

Original Article



Effects of the Combination of Curcumin Supplementation and Aerobic Exercise on Lipid Profile and Oxidative Stress in Type 2 Diabetic Wistar Rats

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ABSTRACT

Objectives: Dyslipidemia and oxidative stress have been reported to play important roles in the pathogenesis of type 2 diabetes mellitus (T2DM) complications. This study aimed to test the hypothesis whether curcumin supplementation combined with aerobic exercise could prevent dyslipidemia and oxidative stress in a rat model of T2DM.

Methods: Male Wistar rats with nicotinamide-streptozotocin-induced T2DM were divided into four groups including untreated diabetes, diabetes treated with curcumin (30 mg/kg, three times weekly), diabetes treated with aerobic exercise (4-week progressive treadmill training), and a combination group. Also, healthy control groups (untreated, curcumin-treated, and curcumin + aerobic-treated) were studied to determine the side effects of the treatments. Fasting blood sugar (FBS), lipid profiles (triglycerides, total cholesterol, LDL, HDL) and antioxidant enzyme activities (catalase, SOD, GPx) were measured by commercial kits after 4 weeks of treatment protocol.

Results: Diabetic rats had significantly elevated serum levels of FBS, triglycerides, total cholesterol, LDL, and reduced antioxidant activities compared to controls. Curcumin and aerobic exercise alone improved these parameters significantly, but their combination was more effective in reducing FBS, improving lipid profiles, and boosting antioxidant activities.

Conclusion: The combination of curcumin and aerobic exercise has more potential to ameliorate dyslipidemia and oxidative stress in T2DM rats, compared to treatments individually. These findings require further exploration in clinical settings.

Keywords: Aerobic exercise, Curcumin, Diabetes mellitus, Lipid profile, Oxidative stress

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Introduction

Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and impaired insulin responsiveness leading to elevated blood glucose level (1). Insulin resistance impairs glucose uptake in peripheral tissues like skeletal muscle, liver, and adipose tissue, causing persistent hyperglycemia. At the same time, pancreatic beta-cells fail to compensate, losing their capacity to secrete adequate insulin in response to glucose challenges (2). Genetic predispositions, such as polymorphisms in the transcription factor 7 like 2 (TCF7L2) gene (3), and environmental factors such as poor diet, physical inactivity, and chronic stress accelerate disease progression (4). The global prevalence of T2DM has surged from 151 million in 2000 to 366 million in 2011, projected to reach 592 million by 2035 (5), imposing a staggering economic toll, with annual global healthcare costs surpassing \$760 billion (6). Low- and middle-income countries bear the brunt of this epidemic, driven by rapid urbanization, dietary shifts toward high-calorie foods, and increasingly sedentary lifestyles. The percent of diabetes prevalence in adult population of Iran has been reported to be 11.4% (5, 7).

There are several T2DM complications, with dyslipidemia and oxidative stress acting as key factors involving in the pathogenesis of the complications. Dyslipidemia affects 60–70% of T2DM patients (8), presenting as high triglycerides, low high density lipoprotein cholesterol (HDL-C) and an existence of small, dense low density lipoprotein (LDL) particles (9). Insulin resistance disrupts lipid metabolism by increasing adipose tissue lipolysis, flooding the liver with free fatty acids, and stimulating excess production of very-low-density lipoprotein (VLDL), which promotes endothelial dysfunction and atherosclerosis, significantly raising the risk of heart disease (10). Oxidative stress results from hyperglycemia-driven overproduction of reactive oxygen species (ROS) through pathways such as mitochondrial dysfunction, the polyol and hexosamine pathways, and advanced glycation end-product (AGE) formation (11). This overwhelms antioxidant defenses like superoxide dismutase and glutathione, causing widespread damage to blood vessel cells, lipids, and proteins (12). Antioxidant enzymes, including catalase, superoxide dismutase, and glutathione peroxidase, help reduce ROS toxicity, with catalase breaking down hydrogen peroxide and glutathione peroxidase reducing glutathione to limit oxidative damage (13, 14). Collectively, dyslipidemia and oxidative stress account for 65–80% of T2DM-related deaths (15), highlighting their vital role in disease progression. Over time, hyperglycemia triggers inflammation and oxidative stress, which further intensify insulin resistance and beta-cell damage, creating a vicious cycle of metabolic

decline (16).

Given the complexity of T2DM complications, interventions targeting metabolic and oxidative pathways are critical. Curcumin, a bioactive compound from turmeric (*Curcuma longa*), exhibits potent antioxidant, anti-inflammatory, and lipid-lowering effects (17). Curcumin can neutralize ROS and activate the nuclear factor erythroid 2-related factor 2 (Nrf2) pathway leading to upregulation of antioxidants genes such as catalase and heme oxygenase-1 (18). This compound also inhibits nuclear factor κ B (NF- κ B) activity resulting in reducing pro-inflammatory cytokines (e.g., TNF- α , IL-6) production (19). In addition, it has been reported that curcumin lowers triglycerides and LDL-C levels while increasing HDL-C level in diabetes (20). Furthermore, it has been believed that the aerobic exercise can improve insulin sensitivity in people with type 2 diabetes. Regular aerobic exercise in patients with T2DM has been shown to improve HbA1c, blood pressure, triglyceride levels, and decrease insulin resistance (21). Aerobic exercise, enhances insulin sensitivity via 5' AMP-activated protein kinase (AMPK)-mediated GLUT4 translocation and improves mitochondrial function through activating the peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1 α) (22). Aerobic exercise also reduces visceral fat, optimizes lipid profiles, and bolsters antioxidant capacity, countering oxidative stress (23). Despite these benefits, the combined effects of curcumin and aerobic exercise remain underexplored. This study aims to evaluate the combined effects of curcumin supplementation and aerobic exercise on lipid profiles and oxidative stress in T2DM Wistar rats.

Materials and Methods

Induction of Type 2 Diabetes

This study was conducted following institutional ethical guidelines for animal research. Male Wistar rats ($n=35$, 180–220 g) were acclimatized for two weeks under controlled conditions (23–27°C, 12-hour light/dark cycle). T2DM was induced via intraperitoneal injection of nicotinamide (110 mg/kg, dissolved in saline), followed by streptozotocin (50 mg/kg, dissolved in citrate buffer, pH 4.5) after 15 minutes. Control rats received saline and citrate buffer. Blood glucose levels were measured one-week post-induction using a glucometer, with levels >200 mg/dL confirming T2DM. Rats were divided into diabetes and control groups. Diabetic groups include untreated (T2DM) ($n=5$), curcumin-treated (T2DM + Cur) ($n=5$), exercise-treated (T2DM + Ex) ($n=5$), curcumin + exercise (T2DM + Cur + Ex) ($n=5$). Control groups include control: untreated (Control) ($n=5$), curcumin-treated (Control + Cur) ($n=5$), curcumin + exercise (Control + Cur + Ex) ($n=5$). After 4 weeks, rats were anesthetized with intraperitoneal ketamine-xylazine, and blood was collected via cardiac puncture. Serum was separated and stored at -80°C for analysis.

Ethical Considerations

Islamic Azad University's Animal Ethical Committee approved the experimental protocol, ensuring it adhered to all relevant national and international standards for the care and use of laboratory animals, as outlined in the NIH Guide (Publication no. 85-23, 1985).

Interventions

Curcumin (30 mg/kg body weight) was administered via gavage three times weekly to the animals. The exercise protocol comprised a 4-week treadmill regimen. Rats were acclimatized with five sessions (5–8 m/min, 0° incline, 5–10 min). The exercise protocol progressed from 10 m/min for 15 min in week 1 to 22 m/min for 30 min by week 4, with a constant 5° incline, including warm-up and cool-down periods (24).

Biochemical Analysis

Fast Blood glucose (FBS), Serum total cholesterol, LDL-C, and high- HDL-C were quantified at a medical diagnostic laboratory by enzymatic-based kits (Pishtazteb, Iran, Tehran) and an autoanalyzer (BT3000, Biotechnica, Italy). Activities of antioxidant enzymes (catalase, superoxide dismutase, glutathione peroxidase) were measured using colorimetric kits (Navand Salamat Company, Iran, Urmia).

Statistical Analysis

Data normality was assessed using the Shapiro-Wilk test, and homogeneity of variances was evaluated with the Levene test. Group differences were analyzed using

one-way analysis of variance (ANOVA) with Tukey's post-hoc test ($p < 0.05$). Statistical analyses were performed using GraphPad Prism version 9.0. Results are reported as means $\pm$ standard deviations.

Results

Body weight and FBS

The untreated T2DM group showed significant weight loss with FBS > 350 mg/dL. Both curcumin and exercise alone partially attenuated these changes. However, the combination group (T2DM+Cur+Ex) exhibited significantly higher body weight than exercise alone ($p < 0.01$) (Fig. 1). However, the difference between the combination and curcumin groups in the diabetic groups was not significant. All diabetic groups treated with curcumin and exercise, alone or combination, could significantly reduce FBS levels, although there was no significant difference between individual and combined treatment groups. In addition, no significant differences were observed among the Control, Control+Cur, and Control+Cur+Ex groups.

Lipid Profile

Fig 2A indicates that serum TG level was significantly higher in the T2DM group compared to the Control group. However, treatment with curcumin or exercise alone and together significantly decreased serum TG in diabetic rats. Combination treatment led to more reduction in TG level compared to curcumin alone ($p < 0.0001$) and exercise alone ($p < 0.0001$).

T2DM+Cur, T2DM+Ex, and T2DM+Cur+Ex

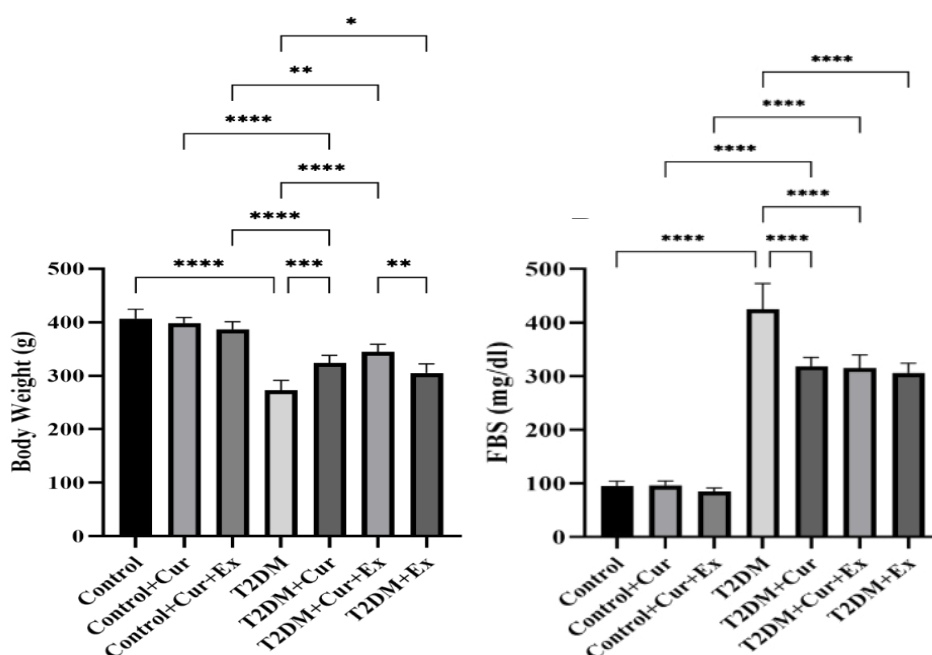


Figure 1. Body Weight and Fasting Blood Sugar (FBS) Levels in Control and T2DM Rats. Illustrating body weight (A) and fasting blood sugar (B) in control and diabetic Wistar rats with curcumin and/or aerobic exercise. T2DM = Type 2 Diabetes Mellitus, Cur = Curcumin, Ex = Exercise. Data are means $\pm$ SD (* $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

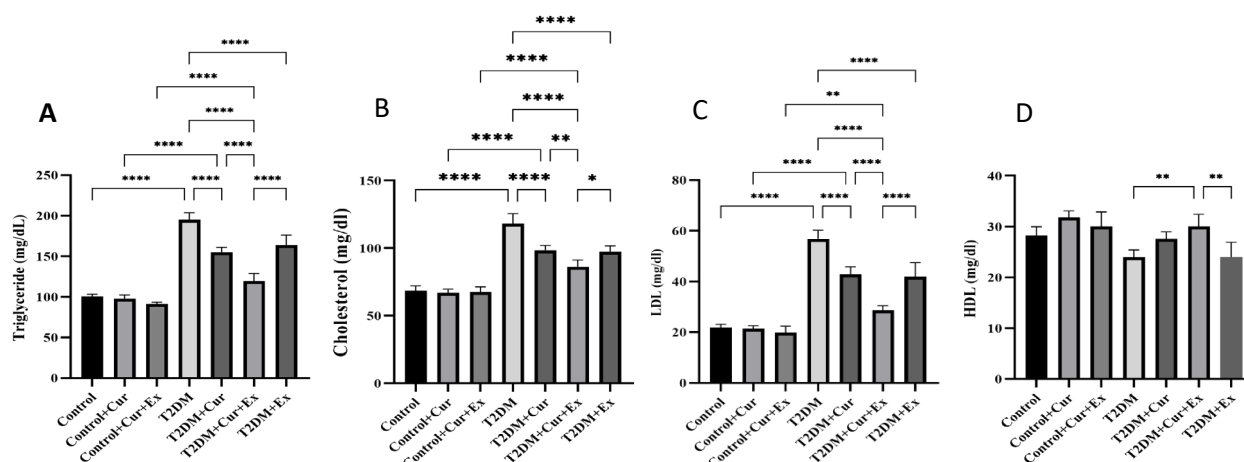


Figure 2. Serum Lipid Profile. (A) Illustrating serum Triglyceride (mg/dL) in control and diabetic Wistar rats after a 4-week intervention with curcumin and/or aerobic exercise. (B) Showing serum total cholesterol (mg/dL). (C) Presenting serum LDL cholesterol (mg/dL). (D) Presenting serum HDL cholesterol (mg/dL). T2DM = Type 2 Diabetes Mellitus, Cur = Curcumin, Ex = Exercise, LDL = Low-Density Lipoprotein, HDL = High-Density Lipoprotein. Data are means $\pm$ SD (* $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

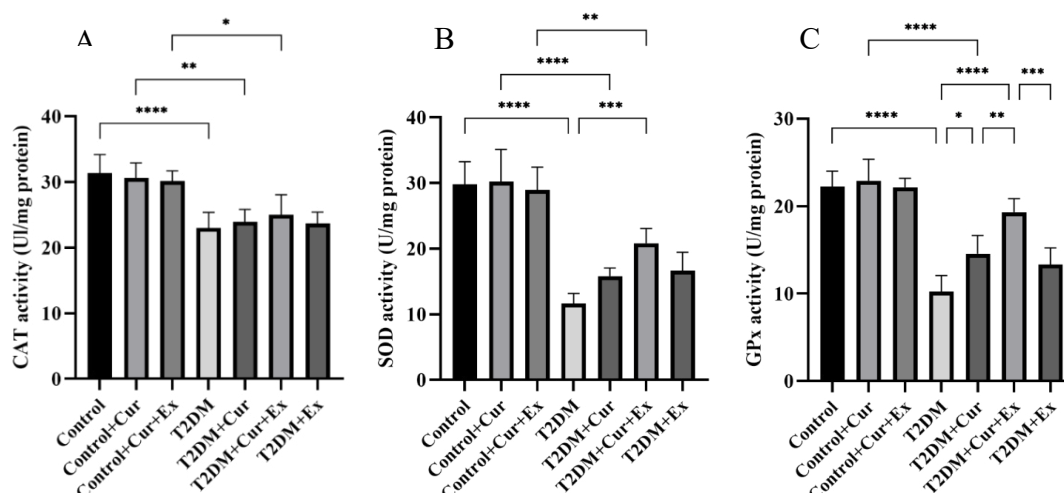


Figure 3. Antioxidant Enzyme Activity. Illustrating antioxidant enzyme activity changes in control and diabetic Wistar rats after a 4-week intervention. (a) Displaying CAT activity (U/mg protein) (b) Illustrating SOD activity (U/mg protein). (c) Showing GPx activity (U/mg protein). T2DM = Type 2 Diabetes Mellitus, Cur = Curcumin, Ex = Exercise, CAT = Catalase, SOD = Superoxide Dismutase, GPx = Glutathione Peroxidase, SD = Standard Deviation. Data are means $\pm$ SD (* $P < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

groups exhibited significantly reduced cholesterol levels compared to the T2DM group. Combination group reduced total cholesterol which was greater than curcumin ($p < 0.01$) and exercise alone groups ($p < 0.05$). The Control+Cur and Control+Cur+Ex groups showed cholesterol levels similar to the Control group, with no significant differences (Fig 2B).

LDL cholesterol level in the T2DM group was significantly higher than that in the Control group. The combination group showed the lowest LDL level, significantly lower than curcumin ($p < 0.0001$) and exercise alone groups ($p < 0.0001$) (Fig. 2C).

Regarding the HDL-C level, T2DM+Cur+Ex group showed a significant increase in HDL levels compared

to the T2DM and T2DM+Ex groups. However, no significant difference was observed between the diabetic groups that received curcumin and aerobic exercise alone.

Antioxidant Enzyme Activity

As shown in Fig. 3A, catalase activity in the T2DM group was significantly lower compared to the Control group. However, no significant differences were observed between curcumin, exercise and combined groups in comparison with the diabetic groups.

SOD activity in the T2DM group was significantly lower compared to the Control group. Only the combination group achieved significant SOD restoration

($p < 0.001$ vs T2DM). Although the differences between the combined group with curcumin and exercise alone groups were observed, those were not significant (Fig. 3 B).

GPx activity in the T2DM group was significantly decreased compared to the Control group. The T2DM+Cur group exhibited a significant elevation in GPx activity compared to T2DM. However, the difference between the T2DM+Ex and the T2DM groups was not significant. Furthermore, the combination group produced the highest GPx activity, significantly greater than curcumin ($p < 0.01$) and exercise alone groups ($p < 0.001$) (Fig. 3C).

Discussion

The present study demonstrates that curcumin supplementation and aerobic exercise, alone and in combination, exert beneficial effects on lipid profiles and oxidative stress in a nicotinamide-streptozotocin-induced T2DM rat model, with the combined intervention yielding the most pronounced improvements. In most parameters, the combination of curcumin and aerobic exercise consistently produced significantly greater improvements than either intervention alone. No significant differences were observed between the healthy groups. These findings demonstrated that curcumin and exercise have no side effects. In our study, curcumin supplementation at 30 mg/kg significantly reduced total cholesterol, LDL, and triglyceride levels in serum of T2DM rats, consistent with the observations by Sadoughi et al., who reported that curcumin improved lipid profiles in diabetic rats (25). Similarly, Panahi et al. found that curcuminoids improved lipid profiles in patients with metabolic syndrome over 8 weeks (20). Asghari et al. demonstrated that nano-curcumin combined with eicosapentaenoic acid enhanced lipid parameters in T2DM patients (26). However, Baum et al. (2007) noted that curcumin reduced total cholesterol in healthy individuals over six months without significantly affecting HDL or triglycerides (27), suggesting that curcumin's lipid-modulating effects may be more robust in the context of diabetic dyslipidemia.

This study showed aerobic exercise in our 4-week treadmill protocol also improved lipid profiles, reduced total cholesterol, LDL, and triglyceride levels in T2DM rats, mirroring the findings from Saghebjo et al., who reported reductions in triglycerides and LDL with increases in HDL following 12 weeks of aerobic exercise in T2DM patients (28). Pedersen et al. (2019) similarly observed the decreases in total cholesterol and triglycerides in overweight patients with cardiovascular disease after 12 weeks of exercise training (29). The combination of curcumin and aerobic exercise in our study resulted in the most significant lipid profile improvements, suggesting a potential effect, though direct comparisons with prior combined intervention studies are limited.

Unlike catalase and superoxide dismutase activity, curcumin supplementation enhanced the activity of glutathione peroxidase in T2DM rats. Hajizadeh et al. found that curcumin at 7.5 and 15 mg/kg increased catalase activity and reduced malondialdehyde levels in the intestines of diabetic rats (30). Funamoto et al. also reported that theracurmin, a curcumin formulation, reduced LDL oxidation, as an oxidative stress parameter, in T2DM patients over six months (31). Aerobic exercise similarly boosted antioxidant enzyme activities in our study, consistent with Nojima et al., who observed a reduction in urinary 8-hydroxy-2'-deoxyguanosine, an oxidative stress marker, after 12 months of aerobic exercise in T2DM patients (32). The combined intervention produced the greatest enhancement in antioxidant enzyme activities, indicating complementary mechanisms that warrant further exploration.

The observed effects on lipid profiles and oxidative stress likely stem from distinct yet overlapping mechanisms. Curcumin may modulate lipid metabolism by inhibiting cholesterol synthesis and enhancing LDL receptor expression, as suggested by prior research (33), while its antioxidant effects are mediated by direct ROS scavenging and activation of Nrf2 leading to upregulating antioxidant defenses (1, 17). Additionally, curcumin inhibits NF- κ B and reduces pro-inflammatory cytokines generation that exacerbate oxidative stress (19). Aerobic exercise improves lipid profiles by increasing lipoprotein lipase activity and fatty acid oxidation, alongside reductions in visceral adiposity (23), and enhances antioxidant capacity through mitochondrial biogenesis and improved insulin sensitivity via AMPK and PGC-1 α (22). The combined intervention may leverage these pathways, with curcumin's anti-inflammatory properties potentially mitigating exercise-induced oxidative stress, though such interactions remain speculative and require molecular-level investigation.

In summary, this study underscores the therapeutic potential of the combination of curcumin and aerobic exercise in ameliorating lipid profiles and oxidative stress in T2DM. Findings suggest that curcumin and aerobic exercise, particularly in combination, hold promise as accessible adjunctive therapies for managing dyslipidemia and oxidative stress in T2DM. However, translating these results to human populations necessitates caution due to differences in metabolism, dosing, and exercise protocols between rats and humans.

Authors contributions

J. Ramezani and M. Mohyadini designed the research; A. Gorji and M. Mohyadini conducted the research; P. Javaherchian handled animals; M. Mohyadini and A. Gorji analyzed the data; and P. Javaherchian, M. Mohyadini, and F. Mahinizadeh wrote the paper. J. Ramezani had primary responsibility for the final content. All authors read and approved the final manuscript.

Conflict of Interest

The authors declare that they have no conflict of interest.

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