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Research Article

Formononetin suppresses hyperglycaemia through activation of GLUT4-AMPK pathway

Esam Qnais[1], Abdelrahim Alqudah[2], Mohammed Wedyan[1], Omar Gammoh[3], Hakam Alkhateeb[4], Mousa Al-Noaimi[5]

1 *Department of Biology and Biotechnology, Faculty of Science, The Hashemite University, Zarqa, Jordan*
2 *Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmaceutical Sciences, The Hashemite University, Zarqa, Jordan*
3 *Department of Clinical Pharmacy and Pharmacy Practice, Faculty of Pharmacy, Yarmouk University, Irbid, Jordan*
4 *Department of Basic Medical Sciences, Faculty of Medicine, Yarmouk University, Irbid, Jordan*
5 *Chemistry Department, Faculty of Science, Kuwait University, P.O. Box 5969, Safat, 13060, Kuwait City, Kuwait*

Corresponding author: Abdelrahim Alqudah (abdelrahim@hu.edu.jo)

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Abstract

Background: Formononetin (FMN) is a flavonoid that has different pharmacological effects. Thus, the anti-diabetic effects of FMN has been investigated in a high-fat diet/Streptozotocin-(HFD/STZ)-induced diabetes mice model.

Methods: Mice were fed with HFD followed by STZ. Diabetic mice were treated orally with FMN or metformin for 28 days before collecting plasma and soleus muscle for further analysis.

Results: FMN reduced serum glucose (p>0.001) and increased serum insulin in diabetic group compared to the vehicle control. Additionally, FMN decreased homeostasis model assessment of insulin resistance (HOMA-IR). Fasting glucose level was also reduced with FMN during the intraperitoneal glucose tolerance test (IPGTT). GLUT4 and p-AMPK-α1 were upregulated following treatment with FMN. LDL, triglyceride, and cholesterol were reduced in diabetic mice treated with FMN. FMN reduced MDA, increased GSH levels , and reduced GSSG levels in diabetic mice.

Conclusion: FMN could represent a promising therapeutic agent to treat T2D.

Keywords

Formononetin, Insulin resistance, Diabetes, Oxidative stress

Introduction

Diabetes is a disorder involving multiple complications affecting more than 537 million people throughout the world and 783 million people are projected to be affected by 2045, out of which 87–91% of the population is suffering from type 2 diabetes mellitus (Sun et al. 2022). Diabetes mellitus type 2 (T2D) is characterized by insulin resistance and insufficient insulin secretion from the pancreatic β cells that generally occurs in adults 40 years old or above who are obese or overweight (Asmat et al. 2016).

Insulin helps to take up glucose from the blood and use it for energy. This happens mainly in the muscles, where the insulin-sensitive glucose transporter-4 (GLUT4) helps to take up glucose from the blood. AMP-activated protein kinase (AMPK) activation helps to increase the number

of GLUT4 proteins on the cell surface, which then helps to facilitate glucose uptake in muscle. Therefore, the activation of the AMPK-GLUT4 pathway is an effective way to improve insulin sensitivity in T2D (Dimitriadis et al. 2011; Vlavcheski et al. 2018).

Type 2 diabetes (T2D) and associated consequences are caused by inflammation and oxidative stress. These problems damage the pancreas and lead to insulin resistance and T2D complications. Obesity is associated with increased levels of pro-inflammatory mediators, which may play a role in the development of type 2 diabetes and its complications (Oguntibeju 2019).

Formononetin (FMN) is found in many medicinal plants, such as *Astragalus membranaceus*, *Trifolium pratense L.* and *Pueraria lobata* (Willd.) (Heinonen et al. 2004; He et al. 2011; Zhang et al. 2018). It is stated that FMN can help the fangchinoline-induced insulin release in streptozotocin-diabetic mice, and it indicated that FMN may exhibit anti-hyperglycemic activity. Furthermore, in vitro study demonstrated the protective effect of FMN by inhibiting pancreatic beta-cell apoptosis. This effect was produced by the downregulation of nuclear factor kappa B (NF-kB) and decreased nitric oxide production.

In addition, another study demonstrated the antihyperglycemic effect of FMN in alloxan-induced type-1 diabetes in mice (Qiu et al. 2016). In this study, the anti-hyperglycemic effect and mechanism of FMN against alloxan-induced type 1 diabetes in mice were studied by determining its activity on several diabetes-related indices in pancreas tissue including body weight, fasting blood glucose, glucose tolerance, hepatic glycogen, serum insulin, serum glucagon, Fas, Caspase-3, pancreatic and duodenal homeobox-1, insulin receptor substrate 2, glucokinase, glucose transporter 2 (GLUT2) mRNA, and proteins levels. GK and glucose-6-phosphatase (G-6-P) mRNA and protein levels were also determined in liver tissue. The results of this work showed that FMN exhibited anti-hyperglycemic activity in alloxan-induced type 1 diabetic mouse by inhibiting islet B cell apoptosis and promoting islet B cell regeneration, insulin secretion, hepatic glycogen synthesis, and hepatic glycolysis.

Moreover, FMN has been reported to have anti-inflammatory and antioxidant activities (Fang et al. 2020). Many mechanisms have been proposed to explain how FMN works to reduce inflammation in vitro such as inhibition of eicosanoid-generating enzymes or the modulation of the production of pro-inflammatory molecules (Machado Dutra et al. 2021). In addition, Oza, M, & Kulkarni, Y reported that the treatment of rats with FMN for 28 days with different concentrations, decreased insulin resistance and reduced hyperglycemia in type 2 diabetes mellitus. The increased expression of SIRT1 in pancreatic tissues is what causes this effect (Oza and Kulkarni 2018).

In the clinical investigation, postmenopausal women and obese men who were treated with FMN and other isoflavones had improved systemic arterial compliance, reduced arterial stiffness, and lowered blood pressure (Nestel et al. 1999, 2007). Shen et al. also reported that FMN is a potent activator of peroxisome proliferator–activated receptors PPAR alpha and PPAR gamma which are important regulators of glucose metabolism and dyslipidemia (Shen et al. 2006). According to the studies stated above, FMN is strongly linked to the control of lipid and glucose metabolism through several pathways.

To date, several natural products have been reported to exhibit significant antidiabetic activity via targeting AMPK and GLUT4. To the best of our knowledge, the stimulation of AMPK and increased GLUT4 translocation by formononetin has not been investigated or reported to date. Based on this observation it has been hypothesized that formononetin may provide a beneficial effect in type 2 diabetes mellitus partly by activating AMPK- GLUT4 pathway in a high-fat diet (HFD)/STZ-induced mouse model in different tissues.

Materials and methods

Induction of T2D and experimental design

Six-week-old male C57BL/6 mice were maintained under standard conditions including 12-hour light/dark cycles and at 22 ± 2° temperature (Al-Ghaithi et al. 2004). T2D was induced by feeding the experimental mice HFD (60% fat, D14292, Research Diets, Inc., New Brunswick, NJ, USA) for 9 weeks followed by intraperitoneal injection of STZ (40 mg/Kg). at week 10, mice were administered another intraperitoneal low dose of STZ (40 mg/kg) to complete the induction of diabetes. The HFD/STZ-induced diabetes model is a well-established model for diabetes in which HFD feeding will lead to obesity, hyperinsulinemia, and altered glucose homeostasis due to insufficient compensation by the beta cells of the pancreatic islets. A single high dose of STZ causes sudden and significant destruction of pancreatic cells, however, progressive multiple low doses of STZ after HFD as the model of this study causes less destruction of pancreatic cells which portraits the same characteristics and mimics the pathogenesis and clinical features of T2D in human (Akinlade et al. 2021; Lertpatipanpong et al. 2021). One week after STZ injection, plasma glucose was measured and mice with a plasma glucose concentration of >200 mg/dl were considered to have developed T2D and selected for the subsequent experiments.

Mice were randomly divided into four groups (n=6 each) as follows: i) the normal control group (non-diabetic, ND) received a normal diet, ii) the vehicle control (VC) diabetic group treated with dimethyl sulfoxide (DMSO, Panreac Quimica SA, Spain) only, iii) diabetic group treated with 20 mg/kg FMN (Qiu et al. 2016) (Sigma-Aldrich, Germany), and iv) diabetic group treated with 200 mg/kg Metformin (MeRCK, Germany). After 28 days of treatment, mice were fasted overnight (16 h) and then sacrificed using a CO_2 chamber, blood and skeletal muscle (soleus muscle) were collected for ex-vivo analysis.

Biochemical investigations

Measurement of serum glucose, insulin, and lipids

Serum glucose was determined using a commercial kit (Glucose assay kit, MyBioSource, USA). Serum insulin was measured by ELISA using a commercial kit (mouse insulin ELISA kit, MyBioSource, USA). Triglyceride (TG, triglyceride assay kit), low-density lipoprotein (LDL, LDL assay kit), and cholesterol (Total Cholesterol assay kit) were determined using commercially available kits (MyBioSource, USA) according to the manufacturer's instructions.

Homeostasis Model Assessment of Insulin Resistance (HOMA-IR)

This model represents the interaction between fasting plasma insulin and fasting plasma glucose which is a useful tool for determining insulin resistance. According to the international diabetes federation, the HOMA-IR cut-off level in healthy individuals is less than 1, in men with diabetes is 1.55, and in women with diabetes is 2.22 (Gayoso-Diz et al. 2013). Studies showed that normal HOMA-IR in healthy mice is 1.9 and in STZ mice is 21.6 (Yu et al. 2013).

In the current study, we used the following formula to compute HOMA-IR:

$$\text{HOMA-IR}=(\text{Fasting glucose (mg/dl)} \times \text{Fasting insulin (μIU/ml)})/405 \text{ (Yoon et al. 2016)}$$

The constant 405 is a normalizing factor representing the result of the multiplication of the normal fasting plasma insulin level (μU/mL) with the normal fasting plasma level (81 mg/dl) (Muniyappa et al. 2008).

Intraperitoneal glucose tolerance test

Mice were given an intraperitoneal injection of glucose (0.5 g/kg) after being fasted for 18 h. Using a glucometer, blood glucose levels were measured from the tail vein at 0, 30, 60, and 120 minutes (Accu-Check Performa, Roche Diagnostics).

Measurement of serum reduced glutathione (GSH), oxidized glutathione (GSSG), and malondialdehyde (MDA)

Reduced glutathione (GSH, GSH assay kit) and oxidized glutathione (GSSG, GSSG assay kit) were measured in the serum using commercially available kits (Mybiosource, USA). Plasma MDA level was determined by using commercial Thiobarbituric acid (TBA) Assay Kit (MyBioSource, USA) according to the manufacturer's instructions.

Western blotting

Skeletal muscle tissues (soleus muscle) were homogenized in radioimmunoprecipitation (RIPA)-lysis buffer, containing a protease inhibitor cocktail (Santa Cruze Biotechnology, USA) using a tissue homogenizer. Homogenates were centrifuged at 12,000 g for 20 minutes at 4 °C and the supernatant was collected. The total protein was quantified using bicinchoninic acid assay kit (Bioquochem, Spain). An equal amount of protein was separated by sodium dodecyl sulfate-polyacrylamide gel and then transferred to a nitrocellulose membrane (Thermo Fisher Scientific, USA). The membrane was blocked for 1 hour at room temperature using 3% bovine serum albumin (BSA) before incubating overnight with either phosphorylated AMPK-α1 (p-AMPK-α1, Abcam, UK) or GLUT4 (MyBioSource, USA) primary antibodies (1:1000 dilution). The membrane was washed three times with washing buffer (Tween-20/Tris-buffered saline) before incubating it with the goat-anti-rabbit secondary antibody (MyBioSource, USA, 1:5000 dilution) for 1 hour at room temperature. Following incubation, the membrane was washed three times before submerging into the ECL substrate (ThermoScientific, USA) for one minute followed by imaging with chemiLITE Chemiluminescence Imaging System (Cleaverscientific, UK). To ensure equal protein gel loading, β-actin was used as a housekeeping gene (MyBioSource, USA, 1:10000 dilution). The intensity of the bands was measured using Image J software and adjusted to β-actin.

Statistical analysis

All zanalyzed parameters were tested for the normality of the data using the Kolmogorov-Smirnov test. Data are represented as mean±SEM. Differences between groups were calculated using one-way analysis of variance (ANOVA) followed by Tukey posthoc using GraphPad Prism software version (9.3.1). The significance value of difference was considered when P value <0.05.

Data availability statement

Data will be made available upon request from the corresponding author.

Results

The hypoglycaemic effect of Formononetin

Serum glucose was significantly higher in the vehicle control diabetic group compared to the non-diabetic group (Fig. 1A, n=6, p<0.001). Treatment with FMN significantly reduced the serum glucose levels compared to the vehicle control group in the presence of T2D (Fig. 1A, n=6, p<0.001). Similarly, the serum glucose level in the diabetic group was significantly reduced with metformin treatment compared to the vehicle control (Fig. 1A, n=6, p<0.001). Insulin levels were significantly reduced in the vehicle control diabetic group compared to the non-diabetic group (Fig. 1B, n=6, p<0.01), however, treating diabetic mice with FMN significantly increased insulin levels compared

to the vehicle control group (Fig. 1B, n=6, p<0.05). Treating diabetic mice with metformin significantly increased insulin levels compared to the vehicle control group (Fig. 1B, n=6, p<0.05). No difference was observed between FMN and metformin in terms of glucose and insulin.

To study the effect of FMN on insulin resistance, HOMA-IR was measured. The presence of T2D was confirmed by HOMA-IR, which was significantly increased in the vehicle control diabetic group compared to the non-diabetic group (Fig. 1C, n=6, p<0.001). Interestingly, FMN was able to restore HOMA-IR in the diabetic group, which was comparable to the non-diabetic group. The same effect on HOMA-IR was observed when diabetic mice were treated with metformin. HOMA-IR was not different between FMN and metformin groups. Moreover, blood glucose level during IPGTT was significantly lower in FMN and metformin groups compared to vehicle control (Fig. 2, n=6, p<0.001).

To determine the mechanism by which FMN improves blood glucose and insulin resistance, GLUT4 and AMPK protein expression in skeletal muscle tissue were measured. GLUT4 protein expression was significantly downregulated in the presence of T2D (Fig. 3A, n=6, p<0.001), however, treating diabetic mice with FMN significantly upregulated GLUT4 expression compared to the vehicle control diabetic group (Fig. 3A, n=6, p<0.001). Metformin also was able to upregulate GLUT4 expression compared to the vehicle control diabetic group (Fig. 3A, n=6, p<0.001). Similarly, p-AMPK-α1 expression was significantly downregulated as a result of T2D (Fig. 3B, n=6, p<0.001), and treating diabetic mice with either FMN or metformin upregulated AMPK expression compared to the vehicle control diabetic group (Fig. 3B, n=6, p<0.01, <0.001, respectively). No difference was observed in GLUT4 and AMPK expression between FMN and metformin groups.

The effect of Formononetin on lipid profile

As depicted in Fig. 4, dyslipidemia was present in diabetic mice. LDL (Fig. 4A, n=6, p<0.001), total cholesterol (Fig. 4B, n=6, p<0.001), and triglycerides (TGs; Fig. 4C, n=6, p<0.001) were significantly higher in the vehicle control diabetic group compared to the non-diabetic group. Treating diabetic mice with FMN significantly reduced serum LDL (Fig. 4A, n=6, p<0.001), total cholesterol (Fig. 4B, n=6, p<0.01), and TGs (Fig. 4C, n=6, p<0.001) levels compared to the vehicle control diabetic group. Similarly, metformin was able to reduce LDL (Fig. 4A, n=6, p<0.05), cholesterol (Fig. 4B, n=6, p<0.01), and triglyceride (Fig. 4C, n=6, p<0.001) levels significantly in diabetic mice in comparison to the vehicle controls.

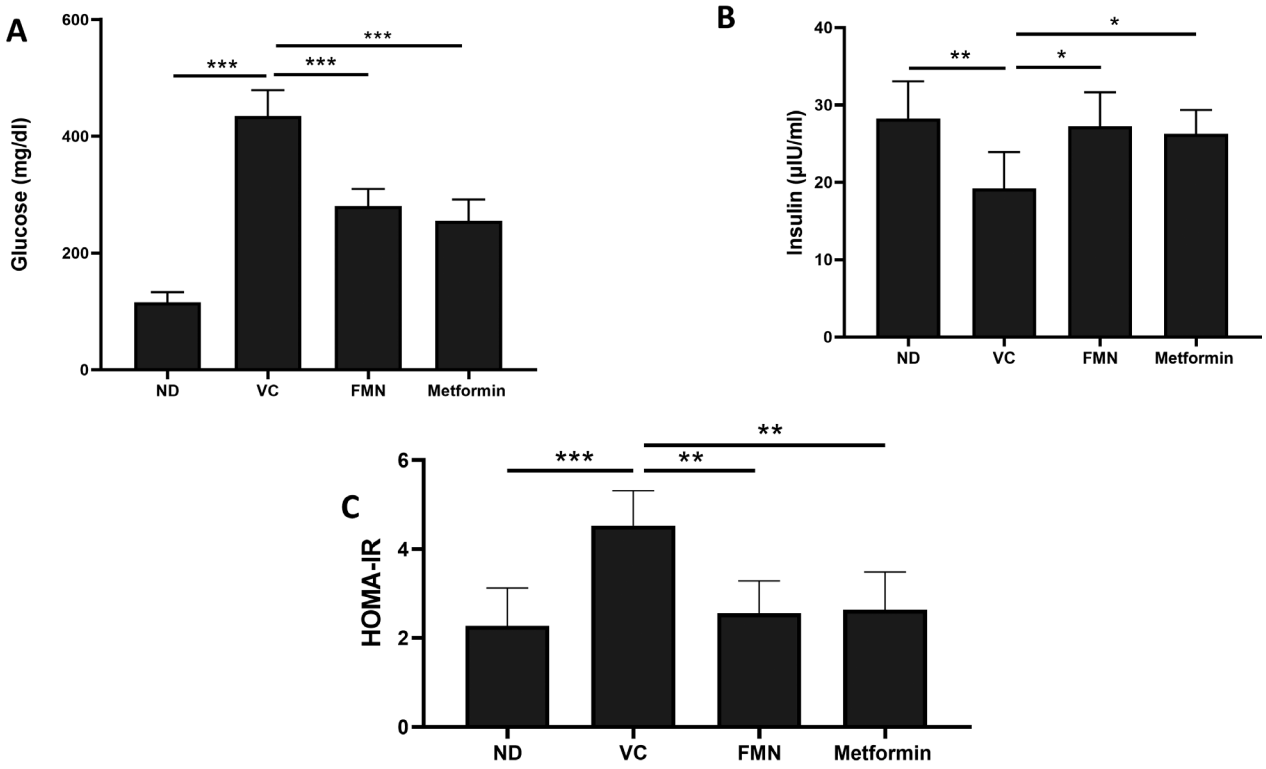

Figure 1. The anti-diabetic effect of FMN. FMN significantly reduced glucose (**A**) and increased insulin (**B**) levels in diabetic mice. HOMA-IR (**C**) was significantly reduced with FMN treatment. Mice were fed with HFD for 9 weeks followed by two low doses of STZ injection (40 mg/kg) after diabetes was confirmed, mice were treated with 20 mg/kg FMN or 200 mg/kg metformin for 28 days, mice were then sacrificed, and serum collected for ELISA analysis. One-way ANOVA followed by Tukey post hoc, *<0.05, **<0.01, ***<0.001. VC; vehicle control.

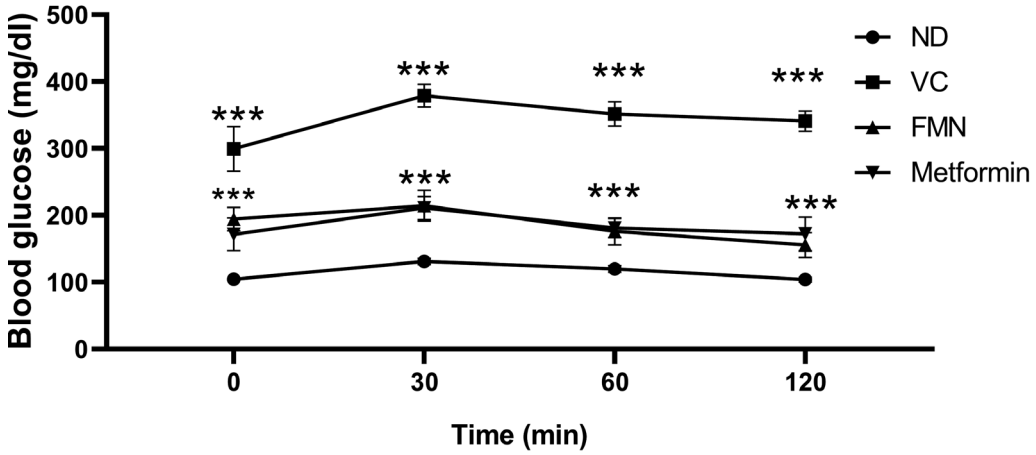

Figure 2. FMN reduced glucose level during IPGTT. Mice were fed with HFD for 9 weeks followed by two low doses of STZ injection (40 mg/kg) after diabetes was confirmed, mice were treated with 20 mg/kg FMN or 200 mg/kg metformin for 28 days, mice were then fasted overnight before injection with 0.5 g/kg glucose intraperitoneally, and glucose level determined at 0, 30, 60, and 120 min. Two-way ANOVA followed by Tukey post hoc, ***<0.001. VC; vehicle control.

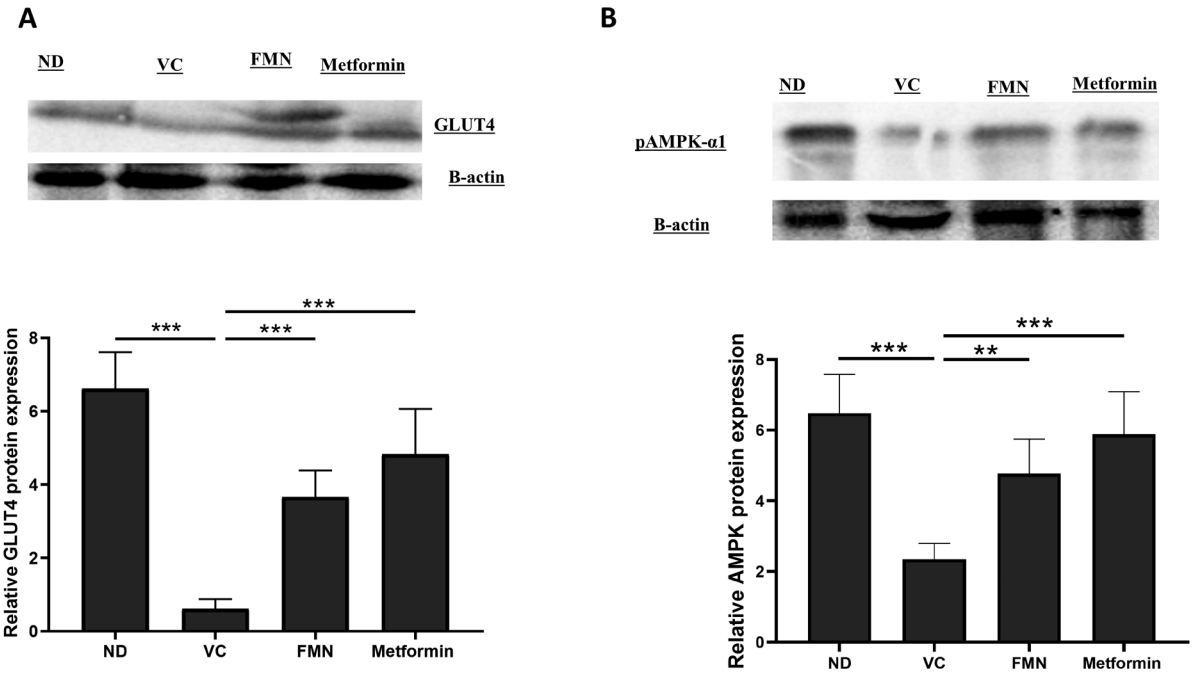

Figure 3. FMN upregulated GLUT4 and AMPK expression. FMN significantly upregulated GLUT4 (A), and AMPK (B) expression in diabetic mice. Mice were fed with HFD for 9 weeks followed by two low doses of STZ injection (40 mg/kg), after diabetes was confirmed, mice were treated with 20 mg/kg FMN or 200 mg/kg metformin for 28 days, mice were then sacrificed, and soleus muscle was isolated and homogenized before western blotting performed. One-way ANOVA followed by Tukey post hoc, **<0.01, ***<0.001. VC; vehicle control.

The effect of Formononetin on GSH, GSSG, and MDA

Serum GSH expression was significantly reduced in vehicle control diabetic mice compared to non-diabetic mice (Fig. 5A, n=6, p<0.001), however, treating diabetic mice with either FMN or metformin demonstrated a significant increase in GSH compared to the vehicle control diabetic group (Fig. 5A, n=6, p<0.01, 0.001, respectively). Moreover, serum GSSG has significantly increased in vehicle control diabetic mice compared to non-diabetic mice (Fig. 5B, n=6, p<0.001), however, FMN and metformin were able to reduce GSSG levels in diabetic mice compared to vehicle control (Fig. 5B, n=6, p<0.001). On the other hand, serum MDA (Fig. 5C, n=6, p<0.001) concentrations were significantly increased in the presence of T2D. Interestingly, FMN showed an ability to reduce serum MDA levels significantly in diabetic mice in comparison to the vehicle control group (Fig. 5C, n=6, p<0.001). The same effect was observed when diabetic mice were treated with metformin (Fig. 5C, n=6, p<0.001).

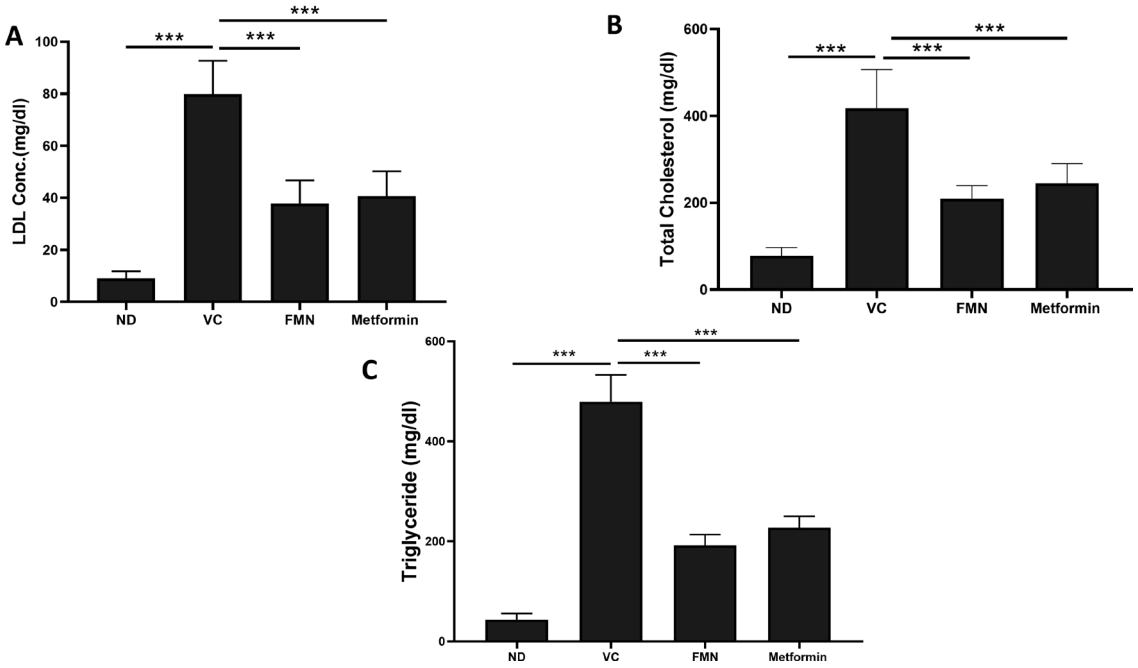

Figure 4. FMN improves lipid profile in diabetes. FMN significantly reduced LDL (**A**), cholesterol (**B**), and triglyceride (**C**) levels in diabetic mice. Mice were fed with HFD for 9 weeks followed by two low doses of STZ injection (40 mg/kg) after diabetes was confirmed, mice were treated with 20 mg/kg FMN or 200 mg/kg metformin for 10 days, mice were then sacrificed, and serum collected for ELISA analysis. One-way ANOVA followed by Tukey post hoc, ***<0.001. VC; vehicle control.

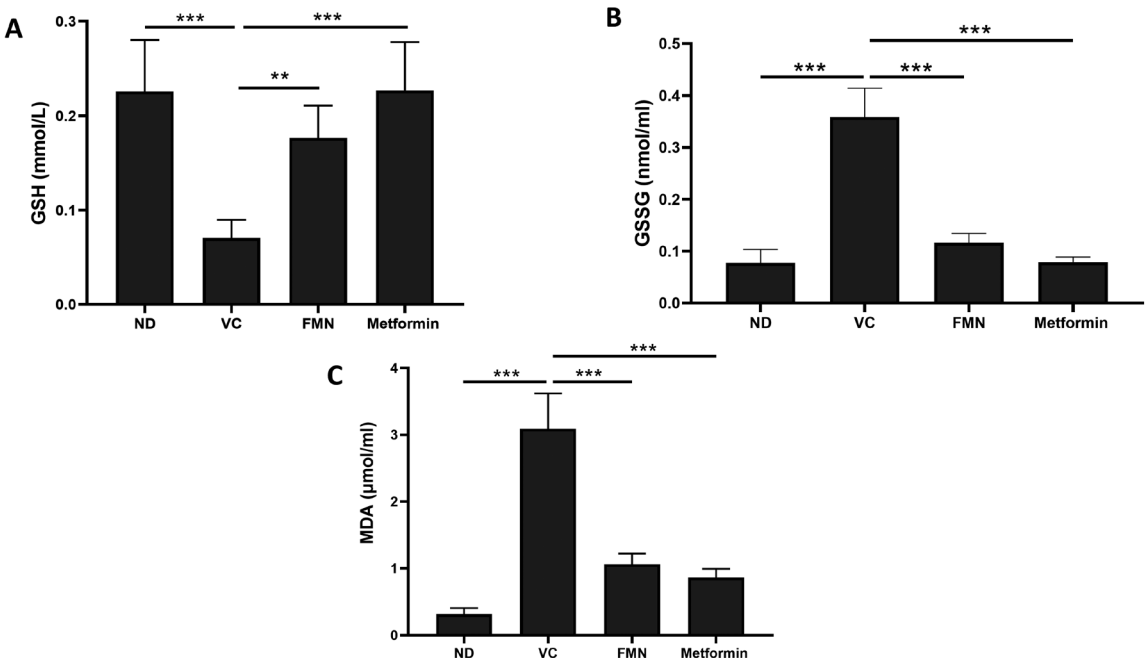

Figure 5. The antioxidant effect of FMN. FMN significantly increased GSH (**A**), reduced GSSG (**B**), and MDA (**C**) levels in diabetic mice. Mice were fed with HFD for 9 weeks followed by two low doses of STZ injection (40 mg/kg) after diabetes was confirmed, mice were treated with 20 mg/kg FMN or 200 mg/kg metformin for 10 days, mice were then sacrificed, and serum collected for ELISA analysis. One-way ANOVA followed by Tukey post hoc, **<0.01, ***<0.001. VC; vehicle control.

Discussion

High blood sugar levels and insulin resistance are the main symptoms of T2D, and these conditions can also lead to nephropathy, retinopathy, neuropathy, and cardiovascular disease (Babiker and Dubayee 2017; Care and Suppl 2019). Our study's findings showed that FMN significantly decreased glucose levels and increased insulin levels, and that was on par with the industry standard drug metformin. This suggests that FMN is a viable new hypoglycemic agent. In addition to lowering blood glucose levels and increasing insulin levels, FMN improved

insulin sensitivity in diabetic mice which was shown by reducing HOMA-IR levels in diabetic mice.

Previous research showed that the high-fat diet (HFD), which is rich in saturated fatty acids, decreased the absorption of glucose by cells and increased insulin resistance. Notably, saturated fatty acids promote the buildup of lipids in the muscles, leading to insulin resistance (Kennedy et al. 2009; Sears and Perry 2015). For instance, palmitate, a saturated fatty acid, encourages the release of cytokines like IL-6 and TNF-, which can cause insulin resistance and glucose intolerance (Korbecki and Bajdak-Rusinek 2019). GLUT4 expression is downregulated by HFD, leading to the development of glucose intolerance (Yasmin et al. 2021). According to reports, the AMP protein kinase (AMPK) is significantly involved in controlling cellular energy metabolism. Insulin resistance and other metabolic diseases are linked to its dysfunction (Rena et al. 2013). The AMP/ATP ratio is changed by metformin, which activates AMPK via phosphorylation and enhances glucose consumption (Rena et al. 2013). HFD, however, is connected to a decline in AMPK phosphorylation, which lowers glucose uptake. It has been demonstrated that metformin increases GLUT4 expression and AMPK activation through phosphorylation, enhancing cellular absorption of glucose. It is necessary to conduct more research to determine whether the mechanism of action of FMN in lowering hyperglycemia is similar to that of metformin. The mammalian target of rapamycin (mTOR) is a serine and threonine protein kinase that has an established role in insulin resistance and AMPK directly phosphorylates Raptor, which is a component of mTORC1, to repress mTORC1 (Tuo and Xiang 2019). FMN may also increase insulin sensitivity by reducing the expression of mTOR, according to a recent study, which needs more research to be conducted in such type of diabetes model (Zhou et al. 2019). The levels of p-AMPK and GLUT4 in skeletal muscle were measured to study the hypoglycemic mode of action of FMN. Skeletal muscle makes up about 45–50% of the body's bulk and transports 80% of the body's glucose (Rivas and Fielding 2012). AMPK controls the transcription of the GLUT-4 gene in skeletal muscles (Zheng et al. 2001). To maintain blood glucose homeostasis, GLUT-4 is a crucial glucose transporter that carries extracellular glucose to cells that are responsive to insulin. In addition, decreased GLUT-4 expression in skeletal muscle and suppression of GLUT4 translocation lead to inadequate glucose transport and consequent insulin resistance. Activation of the AMPK– GLUT4 pathway increases insulin sensitivity which improves glucose control in T2D (Viollet et al. 2009). Additionally, the regulation of insulin signaling and GLUT-4 activity, together with the role of AMPK in the prevention of T2D, have all been studied previously (Entezari et al. 2022). Our findings show that FMN upregulated p-AMPK-α1 and GLUT4 expression in skeletal muscle, suggesting that FMN could activate AMPK-GLUT4 pathway.

On the other hand, T2D is linked to dyslipidemia in addition to hyperglycemia. Diabetic dyslipidemia is defined by high postprandial TGs, total cholesterol, and LDL (Wu and Parhofer 2014). The main causes of the complications linked to T2D are these lipid changes (Eid et al. 2019). In particular, dyslipidemia is a substantial risk factor for macrovascular diabetes complications. Numerous studies have connected dyslipidemia to the microvascular consequences of T2D, including diabetic retinopathy, diabetic nephropathy, and diabetic neuropathy (Wu and Parhofer 2014). According to our research, FMN decreased TGs, total cholesterol, and LDL in our T2D model, indicating that it may reduce the risk of cardiovascular disease that is linked with T2D. Future research ought to examine this.

The development of T2D and its complications are strongly correlated with oxidative stress, according to numerous clinical and experimental investigations (Oguntibeju 2019). Reactive oxygen species (ROS) production and a slowdown in the pace of antioxidant defense mechanisms, such as GSH, cause oxidative stress, which is defined as a decreased tolerance between oxidants and antioxidants (non-enzymatic antioxidant) (Chikezie et al. 2015). Lipid peroxidation, such as that caused by low-density lipoprotein (oxLDL) or polyunsaturated fatty acid peroxidation, can result from the lipids being damaged by ROS (oxPUFAs). Furthermore, MDA, a highly reactive substance that interacts with protein and nucleic acid and damages a variety of tissues and cells, is released as a result of ROS (Su et al. 2019). MDA has been utilized as a blood biomarker for lipid peroxidation and as a sign of damage caused by free radicals (Morales and Munné-Bosch 2019). Our results demonstrate that FMN significantly lowers plasma MDA levels and elevates GSH, suggesting that FMN may be useful as an antioxidant in T2D by lowering lipid peroxidation or elevating free radical scavenging activity.

In conclusion, when compared to the vehicle control group, FMN can lower serum glucose levels and normalize insulin in the diabetic group. Additionally, following IPGTT, the FMN group had a lower fasting glucose level. Additionally, the HOMA-IR value was decreased by FMN in diabetic mice compared to vehicle control. These effects could be attributed to an increase in p-AMPK-α1 levels, which boosts GLUT4 translocation to the cell surface and encourages glucose uptake in skeletal muscles, thus enhancing insulin sensitivity. In addition, when compared to the vehicle control, FMN decreased LDL, cholesterol, and triglyceride levels in diabetic mice. This effect may be attributable to the activation of AMPK, which has been shown to promote glucose and fatty acid catabolism and inhibit protein and fatty acid synthesis (Angin et al. 2016). The improvement in insulin sensitivity seen in our study may be attributable to the decrease in LDL, cholesterol, and triglycerides, which may also play a significant role in lowering insulin resistance (Shirali et al. 2013; Ye 2013). Additionally, FMN increased GSH levels and decreased MDA and GSSG, indicating that it may have an antioxidant effect on T2D. These results are consistent with earlier research that found that FMN improved the activity of the antioxidant enzymes superoxide

dismutase (SOD) and catalase (CAT) in streptozotocin-induced diabetic mice, reducing MDA levels and raising intracellular GSH levels (Mu et al. 2009; Li et al. 2014; Wang et al. 2018). FMN suppressed the NF-κB signaling pathway, according to a different study, which decreased levels of inflammatory mediators such as IL-6, ICAM-1, and TNF-α (Kim et al. 2019). Future research is needed to determine if the antioxidant and anti-inflammatory effects of FMN are due to the activation of antioxidant enzymes and inhibition of the NF-κB signaling pathway.

These are some of how this study has limitations: 1- We only assessed GLUT4 expression in skeletal muscles; liver and adipose tissue should also be used for this., 2- For a brief length of time, FMN was given., 3- GLUT4 expression was assessed using immunoblotting reflective of its total amount, immunohistochemistry may be a better technique to assess its activity and translocation to the cell membrane, 4- More research should be done on the antioxidant and anti-inflammatory properties of FMN in T2D. However, this study's results—the first to do so—indicate the critical part FMN plays in enhancing T2D's usual features.

Conclusions

In conclusion, our results demonstrated that FMN could be a very useful hypoglycaemic agent for the treatment of T2D due to its multifactorial effects including i) the reduction in insulin resistance, ii) increase in glucose uptake by the skeletal muscle, iii) improvement in the lipid profile, iv) reduction in oxidative stress and v) the activation of the GLUT4-AMPK pathway. The effects and mechanisms demonstrated by FMN were very similar to metformin.

Author contributions

Conceptualization, AA, EQ, and MW; methodology, H.A.K and OG software, OG.; validation, EQ., MW. And AA.; formal analysis, M.A.N.; investigation, AA.; resources, M.A.N.; data curation, OG.; writing—original draft preparation, M.A.N.; writing—review and editing, AA.; visualization, EQ.; supervision, A.A..; project administration, A.A., and O.G..; funding acquisition, EQ. All authors have read and agreed to the published version of the manuscript.

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Institutional review board statement

Animal experimental procedures were approved by the animal ethics committee at the Hashemite University (IRB number: 14/4/2021/2022, 24/01/2022) and were in accordance with the guidelines of the U.S. National Institutes of Health on the use and care of laboratory animals and with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines (https://arrive-guidelines.org).

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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