

Vitamin E Augments Metformin Efficacy in Reversing Dexamethasone-Induced Glucose Dysregulation, Dyslipidemia, and Oxidative Stress via GLUT-4 Modulation in Mice

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Article

Keywords: insulin resistance, GLUT-4, Vitamin E, metformin, synergistic effect

Posted Date: October 19th, 2026

DOI: <https://doi.org/10.21203/rs.3.rs-11175270/v1>

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Additional Declarations: No competing interests reported.

Abstract

Insulin resistance, characterized by dyslipidemia, hyperglycemia, and oxidative stress, contributes to the development of type 2 diabetes. Micronutrient Vitamin E as an antioxidant along with Metformin can have synergistic effects on improving insulin resistance. The present study aimed to evaluate whether vitamin E enhances the effects of metformin on GLUT-4 expression, dyslipidemia, and oxidative stress in a mouse model of dexamethasone-induced insulin resistance. Experimental animals were divided into five groups: control; Control, D (dexamethasone 1mg/kg intraperitoneally; IP for 3 weeks), M (metformin 250 mg/kg orally for last 2 weeks of dexamethasone treatment), E, (Vitamin E 250 mg/kg orally for last 2 weeks of dexamethasone treatment), EM (metformin+Vitamin E for last 2 weeks of dexamethasone treatment). At the end of experimental period, oral glucose tolerance test (OGTT) is done. Then Blood samples were collected under deep anesthesia to measure glucose, insulin, lipid profile and liver enzymes. Hepatic oxidative stress markers and muscular GLUT4 Protein level were investigated by immunohistochemistry. Findings demonstrated that the EM group indicated significantly superior improvements in hyperglycemia and oral glucose tolerance (OGTT) ($P < 0.0001$) compared to monotherapy. Furthermore, combined treatment effectively reduced insulin levels and mitigated oxidative stress, evidenced by increased Superoxide Dismutase (SOD) and decreased Malondialdehyde (MDA) markers ($P < 0.01$). Muscular GLUT-4 protein expression was also markedly enhanced in the EM group ($P < 0.001$), although no significant differences were noted in lipid profiles between treatment groups. Overall, these findings suggest that vitamin E supplementation effectively potentiates the beneficial impact of metformin on insulin resistance and its associated metabolic disturbances.

Introduction

Major character of metabolic syndrome is Insulin resistance (IR), which progressively contributes to development of type 2 diabetes mellitus (T2DM). In individuals with IR, the responsiveness of target tissues, including skeletal muscle, adipose tissue, and the liver to insulin is reduced. Insulin resistance is often accompanied by compensatory hyperinsulinemia, hyperglycemia, dyslipidemia, oxidative imbalance, and impaired glucose tolerance (Chandrasekaran and Weiskirchen, 2024, Lee et al., 2022). At the molecular level, insulin resistance is characterized by reduced GLUT-4 expression and impaired GLUT-4 translocation in skeletal muscle, where GLUT-4 plays an essential role in insulin-stimulated glucose uptake (Chandrasekaran and Weiskirchen, 2024, van Gerwen et al., 2023).

Increased secretion of endogenous glucocorticoids or their prolonged use as synthetic drugs for various therapeutic purposes can lead to insulin resistance (Batista et al., 2024, Hamed et al., 2025). So, for induction of insulin resistance, dexamethasone is widely used in laboratory animals. Chronic administration of dexamethasone, a synthetic glucocorticoid that antagonizes insulin action, can lead to significant changes in glucose and lipid metabolism, increased oxidative stress, hepatic dysfunction, and reduced expression of GLUT-4 in skeletal muscle. Oxidative stress and of reactive oxygen species (ROS) are important contributors to cellular damage and metabolic disturbances in this model (Batista et al., 2024, Mahamad et al., 2020, Rouzi et al., 2026).

Antioxidant and anti-inflammatory nutritional agents, such as vitamin E, a potent fat-soluble antioxidant (Guan et al., 2015), have been shown to exert protective effects against oxidative stress induced by obesity, streptozotocin (STZ), and dexamethasone by inhibiting lipid peroxidation and enhancing the body's antioxidant defense system (Williams et al., 2012, Shen et al., 2009, Gren, 2013). On the other hand, It has been reported that dietary Vitamin E in combination with C supplementation in rats can almost completely prevent dexamethasone-induced glucose intolerance by reducing hyperglycemia and hyperinsulinemia (Williams et al., 2012). Additionally, Vitamin E has been shown to reduce glucose, insulin and triglycerides (TG) level in obesity (Shen et al., 2009). In another study, Vitamin E supplementation ameliorated the lipid metabolism in mice model of nonalcoholic fatty liver (He et al., 2019) and obese rats (Picklo and Thyfault, 2015) by reduction of TG, low-density lipoprotein-cholesterol (LDL-c) and total Cholesterol (TC) and elevation of high-density lipoprotein-cholesterol (HDL-c). Also, Vitamin E increased the expression level of GLUT-4 in cell treated with asymmetric dimethyl arginine (ADMA) (Yang et al., 2009).

On the other hand, metformin, a first-line drug for the treatment of type 2 diabetes, is able to regulate glucose and fat metabolism by enhancing insulin sensitivity, activating the AMPK pathway, and increasing GLUT-4 expression (Thomas et al., 1998, Dutta et al., 2023). Given the critical role of skeletal muscle in glucose homeostasis and the known effects of dexamethasone on reducing GLUT-4 expression, investigating the co-administration effect of vitamin E and metformin can provide new insights into the cellular and biochemical mechanisms underlying insulin resistance (Herman et al., 2022).

Therefore, the present study aimed to evaluate whether vitamin E enhances the effects of metformin on skeletal muscle GLUT-4 protein expression, lipid biochemical parameters, and oxidative stress markers in a mouse model of dexamethasone-induced insulin resistance.

Material and methods

Animals and experimental design

Twenty-five albino mice weighing 25–30 g were included in the study and housed in animal polypropylene cage (five mice per cage) under standard condition at $26 \pm 2^\circ\text{C}$ with relative humidity 44–56% along with 12:12 h light/dark cycle. Animals received standard chow and water ad libitum. The methods of the present study have been reported in accordance with ARRIVE guideline (Percie du Sert et al., 2020). All procedures were performed in the present study were in accordance with the local Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (IR.MAZUMS.4.AEC.1404.069) and in accordance with the guidelines for the care and use of laboratory animals (National Institutes of Health Publication No. 80–23, revised 1996).

Prior to the study, all experimental animals were acclimatized for one week under standard laboratory conditions. Insulin resistance was induced by intraperitoneally (IP) injection of dexamethasone (Raha

Pharmaceutical Co., IR) 1mg/kg for 7 days (one week) (Batista et al., 2024). To confirm the induction of insulin resistance by dexamethasone, on the eighth day after placing the mice in fasting conditions, a blood glucose tolerance test was performed in control and dexamethasone groups, and blood glucose was measured by a glucometer. Then, mice with glucose intolerance were included in the study and randomly divided into the five groups (n = 5/group): Control group (C), only received drug solvent daily. Dexamethasone group (D), continued the receiving dexamethasone 1mg/kg/day IP for two next weeks. In the metformin group (M), dexamethasone was administered for three weeks; from the second week onward, metformin (250 mg/kg; Shafa Pharmaceutical Co., Iran) was also provided orally via gavage once daily for two weeks (Mo et al., 2019). The Vitamin E group (E) received dexamethasone for three weeks; starting from the second week of administration, they were also treated with vitamin E (250 mg/kg; Dana Pharmaceutical Co., Iran) orally by gavage once daily for the remaining two weeks (Lin and Zhang, 2023). In the combined Vitamin E and Metformin group (EM), mice received dexamethasone injections for three weeks. Additionally, starting from the second week, they were treated with both Vitamin E (250 mg/kg) and metformin (250 mg/kg) orally by gavage once daily for the remaining two weeks. Gavage of the mice was performed half an hour after dexamethasone injection. All doses of drugs were selected based on were based on previously published animal studies demonstrating their effectiveness in inducing insulin resistance and improving metabolic and oxidative abnormalities.

At the end of the 3-week experimental period, following the glucose tolerance test, the animals were deeply anesthetized with ketamine (80mg/kg) and xylazine (8mg/kg IP), and then were sacrificed and decapitated. Blood samples were collected from heart, and liver and gastrocnemius muscle tissues were separated, and finally the animals were euthanized. Figure 1 provides a schematic representation of the experimental design.

Oral glucose tolerance test (OGTT)

On day 22 and after the last DEX injection, the glucose tolerance of the mice was detected by OGTT. All mice were fasted for 12 hours and orally received (2 g/kg body weight) of 20% glucose solution. Blood glucose levels were measured at 0, 30, 60, 90, and 120 min after oral administration of glucose by glucometer via slight cutting the tail. Glucose area under the curve (AUC) was calculated based on fasting blood glucose levels (Valdez-Palomares et al., 2026). Glucose is detected by glucometer through the cut tail method.

Sample collections

One day after doing OGTT, following a deep anesthesia all mice were sacrificed and decapitated. Then obtained blood samples from heart were centrifuged 15 min at 3000 rpm. Then serum samples were separated and stored in -80°C for analyzing insulin, TG, LDL-c, HDL-c, total Cholesterol (TC), very-low-density lipoprotein-cholesterol (VLDL-c), aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Immediately after blood sampling, liver tissues and gastrocnemius muscles

were isolated and washed in phosphate buffer saline. Then, all livers were weighed, and then frozen in liquid nitrogen and stored at -80°C for measuring oxidative stress factors. The gastrocnemius muscles were immediately fixed in 10% buffer formalin solution.

Serum insulin measurement

Serum insulin levels were detected using a commercial insulin ELISA kit (ZellBio GmbH, Germany; Cat. No. ZB-10707C-M9648) according to the manufacturer's instructions. Briefly, serum samples were added to the ELISA wells of microplate and processed according to the kit protocol. Following incubation and washing, the substrate solution was added, and the absorbance (OD) was measured at 450 nm wavelength using a microplate reader. Insulin concentrations were calculated from the standard curve and reported as mIU/L.

Liver function assessment

The amount of hepatocyte enzymes of ALT and AST in the blood as markers that indicate the level of liver health was assayed using a biochemical spectrophotometry kit (Pars Azmoon, Iran).

Lipid profile assessment

Serum levels of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very-low-density lipoprotein cholesterol (VLDL-C) were measured directly using enzymatic colorimetric kits (Pars Azmoon, Iran), following the manufacturer's instructions and International Federation of Clinical Chemistry (IFCC) guidelines. The results were expressed in mg/dL.

Assessment of hepatic oxidative stress markers (MDA, GSH, and SOD)

The extracted liver tissues were washed with ice-cold phosphate-buffered saline (PBS; pH 7.4) to remove residual blood and then weighed. The samples were homogenized in ice-cold PBS at a ratio of 1:10 (w/v) using a tissue homogenizer 900 rpm. The homogenates were centrifuged at $10,000 \times g$ for 15 min at 4°C , and the supernatants were separated and stored at -80°C until analysis of malondialdehyde (MDA), glutathione (GSH), and superoxide dismutase (SOD) activity. These markers, including MDA levels, GSH levels, and SOD activity, were measured in the liver tissue supernatants using commercial biochemical kits (ZellBio GmbH, Germany).

Determination of lipid peroxidation

Lipid peroxidation and the subsequent generation of malondialdehyde (MDA) were assessed as an indicator of oxidative stress. MDA concentrations in liver tissue homogenates were determined using a commercial assay kit (ZellBio GmbH, Germany; Cat. No. ZB-MDA-48) according to the manufacturer's protocol. The assay was based on a colorimetric reaction performed under acidic conditions at 90–100°C, and absorbance was measured at 535 nm using a microplate reader. The assay sensitivity for MDA detection in biological samples was 0.1 µM.

Determination of Glutathione level

GSH levels in the liver tissue supernatants were determined using a commercial GSH assay kit (ZellBio GmbH, Germany; Cat. No. ZB-GSH-48) according to the manufacturer's instructions. The absorbance was measured at 412 nm using a microplate reader, and GSH levels were calculated accordingly. The assay sensitivity for GSH detection in biological samples was 0.01 mM.

Determination of SOD activity

Total superoxide dismutase (T-SOD) activity was assessed using a commercial colorimetric assay kit (ZellBio GmbH, Germany; Cat. No. ZB-SOD-48) based on the hydroxylamine method. The assay relies on the ability of SOD to inhibit the generation of superoxide anions produced through the xanthine–xanthine oxidase system, thereby reducing nitrite formation. Briefly, liver tissue samples were mixed with the assay reagents and incubated at 37°C for 40 min. Following the addition of the chromogenic reagent and a 10-min incubation at room temperature, absorbance was recorded at 420 nm using a microplate reader. SOD activity was determined according to the manufacturer's instructions and expressed in U/mL.

Immunohistochemical assay for GLUT-4

Gastrocnemius muscle samples were excised, fixed in 10% neutral-buffered formalin, and routinely processed for paraffin embedding. Serial tissue sections (4–5 µm thick) were prepared using a microtome and mounted on glass slides for immunohistochemical analysis of GLUT4 protein. Immunohistochemical detection of GLUT4 was performed using the Master Polymer Plus Detection System (Peroxidase, including DAB Chromogen; Vitro Master Diagnostica, Spain), according to the manufacturer's instructions. Briefly, tissue sections were deparaffinized in xylene and rehydrated through a graded series of ethanol. Endogenous peroxidase activity was blocked using Peroxidase Blocking Reagent. After washing with TBS, sections were incubated overnight with a rabbit polyclonal anti-GLUT4 primary antibody (1:75; ZellBio, Germany). The sections were subsequently incubated with Primary Antibody Amplifier Master at room temperature for 10–15 min, followed by incubation with Master Polymer Plus HRP for 20–30 min. Immunoreactivity was visualized using DAB substrate/chromogen, followed by incubation with DAB enhancer for 2 min. GLUT4 immunoreactivity was quantitatively analyzed using ImageJ software (Mac Biophotonics ImageJ, version 1.41a) (Taghizadeh et al., 2020).

Statistical analysis

All data were reported as standard error of the mean (mean $\pm$ SEM). The data were analyzed by Graph Pad Prism 8 statistical software. The Kolmogorov-Simonov normality test was used for variables in this study. Data for fasting glucose, insulin, lipid profile, liver enzymes, oxidative stress markers (SOD and MDA), and GLUT-4 immunohistochemical expression were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. For the oral glucose tolerance test (OGTT), glucose concentrations at different time points were analyzed using two-way ANOVA, with treatment group and time as the two factors followed by Tukey's multiple-comparison test. P value < 0.05 was considered statistically significant.

Results

Combined effect of vitamin E with metformin on blood glucose and insulin

The results of one-way ANOVA showed that fasting glucose level after 3 weeks treatment of mice with dexamethasone in the D group had a significant increase compared to the control group ($P < 0.0001$). Mice treated with metformin alone significantly reduced glucose level compared to D group ($P < 0.05$). Vitamin E alone also significantly decreased glucose ($P < 0.01$) but this reduction effect was more considerable in the EM group ($P < 0.0001$) (Fig. 2A).

Insulin concentration in the fasting condition in the D group decreased non-significantly compared to control group, while metformin and Vitamin E treatment, each alone, increased insulin level compared to the D group ($P < 0.001$ and $P < 0.05$, respectively). However, combined therapy with metformin and Vitamin E did not produce a significant change in insulin concentration compared to the D group (Fig. 2B).

Combined effect of vitamin E with metformin on oral glucose tolerance test (OGTT)

AUC of blood glucose levels for OGTT following chronic dexamethasone administration significantly increased in the D group compared to the control group ($P < 0.01$). Mice treated with metformin showed no change while mice treated with Vitamin E showed an improvement in glucose intolerance, as indicated by a significant decrease in AUC of glucose in the E group ($P < 0.01$). In the EM group combination therapy with metformin and vitamin E showed a more notable reduction in glucose AUC compared to monotherapy in glucose AUC compared to the D group ($P < 0.001$) (Fig. 3A and B).

Combined effect of vitamin E with metformin on serum lipid profile

Dexamethasone treatment for 3 weeks significantly enhanced serum total cholesterol compared to the C group ($P < 0.05$). All treatments significantly reduced cholesterol (Metformin $P < 0.01$. Vitamin E $P <$

0.001 and EM $P < 0.05$) (Fig. 4A). Serum TG levels in the D group was higher than those in the C group ($P < 0.05$) and all treatments decreased the elevated TG levels ($P < 0.05$) (Fig. 4B). There was a trend toward an increase in serum VLDL-c in the D group and a decrease in the treatment groups; however, these changes were not significant (Fig. 4C). LDL-c concentration in the D group showed a significant increase compared to the C group ($P < 0.01$). Metformin, Vitamin E and combined treatment all lowered LDL-c levels ($P < 0.01$, $P < 0.001$ and $P < 0.01$ respectively) (Fig. 4D). Although a trend toward an elevation in HDL-c was shown after dexamethasone treatment, neither monotherapy nor combined therapy produced significant changes (Fig. 4E). As shown, the combined effect of the drugs on lowering lipid levels was not greater.

Combined effect of vitamin E with metformin on serum ALT and AST

Serum ALT and AST levels, as liver function enzymes were measured. Although was not shown significant changes in ALT level in D group but there was a trend toward a reduction in ALT levels in the E and EM group (Fig. 5A). Serum AST levels in the D group were non-significantly increased compared to the C group. Only the combined treatment was able to lower AST levels compared to the D group ($P < 0.05$) (Fig. 5B).

Combined effect of vitamin E with metformin on oxidative stress markers of liver tissue

For assessment of the antioxidant potential of these treatments, hepatic oxidative biomarkers, such as MDA level, SOD activity, and GSH content, were measured. MDA level, as an indicator of lipid peroxidation, increased after 3 weeks of dexamethasone administration ($P < 0.01$). Vitamin E alone, unlike metformin, was able to reduce MDA levels compared to the D group ($P < 0.05$). Vitamin E in combination with metformin produced a greater reduction in MDA level ($P < 0.01$) (Fig. 6A). SOD activity in the liver tissue homogenate in the D group was reduced by dexamethasone ($P < 0.01$). Similar to its effect on MDA, Vitamin E alone, unlike metformin, was able to significantly increase SOD activity compared to the D group ($P < 0.05$). In addition, Vitamin E in combination with metformin produced a greater increase in SOD activity ($P < 0.01$) (Fig. 6B). Among the groups, only the E group showed an increase in hepatic GSH content ($P < 0.05$) (Fig. 6C).

Combined effect of vitamin E with metformin on immunoreactivity of GLUT-4 protein expression of skeletal muscle tissue

Immunohistochemical analysis of the soleus muscle was performed to measure GLUT-4 protein expression level in the experimental animals of this study. Results indicated that expression levels of GLUT-4 in muscle of mice receiving dexamethasone significantly decreased compared to C group ($P < 0.01$). Monotherapy with metformin increased GLUT-4 level ($P < 0.01$) and monotherapy with Vitamin E

increased that ($P < 0.05$). Metformin in combination with Vitamin E in the EM group induced a greater increase as compared to the D group ($P < 0.001$) (Figs. 7 and 8).

Discussion

The results of the current study showed that chronic dexamethasone administration induced insulin resistance as shown by hyperglycemia, glucose intolerance, dyslipidemia and liver oxidative stress as well as reduced GLUT-4 protein expression. Monotherapy with metformin and Vitamin E for 2 weeks was able to improve these metabolic changes produced by dexamethasone. While combined treatment exhibited a greater improvement compared to monotherapy. In fact, the main goal of this research was to investigate the potential impact of combination therapy with Vitamin E, a potent antioxidant and metformin, the first line drug for the treatment of type 2 diabetes. It is recognized that dexamethasone as a potent glucocorticoid has an antagonistic effect on insulin action that leads to induction of insulin resistance, which is demonstrated by glucose intolerance (Pandey et al., 2014), hyperlipidemia and hyperglycemia (Batista et al., 2024, Mahamad et al., 2020, Shen et al., 2009, Bhujbal et al., 2012). It seems that dexamethasone induces this effect via suppressing hepatic glucose output and reducing peripheral glucose utilization (Ghaisas et al., 2011, Bhujbal et al., 2012). These two factors impair the balance between antioxidant and oxidative responses via an increment in the most important oxidative marker called MDA, a final product of lipid peroxidation, and alterations in the key antioxidant enzyme called SOD (Batista et al., 2024, Mahamad et al., 2020, Ghaisas et al., 2011, Tayade et al., 2012). Oxidative stress in turn may further exacerbate insulin resistance. Hyperglycemia can result from stimulation of gluconeogenesis in the liver and inhibition of glucose uptake in peripheral tissues such as adipose tissue and muscle. Impairment of the insulin-signaling pathway may result in inhibition of GLUT-4 translocation (Pandey et al., 2014) or reduced GLUT-4 expression as shown in our data. In the present study, monotherapy with Vitamin E and/or metformin could decrease the fasting blood glucose elevated by dexamethasone. Surprisingly, combined therapy with metformin and Vitamin E produced a greater hypoglycemic response. In addition, treatment with Vitamin E but not metformin alone decreased the AUC of glucose during OGTT. Furthermore, metformin in combination with Vitamin E was able to reduce glucose AUC more effectively than monotherapy. Therefore, we observed that Vitamin E augmented the effect of metformin in improving hyperglycemia and glucose tolerance. Previous studies showed that metformin reduced hyperglycemia induced by dexamethasone (Mahamad et al., 2020, Savych et al., 2020). Also, metformin has been demonstrated to reduce dexamethasone-induced insulin resistance in peripheral tissues (Mahamad et al., 2020, Mahmoud et al., 2022) by inhibiting hepatic gluconeogenesis through AMPK activation. It leads to fat oxidation and low glucose uptake in muscle (Foretz et al., 2023, LaMoia and Shulman, 2021). In accordance with our findings, the hypoglycemic effect of Vitamin E has been demonstrated in previous studies. For example, 10 of weeks administration of Vitamin E at 350 mg/kg in obese rats decreased blood glucose (Shen et al., 2009). Additionally, another study found that administration of twice a week administration of Vitamin E lowered blood glucose in obese mice (Kamimura et al., 2013). On the other hand, Vitamin E improved glucose intolerance, which is consistent with our findings (He et al., 2019). Although, mice treated with dexamethasone tended to show a decline

in fasting insulin concentration but administration of Vitamin E and metformin individually increased the serum level of insulin, while co-treatment ameliorated this effect. Previous studies have reported that treatment with glucocorticoids such as dexamethasone in inducing the insulin resistance model has shown conflicting evidence regarding insulinemia. Serum insulin level can be decreased (Toriumi and Imai, 2003, Martínez et al., 2016), increased (Bönisch et al., 2016, Mahmoud et al., 2022) or unchanged like our data (O'Brien et al., 1991). It seems that the different pancreatic responses to dexamethasone in various studies depend on several factors, including the animal model, duration and dose of glucocorticoid treatment. Hypoinsulinemia can be due to the direct inhibition of dexamethasone on the β -cell function. Another possible mechanism can be apoptosis and/or toxicity in β -cells exposed to dexamethasone resulting from the oxidative stress induction (Toriumi and Imai, 2003, Martínez et al., 2016). In the present study, the observed elevation in fasting insulin concentration in experimental animals exposed to metformin may be related to increased insulin secretion by beta cells as a compensatory pancreatic response to reduce the hyperglycemia as shown by its hypoglycemic effect (Kupsal et al., 2016). In agreement with our data, the study conducted by Mona El-Agamy (2019) (El-Agamy et al., 2020) showed that Vitamin E supplement for 30 days in STZ-diabetic rats increased fasting insulin level. It appears that this increase may be associated with increased production of insulin by pancreatic endocrine cells (Picklo and Thyfault, 2015). Various impacts of metformin or Vitamin E on insulin level reported in previous studies may be attributed to the animal species used, the duration or dose of the treatment, and particularly the different experimental models of diabetes induction, including type 1 and type 2. Vitamin E or metformin may have a modulatory role in regulating insulin secretion from β -cells. In one study, for example, metformin did not change the insulin level increased by dexamethasone but it could reduce the blood glucose levels (Thomas et al., 1998). Additionally, in another research, in high fat/STZ-diabetic rats, metformin has been shown to have a remarkable lowering effect on fasting blood glucose and insulin concentration (Abdulmalek and Balbaa, 2019). A possible suggestion is that Vitamin E, as a potent antioxidant, can protect against hyperglycemia-induced oxidative stress and preserve β -cell secretory function. In animal studies, combining these micronutrients with metformin has shown additive antihyperglycemic and antioxidant effects. Recent evidence from preclinical and clinical studies also supports the potential synergistic metabolic and antioxidant effects of combining metformin with essential micronutrients and antioxidant compound (Ninsiima et al., 2026). The co-treatment of metformin with vitamin E may significantly improve insulin sensitivity and inhibit hepatic glucose production; in this case, despite lower glucose, the need for insulin is reduced and therefore plasma insulin level is lower. Vitamin E as a potent antioxidant alone reduced liver MDA levels and increased GSH and SOD activities altered by dexamethasone. However, metformin did not induce significant changes in these markers. In contrast, when vitamin E was co-administered with metformin in dexamethasone-treated mice, the combined therapy produced a more pronounced antioxidant effect. It has been reported in several previous studies, consistent with our findings that metformin reduced liver MDA and increased SOD levels altered by dexamethasone (Mahamad et al., 2020, Savych et al., 2020, Mahmoud et al., 2022). It was found that Vitamin E supplementation led to an increase in GPX and SOD activity due to its antioxidant properties, enabling it to reduce the glycemic index in diabetes, which in turn resulted in an increase in insulin levels, as shown by our results (El-

Agamy et al., 2020). Vitamin E acts by potentiating the generation of the SOD enzyme, which is involved in the antioxidant defense of the whole body (El-Agamy et al., 2020, Williams et al., 2012, Gray et al., 2011, Alcalá et al., 2015). The final product of lipid peroxidation is MDA which its enhancement indicates the installation of oxidative stress. SOD functions by disproportioning cytotoxic superoxide to hydrolyze to hydrogen peroxide (Mahamad et al., 2020). Glutathione as a non-enzymatic antioxidant acts like catalase through decreasing hydrogen peroxide that in turn leads to reduce lipid peroxidation, a factor associated with oxidative stress (Mahamad et al., 2020). In the present research, co-administration of metformin and vitamin E did not exert additive effects on the regulation of dexamethasone-induced dyslipidemia. Dysregulation of the lipid profile is the main cause of cardiovascular diseases (Sagha et al., 2023). Although Vitamin E alone and also in combination with metformin showed a reduction effect in VLDL-c concentration, this reduction was not significant. Previous studies have shown that metformin reduces dexamethasone-induced hyperlipidemia, (Mahamad et al., 2020, Savych et al., 2020) such as LDL-c and TG (Mahmoud et al., 2022). Meanwhile, several studies have confirmed the antihyperlipidemic effects of Vitamin E (El-Agamy et al., 2020, Shen et al., 2009, He et al., 2019, Alcalá et al., 2015). Whereas, co-administration of vitamin E with metformin produced no a stronger impact. Perhaps this phenomenon can be attributed a ceiling effect, in which the pathways and mechanisms of Vitamin E and metformin have already activated most of the metabolic responses. So, additive effect was not observed. Another additional effect of the combined therapy with vitamin E and metformin was observed in the reduction of AST levels in blood serum, but not ALT. Biomarkers of ALT and AST are hepatic transaminases linked to liver dysfunction (Abdulmalek and Balbaa, 2019). So, it be concluded that metformin in combination with Vitamin E is able to improve dexamethasone-induced hepatic dysfunction, which is consistent with the marked decrease in oxidative stress markers in the combined treatment group (EM). Monotherapy with Vitamin E and metformin increased the level of GLUT-4 protein reduced by dexamethasone in muscle tissue of mice. Whereas, combination therapy with Vitamin E and metformin showed an additive effect on the increased expression of GLUT-4 protein. According to our findings, studies reported that dexamethasone could inhibit the insulin signaling pathway leading to reduced translocation of GLUT-4 to the cell membrane in muscle (Mahamad et al., 2020, Ghaisas et al., 2011). This insulin-antagonist action of dexamethasone causes a reduction in glucose uptake in peripheral tissues which resulted to insulin resistance and hyperglycemia (Ghaisas et al., 2011). On the other hand, previous reports have suggested the possible mechanisms underlying the hypoglycemic effects of metformin and vitamin E, which include enhanced insulin sensitivity through increased GLUT-4 translocation or expression (Thomas et al., 1998, Dutta et al., 2023). Recent findings from an experimental type 2 diabetes study further support the beneficial effects of combined antidiabetic and antioxidant strategies, demonstrating improved insulin-related metabolic responses and restoration of GLUT4 expression in skeletal muscle (Akarslan et al., 2025). In this study, the better improvement in insulin resistance as shown by OGTT in combined group, could be attributed to greater expression of GLUT-4 m-RNA (Ghaisas et al., 2011). This augmented effect of metformin through co-treatment with vitamin E resulted in better regulation of glucose homeostasis compared with metformin monotherapy.

Although the co-administration of metformin and vitamin E in the present study showed favorable effects on metabolic indices, the safety of high-dose vitamin E, especially over long-term periods, should be considered. Long-term use of high doses of vitamin E may be associated with some side effects, including impaired coagulation and an increased risk of bleeding and gastrointestinal complications (Miller et al., 2005). Given that the present study was conducted in an animal model and during a relatively short treatment period, it is not possible to draw conclusions about the safety of long-term use of this combination in humans based on the current results. Therefore, long-term and toxicological studies are necessary to evaluate the safety and determine the appropriate therapeutic range of this combination.

Conclusion

In summary, the data resulting from this study suggest that hypoinsulinemia produced by dexamethasone, accompanied by an increase in blood glucose, likely reflects impaired insulin secretion and increased insulin resistance. Although monotherapy with either vitamin E or metformin attenuated oxidative stress, improved glucose tolerance, and reduced blood glucose levels, along with increased GLUT-4 expression, these effects were significantly amplified by their combined administration. These results suggest that metformin is more effective in improving insulin resistance-related parameters when co-administered with vitamin E. Notably, vitamin E appears to enhance the metabolic actions of metformin, resulting in a more pronounced improvement in insulin sensitivity and glucose homeostasis. In fact, co-administration of metformin and Vitamin E in improving insulin resistance may be a hopeful and more effective supplemental treatment for T2DM. Overall, metformin and vitamin E demonstrated protective and modulatory effects on metabolic parameters.

Limitations of study

Based on the current findings following complementary studies are proposed: Vitamin E Enhances Metformin-Induced GLUT4 Translocation in Skeletal Muscle of Dexamethasone-Induced Insulin-Resistant Mice. Vitamin E Potentiates Metformin-Induced AMPK Signaling and GLUT4 Gene Expression in Dexamethasone-Induced Insulin Resistance.

Declarations

Acknowledgments

This article has been extracted from the thesis written by Mrs. Hanieh Baghaeishiva in School of Medicine of Mazandaran University of Medical Science. This research was supported by the grant (grant number: 17698) from the Immunogenetics Research Center, Mazandaran University of Medical Sciences, Sari, Iran.

Ethical approval: Ethical approval for the present study was obtained from the Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran (IR.MAZUMS.4.AEC.1404.069).

Accordance statement: All procedures performed in the present study were conducted in accordance with the ethical standards and approval of the Ethics Committee of Mazandaran University of Medical Sciences, Sari, Iran, and in accordance with the guidelines for the Care and Use of Laboratory Animals (National Institutes of Health Publication No. 80–23, revised 1996). The methods of the present study have been reported in accordance with the ARRIVE guidelines (Percie du Sert et al., 2020).

Author contributions

F Sadeghimahalli: Conceptualization, Data curation, Investigation, Methodology, visualization, writing – original draft, Writing – review & editing, Project administration; **R Zare ghashti:** Conceptualization, Data curation, Investigation, Methodology; **M Mirzaei:** Data curation, Methodology; **H Baghaeishiva:** Investigation, Data curation, Methodology; **M Esmaili:** Investigation, Software.

Data availability

All data generated or analyzed during this study are included in this article. Further enquiries can be directed to the corresponding author.

Competing interests

No potential conflict of interest was reported by the author(s).

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Figures

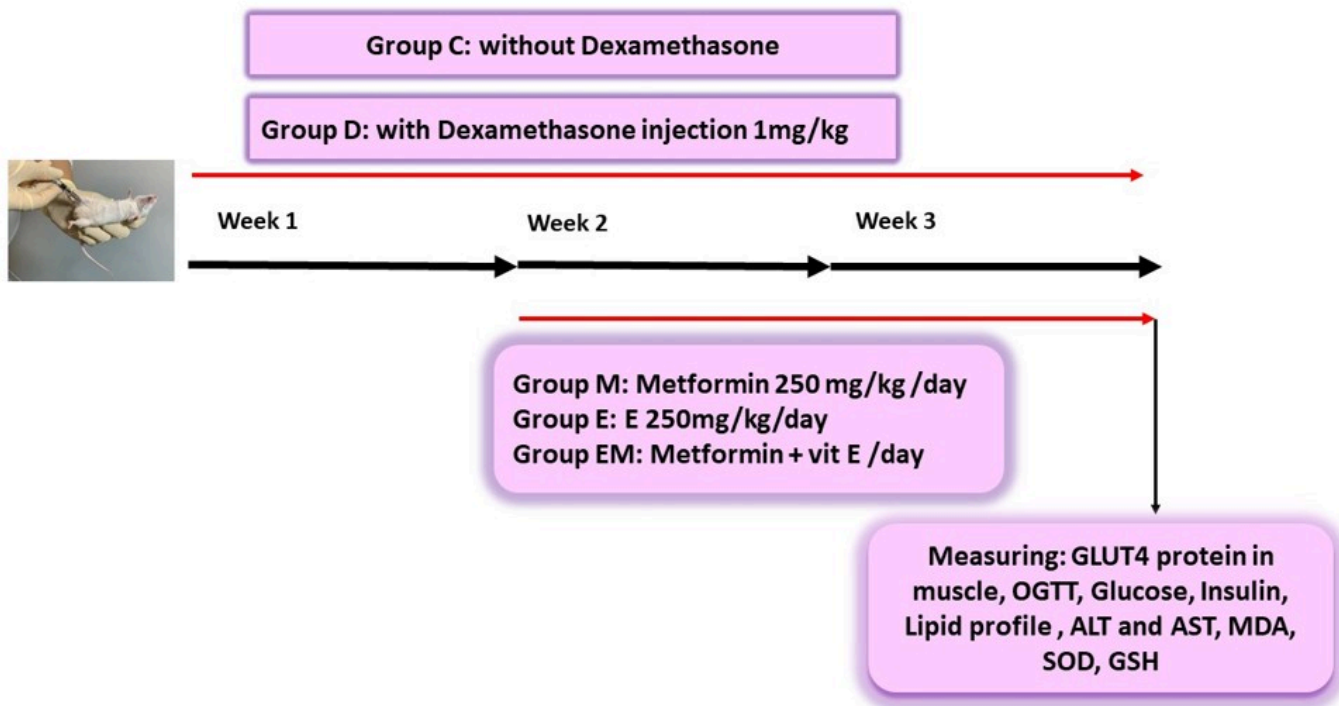


Figure 1

Study design for co-administration of vitamin E + metformin on metabolic parameters in insulin-resistant mice induced by dexamethasone

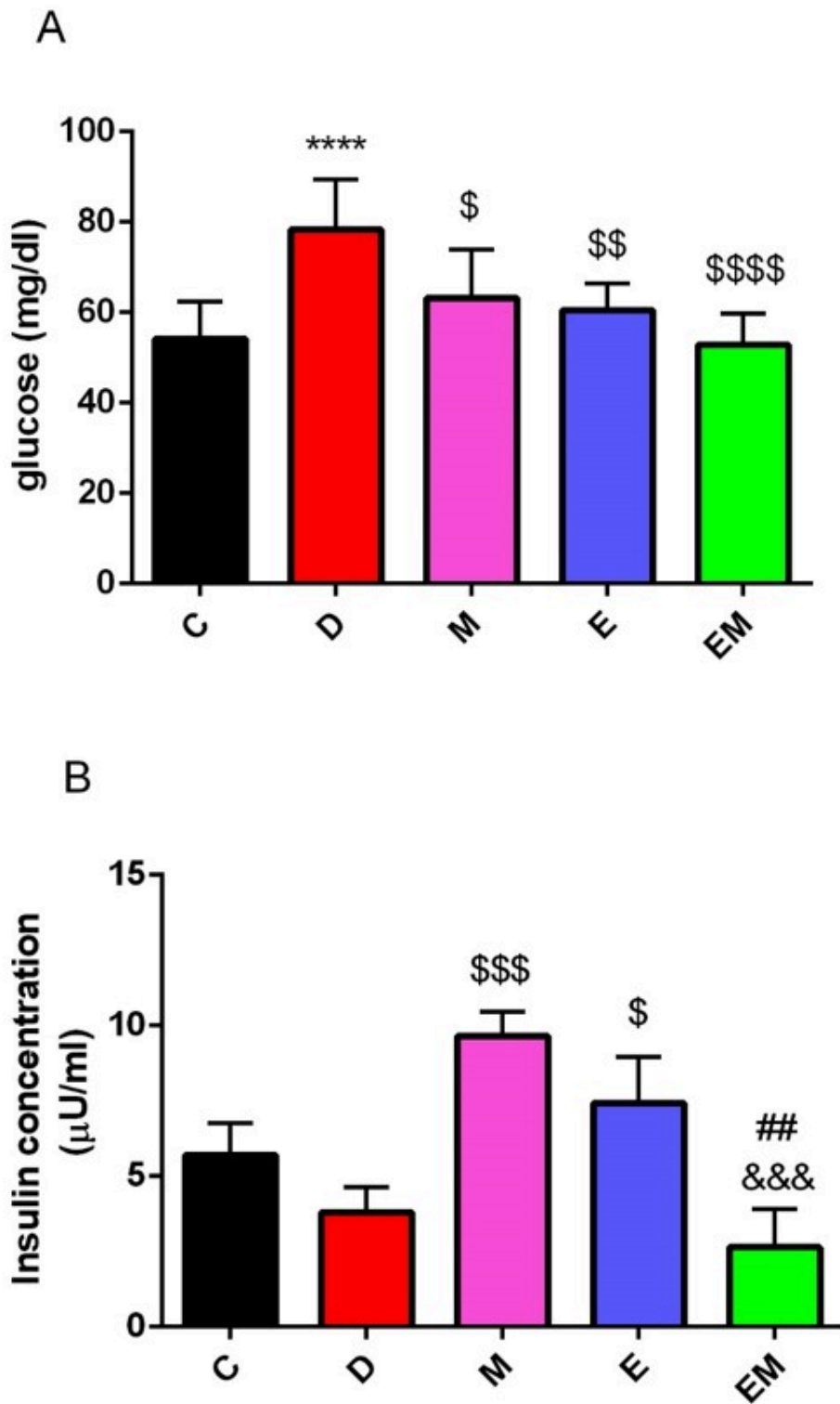


Figure 2

The effects of Metformin or Vitamin E on the serum concentration of glucose (A) and insulin (B). Groups: Control (C), Dexamethasone (D), metformin E (M), Vitamin E (E), Metformin +Vitamin E (EM). Data are expressed as the mean $\pm$ SEM (n = 5).

*** P<0.0001 vs the C group.

$P < 0.0001$, $^{\$\$}P < 0.01$, $^{\$}P < 0.05$ vs the D group.

$^{\&\&\&}P < 0.001$ vs the M group.

$^{##}P < 0.01$ vs the E group.

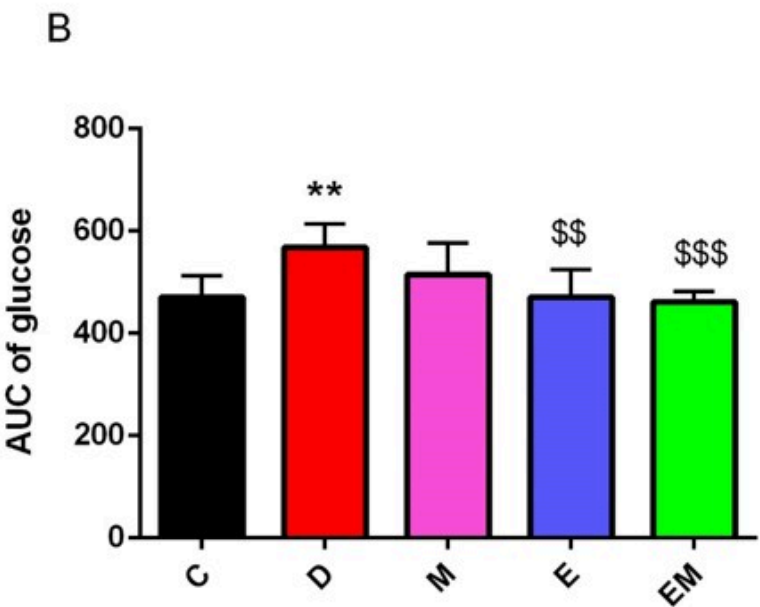
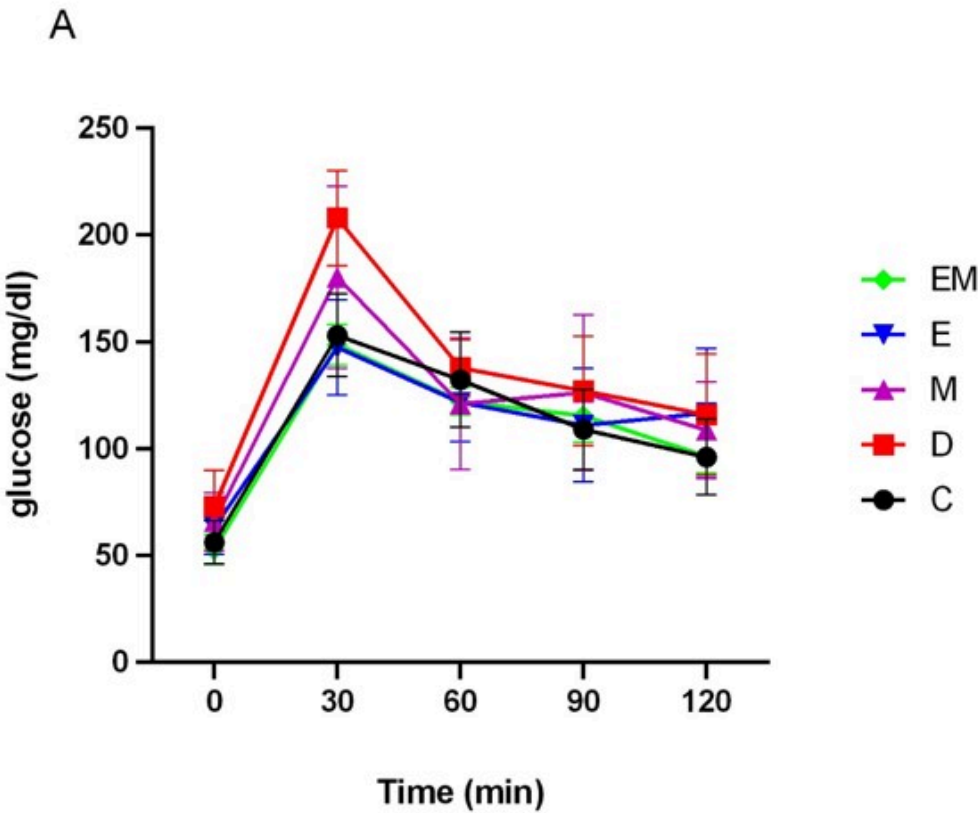


Figure 3

The effects of Metformin or Vitamin E on the OGTT. Linear curve (A) and area under curve AUC (B). Groups: Control (C), Dexamethasone (D), metformin (M), Vitamin E (E), Metformin +Vitamin E (EM). Data are expressed as the mean $\pm$ SEM (n = 5).

$P < 0.0001$, $^{\$} P < 0.05$ vs the D group.

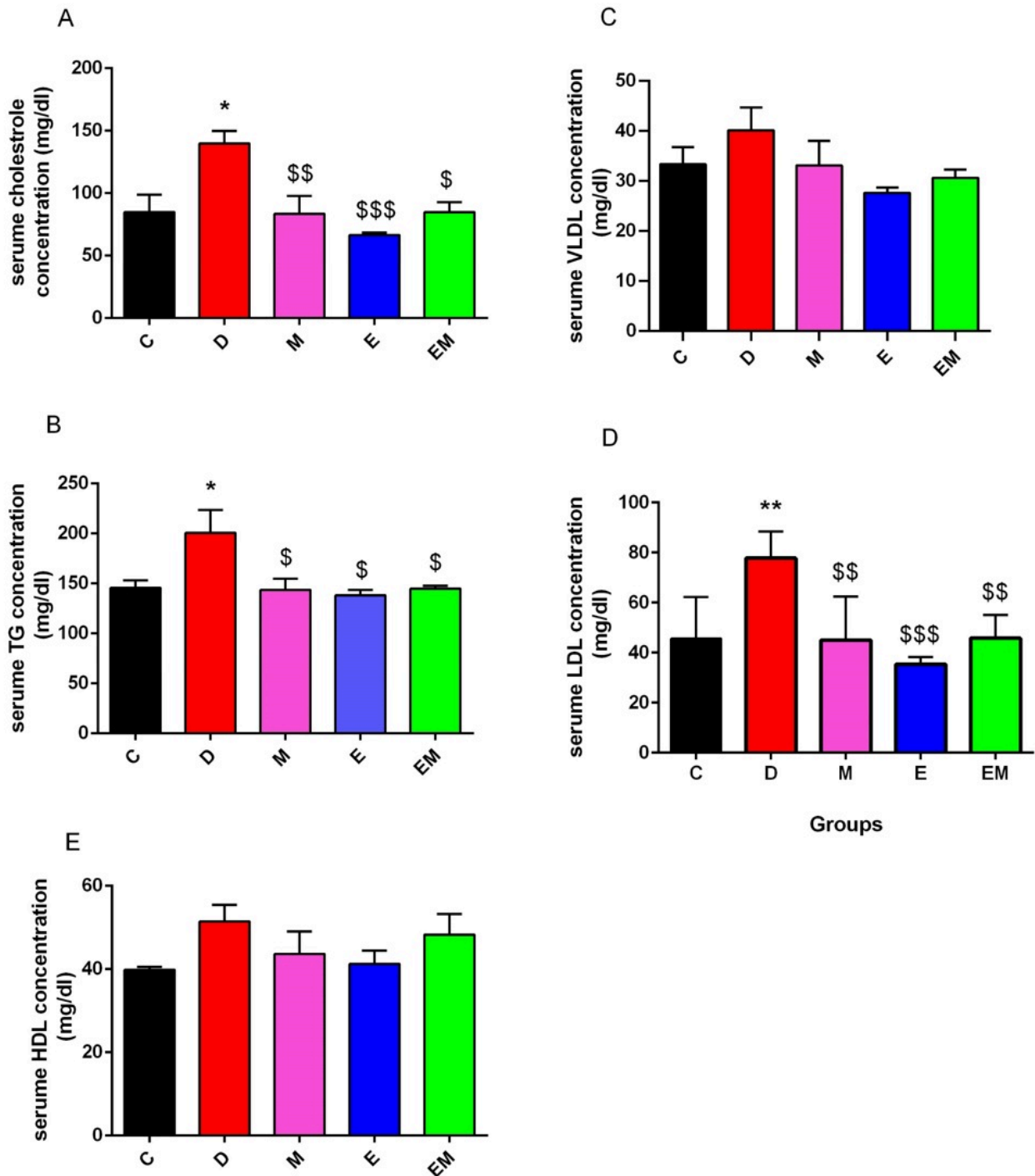


Figure 4

The effects of Metformin or Vitamin E on the serum lipid profile; total cholesterol (A), triglyceride TG (B), VLDL-C (C), LDL cholesterol LDL-C (D), HDL cholesterol HDL-C (E) and TG/HDL-C ratio. Groups: Control (C), Dexamethasone (D), metformin (M), Vitamin E (E), Metformin +Vitamin E (EM). Data are expressed as the mean $\pm$ SEM (n = 5).

**P<0.01, *P<0.05 vs the C group

P<0.0001, \$\$P<0.01, \$ P<0.05 vs the D group.

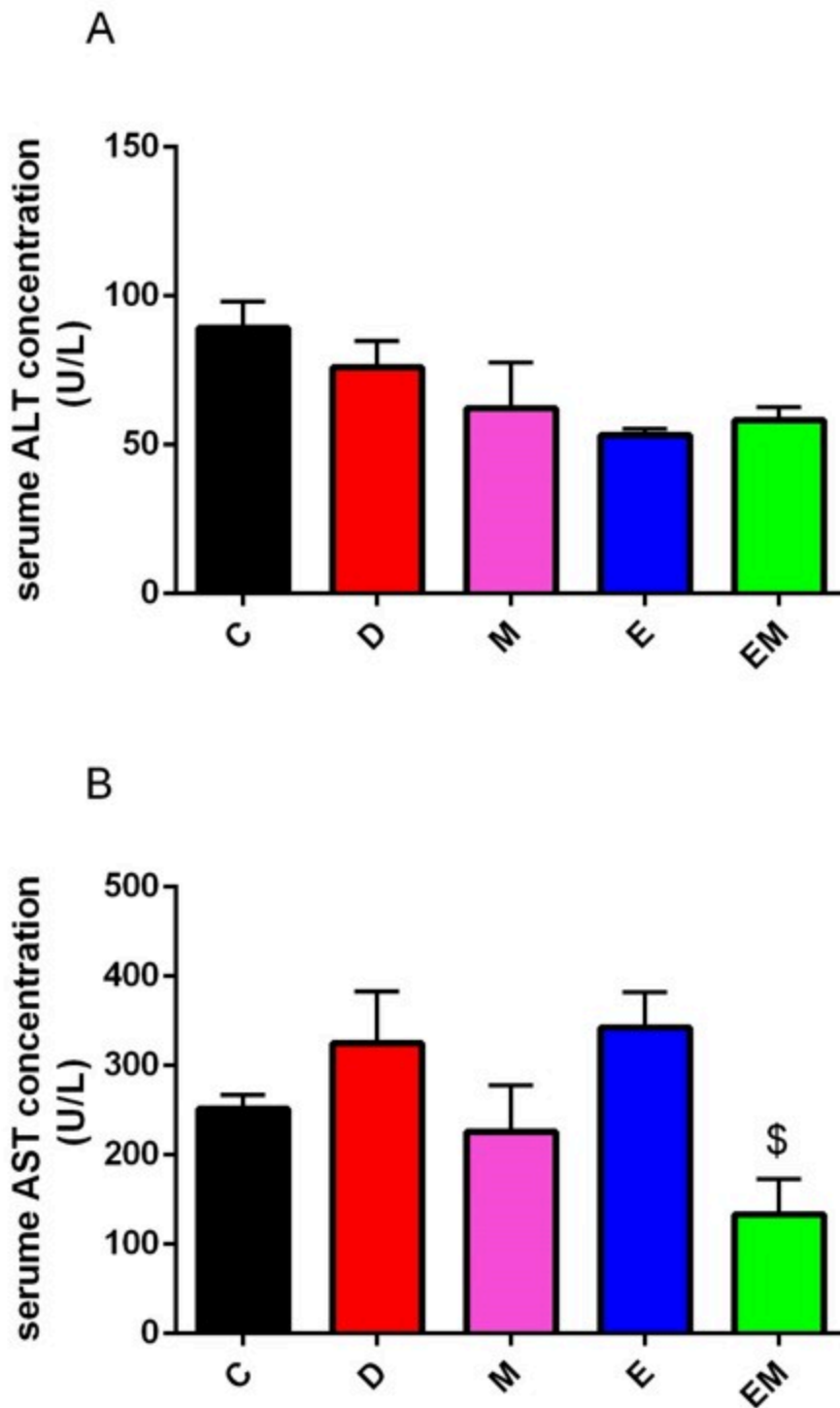


Figure 5

The effects of Metformin or Vitamin E on the serum ALT (A) and AST (B). Groups: Control (C), Dexamethasone (D), metformin (M), Vitamin E (E), Metformin +Vitamin E (EM). Data are expressed as the mean $\pm$ SEM (n = 5).

$\$ P < 0.05$ vs the D group.

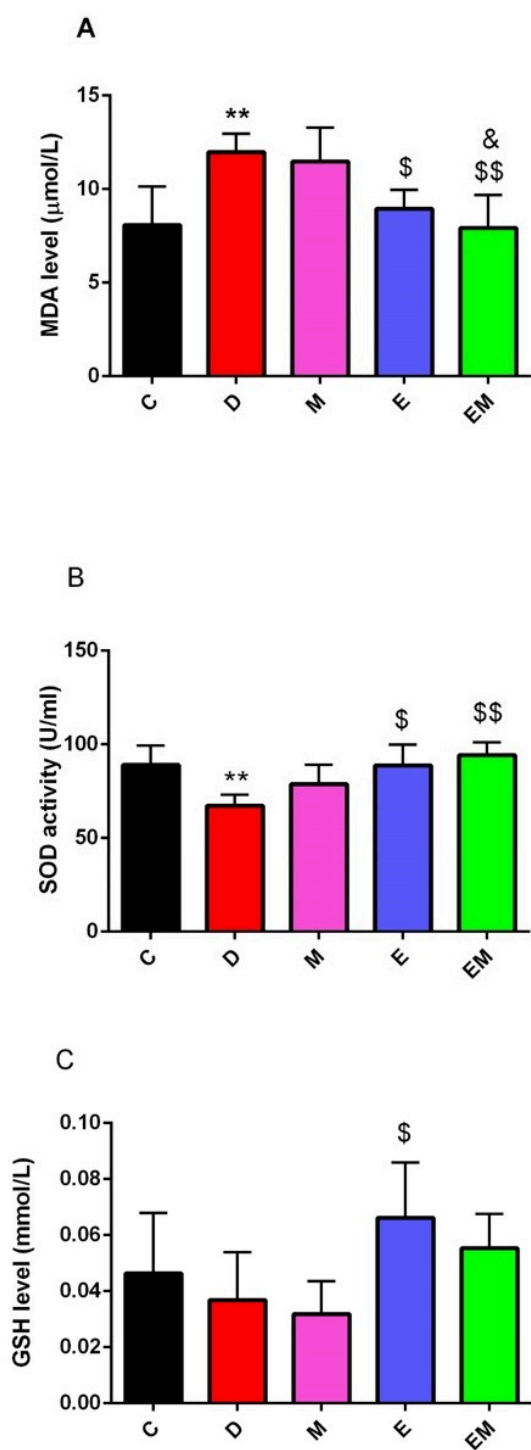


Figure 6

The effects of Metformin or Vitamin E on the hepatic MDA (A), SOD (B), GSH (C). Groups: Control (C), Dexamethasone (D), metformin (M), Vitamin E (E), Metformin +Vitamin E (EM). Data are expressed as the mean $\pm$ SEM ($n = 5$).

* $P < 0.05$ vs the control group.

\$\$ P<0.01, \$ P<0.05 vs the D group.

&P<0.05 vs the M group.

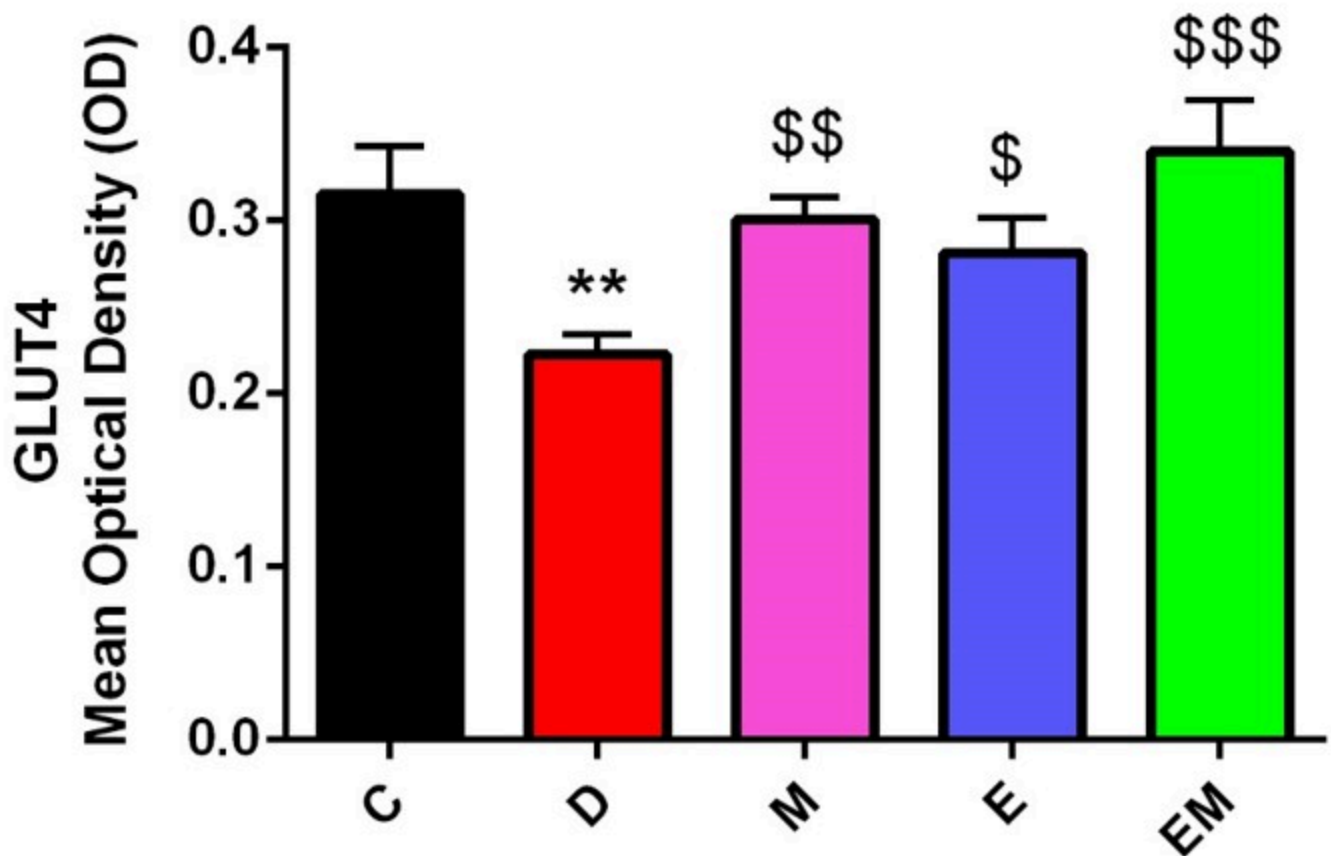


Figure 7

The effects of Metformin or Vitamin E on the level of expression GLUT-4 protein in muscle tissue detected by immunohistochemistry technique. Groups: Control (C), Dexamethasone (D), metformin (M), Vitamin E (E), Metformin +Vitamin E (EM). Data are expressed as the mean $\pm$ SEM (n = 5).

**P<0.01 vs the control group.

\$\$\$ P<0.001, \$\$ P<0.01, \$ P<0.05 vs the D group.

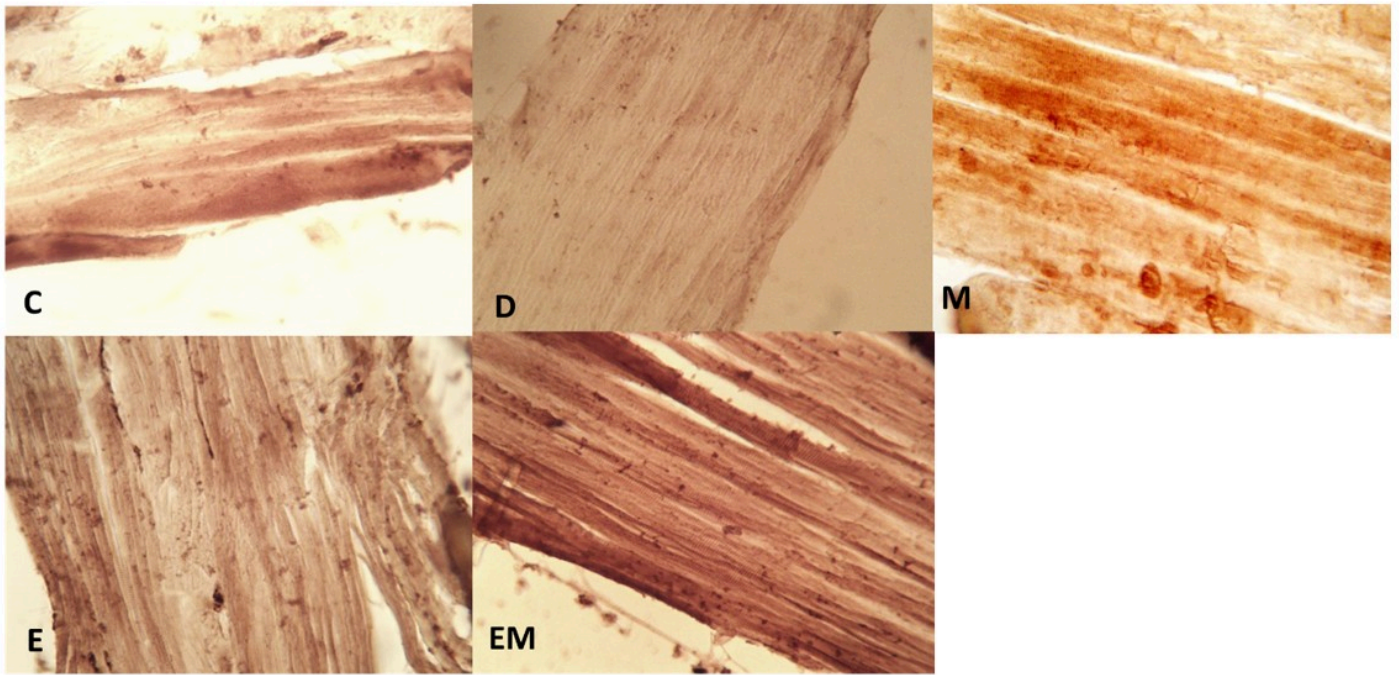


Figure 8

Immunohistochemical staining of GLUT-4 protein in skeletal muscle tissue of mice

Groups: Control (C), Dexamethasone (D), metformin (M), Vitamin E (E), Metformin +Vitamin E (EM). The brown color shows GLUT-4 positive cells. 40× magnification; Scale bar = 100 μm.