92. doi: 10.1172/JCI8538, PMID 10675354.
29. Kitada M, Kume S, Kanasaki K, Takeda-Watanabe A, Koya D. Sirtuins as possible drug targets in type 2 diabetes. Curr Drug Targets. 2013;14(6):622-36. doi: 10.2174/1389450111314060002, PMID 23445543.
30. Kitada M, Koya D. SIRT1 in type 2 diabetes: Mechanisms and therapeutic potential. Diabetes Metab J. 2013;37(5):315-25. doi: 10.4093/dmj.2013.37.5.315, PMID 24199159.
31. Sun K, Zhu LG, Wei X, Zhang P, Zhan JW, Wang Y, *et al*. [Study on mechanism of "*Epimedii folium*-Paeoniae Radix Alba" in treatment of lumbar disc herniation based on network pharmacology]. Zhongguo Zhong Yao Za Zhi. 2020;45(3):609-16. doi: 10.19540/j.cnki.cjcmm.20190905.401, PMID 32237520.

PICTORIAL ABSTRACT



SUMMARY

The objective of the present study was to investigate the effects of *Epimedii folium* polysaccharide (EFP) in db/db mice with spontaneous glucose metabolic disorder. The results showed that administration of EFP significantly ameliorated the metabolic disorders and reduced the amount of MDA, as well as increased the levels of antioxidant enzymes, including SOD, CAT, and GSH-Px. Further, EFP increased the mRNA and protein expression levels of SIRT1 and PPARγ in the liver tissues of db/db mice. In summary, EFP exerts the protective effect against metabolic disorder through restoring the SIRT1/PPARγ signaling pathway.

About Authors

Ruo-Fei Huang: She is currently working as an attending doctor at the Department of Endocrinology, the First People's Hospital of Yongkang, Yongkang City, Zhejiang Province, China.

Lin-Lin Xu : She is currently working as an attending doctor at the Department of Endocrinology, the First People's Hospital of Yongkang, Yongkang City, Zhejiang Province, China.

Tian-Xi Chen: He is currently working as an attending doctor at the Department of Nephrology , the First People's Hospital of Yongkang, Yongkang City, Zhejiang Province, China.

Ke Li: She is currently working as a chief physician at the Department of Endocrinology, the First People's Hospital of Yongkang,Yongkang City, Zhejiang Province, China.

Jing-Yao Xia: She is currently working as an attending doctor at the Department of Endocrinology, the First People's Hospital of Yongkang, Yongkang City , Zhejiang Province, China.

Hui-Shang Wang: She is currently working as an attending doctor at the Department of Nephrology, the First People's Hospital of Yongkang, Yongkang City, Zhejiang Province, China

Cite this article: Huang R, Xu L, Chen T, Li K, Xia J, Wang H. *Epimedii folium* Polysaccharide Ameliorated Glucose Metabolic Disorder in Type 2 Diabetic Mice by Regulating the SIRT1/PPARγ Signaling Pathway. Indian J of Pharmaceutical Education and Research. 2022;56(2s):s274-s280.