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CURCUMIN, THYMOQUINONE, AND METFORMIN: IMPACT ON SERUM LEVELS OF AGES, SDF-1, MIR-192 AND MIR-497, AND HEPATIC OXIDATIVE STRESS AND *G6PD* EXPRESSION IN DIABETIC RATS

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Background: *In a rat model of diabetes, this study investigated the impacts of curcumin (CUR), thymoquinone (TQ), and metformin (Met) on glycemic control, lipid profile, liver and kidney functions, serum advanced glycation end products (AGEs), stromal cell-derived factor-1 (SDF-1), miR-192 and miR-497 levels. Also, it evaluated their effects on hepatic oxidative stress markers [glutathione peroxidase-1 (GPx-1), superoxide dismutase (SOD), and malondialdehyde (MDA)] and hepatic G6PD expression.* ***Methods:*** *Fifty adult male Wistar rats (200-250g) were used. Diabetes was induced in 40 rats with a single dose of intraperitoneal streptozotocin (65 mg/kg), while 10 received citrate buffer (G1; control). The diabetic rats were divided into: G2 (untreated; positive control), G3 (CUR 100mg/kg/day), G4 (TQ 50mg/kg/day), and G5 (Met 50mg/kg/day). After 28 days, rats fasted for 12h before being euthanized. Glycemic indices, lipid profile, and liver and kidney function tests were performed. Serum AGEs, SDF-1, and miRNAs levels were measured using fluorescence assay, ELISA, and qPCR, respectively. Hepatic GPx-1 and SOD activity, MDA levels, and G6PD expression were assessed by ELISA, TBARS assay, and Western blotting, respectively)* **.** ***Results:*** *All treatments improved glycemic control, lipid profile, and liver and kidney functions. They significantly reduced serum AGEs, SDF-1, and miR-192 levels, while restoring miR-497 levels. Additionally, they significantly increased hepatic GPX-1 and SOD activity, while decreased MDA levels, and G6PD expression. Notably, CUR demonstrated the most pronounced effect.* ***Conclusion:*** *CUR, TQ, and Met all demonstrated potential as adjunctive diabetes therapies by modulating metabolic and oxidative stress parameters, alongside gene expression.*

Keywords: *Curcumin; Thymoquinone; Metformin; Advanced glycation end products; Stromal cell-derived factor-1; MicroRNA-192; MicroRNA-497; Glucose-6-phosphate dehydrogenase; Oxidative stress; Diabetes*

INTRODUCTION

Intervention for diabetes mellitus (DM) and its associated comorbidities is prompting exploration of novel therapeutic avenues. These strategies focus on utilizing bioactive agents to target and inhibit the deleterious biochemical pathways initiated by reactive oxygen species (ROS), advanced glycation end products (AGEs), and inflammation[1, 2]. Natural products, recognized for their therapeutic efficacy and favorable safety profile, hold promise in this regard. They may empower cells to mitigate the detrimental effects of chronic hyperglycemia[3-6].

Curcumin (CUR), [(1E,6E)-1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione], is the chief bioactive constituent of turmeric. Extracted from the dried rhizomes or synthesized in the lab, this versatile natural polyphenol exhibits pleiotropic effects. Notably, it possesses antioxidant, anti-

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inflammatory, and anti-cancer properties, suggesting therapeutic potential in various diseases like diabetic complications and chronic inflammatory conditions. CUR's safety profile is generally favorable, with minimal to no reported side effects [7-9].

Thymoquinone (TQ), [2-Isopropyl-5-methyl-1,4-benzoquinone], the principal bioactive component of Nigella sativa oil, exhibits a broad spectrum of therapeutic effects. This quinonoid compound demonstrates potent inhibitor of oxidative stress and inflammatory processes activities. Notably, TQ's lipophilicity (fat-solubility) facilitates cellular penetration, enabling it to scavenge free radicals within intracellular compartments. Furthermore, TQ can interact with essential antioxidant molecules like glutathione, NADH, and NADPH, forming a reduced metabolite (glutathione-dihydro-TQ) that further enhances its free radical scavenging capacity. This multifaceted mechanism of action against various stressors positions TQ as a promising natural therapeutic candidate for managing diverse diseases including DM [10-12].

Metformin (Met), the most used medication to begin treatment for type 2 diabetes (T2DM), is lauded for its glycemic control properties. It exerts its effects through both blood sugar-lowering and insulin-sensitizing mechanisms. Met primarily functions by suppressing hepatic gluconeogenesis and enhancing peripheral tissues' glucose uptake. Additionally, it modulates glucagon secretion, a hormone that antagonizes insulin's action. While primarily indicated for T2DM, Met can be used adjunctively with insulin in some type 1 diabetes (T1DM) cases, potentially reducing insulin requirements and improving overall glycemic control[13].

This study examined the potential of CUR, TQ, and Met to combat DM and oxidative stress in diabetic rats. We evaluated their effects on serum levels of AGEs and stromal cell-derived factor-1 (SDF-1), a crucial regulator of pancreatic β-cell function and insulin sensitivity[14, 15]. Additionally, we assessed their influence on the levels two circulating microRNAs (miR-192 and miR-497), known to be dysregulated in DM [16, 17]. Furthermore, the study examined the impact of these compounds on hepatic oxidative stress markers [glutathione peroxidase-1 (GPx-1), superoxide dismutase (SOD), and malondialdehyde (MDA)] and expression of glucose-6-phosphate dehydrogenase (*G6PD*). By this analysis, we aimed to elucidate the mechanisms by which these compounds might exert beneficial effects in diabetes management.

MATERIALS AND METHODS

Animals

The study utilized fifty adults male Wistar rats (200 to 250 g). The rats were purchased from the animal housing unit of Assiut University's Faculty of Veterinary Medicine. Housed in standard animal-grade rooms (20-22°C, 50-60% moisture, 12h light/dark cycle), ten rats occupied each cage with ad libitum access to laboratory chow and water. Acclimatization for one week in the animal house occurred before the experiments began.

Of an initial 50 rats, 40 were randomly selected for induction of DM by a lone dose of streptozotocin (65 mg/kg, solubilized in 100 mmol/L citrate buffer), injected intraperitoneally (Sigma-Aldrich, St. Louis, MO, USA). A tail blood glucose value of 200 mg/dL or more within a specified timeframe served as confirmation of successful diabetes induction. The remaining ten rats received an equal quantity of the citrate buffer alone and served as the control group (G1).

Following the injections, all fifty rats were kept on ad libitum food and water for four weeks. During this period, their fasting blood glucose levels, body weights, and food and water intake were monitored regularly. This provided important baseline data for evaluating the subsequent effects of the drug treatments.

After the four-week baseline observation period, the 40 diabetic rats were separated into 4 groups (G2-G5) of ten rats each. The G2 group (positive control) received no intervention. G3 received CUR (100 mg /kg/day)[18]. G4 received TQ (50 mg/kg/day)[6, 19]. G5 received Met (50 mg/kg/day)[20]. All treatments were purchased from Sigma-Aldrich (St. Louis, MO, USA). Each calculated dosage was dissolved in normal saline and administered as a single daily dose via gastric gavage for 28 days. The control group (G1) and the diabetic mellitus group (G2) received 1ml

distilled water/100gm body weight daily via the same route[6]. Upon completion of the experimentation, the rats, having fasted for 12 hours, were euthanized in compliance with approved ethical guidelines.

Sampling and methods

Blood samples

They were obtained from the medial eye canthus into plain tubes. These were left to clot for 10 min at °٢٢-٢٠C. Centrifugation (3000 rpm for 15 min) separated the serum, which was then aliquoted and kept at -80°C till use [21]. Glycated hemoglobin percentage (HbA1c%) in red blood cells was measured using the HbA1c% ELISA Kit (Cat# ab289836) from Abcam, UK, utilizing a blood sample collected in an EDTA tube.

Analysis of serum lipid profile, and liver and renal functions

Serum triglycerides (TG) and total cholesterol (TC) levels were assayed calorimetrically by their corresponding kits from Sigma-Aldrich (Cat# MAK266 & MAK043, respectively). HDL cholesterol was measured with the HDL-c colorimetric assay kit from ThermoFisher Scientific (Invitrogen™, Cat# EEA012). LDL-c was then determined indirectly via the Friedewald equation LDL-c = TC – (HDL-c + TG/5)] [6]. All kits employed met the relevant specifications, and all results are expressed in mg/dL.

Blood urea nitrogen (BUN), and serum creatinine (sCr), alanine transaminase (sALT), and aspartate transaminase (sAST), were measured calorimetrically using their corresponding kits from Sigma-Aldrich (Cat# MAK006 & MAK080, MAK052, and MAK055, respectively).

Assay of the AGEs and SDF-1

A commercially available fluorescence assay kit (ab273298, UK) was used to estimate the serum AGEs based on their natural fluorescent properties, exhibiting peak emission at 460 nm upon excitation at 360 nm, a characteristic shared by most AGEs. A proprietary buffer formulation within the kit ensures selective detection of AGEs by preventing interference from non-oxidized proteins. To validate assay performance, oxidized bovine serum albumin (AGE-BSA) served as a positive control, representing elevated AGEs levels, while non-oxidized BSA provided a background reference. Subsequently, the fluorescence intensity of the samples was normalized against the background signal, yielding a relative measurement of AGEs levels compared to the control. The relative fluorescence intensity (RFI) measured in arbitrary units (AU) under assay conditions is set to 1, where RFI = relative fluorescence unit (RFU) sample / RFU Background. Serum SDF-1 levels were estimated calorimetrically using a mouse SDF-1 ELISA kit from Sigma-Aldrich (cat# RAB0125).

Analysis of serum miR-192 and miR-497 relative expression

RNA extraction

Total RNA was isolated from serum samples by the Applied Biotechnology Total RNA Extraction Kit (spin column) (Cat. No. ABT002, Egypt). All steps were performed in an RNase-free environment at the medical research center, Assiut University. Briefly, 200 µL of serum was added to 700 µL of RNA lysis buffer by pipetting and vertexing. After the addition of 0.2 mL of chloroform, centrifugation was done at 12,000 rpm for 5 min at 20-22°C . The aqueous phase was transferred to a tube, followed by adding 700 µL of 70% ethanol. After mixing by inversion, the mixture and precipitate were loaded into a spin column and centrifugated at 12,000 rpm for 30 sec at 20-22 °C.

A 500 µL washing buffer was introduced to the mixture. The mixture was then centrifugated at 12,000 rpm for 30 sec twice. To remove any remaining washing buffer, the empty column was centrifugated at 12,000 rpm for 1 min. Finally, the spin column was transferred to a 1.5 mL RNase-free centrifugation tubes, and 50 µL of elution buffer was mixed and centrifugated at 12,000 rpm for 1 min. RNA quality and concentration were assessed using a DNA-RNA calculator on the BioTek Epoch Microplate Spectrophotometer (Inventory number MRC 4-23, serial number 130125E). The RNA was frozen at -80°C for later use.

Complementary DNA (cDNA) synthesis

Reverse transcription was performed using cDNA ABT 2X RT Mix (Applied Biotechnology, Egypt, Cat. No: ABT002), following the manufacturer's instructions. Ten microliters of ABT 2X RT Mix were combined with 0.2-2 micrograms of template RNA and up to 20 microliters of nuclease-free water. The mixture was kept at 42°C for 30 min in a thermal cycler. The reaction was then stopped by heating it at 70°C for 5 min. The cDNA product was kept at -20°C.

Quantitative real time polymerase chain reaction (qPCR)

The qPCR was done using ABT 2X qPCR Mix (SYBR) (Applied Biotechnology, Egypt, Cat. No: ABT002). The reaction mix contained 10 µL of ABT 2X qPCR Mix, 15 pmol each of forward and reverse primers, 80-200 ng of cDNA, and nuclease-free water up to a volume of 20 µL. The program began with a denaturation step at 95°C for 3 min then 45 cycles with three stages each: 15 sec at 95°C, 30 sec at 50-60°C (based on primer's melting temperature), and 30 sec at 72°C for extension.

The primers were obtained from Willow Fort, UK. For miR-192, the forward primer (5′-CTGACCTATGAATTGACAGCCA-3′) has a melting temperature (Tm) of 54.7°C, and the reverse primer (5′-GCTGTCAACGATACGCTACGT-3′) has a Tm of 57.2°C. For miR-497, the forward primer (5′-GCAGCACACTGTGGTTT-3′) has a Tm of 55.6°C, and the reverse primer (5′-GAACATGTCTGCGTATCTC-3′) has a Tm of 58.3°C. The U6 snRNA forward primer (5′-CGCTTCGGCAGCACATATAC-3′) has a Tm of 56.3°C, while the reverse primer (5′-TTCACGAATTTGCGTGTCAT-3′) has a Tm of 53.1°C[22]. U6-snRNA served as the internal control for normalization. miR-192, and miR-497 expression levels were subsequently standardized to U6-snRNA and fold changes (relative expressions) were determined by the $2^{-\Delta\Delta Ct}$ method [23].

Liver samples

Analysis of hepatic oxidative status

Some liver specimens were homogenized and centrifugated at 1000 rpm for 20 min. The supernatants were gathered in aliquots and kept at -80°C. Total protein concentrations were assayed using the Bradford method [24]. The levels of GPx-1 and SOD were assayed in the supernatants using ELISA kits (Cat# ab102530 and ab65354, respectively) from Abcam, UK, following the manufacturer's directions. The lipid peroxidation product MDA was assayed by the thiobarbituric acid reactive substances (TBARS) assay. Under acidic and hot conditions (around 95°C), MDA and TBA readily react, forming a distinctive pink chromophore. The intensity of this pink color, measured by its absorbance at 535 nm, directly reflects the amount of MDA present in the sample and indirectly serves as a marker for overall lipid peroxidation levels[25].

Assessment of hepatic *G6PD* expression by Western blotting

Other liver specimens were carefully excised, homogenized, and lysed on ice-cold radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris–Cl, 5 mM EDTA, 150 mM NaCl, 0.5% NP-40, and 0.5% Triton-X-100, pH 7.6) supplemented with 1 µg/ml leupeptin, aprotinin, and 0.5 mM phenylmethylsulfonyl fluoride. After being centrifugated at 2500 rpm (10 min at 4°C), supernatants were gathered. Aliquots of 30 µg protein were then separated by 10% SDS-PAGE. The separated proteins were then blotted to nitrocellulose membranes using a semi-dry BioRad blotter. The membranes were then blocked with 2% BSA to avoid unfavorable binding. Subsequently, they were kept for 12h at 4°C with primary antibodies: mouse anti-G6PD IgG (1:5000, Develop, China, Cat. No. DL96832A) and rabbit anti-β-actin IgG (1:5000, Abcam, Cambridge, UK). After washing the membranes the next day, they were incubated with HRP-conjugated secondary antibodies (goat anti-mouse IgG and goat anti-rabbit IgG, Santa Cruz Biotechnology, 1:10,000) for 1h at 20-22°C. Immunoreactive bands were envisioned by the electrochemiluminescence substrate. Protein band densities were quantified using ImageJ freeware [24].

Statistical analysis

We used statistical software (SPSS version 26) to analyze the data. To compare the variables between different groups, we performed a one-way analysis of variance (ANOVA). In this analysis, we considered

results statistically significant if the p-value was less than or equal to 0.05.

RESULTS AND DISCUSSION

Fasting blood glucose (FBG), HbA1c%, and lipid profile

Treatment with CUR (G3) in diabetic rats significantly reduced FBG and HbA1c% compared to the untreated diabetic group (G2). Both TQ (G4) and Met (G5) also showed significant improvements, though to a lesser extent. None of the treatments, however, fully normalized these markers to control levels (**Table 1**).

While TG and TC showed non-significant difference between the groups (**Table 1**), pairwise comparisons (not shown) revealed a significant decrease in TC for TQ treated rats (G4) compared to the untreated diabetic rats (G2) ($p = 0.34$).

Untreated diabetic rats (G2) exhibited significantly lower HDL-c and higher LDL-c levels than healthy controls (G1). Notably, all treatments significantly reversed the reduced HDL-c, with CUR showing the most pronounced effect (**Table 1**). Similarly, treatment with TQ (yielding the most significant improvement) followed by CUR and Met significantly alleviated the elevated LDL-c in diabetic rats (**Table 1**).

Liver and renal function tests

Compared to the nondiabetic rats (G1), untreated diabetic rats (G2) exhibited significantly elevated sALT and sAST levels, and these liver enzyme levels were significantly lowered in diabetic rats treated with CUR, TQ, or Met (all $p < 0.001$, **Table 2**). Similarly, sCr levels followed this trend (**Table 2**). However, BUN showed nonsignificant differences among the study groups (**Table 2**).

Serum AGEs and SDF-1 levels

Untreated diabetic rats (G2) exhibited significantly higher AGEs levels compared to the control group (G1) ($p < 0.001$). Notably, treatment with CUR (G3), TQ (G4), and Met (G5) all significantly decreased AGEs levels in diabetic rats **(Fig. 1a, AGEs**). Similar findings were observed for serum SDF-1 levels with Met (G5) had the least pronounced effect (**Fig. 1b**).

Table 1: FBG, HbA1c%, and lipid profile in the study groups.

	G1: CNT n = 10	**G2: DM n = 10**	**G3: CUR n = 10**	**G4: TQ n = 10**	**G5: MET n = 10**	**p Value**
FBG (mg/dl)	87.5 ± 11.6	257 ± 39.3	166.9 ± 26.3	186.2 ±25.7	192.3 ± 16.7	< 0.001
HbA1C %	5.2 ± 0.3	10.3 ± 1.2	7.1 ± 0.8	8.1 ± 0.7	8.3 ± 0.5	< 0.001
TG (mg/dl)	107 ± 13.8	120 ± 19.2	118 ± 13.6	118 ± 19.3	119 ± 16.8	0.388
TC (mg/dl)	166.2 ± 18.1	184 ± 7	175 ± 17	168 ± 22.4	178 ± 15	0.108
HDL-c (mg/dl)	49.4 ± 3.3	34.2 ± 3.7	44.2 ± 2.4	42.8 ± 2.8	39.4 ± 2.9	< 0.001
LDL-c (mg/dl)	95.4 ± 17.2	125.8 ± 11.3	106.9 ± 17.3	101.3 ± 23.2	115.5 ± 16.9	0.003

FBG: fasting blood glucose**, HbA1C** %**:** glycated hemoglobin percentage, **TG**: triglycerides, **TC:** serum total cholesterol, **HDL-c:** high density lipoprotein-cholesterol, **LDL-c**: low density lipoprotein cholesterol, **CNT:** control group, **DM:** diabetic untreated rats, **CUR:** diabetic rats treated with curcumin, **TQ:** diabetic rats treated with thymoquinone, **Met**: diabetic rats treated with metformin.

Table 2: Serum ALT, AST, and Cr, and BUN in the study groups**.**

	G1: CNT n = 10	**G2: DM n = 10**	**G3: CUR n = 10**	**G4: TQ n = 10**	**G5: MET n = 10**	**p Value**
sALT (U/L)	24.4 ± 4.9	58.9 ± 23.7	27.3 ± 7.1	31.1 ± 8.6	43.5 ± 20.7	< 0.001
sAST (U/L)	23.3 ± 3.9	51.8 ± 21.7	30 ± 6.8	37 ± 18	30 ± 7.2	< 0.001
BUN (mg/dl)	15.7 ± 4.4	16.3 ± 3.2	13.9 ± 3.6	13.0 ± 3.8	14.1 ± 3.8	0.348
sCr (mg/dl)	0.87 ± 0.08	1.06 ± 0.12	1.05 ± 0.08	1.04 ± 0.08	1.05 ± 0.1	< 0.001

sALT: serum alanine transaminase, **sAST:** serum aspartate transaminase, **BUN:** blood urea nitrogen, **sCr:** serum creatinine**, CNT:** control group, **DM:** diabetic untreated rats, **CUR:** diabetic rats treated with curcumin, **TQ:** diabetic rats treated with thymoquinone, **Met**: diabetic rats treated with metformin.

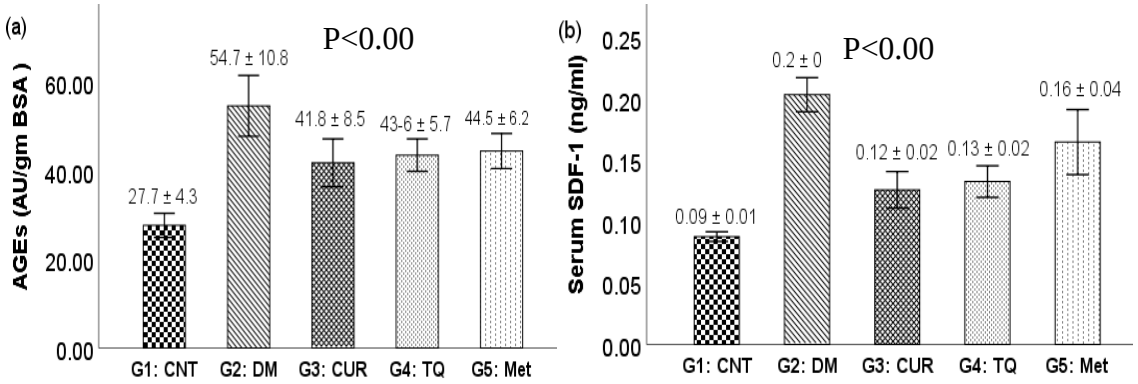

Fig. 1: (a) Serum advanced glycation end products **(AGEs)** and **(b)** serum stromal cell derived factor-1**(SDF-1)** levels in different study groups. **CNT:** control group, **DM:** diabetic untreated rats, **CUR:** diabetic rats treated with curcumin, **TQ:** diabetic rats treated with thymoquinone, **Met**: diabetic rats treated with metformin.

Circulating miR-192 and miR-497 levels

The untreated diabetic group (G2) exhibited significantly higher circulating miR-192 levels compared to the nondiabetic control (G1). Interventions with CUR (G3), TQ (G4), and Met (G5) all significantly reduced circulating miR-192 ($p< 0.001$). CUR (G3) showed the most pronounced effect, restoring miR-192 expression in diabetic rats to near-control levels (**Fig. 2a**).

Additionally, G2 showed significantly lower miR-497 expression compared to G1 ($p< 0.001$). Treatment with CUR (G3), TQ (G4), and Met (G5) significantly restored miR-497 expression ($p< 0.001$), with CUR demonstrating the greatest increase (**Fig. 2b**). TQ and Met were also significantly effective, but to a lesser extent.

The levels of hepatic oxidative status biomarkers (GPX-1, SOD, and MDA)

Compared to the nondiabetic control group (G1), GPX-1 levels were significantly low in the untreated diabetic rats (G2). Treatment with CUR (G3), TQ (G4), and Met (G5) significantly increased GPX-1 levels in diabetic rats. CUR (G3) nearly restored GPX-1 levels to those of the control group. TQ (G4) treatment achieved similar levels to CUR (G3). Met (G5) increased GPX-1 less than CUR (G3) and TQ (G4), but still significantly higher than untreated diabetic rats (G2) (p-values were <0.001 for all) (**Fig. 3a**).

Similar results were observed regarding the hepatic SOD levels but the increase in its levels were nearly the same in the diabetic rats treated with TQ (G4) and Met (G5) (**Fig.** 3b).

Furthermore, untreated diabetic rats (G2) exhibited a significant elevation in hepatic MDA levels compared to nondiabetic controls (G1). Treatment with CUR (G3), TQ (G3), or Met (G5) significantly reduced MDA levels in diabetic rats ($p < 0.001$), with no significant differences observed among the treatment groups (**Fig. 3C**).

Western blotting analysis of the expression level of *G6PD* in liver tissue

Our results showed a significant increase in *G6PD* expression in the liver tissue of the untreated diabetic rats (G2) compared to nondiabetic controls (G1). Treatment with CUR, TQ, or Met significantly downregulated *G6PD* levels ($p < 0.001$). Notably, CUR and Met had the most pronounced effect, reducing *G6PD* expression to even below control levels (**Fig. 4a & 4b**).

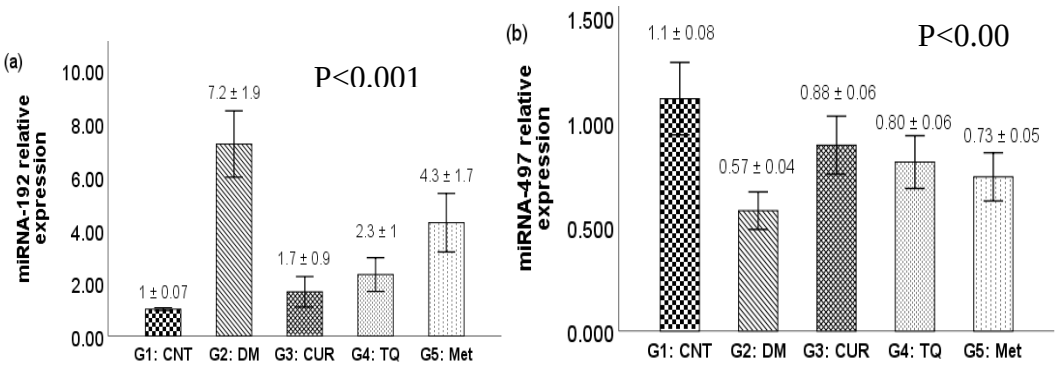

Fig. 2: miRNA-192 and miRNA-497 relative expressions in different study groups. **CNT:** control group, **DM:** diabetic untreated rats, **CUR:** diabetic rats treated with curcumin, **TQ:** diabetic rats treated with thymoquinone, **Met**: diabetic rats treated with metformin.

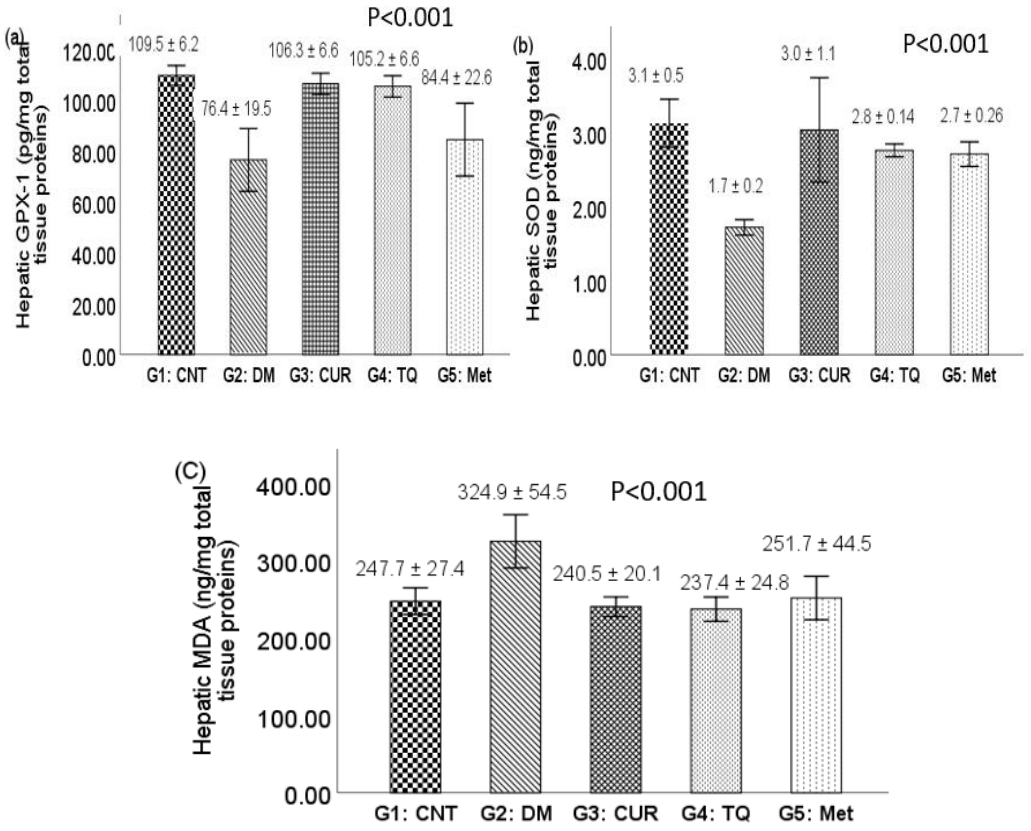

Fig. 3: Hepatic oxidative status showing the levels of **(a)** glutathione peroxidase-1 (GPX-1) **(b)** superoxide dismutase (SOD), and **(c)** malondialdehyde (MDA). **CNT:** control group, **DM:** diabetic untreated rats, **CUR:** diabetic rats treated with curcumin, **TQ:** diabetic rats treated with thymoquinone, **Met**: diabetic rats treated with metformin.

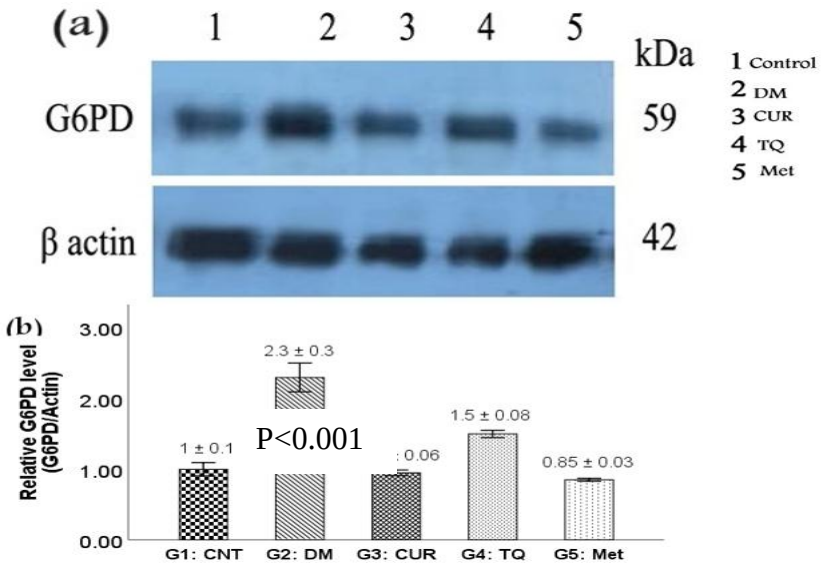

Fig. 4 : Western blotting analysis of the expression level of glucose-6-phosphate dehydrogenase (*G6PD*) in liver tissue of different groups; **(a)** a representative figure and **(b)** a densitometric estimation of bands. β action was used as an equal loading reference. **CNT:** control group, **DM:** diabetic untreated rats, **CUR:** diabetic rats treated with curcumin, **TQ:** diabetic rats treated with thymoquinone, **Met**: diabetic rats treated with metformin.

Discussion

The global rise of DM and its long-term complications pose a major public health threat. Even with optimal blood sugar control, some tissue damage may linger. This "metabolic memory" refers to persistent complications despite good management. It's likely driven by oxidative stress and inflammation. High blood sugar environments lead to excessive ROS production, which disrupts cellular functions. This includes mitochondrial dysfunction, formation of AGEs, and activation of stress pathways like the polyol pathway and protein kinase C signaling. Ultimately, these overproduced ROS trigger cellular stress responses, causing lasting damage[26, 27].

In this study, we explored the potential of CUR, TQ, and Met to combat DM and oxidative stress in diabetic rats. We investigated the impact of these compounds on serum levels of AGEs, SDF-1, miR-192, and miR-497, along with hepatic oxidative stress markers and the expression of *G6PD*.

Glycemic control and dyslipidemia

All three interventions demonstrated positive effects. While none completely normalized all parameters, our study demonstrated the efficacy of CUR, TQ, and Met in improving glycemic control in diabetic rats, evidenced by reductions in FBG levels and HbA1c%. While all treatments exerted beneficial effects, CUR displayed the most potent hypoglycemic activity, suggesting its potential as a valuable adjunct therapy for DM management.

Interestingly, although serum TG and TC levels did not statistically differ between groups, pairwise comparisons revealed a significant reduction in TC for TQ-treated rats. This suggests that TQ might offer lipid-modulating benefits beyond glycemic control, warranting further investigation.

More prominently, all treatments significantly reversed the decline in HDL-c and elevation in LDL-c observed in untreated diabetic rats. CUR again emerged as the strongest modulator, highlighting its potential in managing diabetic dyslipidemia and potentially reducing cardiovascular risks.

Liver and renal functions

Untreated DM led to elevated levels of liver enzymes ALT and AST, indicating possible hepatic injury. Notably, all interventions effectively reduced these enzyme levels, demonstrating their protective effects on liver function. Similarly, serum Cr levels, a marker of renal function, showed improvement

with all treatments, suggesting potential benefits for preserving kidney health in diabetic individuals.

However, blood urea nitrogen (BUN) remained unaffected by interventions. This finding warrants further exploration to elucidate the underlying mechanisms and differential effects of treatments on specific renal parameters.

Serum levels of AGEs and SDF-1

Our study revealed significantly higher serum AGEs and SDF-1 levels in untreated diabetic rats compared to controls. These markers play significant roles in diabetic complications, and their reduction by CUR, TQ, and Met suggests promising therapeutic potential. In DM, chronic hyperglycemia fuels the excessive formation of AGEs through reactions between glucose and other molecules. AGEs can cross-link macromolecules (proteins/lipids), causing detrimental effects. The accumulation of AGEs in various tissues induces oxidative stress and the production of ROS. Additionally, AGEs activate inflammatory pathways, promoting sustained, low-level inflammation, a key player in diabetic complications[28,29]. While there are currently no specific medications directly targeting AGEs, managing their impact and the oxidative stress they cause is crucial. Antioxidant therapy might be helpful in this regard[30, 31]. Looking ahead, research is actively exploring therapies that target AGEs or their receptors, which could hold promise as new avenues to prevent or slow the progression of diabetic complications.

SDF-1, also known as CXCL12, is a chemokine playing a crucial role in cell migration, tissue development, repair, and regeneration[32]. It triggers the chemotactic mobilization of various immune cells, including neutrophils, lymphocytes, and monocytes, following stimulation by inflammatory mediators like TNF, LPS, and IL1[33]. Beyond its immune functions, SDF-1 affects pancreatic β-cell development, insulin secretion, and sensitivity[14, 15]. Dysregulation of SDF-1 disrupts β-cell function and modulates insulin sensitivity in peripheral tissues (insulin resistance). SDF-1 further amplifies this metabolic imbalance by influencing inflammatory pathways and oxidative stress[34, 35]. Studies link elevated SDF-1 in diabetic patients to worsened insulin resistance, chronic inflammation (a hallmark of DM complications), and potentially diabetic kidney disease development[14, 36].

Circulating miR-192 and miR-497 levels

Additionally, CUR, TQ, and Met significantly altered microRNA levels. They reduced elevated levels of miR-192 and restored low levels of miR-497 in diabetic rats, with CUR again demonstrating the strongest effect. This finding highlights the potential of these interventions in modulating miRNA expression and thereby influencing disease progression.

MicroRNAs are tiny, typically about 22 nucleotides long, non-coding RNA molecules that have emerged as powerful regulators of gene expression. They play a major role in numerous biological processes, making them key players in human health and disease, including potentially influencing diabetic complications[37]. MicroRNAs orchestrate both insulin production and response, playing a crucial role in DM. Their dysregulation can disrupt the dance of healthy glucose control, leading to islet dysfunction and insulin resistance[38].

The role of miR-192 and miR-497 in various aspects of DM and its complications is complex and remains under investigation. Elevated blood levels of miR-192 have been related with an increased risk of developing both types of DM. This suggests its potential as a biomarker for predicting or monitoring the progression of diabetic complications, such as diabetic nephropathy[39-42]. Research on miR-497 in DM shows promise in furthering the understanding of diabetic complications. High blood sugar levels appear to suppress miR-497 expression, potentially linking it to the development of diabetic neuropathic pain[17, 43]. In diabetic wounds, miR-497 exhibits anti-inflammatory properties by defeating the activity of pro-inflammatory cytokines, making it a promising candidate for therapeutic intervention[44]. Extra studies are required to reveal the mechanisms by which these two microRNAs act and to explore their potential as therapeutic targets in diabetic patients.

Hepatic oxidative stress status

Hepatic GPX-1 levels, an antioxidant enzyme, were significantly lower in untreated diabetic rats, indicating increased oxidative stress. Treatment with all three interventions significantly increased GPX-1 activity, with CUR and TQ showing the most remarkable restorations. Similar trends were observed for SOD, another antioxidant enzyme. These findings suggest that the studied compounds act by modulating antioxidant machinery and reducing oxidative stress, which likely contributes to their overall beneficial effects.

Interestingly, MDA showed significant reductions in all treatment groups, with no significant differences among them. This suggests that despite variations in the extent of GPX-1 and SOD induction, all interventions effectively mitigated oxidative stress markers in the liver.

Hepatic *G6PD* Expression

Finally, untreated diabetic rats exhibited significantly elevated *G6PD* expression, a key enzyme in the pentose phosphate pathway. This suggests a potential compensatory mechanism against oxidative stress. *G6PD* plays a crucial role in regulating nicotinamide adenine dinucleotide phosphate (NADPH) levels, a molecule essential for the antioxidant system. Interestingly, G6PD activity measurements have been reported as a potential marker for both oxidative stress and inadequate blood sugar control in diabetic patients[45, 46]. Notably, all interventions significantly downregulated *G6PD* expression, with CUR and Met achieving reductions even below control levels. This finding warrants further investigation to understand the complex interplay between oxidative stress, *G6PD* activity, and the specific effects of the tested compounds.

Conclusion

Our study provided compelling evidence for the therapeutic potential of CUR, TQ, and Met in managing DM and its associated complications. All three compounds (CUR, TQ, and Met) demonstrated significant modulatory effects on various metabolic and oxidative stress parameters, alongside gene expression, suggesting their promise as adjunct therapies in DM treatment.

Limitations of the study

While this study offers promising results, translating these findings to human application necessitates careful consideration. Physiological and metabolic differences between rats and humans necessitate confirmation of these observations in well-designed clinical trials. Furthermore, establishing safe and effective dosing regimens for each agent in humans remains a crucial next step. Finally, while the study sheds light on potential mechanisms, further investigation is required to definitively elucidate the precise mechanisms by which these compounds exert their effects.

Abbreviations

DM: Diabetes mellitus, **ROS**: reactive oxygen species, **AGEs**: Advanced glycation end products, **CUR:** Curcumin, **TQ:** Thymoquinone, **NADH:** Reduced nicotinamide adenine dinucleotide, **NADPH:** Reduced nicotinamide adenine dinucleotide phosphate, **Met:** Metformin, **T2DM:** Type 2 diabetes mellitus, **T1DM:** Type 1 diabetes mellitus, **SDF-1**: stromal cell-derived factor-1, **RNA:** Ribonucleic acid, **miR**: microRNA, **GPx-1:** Glutathione peroxidase-1, **SOD**: Superoxide dismutase, **MDA**: Malondialdehyde, ***G6PD*:** glucose-6-phosphate dehydrogenase, **IRB:** Institutional review board, **USA**: United States of America, **HbA1c**: Glycated hemoglobin, **rpm**: Revolutions per minute, **TG**: Triglycerides, **TC:** total cholesterol, **HDL-c:** High density lipoprotein-cholesterol, **LDL-c:** Low density lipoprotein-cholesterol, **TC:** Total cholesterol, **Cat:** Catalog, **BUN**: Blood urea nitrogen, **sCr**: Serum creatinine, **sALT:** Serum alanine transaminase, **sAST:** Serum aspartate transaminase, **BSA:** Bovine serum albumin, **RFI**: Relative fluorescence intensity, **AU:** Arbitrary units **RFU**: Relative fluorescence unit, **ELISA:** Enzyme-linked immunosorbent assay, **DNA:** Deoxyribonucleic acid, **cDNA:** Complementary DNA, **qPCR:** Quantitative real time polymerase chain reaction, **UK:** United Kingdom, **snRNA**: Small nuclear RNA, **TBARS:** Thiobarbituric acid reactive substances, **RIPA**: Radioimmunoprecipitation assay, **EDTA:** Ethylenediaminetetraacetic acid, **SDS-PAGE**: Sodium dodecyl-sulfate polyacrylamide gel electrophoresis, **SPSS:** Statistical Package for the Social Sciences,

FBG: Fasting blood glucose, **CNT:** control group, **CXCL12:** C-X-C motif chemokine ligand 12, **TNF:** Tumor necrosis factor, **IL1**: Interleukin-1.

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الكركمين والثيموكينون والميتفورمين: التأثير على مستويات AGEs وSDF-1 وmiR-192 وmiR-497 في المصل والإجهاد التأكسدي الكبدي وتعبير G6PD في الفئران المصابة بمرض السكري

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خلفية: في نموذج الفئران لمرض السكري، بحثت هذه الدراسة في تأثيرات الكركمين (CUR) والثيموكينون (TQ) والميتفورمين (Met) على التحكم في نسبة السكر في الدم، ومستوى الدهون، ووظائف الكبد والكلى، ومنتجات نهاية الارتباط المتقدم بالجلوكوز في المصل (AGEs)، وعامل مشتق من الخلايا السدوية-١ (SDF-١)، ومستويات miR-١٩٢ وmiR-٤٩٧. كما قامت بتقييم تأثيراتها على علامات الإجهاد التأكسدي الكبدي [الجلوتاثيون بيروكسيديز-١ (GPx-١)، وسوبر أكسيد ديسموتاز (SOD)، والمالونديالدهيد (MDA)] وتعبير PD٦G الكبدي.

طُرق: تم استخدام خمسين من ذكور جرذان ويستار البالغة (٢٠٠-٢٥٠ جرام). تم إحداث مرض السكري في ٤٠ جرذًا بجرعة واحدة من ستربتوزوتوسين داخل الصفاق (٦٥ مجم / كجم)، بينما تلقى ١٠ جرذان محلول سترات (G١؛ مجموعة التحكم). تم تقسيم الجرذان المصابة بمرض السكري إلى: G٢ (غير معالجة؛ مجموعة التحكم الإيجابية)، G٣ (CUR١٠٠ مجم / كجم / يوم)، G٤ (TQ٥٠ مجم / كجم / يوم)، وG5 (Met٥٠ مجم / كجم / يوم). بعد ٢٨ يومًا، صامت الجرذان لمدة ١٢ ساعة قبل إعدامها. تم إجراء مؤشرات نسبة السكر في الدم، ومستوى الدهون، واختبارات، وظائف الكبد، والكلى. تم قياس مستويات AGEs وSDF-١ وmiRNAs في المصل باستخدام اختبار الفلورسنت وELISA وqPCR على التوالي. تم تقييم نشاط GPx-١ وSOD الكبدي ومستويات MDA وتعبير PD٦G بواسطة ELISA واختبار RSTBA واختبار Western blotting على التوالي)

نتائج: وقد أدت جميع العلاجات إلى تحسين التحكم في نسبة السكر في الدم، ومستوى الدهون، ووظائف الكبد، والكلى. كما خفضت بشكل ملحوظ مستويات AGEs وSDF-١ وmiR-١٩٢ في المصل، مع استعادة مستويات miR-٤٩٧. بالإضافة إلى ذلك، فقد زادت بشكل ملحوظ نشاط GPX-١ وSOD في الكبد، بينما خفضت مستويات MDA وتعبير PD٦G. والجدير بالذكر أن CUR أظهر التأثير الأكثر وضوحًا.

خاتمة: أظهرت كل من CUR وTQ وMet إمكانات كبيرة كعلاجات مساعدة لمرض السكري من خلال تعديل معايير الإجهاد الأيضي والتأكسدي، إلى جانب التعبير الجيني.