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Emodin attenuates insulin resistance in the gastrocnemius muscle by modulating the expression of IL-1βTNF-α/NF-kB-mediated signalling in streptozotocin-induced diabetic Rats

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ABSTRACT

Diabetes mellitus, particularly type 2 diabetes (T2DM), is characterized by insulin resistance, oxidative stress, and inflammation, which contribute to metabolic dysfunction. This study aimed to evaluate the effects of emodin on oxidative stress, inflammatory cytokines, and insulin signaling in a Streptozotocin (STZ)-induced diabetic rat model. Type 2 diabetes was induced by an intraperitoneal injection of streptozotocin (35 mg/kg body weight in 0.1 M citrate buffer, pH 4.5). The rats were divided into five groups, each consisting of six animals: Group I (normal rats, vehicle control), Group II (Type 2 diabetic rats), Group III (Type 2 diabetic rats treated with Emodin at 40 mg/kg b.wt/day orally for 45 days), Group IV (Type 2 diabetic rats treated with metformin), and Group V (control with Emodin). Emodin treatment significantly reduced oxidative stress markers (H_2O_2: 32±2.45 µm, OH: 40±2.9 µm), restored antioxidant enzyme activities (CAT: 15±0.84 ng/L, GPX: 27±1.8 pmol/ml), and reduced inflammatory cytokines (TNF-α: 180±11.64 pg/ml, IL-1β: 360±28.63 pg/ml, NF-kB: 140±8.9 ng/L). Additionally, emodin treatment enhanced the expression of insulin signaling molecules, including IR, IRS-1, PI3K, AKT, GLUT4, and AS160, indicating improved insulin sensitivity. These findings suggest that emodin can modulate oxidative stress, inflammation, and insulin resistance, providing a promising therapeutic approach for type 2 diabetes. Further studies are necessary to explore its clinical relevance and underlying molecular mechanisms.

Keywords: *Type 2 diabetes, Emodin, Insulin resistance, Gastrocnemius muscle and IL-1beta/TNF-α/NF-kB signaling pathway.*

1. INTRODUCTION

Diabetes mellitus, a significant global health concern, is expected to rise sharply in regions such as Asia, the Middle East, and Africa due to genetic, environmental, and lifestyle factors [1]. Type 2 diabetes mellitus (T2DM) is characterized by insulin resistance, β-cell dysfunction, and metabolic disruptions exacerbated by oxidative stress from high-fat diets [2]. Insulin resistance in skeletal muscle, the primary site for postprandial glucose uptake, impairs glucose metabolism due to defects in insulin signaling, glycogen synthesis, and GLUT4 translocation [3]. The consumption of high-fat and high-sucrose diets is a major contributor to insulin resistance, disrupted gluconeogenesis, oxidative stress, and the development of complications associated with type 2 diabetes, including both macro- and microvascular dysfunctions [4].

Oxidative stress and inflammatory cytokines disrupt IRS-1/PI3K/Akt pathways, further diminishing insulin sensitivity and glucose uptake [5]. Hyper glycemia-induced oxidative damage further exacerbates metabolic inflexibility and insulin resistance. Akt activation, essential for glucose uptake, is compromised in insulin resistance, highlighting its critical role in maintaining normal glucose metabolism [6]. Estradiol replacement in ovariectomized rats enhances insulin signaling, GLUT4 expression, and glucose oxidation in skeletal muscle, preventing insulin resistance caused by estradiol deficiency [7]. Calotropin from *Calotropis gigantea* inhibits HSC-3 oral cancer cell growth, migration, and invasion by inducing apoptosis, cell cycle arrest, and metabolic alterations, showing potential as an anti-cancer therapy [8].

Emodin, a natural anthraquinone derivative from *Rheum palmatum*, has shown promising anti-diabetic effects in preclinical studies. It improves insulin sensitivity by activating AMPK and modulating the IRS/PI3K/Akt pathway, leading to increased GLUT4 translocation and glucose utilization [9,10]. Additionally, Emodin exhibits anti-inflammatory properties by reducing serum levels of pro-inflammatory cytokines like TNF-α and IL-6, which are implicated in insulin resistance [11]. In diabetic mouse models, Emodin alleviates hyper glycemia, improves lipid metabolism, and protects against complications like cardiomyopathy through Akt/GSK-3β signaling [12]. Significantly higher salivary MMP-9 levels were found in OSCC and severe oral epithelial dysplasia, indicating that it may be used as a biomarker for early diagnosis and the prediction of malignant transformation [13].

Emodin, a compound derived from Rheum palmatum, has shown promising anti-diabetic effects in various studies. It ameliorates insulin resistance by reducing lipid accumulation in skeletal muscle through decreased FATP1 expression [14]. In high-fat diet-fed and STZ-induced diabetic mice, emodin activates PPARγ and modulates metabolism-related genes, leading to decreased blood glucose, triglycerides, and total cholesterol, while increasing HDL cholesterol [15]. IL-17A levels and salivary 1-25dihydroxycholecalciferol were found to be negatively correlated during orthodontic treatment, indicating that vitamin D administration may improve tooth movement with little harm to surrounding tissue [16]. In diabetes, chronic inflammation driven by cytokines such as IL-1β and TNF-α activates NF-kB signaling, contributing to insulin resistance and metabolic dysfunction in skeletal muscle [17]. Streptozotocin (STZ)-induced diabetes significantly alters protein expression in the gastrocnemius muscle, impairing glucose metabolism [18]. The aim of this study is to evaluate the impact of emodin on IL-1β/TNF-α/NF-kB-mediated signaling pathways and its effects on glucose metabolism in the gastrocnemius muscle of STZ-induced diabetic rats. This investigation seeks to understand emodin's potential as an anti-inflammatory and insulin-sensitizing agent in diabetes.

2. MATERIALS AND METHODS

Chemicals and Reagents

RNA isolation reagents, reverse-transcriptase enzymes (e.g., SuperScript™ III Reverse Transcriptase), and Go Taq Green master mix (e.g., Promega GoTaq® Green Master Mix) were acquired from different suppliers. Primers for IR, IRS-1, AKT, and β-actin and ELISA kits for glutathione peroxidase (e.g., Abcam Glutathione Peroxidase ELISA Kit) and LPO (e.g., Thermo Fisher Scientific Lipid Peroxidation Assay Kit) were procured.

Experimental design

Healthy adult male Wistar albino rats, weighing 150-180 g and aged 150-180 days, were used in this study. The animals were maintained under standard environmental conditions with free access to food and water, in compliance with the National Guidelines and approved by the Institutional Animal Ethical Committee (IAEC No: 19/23-24). Type 2 diabetes was induced by an intraperitoneal injection of streptozotocin (35 mg/kg body weight in 0.1 M citrate buffer, pH 4.5). The rats were divided into five groups, each consisting of six animals: Group I (normal rats, vehicle control), Group II (Type 2 diabetic rats), Group III (Type 2 diabetic rats treated with Emodin at 40 mg/kg b.wt/day orally for 45 days), Group IV (Type 2 diabetic rats treated with metformin), and Group V (control with Emodin). The Emodin dose was selected based on previous studies.

Lipid Peroxidation and Reactive Oxygen Species (ROS)

Lipid peroxidation (LPO) was measured using the method described by Devasagayam and Tarachand (1987), with the malondialdehyde (MDA) level reported as n moles of MDA formed/min/mg protein [19]. Hydrogen peroxide was quantified using the spectrophotometric method of Pick and Keisari (1981), with results expressed as µ moles/min/mg protein [20]. Hydroxyl radical (OH*) formation was assessed using Puntarulo and Cederbaum's (1988) technique and expressed as µ moles/min/mg protein [21].

Antioxidant Enzyme Analysis

Standard protocols were followed to evaluate antioxidant enzyme activities.

Gene Expression Analysis: RNA Isolation

Total RNA was isolated from the adipose tissues of rats under study by the method explained by Fourney et al. (1988) using a TRIR (total RNA isolation reagent) obtained from Ab gene house, United Kingdom [22]. With the reverse transcriptase RT kit from Eurogentec (Seraing, Belgium)2µg of RNA was reverse transcribed. The sequence of the primers used in this study and β-actin is used as reference gene. Using SYBR green master mix (Takara, Japan), genes were amplified in real time PCR system (Stratagene MX 3000P, Agilent Technologies, 530l, Stevens Creek Blvd, Santa Clara, CA 95051, USA) under the following reaction conditions: initial denaturation at 95 °C for 5 min followed by 40 cycles of 95 °C for 30 s, 59–60 °C for 30 s and 72 °C for 30 s. Relative quantification was calculated from the melt and amplification curves analysis. List of primers used in this study: TNF-α: FW: 5'-GTCGTAGCAAACCACCAAGC-3'; RW: 5'-CTCCTGGTATGAA ATGGCAAA-3', NFkB-FW: 5' -CATGAAGAGAAGACACTGACCATGGAAA-3'; NFkB-RW: 5' -TGGATAGAGGCTAAGTGT AGACACG-3', β-actin FW: 5'-AAG TCC CTC ACC CTC CCA AAA G-3'; RW: 5'-AAG

CAA TGC TGT CAC CTT CCC-3', IR-FW: 5′- GCC ATC CCG AAA GCG AAG ATC-3′;RW: 5′- TCT GGG TCC TGA TTG CAT-3′, AKT:FW- 5′- GGA AGC CTT CAG TTT GGA TCC CAA-3′ ;RW: 5′- AGT GGA AAT CCA GTT CCG AGC TTG-3′, GLUT4-FW-5′- GGG CTG TGA GTG AGT GCT TTC - 3′ ;RW- 5′- CAG CGA GGC AAG GCT AGA – 3', IRS-1:5' -GCC AAT CTT CAT CCA GTT GCT-3';RW- 5' -CAT CGT GAA GAA GGC ATA GGG-3'.

Statistical Analysis

The triplicate analysis results of the experiments performed on control and treated rats were expressed as mean ± standard deviation. Results were analyzed statistically by a one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Duncan's multiple range test using Graph Pad Prism version 5. The results with the $p < 0.05$ level were considered to be statistically significant.

3. RESULTS

Effect of emodin on oxidative stress markers and antioxidant enzyme activity

The study assessed the effect of emodin in diabetic model. The given table 1 shows, compared to control group (G1) the disease group (G2) shows increased level of H_2O_2 and OH. Emodin treatment significantly reduces the H_2O_2 and OH level (G3), values that were comparable to those seen in the metformin treatment diabetic group (G4). These findings suggest that emodin may help modulate ROS activity in type 2 diabetes. In diabetes conditions CAT and GPX activity are impaired. These reductions in antioxidant defences cause oxidative damage in cells. The given results shows that CAT and GPX level are reduces in diabetic conditions compare (G2) to control group (G1), emodin treated model results shows, that this compound increases the level of CAT and GPX (G3) almost the equal result of metformin treatment group (G4) (table 1 & Figure 1 &2).

Parameter	CON	DIAB	DIAB+EMOD	DIAB+MET	CON+EMOD
H_2O_2 (µm)	20±1.19	$45±2.61^{a}$	$32±2.45^{ab}$	$29±1.42^{ab}$	$24±1.72^{bc}$
OH (µm)	32±2.14	$59±3.54^{a}$	$40±2.9^{ab}$	$38±1.78^{ab}$	$30±1.79^{bcd}$
CAT (ng/L)	17±1.12	$9±0.45^{a}$	$15±0.84^{ab}$	$15±0.95^{ab}$	$16±1.12^{bcd}$
GPX (pmol/ml)	29±1.21	$16±0.89^{a}$	$27±1.8^{ab}$	$27±1.61^{ab}$	$31±2.7^{b}$

Table 1: G1 is control group, compared with diabetic control (G2), the Emodin treated diabetic group (G3) reduces the level of H_2O_2, OH and increase a level of CAT and GPX compared to G3, G4 are metformin-treated diabetic control, which shows emodin have significant effect in diabetic model.

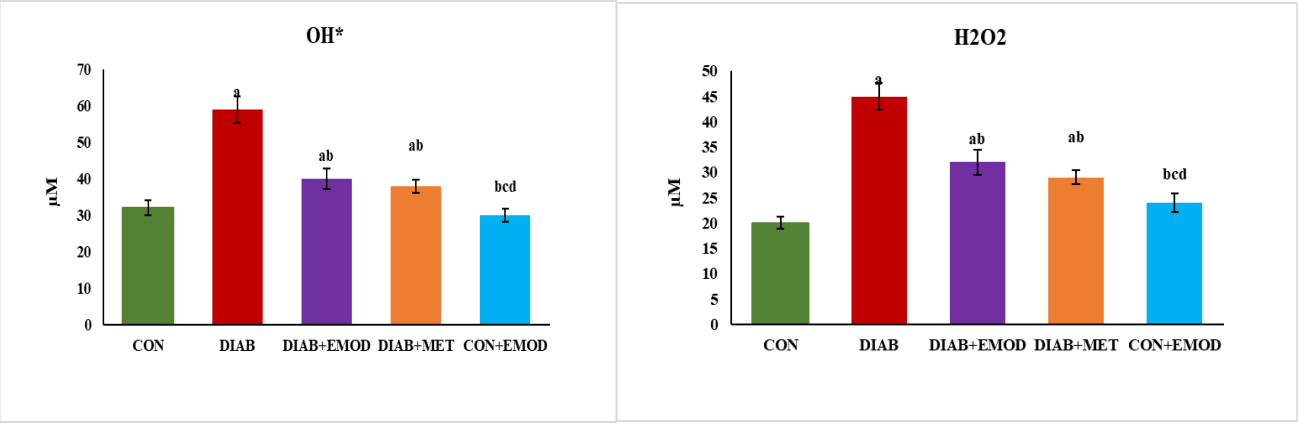


Figure 1& 2: G1 is control group, compared with diabetic control (G2), the Emodin treated diabetic group (G3) reduces the level of H_2O_2, OH and increase a level of CAT and GPX compared to G3, G4 are metformin-treated diabetic control, which shows emodin have significant effect in diabetic model.

Effect of emodin in inflammatory parameters

The pro inflammatory cytokines like TNF-α, IL-1β, NF-kB are elevated in diabetic conditions. Table 2 represent the treatment of emodin is reduces the elevation of this cytokines (TNF-α, IL-1β, NF-kB) (G3) compared to Diabetic control (G2), emodin shows nearby effect like metformin in type 2 diabetic conditions (Table 2).

Parameter	CON	DIAB	DIAB+EMOD	DIAB+MET	CON+EMOD
TNF-α (pg/ml)	150±11.21	250±16.15^{a}	180±11.64^{ab}	175±12.46^{ab}	160±13.12^{bc}
IL-1β (pg/ml)	350±25.66	490±30.64^{a}	360±28.63^{ab}	340±24.12^{abc}	340±24.64^{abc}
NF-kB (ng/L)	100±6.5	210±14.62^{a}	140±8.9^{ab}	120±6.42^{abc}	110±7.4^{abc}

Table 2: This indicates emodin's potential anti-inflammatory role in mitigating diabetic inflammation. The control group (G1) and diabetic control group (G2) shows the elevation of cytokines in normal and diabetic conditions. (G3) is emodin treated diabetic group and (G4) is metformin treated diabetic group.

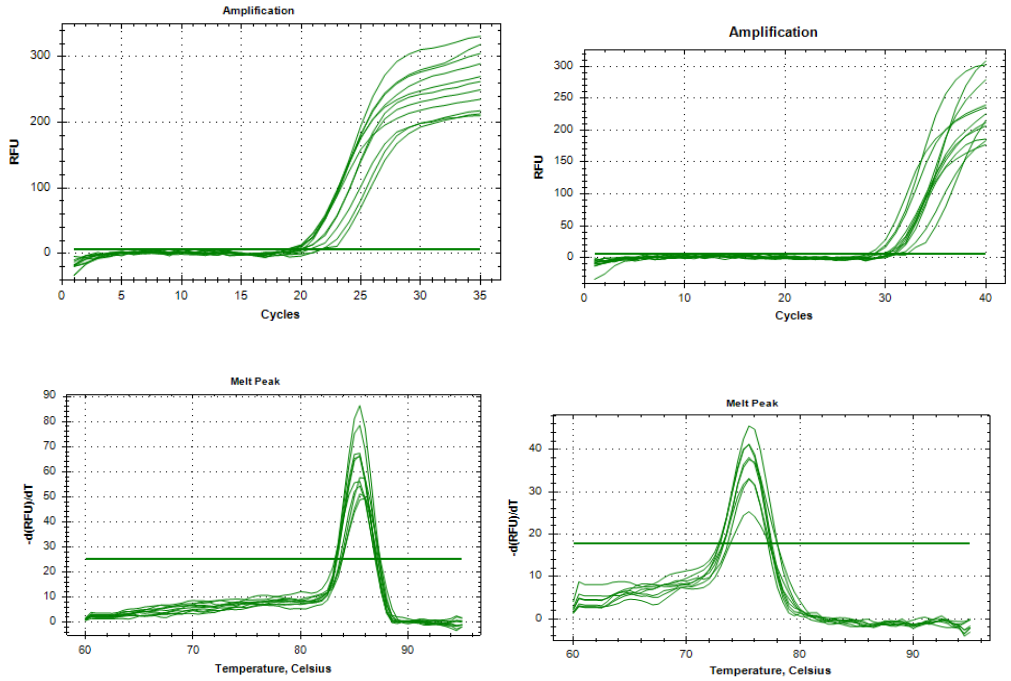
4. GENE EXPRESSION ANALYSIS

Effect of emodin on insulin signaling molecules

Gene expression analysis showed significant downregulation of IR, IRS-1, PI3K, AKT, GLUT4, and AS160 in G2 compared to G1, indicating impaired insulin signaling in this group. Treatment in G3, G4, and G5 progressively restored the expression of these genes, with G5 showing near-normal or slightly enhanced levels compared to G1. The recovery of IR, IRS-1, and downstream molecules (PI3K, AKT, GLUT4, and AS160) highlights the improved insulin signaling and glucose transport, particularly in G4 and G5. These findings suggest the treatment effectively reverses the molecular defects in insulin signaling pathways (Table 3, Figure 3-7).

Parameter	CON	DIAB	DIAB+EMOD	DIAB+MET	CON+EMOD
IR	1	0.45±0.03^{a}	0.89±0.05^{ab}	0.91±0.06^{abc}	1.1±0.09^{abcd}
IRS-1	1	0.52±0.03	0.79±0.04^{a}	0.89±0.04^{ab}	1±0.05^{abc}
AKT	1	0.71±0.04	0.92±0.06	1.0±0.07	1.1±0.06
GLUT4	1	0.41±0.02	0.82±0.03	0.95±0.04	0.95±0.04
AS160	1	0.54±0.03	0.87±0.05	0.96±0.05	1.05±0.07

Table 3: It shows that emodin treatment significantly restores the expression of insulin signaling molecules (IR, IRS-1, PI3K, AKT, GLUT4, AS160) in diabetic rats, with G3, G4, and G5 demonstrating progressive improvements compared to the diabetic control (G2).



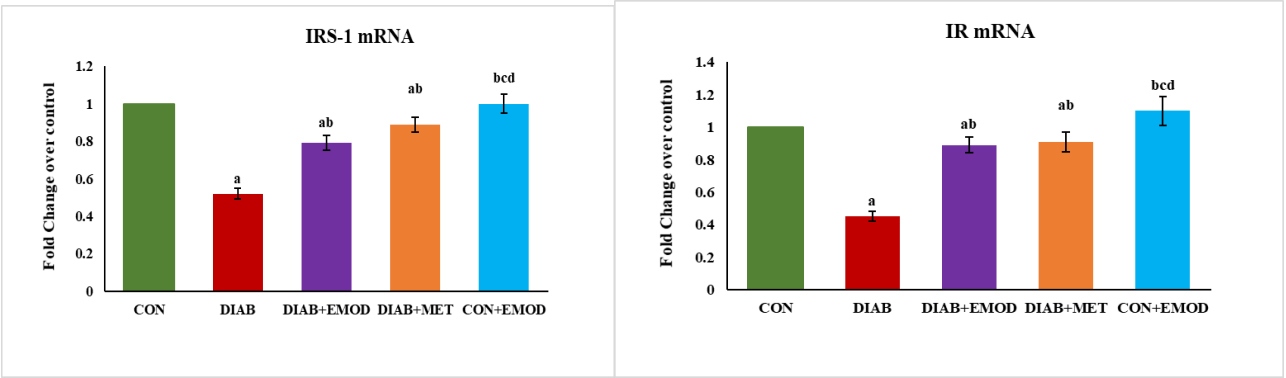
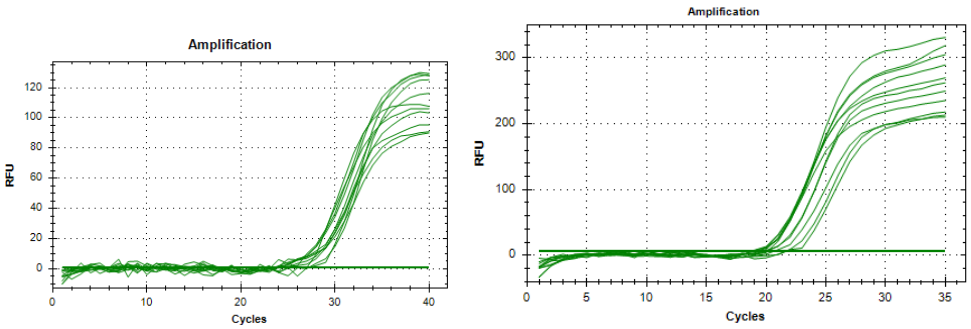


Figure 3 & 4: It shows that emodin treatment significantly restores the expression of insulin signaling molecules (IR, & IRS-1) in diabetic rats, with G3, G4, and G5 demonstrating progressive improvements compared to the diabetic control (G2).



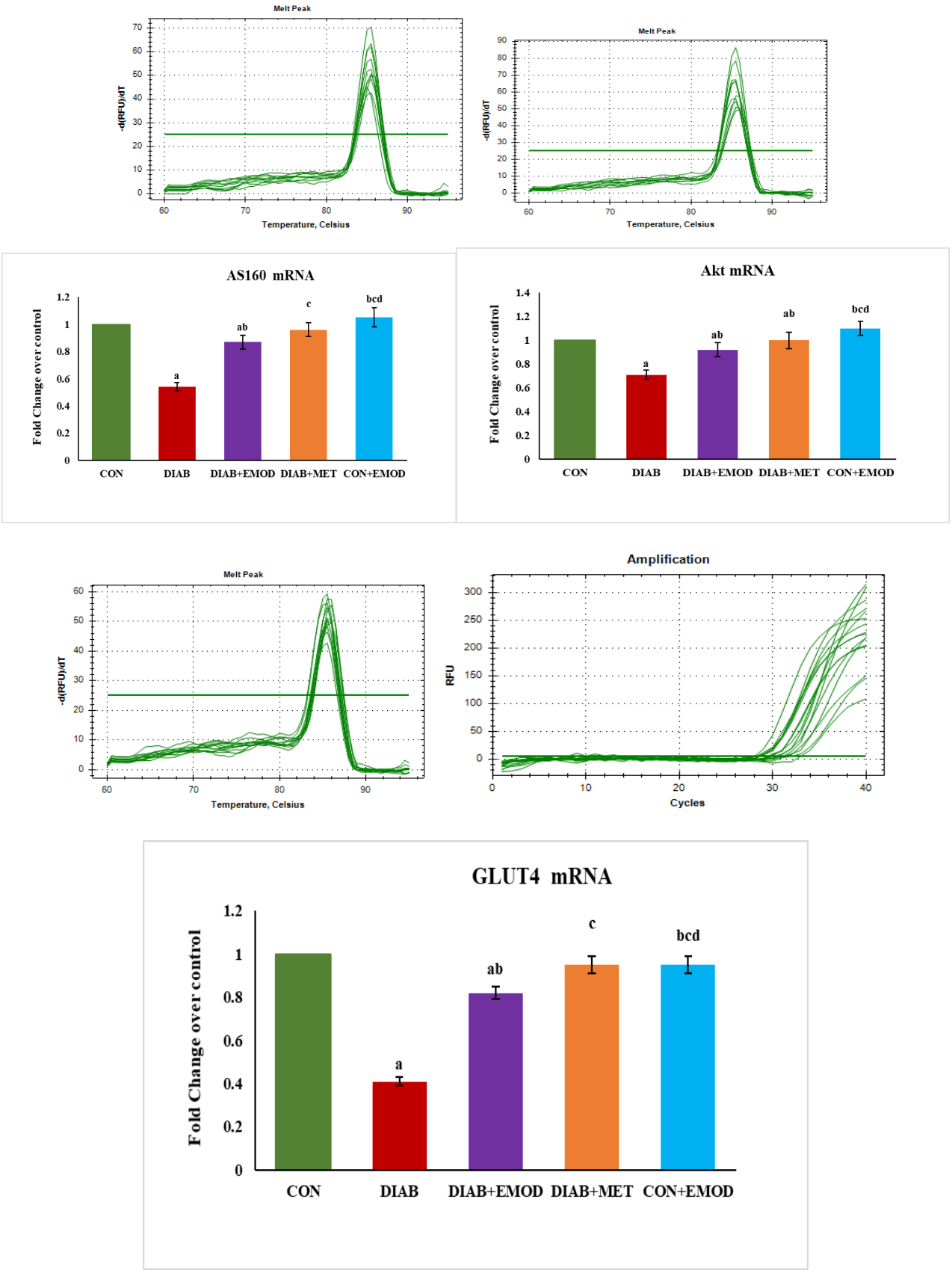


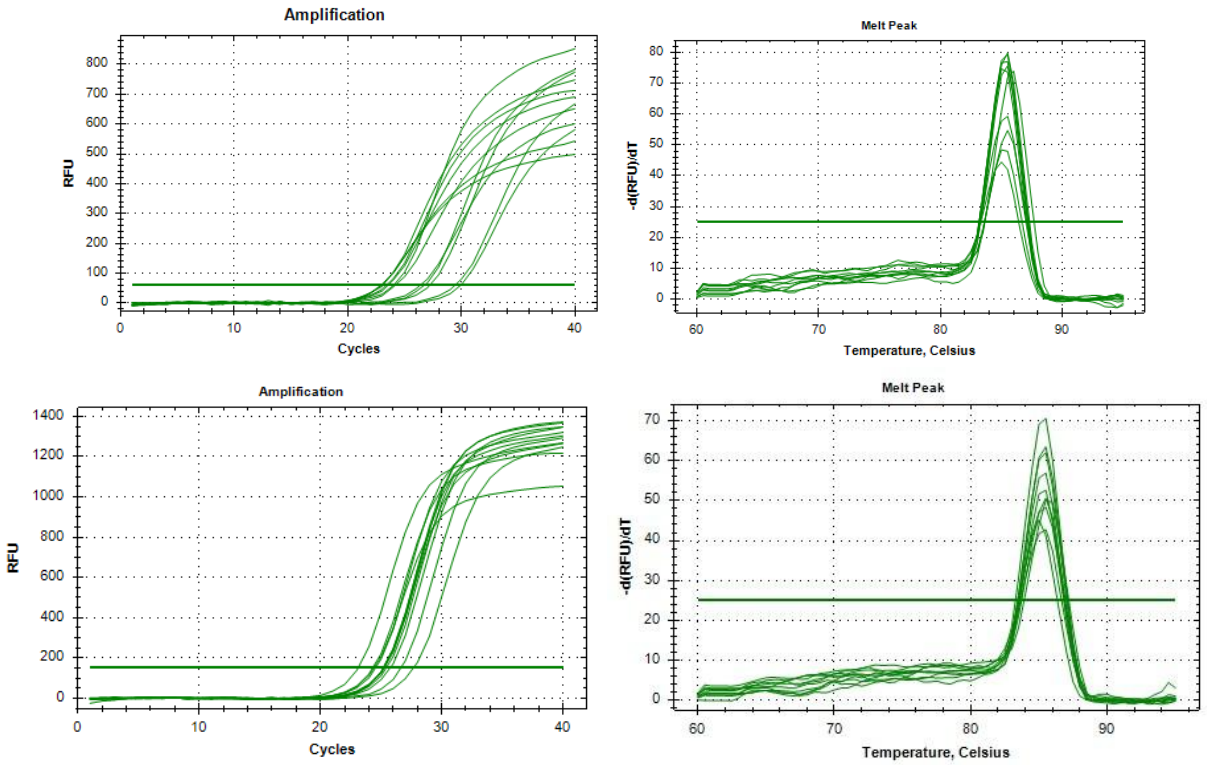
Figure 5-7: It shows that emodin treatment significantly restores the expression of insulin signaling molecules (IRS-1, AKT, GLUT4, AS160) in diabetic rats, with G3, G4, and G5 demonstrating progressive improvements compared to the diabetic control (G2).

Effects of emodin on inflammatory markers

The effects of emodin on inflammatory markers indicate that NF-kB, IL-1β, and TNF-α levels are significantly elevated in G2 compared to G1, reflecting increased inflammation. Treatment with emodin in G3, G4, and G5 progressively reduces these inflammatory markers. Notably, NF-kB and TNF-α levels in G5 are restored close to or slightly below baseline levels, while IL-1β shows partial reduction. These outcomes suggest that emodin effectively attenuates inflammation by modulating key inflammatory pathways (Table 4, Figure 8-10).

Parameter	CON	DIAB	DIAB+EMOD	DIAB+MET	CON+EMOD
NF-kB	1	1.7±0.09	1.3±0.02	1.1±0.07	0.97±0.07
IL-I β	1	1.4±0.08	1.2±0.06	0.92±0.05	1.1±0.08
TNF- α	1	1.7±0.12	1.3±0.05	1.1±0.07	1.1±0.06

Table 4: It shows that emodin treatment significantly reduces the elevated levels of inflammatory markers (NF-kB, IL-1β, TNF-α) in diabetic rats, with G5 demonstrating near-normal levels, suggesting emodin's potential to modulate inflammation effectively.



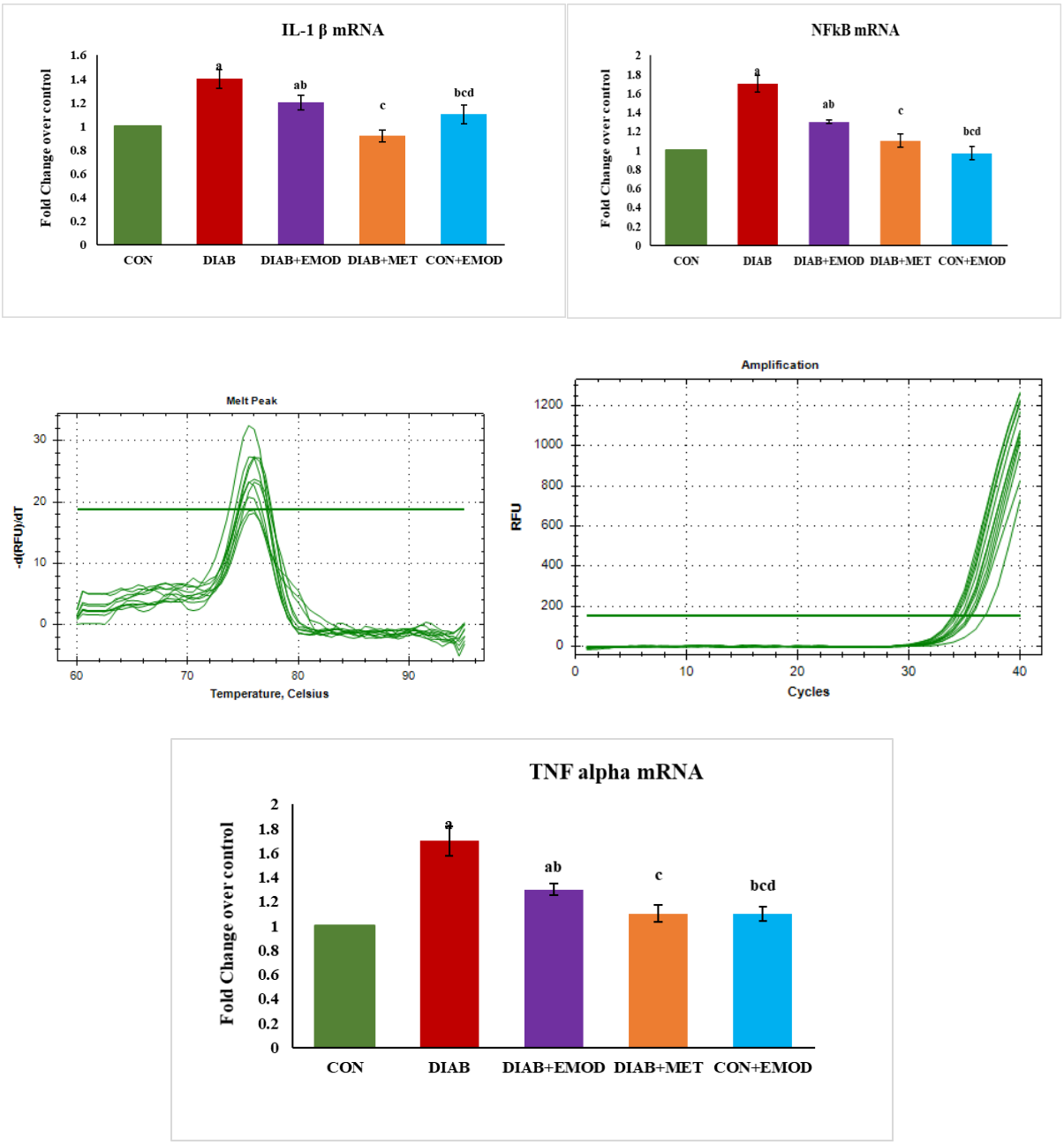


Figure 8-10: It shows that emodin treatment significantly reduces the elevated levels of inflammatory markers (NF-kB, IL-1β, TNF-α) in diabetic rats, with G5 demonstrating near-normal levels, suggesting emodin's potential to modulate inflammation effectively.

5. DISCUSSION

Diabetes mellitus is predicted to rise by 50% across Asia, the Middle East, and Africa by 2030 as a result of lifestyle, environmental, and genetic factors, making it a global health emergency. Insulin resistance damages pancreatic beta cells in type 2 diabetes, which lowers insulin production and results in malfunction. This is made worse by high-fat diets, which alter lipid metabolism, cause inflammation, and raise the formation of reactive oxygen species (ROS) [23,24]. In this study, the effects of emodin on oxidative stress, inflammation, and insulin signaling in a diabetic model were evaluated. Our findings indicate that emodin treatment significantly reduced oxidative stress markers, such as H_2O_2 and OH, in diabetic rats, which were elevated in the diabetic control group (G2) compared to the control group (G1). This reduction was similar to the effects of metformin treatment, suggesting emodin's potential to modulate reactive oxygen species (ROS) activity in type 2 diabetes. Furthermore, emodin treatment restored the activities of key antioxidant enzymes, CAT and GPX, which were significantly reduced in the diabetic control group. This aligns with previous studies showing that emodin enhances antioxidant defenses by modulating ROS levels.

Rats treated with emodin had considerably lower levels of LDL-C, total cholesterol, triglycerides, and blood glucose. By lowering FATP1-mediated lipid accumulation in the skeletal muscles of rats given a high-fat diet, it also reduced lipid accumulation in L6 cells and skeletal muscle, hence easing insulin resistance [25,14]. According to these results, emodin may help reduce oxidative stress in diabetics, possibly guarding against the harm that high ROS levels can do to cells. Furthermore, cavitated carious lesions in children have been found to have *H. pylori*, which is linked to more severe caries and a disturbed plaque ecology that favors Streptococcus mutans [26]. Circulating exosomal miRNAs, including miR-21, miR-184, and miR-145, have demonstrated promise as biomarkers for identifying patients at high risk of malignant transformation in cases of oral squamous cell carcinoma, OSMF, and leukoplakia [27].

Our research showed that the diabetic control group (G2) had considerably higher levels of pro-inflammatory cytokines, such as TNF-α, IL-1β, and NF-kB, than the control group (G1), suggesting increased inflammation. These inflammatory markers were gradually decreased by emodin treatment (G3, G4, G5), with NF-kB and TNF-α levels in G5 approaching baseline, indicating emodin's anti-inflammatory ability. These findings are consistent with past research demonstrating that emodin dramatically reduces the mRNA expression of inflammatory cytokines, including IL-6 and TNF-α, in a variety of animals [28]. This demonstrates emodin's ability to alter inflammatory pathways, which may help control the chronic inflammation linked to type 2 diabetes. Furthermore, miRNAs' potential as biomarkers and therapeutic targets is highlighted by the pathogenesis, diagnosis, and therapy of oral premalignant diseases (OPMDs) [29].

In terms of insulin signaling, gene expression analysis revealed significant downregulation of insulin resistance markers such as IR, IRS-1, PI3K, AKT, GLUT4, and AS160 in the diabetic control group (G2), reflecting impaired insulin signaling in diabetic conditions. However, emodin treatment restored the expression of these genes, with G5 demonstrating near-normal or slightly enhanced levels compared to G1. This progressive improvement in insulin signaling suggests that emodin could reverse molecular defects associated with insulin resistance. Supporting this, studies have shown that emodin enhances insulin sensitivity by activating AMPK, modulating lipid metabolism, and improving glucose uptake in skeletal muscle [9,14]. Additionally, emodin's ability to activate AMPK by inhibiting mitochondrial respiratory complex I and increasing ROS production was previously reported, contributing to enhanced glucose tolerance and insulin sensitivity [17]. NGS analysis helped with individualized therapy planning for patients with OSCC by identifying a variety of genetic variants in different grades of the disease, such as TP53, APC, and CTNNB1 [30].

Overall, our results corroborate findings from previous studies, suggesting that emodin effectively reduces oxidative stress, inflammation, and insulin resistance in diabetic models. The beneficial effects of emodin on lipid metabolism and insulin sensitivity have been demonstrated in various animal models, including KKAy diabetic mice and skeletal muscle cells [15,28]. These findings underscore emodin's therapeutic potential as an anti-diabetic agent, capable of modulating multiple pathways implicated in diabetes pathophysiology. Further research is warranted to explore its molecular mechanisms and evaluate its potential for clinical application in managing type 2 diabetes.

6. CONCLUSION

Emodin demonstrates significant therapeutic potential in mitigating oxidative stress, inflammation, and insulin resistance in a diabetic model. The study showed that emodin effectively reduced oxidative stress markers and inflammatory cytokines, while enhancing antioxidant enzyme activities. Moreover, emodin restored insulin signaling pathways, improving glucose uptake and insulin sensitivity. These findings support the role of emodin in managing type 2 diabetes through its multi-targeted actions. Further investigation is needed to explore its clinical applicability and molecular mechanisms in diabetes treatment.

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