

Effect of Quercetin and Metformin on Glucose Transporter-4 Expression, Oxidative Stress, Inflammation Markers and Insulin Resistance in Type 2 Diabetes Mellitus

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Keywords

- Glucose transporter-4
- Quercetin, Metformin
- Insulin resistance
- Type 2 diabetes mellitus

Abstract

Background: Defects in glucose transporter-4 (GLUT-4) expression and function in the skeletal muscles and adipose tissue can be considered as the main cause of insulin resistance (IR) in type 2 diabetes mellitus (T2DM). Also, Oxidative stress and inflammation play a very important role in the development of IR and T2DM. **Aim:** Studying the effect of quercetin and metformin on GLUT-4 expression, oxidative stress, inflammation markers and IR in T2DM. **Method:** This study was carried out on 50 male Wistar rats which were divided into five groups; control, untreated diabetic rats, diabetic rats treated with metformin, diabetic rats treated with quercetin and diabetic rats treated with metformin and quercetin. **Results:** The use of metformin and quercetin separately produced significant decrease in plasma glucose, insulin, IL-6, TNF- α levels, HOMA-IR and TBARS level in skeletal muscle. Also, they caused significant elevation in the antioxidant enzyme activities in skeletal muscles with increased expression of GLUT-4 in the skeletal muscles and adipose tissue compared to the diabetic rats. Evidently, the quercetin effects were more significant than that of metformin on all the parameters except on HOMA-IR (similar significant improvement). Moreover, the combined use of quercetin and metformin produced highly significant improvement approaching control level in all the parameters than that observed by using either of them. **Conclusion:** The combined use of QC and MF improved hyperglycemia and IR by increasing the expression of GLUT-4 in skeletal muscles and adipose tissue together with the reversal of the oxidative stress and inflammatory states.

INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic disorder caused by deficiency of insulin secretion by the beta cells of the pancreas or diminished action of insulin on different tissues cells or both. Long term hyperglycemia and accompanying metabolic disturbances are the main causes of development of various diabetic complications as retinopathy, nephropathy, peripheral neuritis, cardiovascular diseases and sexual impairment (1). DM is the most prevalent endocrine disease in the world and its prevalence was estimated to be increased from 382 million in 2013 to 592 million in 2035 (2). According to American Diabetes Association (ADA), type 2 DM (T2DM) accounts for ~90-95 % of all diabetic patients worldwide. It is caused by a mixture of peripheral insulin resistance (IR) and insufficient compensatory insulin output by the pancreatic beta cells (3).

It was confirmed that inflammation plays a major role in the pathogenesis of T2DM mediated by pro-inflammatory cytokines as interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF- α) that are secreted in response to excess intracellular triglycerides which enhances IR (4).

Also, reactive oxygen species (ROS) induced damage has been implicated in the pathogenesis of T2DM (5). Moreover, it was reported that the free radical scavenging ability of diabetic patients is lower than that of healthy people which may be due to lower concentration of natural antioxidants (6).

As DM is a chronic disease and antidiabetic drugs have many side effects, the use of natural products as functional foods and herbal medicine is now emerging as an alternative and

complementary line of management due to its apparent effectiveness, minimal side effects and relatively low cost (7).

Quercetin (QC) Flavonoids are a family of plant compounds that occurs naturally in fruits and vegetables as onions, apples, grapes and nuts which are commonly included in human diets (8). A previous study showed that administration of QC to streptozotocin (STZ) treated rats showed a significant reduction in fasting and post-prandial blood glucose levels (9). There was a significant evidence that QC has a role in the treatment of oxidative damage, DM, cancer, inflammation, bacterial and viral infections and cardiovascular diseases (10).

Metformin (MF) is an oral hypoglycemic drug of the biguanide class which has the ability to decrease glucose production by the liver, increase glucose consumption by peripheral tissues and increase expression of insulin receptors and tyrosine kinase enzyme activity in liver and muscles (11). Moreover, it influences translocation of glucose transporter 4 (GLUT-4) (12) besides its powerful antioxidant actions (13). These effects result in enhancement in peripheral insulin sensitivity with no danger of hypoglycaemia or body weight increase (14). Therefore, the American Diabetes Association and the European Association for the Study of Diabetes suggested MF as the first choice for T2DM treatment (15).

GLUT-4 is an insulin-dependent glucose transporter which is present in the myocardium, skeletal muscles, adipose tissue and brain. It exists in vesicles inside the cells cytoplasm from which it is translocated to the cell membrane under the effect of insulin. It causes rise of the cells glucose uptake by about 10-20 fold (16). Insulin promotes

glucose entry to cells by binding to its receptor resulting in activation of phosphatidylinositol-3 kinase (PI3K) pathway which increases GLUT-4 translocation from intracellular vesicles to the plasma membrane of skeletal muscle (17,18). It was found that defects in signaling pathway which lead to defects in GLUT-4 expression and function can be considered as the major cause of IR (19). So, GLUT-4 could be a very important target for the different methods of T2DM treatment (20).

Accordingly, the treatment of T2DM should be directed to increase the expression of GLUT-4 in the skeletal muscles and adipose tissue cells and improve the oxidative stress and inflammatory states to decrease IR and lower the blood glucose level.

So, the aim of the present work is to study the effect of quercetin and metformin on GLUT-4 expression in skeletal muscles and adipose tissue, oxidative stress, inflammatory markers and insulin resistance in T2DM. We chose metformin as it is a cheap standard drug for treatment of T2DM and quercetin as it is a natural flavonoid available in many foods and easy to be consumed by the diabetic patients.

Material and Methods:

Experimental animals

After approval from local ethical committee of Faculty of Medicine, Tanta University, this study was carried out on fifty male Wistar rats weighing 180–200 g. The animals were obtained from the animal house of Faculty of Medicine, Tanta University. All animals were kept under the same environmental conditions, fed standard commercial rat chow and had free access to tap water throughout the period of the study.

The rats were housed in isolated animal cages and kept under a 12-hour light–dark cycle at controlled temperature ($23\pm 1^\circ\text{C}$) and humidity 70–75%. They were divided into five groups of 10 rats each and allowed to acclimatize to their environment for five days before the start of experiment. The handling of the animals was carried out in accordance with the ethical guidelines for investigations.

Research design

At the beginning of the present study, 10 rats were separated as a control group (group I) and fed ad libitum standard commercial chow with tap water for 8 weeks. For the induction of T2DM, 50 rats were injected with single intravenous injection of 45 mg/kg streptozotocin (STZ) (Sigma, Chemical Co., St. Louis, MO, USA) dissolved in sodium citrate buffer (0.1 mol/liter, pH adjusted to 4.5) at a concentration of 20 mg/ml (21). After 3 days, fasting blood samples were obtained from the tail vein and fasting blood sugar was measured using a glucometer (Aquo-Check, Roche). Rats having fasting blood sugar more than 200 mg/dl were considered as diabetic (21) and used for the study. Then, 40 diabetic rats were randomly chosen and distributed among 4 groups (each group consisted of 10 rats) as follows:

Group II (Untreated diabetic rats): The rats had free access to food and water and treated orally with 1 ml normal saline by orogastric tube (oral gavage) for 8 weeks.

Group III (Metformin treated diabetic rats): The rats received MF orally in a dose of 10 mg/kg/day dissolved in 1 ml normal saline by orogastric tube (oral gavage) for 8 weeks.

Group IV (Quercetin treated diabetic rats): QC was given to the rats orally in a dose of 25 mg/kg/day dissolved in 1 ml normal saline by orogastric tube (oral gavage) for 8 weeks.

Group V (Metformin and quercetin treated diabetic rats): The rats were given combined treatment with MF and QC orally in the same doses in group III and IV for 8 weeks.

At the end of the experimental period, the rats were fasted for 12 hours overnight and then fasting blood samples were taken from retro-orbital venous plexus immediately in heparinized capillary tubes under light ether anesthesia. Thereafter, the blood was centrifuged at 3000 rpm for 15 min to separate plasma for different biochemical assays. The animals were then decapitated under ether anesthesia, and tissue samples from the soleus muscle were rapidly excised and stored at -20°C for subsequent biochemical assays. For determination of GLUT-4 expression, samples from soleus muscle and its adjacent adipose tissue were preserved in formalin 10% solution and processed in usual way until paraffin blocks were obtained.

Chemicals:

Quercetin and metformin were purchased from Sigma-Aldrich Co., USA.

The techniques:

The different techniques of the present study were carried out in Physiology department, immunohistochemistry unit, Pathology department, Faculty of Medicine and central research laboratory of Tanta University.

Biochemical assays:

Fasting plasma glucose was measured using Roche Hitachi 912 Chemistry Analyzer. Insulin plasma level was determined by radioimmunoassay (RIA-Immulite, IML2000, IML 2500 insulin) provided by Siemens Medical Solutions Diagnostics. Insulin resistance was calculated using the formula of the Homeostasis Model Assessment (HOMA-IR) = fasting insulin (uIU/ml) x fasting glucose (mmol/liter) / 22.5. Plasma IL-6 level was determined using ELISA kits (R&D Systems, Minneapolis, MN, USA). Plasma TNF- α level was assessed using ELISA kits (TiterZyme EIA kit, Assay Designs, Inc., Ann Arbor, MI, USA).

The oxidative stress markers were estimated in the skeletal muscle tissue as follows: Thiobarbituric acid reactive substance (TBARS) by the method of Ohkawa et al. (22), superoxide dismutase (SOD) activity by the method of Marklund and Marklund (23), catalase (CAT) activity by the method of Aebi (24) and activity of glutathione peroxidase (GSH-Px) by the method of Lawrence and Burk (25).

Immunohistochemical detection of GLUT-4 expression in skeletal muscle and adipose tissue:

Sections of 4- μm thickness were cut from the blocks of formalin-fixed paraffin-embedded tissues, deparaffinized and rehydrated as usual. To reduce nonspecific background staining due to endogenous peroxidase, slides were incubated in H_2O_2 block for 15 min. Slides were microwaved in 10 mmol/l of citric acid at pH 6.0 for antigen retrieval. After being rinsed with phosphate buffer solution (PBS), the sections were incubated with

anti-GLUT-4 primary antibody (1:100; Servicebio, USA) overnight at 4°C. A further wash in PBS was followed by treatment with peroxidase-labeled polymer conjugated to a secondary antibody for 30 minutes at room temperature. The staining was visualized with diaminobenzidine chromogen, followed by counterstaining with Mayer's hematoxylin. Normal cardiac muscles were used as GLUT-4 positive control. For negative control, the primary antibody was omitted for each run.

Evaluation of immunohistochemical results:

GLUT-4 immunoreactivity was evaluated semi-quantitatively in skeletal and adipose cells demonstrating distinct membranous and/or cytoplasmic staining. Immunoreactivity was assessed in at least 5 high-power fields at $\times 400$ magnification and was assessed as: 0% of cells positive (-, negative), <25% of cells positive (+, weakly positive), 25%–50% of cells positive (++ , moderately positive), >50% of cells positive (+++ , strongly positive). Negative and weakly positive were regarded as low expression, and moderately and strongly positive were regarded as high expression (26).

Statistical analysis

Data were processed using SPSS program V. 20. They were expressed as mean (M) and standard deviation (SD). Comparisons between groups were done using one-way analysis of variance (ANOVA) test followed by inter-group comparisons using Post hoc test. Statistical analysis of the immunohistochemical results was conducted using χ^2 test. The results were considered as statistically significant if the p value was <0.05.

Results:

- Effect of DM and its treatment with MF and QC separately and in combination on plasma levels of glucose, insulin and Homeostatic Model Assessment for Insulin Resistance (HOMA-IR):-

The induction of DM in group II caused significant elevation in plasma glucose, insulin levels and HOMA-IR as compared to control group (I). In group III (MF treated diabetic rats), a significant decrease in the previous parameters was detected ($p < 0.05$ for glucose and insulin and $p < 0.001$ for HOMA-IR) when compared to group II. In group IV, QC produced a similar effect on HOMA-IR ($p < 0.001$) as that of MF but caused more significant reduction in the plasma glucose and insulin levels ($p < 0.001$) than that made by MF. Additionally, in group V, the combined use of MF and QC caused more significant decrease in all the parameters when compared to group II ($p < 0.001$) and approaching nearly the control levels when compared to group I ($p > 0.05$) (Table 1).

- Effect of DM and its treatment with MF and QC separately and in combination on oxidative stress markers levels in the skeletal muscles.

Comparing the results of group II with that of the control group revealed significant increase in TBARS level with significant decrease in SOD, CAT and GSH-Px antioxidant enzyme activities in the skeletal muscle. While, the use of MF in group III caused significant reduction in

TBARS level with significant elevation of the antioxidant enzymes activities in skeletal muscles ($p < 0.05$) as compared to the diabetic rats. Also, QC administration in group IV produced more significant results when compared to group II ($p < 0.001$). In addition, in group V, treatment

of diabetic rats with both QC and MF resulted in more significant improvement ($p < 0.001$ compared with group II) in the oxidative stress markers with restoration of the antioxidant enzymes activities to near-control values ($p > 0.05$ when compared to control group) (Table 2).

Table 1: Effect of DM and its treatment with MF and QC separately and in combination on plasma levels of glucose, insulin and HOMA-IR.

| Parameter | Group I (Control) | Group II (DM) | Group III (DM+MF) | Group IV (DM+QC) | Group V (DM+ MF & QC) |
|--------------------------------|-------------------|----------------|-------------------|------------------|-----------------------|
| Fasting plasma glucose (mg/dl) | 81.4±8.93 | 226.8 ± 10.57* | 214.5 ± 10.30 # | 132 ± 8.60 ** | 86.3 ± 6.50 ** † |
| Plasma Insulin (uIU/ml) | 4.89±0.85 | 16.82±0.93* | 15.59±0.42 # | 6.38±0.81 ** | 5.26±0.76 ** † |
| HOMA-IR | 1±0.04 | 9.42±1.9* | 8.26±0.75 ** | 2.08±0.8 ** | 1.12±0.15 ** † |

Data are expressed as mean±SD. DM= Diabetes Mellitus, MF= Metformin, QC= Quercetin

* $p < 0.05$ compared to group I.

** $p < 0.001$ compared to group II.

$p < 0.05$ compared to group II.

† $p > 0.05$ compared to group I.

Table 2: Effect of DM and its treatment with MF and QC separately and in combination on tissue levels of oxidative stress markers in the skeletal muscles.

| Parameter | Group I (Control) | Group II (DM) | Group III (DM+MF) | Group IV (DM+QC) | Group V (DM+ MF & QC) |
|---------------------------|-------------------|---------------|-------------------|------------------|-----------------------|
| TBARS (nmol/l/mg protein) | 1.54 ± 0.11 | 4.44 ± 0.83* | 3.59 ± 0.65# | 2.55 ± 0.72** | 1.64 ± 0.10 ** † |
| SOD (U/mg protein) | 6.32 ± 0.82 | 2.55 ± 0.90* | 3.71 ± 0.92# | 5.5 ± 0.84** | 6.02 ± 0.74 ** † |
| CAT (U/mg protein/min) | 1.53± 0.10 | 0.62 ± 0.12* | 0.76 ± 0.10# | 1.35 ± 0.08** | 1.48 ± 0.12 ** † |
| GSH-Px (U/mg protein/min) | 131± 10.30 | 76.2 ± 7.90* | 85.5 ± 9.43# | 101.35± 9.50** | 127.3 ± 9.12 ** † |

Data are expressed as mean±SD. DM= Diabetes Mellitus, MF= Metformin, QC= Quercetin

* $p < 0.05$ compared to group I.

** $p < 0.001$ compared to group II.

$p < 0.05$ compared to group II.

† $p > 0.05$ compared to group I.

- Effect of DM and its treatment with MF and QC separately and in combination on IL-6 and TNF- α plasma levels.

In group II, the diabetic rats showed significant elevation in IL-6 and TNF- α plasma levels as compared to the control group. In contrast, the treatment of

diabetic rats with MF in group III caused significant reduction in the plasma level of these proinflammatory cytokines ($p < 0.05$) as compared to the untreated diabetic rats. While, the use of QC in group IV resulted in more significant decrease in the plasma level of IL-6 and TNF- α ($p <$

0.001) when compared to group II. Moreover, the combined treatment with both QC and MF more significantly decreased the plasma level of IL-6 and TNF- α to near the control level ($p < 0.001$ when compared to group II and $p > 0.05$ when compared to group I) (Table 3).

- Effect of DM and its treatment with MF and QC separately and in combination on GLUT-4 expression in skeletal muscles and adipose tissue:

In group I (control group), there was a high GLUT-4 membranous and cytoplasmic expression in skeletal muscles and high membranous expression in adipose tissue cells [100% of cases] (Figure 1 A & B), in contrary to group II (diabetic rats) in which the expression of GLUT-4 was low either in the cell

membrane or cytoplasm in both skeletal and adipose tissue cells [100% of cases] (Figure 1 C & D). In group III and IV (MF and QC treated rats separately), the GLUT-4 expression was ranged from high expression in group III and IV [60% and 70% of cases respectively] to low in 40% and 30% of cases respectively (Figure 1E,F,G & H). While, in group V (combined MF and QC treated rats), most cases show high GLUT-4 expression in skeletal muscles and adipose tissue [90% of cases] (Figure 1 I & J). There was a strong significant statistical difference in GLUT-4 immunohistochemical expression between the five studied groups ($p < 0.001$) (Table 4).

Table 3: Effect of DM and its treatment with MF and QC separately and in combination on plasma levels of proinflammatory cytokines (IL-6 and TNF- α).

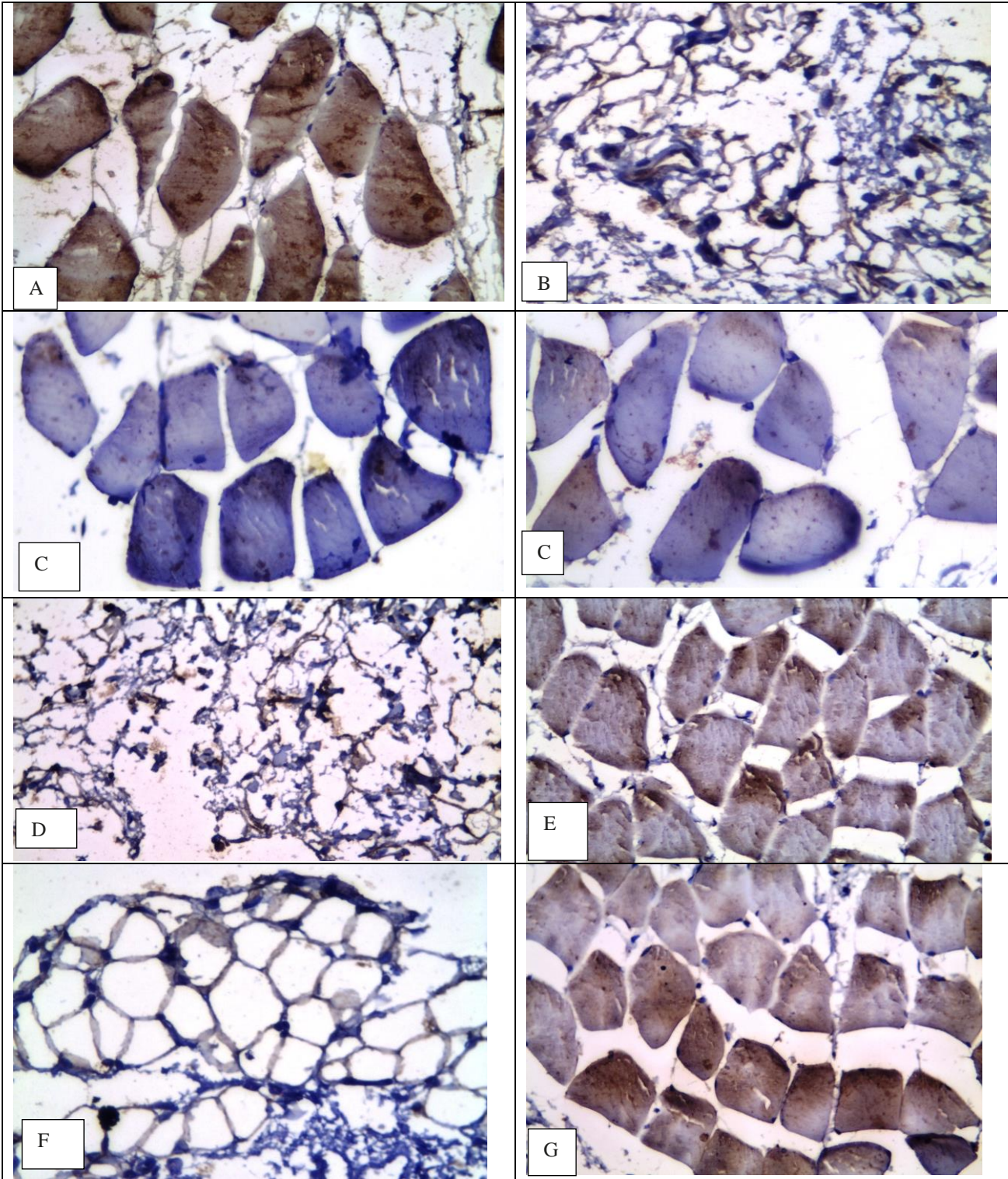
| Parameter | Group I (Control) | Group II (DM) | Group III (DM+MF) | Group IV (DM+QC) | Group V (DM+ MF & QC) |
|-----------------------|-------------------|--------------------|---------------------|--------------------|----------------------------------|
| IL-6 (pg/ml) | 142.90 \pm 7.83 | 234.5 \pm 8.55* | 221.60 \pm 10.78# | 166.5 \pm 8.84** | 144.13 \pm 6.66** [†] |
| TNF- α (pg/ml) | 68.80 \pm 7.32 | 137.30 \pm 6.86* | 127.70 \pm 8.76# | 85.10 \pm 9.42** | 74.10 \pm 8.63 ** [†] |

Data are expressed as mean \pm SD. DM= Diabetes Mellitus, MF= Metformin, QC= Quercetin
 * $p < 0.05$ compared to group I. # $p < 0.05$ compared to group II.
 ** $p < 0.001$ compared to group I. [†] $p > 0.05$ compared to group I

Table 4: Effect of DM and its treatment with MF and QC separately and in combination on glucose transporter- 4 (GLUT-4) expression in skeletal muscles and adipose tissue.

| Groups | GLUT-4 expression | | X ² | P value |
|--------------------|-------------------|----------|----------------|---------|
| | Low | High | | |
| Group I (Control) | 0 (0%) | 10(100%) | 26.562 | <0.001* |
| Group II (DM) | 10(100%) | 0(0%) | | |
| Group III (DM+MF) | 4 (40%) | 6 (60%) | | |
| Group IV (DM+QC) | 3 (30%) | 7 (70%) | | |
| Group V (DM+MF+QC) | 1 (10%) | 9 (90%) | | |

DM= Diabetes Mellitus, MF= Metformin, QC= Quercetin
 *Significant $p < 0.05$



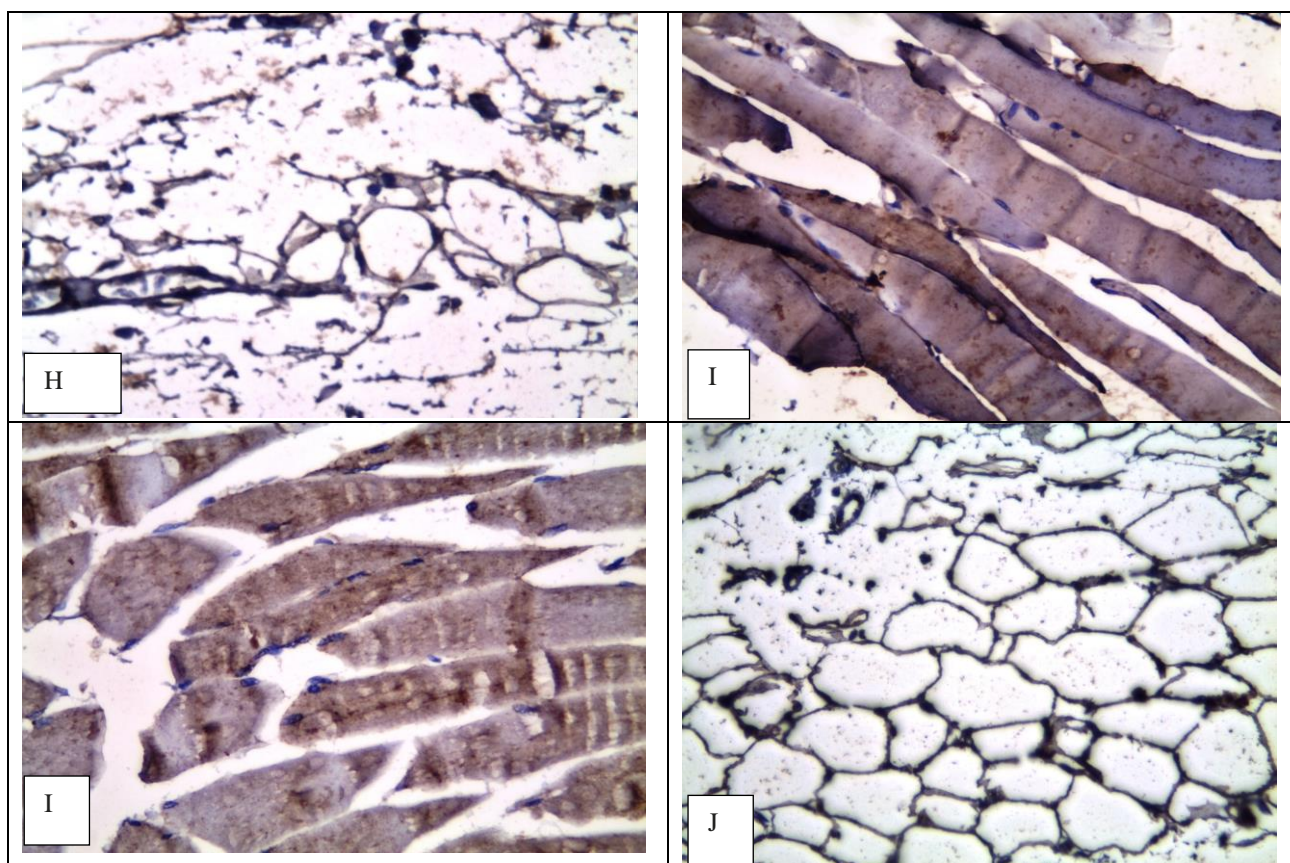


Figure 1: Group I showed high immunohistochemical expression of GLUT-4 in cell membrane and cytoplasm of skeletal muscles (A) and in cell membrane of adipose tissue (B) (x400). Group II showed negative and low expression of GLUT -4 in skeletal muscle (C) (x400) and low expression in adipose tissue (D) (x200). Group III showed high GLUT -4 expression in skeletal muscles (E) and adipose tissue (F)(x400). Group IV showed high immunohistochemical staining of GLUT -4 in skeletal muscles (G) and adipose tissue (H) (x400). Group V showed strong GLUT-4 immunohistochemical expression in skeletal muscles (I) and adipose tissue (J) (x400).

DISCUSSION:

Diabetes mellitus is a major health problem and the most prevalent endocrine disease that affects increasing numbers of people throughout the world (2). It is now known that defects in GLUT-4 expression and function (19) and an inflammatory milieu mediated by proinflammatory cytokines and oxidative stress result in both IR and β -cell dysfunction leading to chronic hyperglycemia and Type 2 DM (1).

The aim of the present work was to study the effect of QC and MF on GLUT-4 expression in skeletal muscles and adiposed tissue, oxidative stress, inflammation markers and IR in T2DM.

The results of the current study revealed

that treatment of diabetic rats with either MF or QC produced significant reduction in plasma glucose, insulin levels and HOMA-IR in comparison to its significant increase in untreated diabetic rats. The effect of QC was more prominent than MF on plasma levels of glucose and insulin. Moreover, the combined treatment by MF and QC produced more significant improvement of the studied parameters.

In accordance with our results several studies proved the effect of MF on the same parameters (11,27,28,29). The hypoglycemic effect of MF could be explained by its ability to inhibit gluconeogenesis in the liver and facilitate glucose uptake and use by peripheral tissues (11). In the liver, MF stimulates adenosine monophosphate-

activated protein Kinase (AMPK), increases AMP/ATP ratio as it inhibits the respiratory chain complex I in the mitochondria, inhibits glucagon receptor-cAMP signaling and fructose-1,6-bisphosphatase with subsequent decrease in hepatic gluconeogenesis. Besides, MF decreases the conversion of lactate and glycerol to glucose through a redox change by decreasing the activity of glycerophosphate dehydrogenase in the mitochondria (27,28,29).

Also, it was reported that AMPK modulates adipokine secretion, inhibits Acetyl Co A Carboxylase activity and suppresses steroid regulatory element binding protein 1 (SREPB 1) leading to increased fatty acid oxidation. The resultant decrease in lipotoxicity and glucotoxicity (by increasing glucose uptake in skeletal muscles) improves IR and hyperglycemia (30,31).

A previous study proved that lipid phosphatase Src homology inositol-5-phosphatase 2 (SHIP2) is upregulated in the muscle and adipose tissue of diabetic mice (32). This in turn reduces Akt/PKB (protein kinase B) activation leading to IR, inhibition of GLUT-4 translocation to the plasma membrane and decrease glucose uptake (33). It was shown that MF increases glucose uptake through inhibiting the activity of SHIP2 in the muscles and adipose tissue (34) and this could be another mechanism by which MF decreases the plasma glucose level observed in our study.

Regarding the effects of QC on plasma glucose, insulin levels and HOMA-IR in the present work, a marked significant reduction was detected in QC treated diabetic rats. With agreement of our results, Arias et al. (35) reported that QC significantly decreased the elevated blood glucose levels, insulin levels and HOMA-IR in

diabetic rats. Also, Srinivasan et al. (36) proved that QC significantly reduce the blood and urine glucose levels in STZ-induced diabetic rats.

The hypoglycemic effect of QC could be explained by its ability to improve insulin sensitivity (37), decrease the activity of glycogen phosphorylase (increased in DM) and increase the activity of glycogen synthase (decreased in DM) (35), besides its effect in maintaining β -cells proliferation (38). Reduction of insulin level in QC treated diabetic rats may be due to increasing expression and phosphorylation of insulin receptors (IR) and glucose transporters (GLUTs) (39). Furthermore, QC markedly increased serum adiponectin levels and decreased serum TNF- α levels (40). All these changes will improve the plasma glucose level. The superior effect of QC over MF in reducing plasma glucose level could be attributed mainly to its effect on maintaining β -cells mass, proliferation and function and increased serum insulin effect by reducing IR (41).

The decrease of HOMA-IR with improvement of insulin sensitivity in QC treated diabetic rats which was found in our study may be related to the ability of QC to decrease the free fatty acids and lipids plasma level, increase intracellular cAMP and activate protein kinase A (PKA) (39).

As regard the association between DM and oxidative stress, it was found that the induction of DM in group II produced significant elevation in TBARS level with significant decrease in SOD, CAT and GSH-Px antioxidant enzyme activities in skeletal muscles. While, treatment of diabetic rats with MF and QC in group III and IV caused reversal of these effects and QC produced more pronounced effects than MF. Interestingly, the

concomitant use of both MF and QC provoked more significant results approaching near the control levels.

These results are in accordance with a previous study which stated that DM is associated with increased oxidative stress that constitutes one of the main factors responsible for the development of DM and its complications (42).

The significant improvement in oxidative stress markers observed in our study could be explained by the ability of MF to decrease the production of free oxygen radicals by direct inhibition of the complex I chain (43) which is involved in inducing the production of IL-1 β through ROS. Thus, MF inhibited the production of IL-1 β (44).

It was proved that the administration of MF in diabetic rats caused significant decrease in the malondialdehyde (MDA) concentration and significant improvement in the reduced activities of serum antioxidant enzymes as superoxide dismutase (SOD), catalase and glutathione (GSH) activity (45). Similarly, another study showed that MF with life style modification significantly improved the oxidative stress in diabetic patients and this was associated with significant reduction in body mass index (BMI), glycosylated hemoglobin (HBA1c) and TNF- α plasma levels (46).

As regard the antioxidant effect of QC observed in group IV, our results are in accordance with Periera et al. (47) who reported that QC given to diabetic rats adjusted plasma lipid profiles, improved oxidative stress by decreasing lipid hydroperoxides synthesis and elevating the glutathione peroxidase enzyme activity. In addition, it was proved that QC is a powerful free

radical scavenging with a strong antioxidant activity by increasing glutathione concentrations (48).

The present study showed that there was reduced expression of GLUT-4 in the skeletal muscle and adipose tissue in diabetic rats. While, the treatment of diabetic rats with MF in group III and QC in group IV caused increased expression of GLUT-4 in both tissues with more significant results caused by QC. In group V, the combined use of both MF and QC caused stronger expression of GLUT-4 than that produced by each one alone.

A previous study proved that MF increased glucose uptake in human adipocytes by increasing GLUT-4 mRNA expression and GLUT-4 protein content in the plasma membrane (49). Also, Lee et al. (12) showed that MF influences translocation of glucose transporter-4 (GLUT-4) through its stimulatory effect on AMP-activated Protein Kinase (AMPK).

With reference to QC effect on GLUT-4 expression, it was shown that QC upregulated the levels of GLUT-4 mRNA and promoted the translocation of GLUT-4 to the cell membrane of adipocytes and skeletal muscle cells (50). This effect of increased GLUT-4 expression could be explained the ability of QC to stimulate AMPK (51).

In relation to the proinflammatory cytokines in the present study, it was found that induction of diabetes in group II caused significant elevation in the plasma level of IL-6 and TNF- α proving the association of inflammatory process with T2DM. With respect to the use of MF in group III and QC in group IV to treat diabetic rats, a significant decrease in the plasma levels of these proinflammatory cytokines was detected in both

groups. It was clear that QC produced more significant results than MF. Eventually, the combined use of both MF and QC in group V produced marked significant effect (approaching control level) than the separate use of them.

The results of our study are in accordance with Lee et al. (52) who proved that T2DM is associated with increased expression of both IL-6 and TNF- α and this leads to exacerbation of oxidative stress with the development of diabetic complications. Also, a previous study confirmed the association of increased level of proinflammatory cytokines; IL-6 and TNF- α with the development of T2DM (4).

Previous studies reported that MF treatment of T2DM significantly decreased the levels of serum IL-6 and TNF- α of macrophages and induces the production of anti-inflammatory cytokines such as IL-4 and IL-10 (53,54). The effect of MF on these inflammatory cytokines with improvement of inflammation could be mediated through AMPK activation (27).

Quercetin is an enduring anti-inflammatory flavonoid that inhibits inflammatory intermediaries and decreased the plasma levels of TNF- α and IL-6 with improvement of the symptoms of diabetes (55,56). These results are similar to ours and indicate the improvement of the inflammatory process in T2DM by the use of QC.

Ultimately, in group V, the highly significant improvement of all the tested parameters by the combined use of both QC and MF could be explained by their additive effects in increasing the expression of GLUT-4, improving the oxidative, inflammatory stress and insulin resistance in addition to the beneficial effect of

quercetin in maintaining β -cells mass, proliferation and function.

Conclusion:

From the previous findings of this study, it was concluded that the combined use of QC and MF improved T2DM hyperglycemia and IR by increasing the expression of GLUT-4 in skeletal muscles and adipose tissue together with the reversal of the oxidative stress and inflammatory states. Therefore, it is recommended for type 2 diabetic patients to use quercetin as a complementary treatment with metformin and eat quercetin rich food.

Conflict of interest:

The authors declare no conflict of interest.

References:

1. **American Diabetes Association.** Diagnosis and Classification of Diabetes Mellitus. *Diabetes Care* 2013; 36(Supplement1):S76-S74.
2. **Guariguata L, Whiting D, Hambleton I, Beagley J, Linnenkamp U, Shaw J.** Global estimates of diabetes prevalence for 2013 and projections for 2035. *Diabetes Res Clin Practice* 2014;103(2):137-49.
3. **American Diabetes Association.** Classification and diagnosis of diabetes: Standards of medical care in diabetes-2018. *Diabetes Care* 2018;41(Supplement 1):S13-27.
4. **Domingueti CP, Dusse LM, Carvalho MD, de Sousa LP, Gomes KB, Fernandes AP.** Diabetes mellitus: the linkage between oxidative stress, inflammation, hypercoagulability and vascular complications. *J Diabetes Complications* 2016;30:738-45.

5. **Pitocco D, Zaccardi F, Di Stasio E, Romitelli F, Santini SA, Zuppi C, et al.** Oxidative stress, nitric oxide, and diabetes. *Rev Diabet Stud* 2010;7(1):15–25.
6. **Grossman LD, Roscoe R, Shack AR.** Complementary and Alternative Medicine for Diabetes. *Canadian Journal of Diabetes* 2018; 42(1):S154-S161.
7. **Pietta PG.** Flavonoids as antioxidants – review. *J Nat Prod* 2000;63(7):1035-1042.
8. **Smith AJ, Oertle J, Warren D, Prato D.** **Quercetin:** A Promising Flavonoid with a Dynamic Ability to Treat Various Diseases, Infections, and Cancers. *Journal of Cancer Therapy* 2016;7:83-95.
9. **Hemmati M, Mostafavi SE, Zarban A, Hoshyar R.** Protective Effects of Quercetin on Hyperglycemia and Stress Proteins Expression in Rats with Streptozocin-Induced Diabetes. *Mod Care J* 2018; 15(2):e64964.
10. **Mazloom Z, Abdollahzadeh SM, Dabbaghmanesh MH, Rezaianzadeh A.** The Effect of Quercetin Supplementation on Oxidative Stress, Glycemic Control, Lipid Profile and Insulin Resistance in Type 2 Diabetes: A Randomized Clinical Trial. *J Health Sci Surveillance Sys* 2014; 2(1):8-14.
11. **Gunton JE, Delhanty PJ, Takahashi S, Baxter RC.** Metformin rapidly increases insulin receptor activation in human liver and signals preferentially through insulin-receptor substrate-2. *J. Clin. Endocrinol. Metab* 2003;88:1323–1332.
12. **Lee JO, Lee SK, Kim JH, Kim N, You GY, Moon JW, et al.** Metformin Regulates Glucose Transporter 4 (GLUT4) Translocation through AMP-activated Protein Kinase (AMPK)-mediated Cbl/CAP Signaling in 3T3-L1 Preadipocyte Cells. *The Journal Of Biological Chemistry* 2012;287(53):44121–44129.
13. **Esteghamati A, Eskandari D, Mirmiranpour H, Noshad S, Mousavizadeh M, Hedayati M, et al.** Effects of metformin on markers of oxidative stress and antioxidant reserve in patients with newly diagnosed type 2 diabetes: a randomized clinical trial. *Clin Nutr.* 2013;32(2):179-85.
14. **Knowler WC, Barrett-Connor E, Fowler SE, Hamman RF, Lachin JM, Walker EA, et al.** Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *The New England Journal of Medicine* 2002;346(6):393–403.
15. **Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al.** Management of hyperglycaemia in type 2 diabetes: a patient-centered approach. Position statement of the American Diabetes Association (ADA) and the European Association for the Study of Diabetes (EASD). *Diabetologia* 2012;55(6):1577–96.
16. **Navale AM, Paranjape AN.** Glucose transporters: physiological and pathological roles. *Biophys Rev* 2016; 8(1):5-9.
17. **Lee YK, Kim JE, Nam SH, Goo JS, Choi SI, Choi YH, et al.** Differential regulation of the biosynthesis of glucose transporters by the PI3-K and MAPK pathways of insulin signalling by treatment with novel compounds from *Liriope platyphylla*. *International Journal of Molecular Medicine* 2011; 27(3):319-27.
18. **Xiong H, Zhang S, Zhao Z, Zhao P, Chen L, Mei Z.** Antidiabetic activities of antigenic acid

- in type 2 diabetic db/db mice and L6 myotubes via AMPK/GLUT-4 pathway. *Journal of Ethno pharmacology* 2017; 211:366-74.
19. **Gutiérrez-Rodelo C, Roura-Guiberna A, Olivares-Reyes JA.** Molecular Mechanisms of Insulin Resistance: An Update. *Gac Med Mex* 2017;153:197-209.
20. **Alam F, Islam MA, Khalil MI, Gan SH.** Metabolic Control of Type 2 Diabetes by Targeting the GLUT4 Glucose Transporter: Intervention Approaches. *Current Pharmaceutical Design* 2016;22:3034-3049.
21. **Srinivasan K, Ramarao P.** Animal models in type 2 diabetes research: an overview. *Indian J Med Res.* 2007;125:451–742.
22. **Ohkawa H, Ohishi N, Yagi K.** Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 1979;95:351–358.
23. **Marklund S, Marklund G.** Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *Eur J Biochem* 1974;47:469–474.
24. **Aebi H.** Catalase in vitro. *Methods Enzymol* 1983;105:121–126.
25. **Lawrence A, Burk F.** Glutathione peroxidase activity in selenium-deficient rat liver. *Biochem Biophys Res Commun* 1976;71:952–958.
26. **Zhai J, xi Liu C, Tian Z, Jiang Q, Sun Y.** Effects of Metformin on the Expression of GLUT4 in Endometrium of Obese Women with Polycystic Ovary Syndrome. *Biology of Reproduction* 2012; 87(2):29.
27. **Foretz M, Guigas B, Bertrand L, Pollak M, Violette B.** Metformin: from mechanisms of action to therapies. *Cell Metab* 2014;20:953–966.
28. **Aatsinki SM, Buler M, Salomaki , Koulou M, Pavek P, Hakkola J.** Metformin induces PGC-1 α expression and selectively affects hepatic PGC-1 α functions. *Br. J. Pharmacol* 2014;171:2351–2363.
29. **Madiraju AK, Erion DM, Rahimi Y, Zhang XM, Braddock DT, Albright RA, et al.** Metformin suppresses gluconeogenesis by inhibiting mitochondrial glycerophosphate dehydrogenase. *Nature* 2014;510:542–546
30. **Somasundaram NP, Wijesinghe AM.** Metformin – A Mini Review. *Ann Clin Exp Metabol* 2016;1(1): 1003.
31. **Hur KY, Lee MS.** New mechanisms of metformin action: Focusing on mitochondria and the gut. *J Diabetes Investig* 2015;6:600–609
32. **Hyvonen ME, Saurus P, Wasik A, Heikkilä E, Havana M, Trokovic R, et al.** Lipid phosphatase SHIP2 downregulates insulin signalling in podocytes. *Mol Cell Endocrinol* 2010;328:70–79.
33. **Gonzalez E, McGraw TE.** Insulin Signaling Diverges into Akt-dependent and -independent Signals to Regulate the Recruitment/Docking and the Fusion of GLUT4 Vesicles to the Plasma Membrane. *Molecular Biology of the Cell* 2006;17:4484- 4493.
34. **Polianskyte-Prause Z, Tolvanen TA, Lindfors S, Dumont V, Van M, Wang H, et al.** Metformin increases glucose uptake and acts renoprotectively by reducing SHIP2 activity. *FASEB J* 2019;33(2):2858-2869.
35. **Arias N, Macarulla MT, Aguirre L, Martínez-Castaño MG, Portillo MP.**

- Quercetin can reduce insulin resistance without decreasing adipose tissue and skeletal muscle fat accumulation, *Genes Nutr* 2014;9 (1):361.
36. **Srinivasan P, Vijayakumar S, Kothandaraman S, Palani M.** Anti-diabetic activity of quercetin extracted from *Phyllanthus emblica* L. fruit: In silico and in vivo approaches. *Journal of Pharmaceutical Analysis* 2018;8(2):109–118.
37. **Anhê GF, Okamoto MM, Kinote A, Sollon C, Lellis-Santos C, Anhê FF, et al.** Quercetin decreases inflammatory response and increases insulin action in skeletal muscle of ob/ob mice and in L6 myotubes. *Eur J Pharmacol* 2012;689(1-3):285-93.
38. **Adewole SO, Caxtonmartins EA, Ojewole JAO.** Protective effect of quercetin on the morphology of pancreatic β -Cells of streptozotocin-treated diabetic rats. *Afr. J. Trad. CAM* 2007;4(1):64-74.
39. **Shi GJ, Li Y, Cao QH, Wu HX, Tang XY, Gao XH, et al.** In vitro and in vivo evidence that quercetin protects against diabetes and its complications: A systematic review of the literature. *Biomedicine & Pharmacotherapy* 2019;109:1085–1099.
40. **Choi HN, Jeong SM, Huh GH, Kim JI.** Quercetin ameliorates insulin sensitivity and liver steatosis partly by increasing adiponectin expression in ob/ob mice, *Food Sci. Biotechnol* 2015; 24:273–279.
41. **Youl E, Bardy G, Magous R, Cros G, Sejalon F, Virsolvy A, et al.** Quercetin potentiates insulin secretion and protects INS-1 pancreatic β -cells against oxidative damage via the ERK1/2 pathway, *Br. J. Pharmacol* 2010;161:799–814.
42. **Oguntibeju OO.** Type 2 diabetes mellitus, oxidative stress and inflammation: examining the links *Int J Physiol Pathophysiol Pharmacol* 2019;11(3):45-63.
43. **Diniz Vilela D, Gomes Peixoto L, Teixeira RR, Belele Baptista N, Carvalho Caixeta D, Vieira de Souza A, et al.** The Role of Metformin in Controlling Oxidative Stress in Muscle of Diabetic Rats. *Oxid Med Cell Longev.* 2016;2016:9 pages.
44. **Kelly B, Tannahill GM, Murphy MP, O'Neill LA.** Metformin inhibits the production of reactive oxygen species from NADH: ubiquinone oxidoreductase to limit induction of interleukin-1 β (IL-1 β) and boosts interleukin-10 (IL-10) in lipopolysaccharide (LPS)-activated macrophages. *J Biol Chem* 2015;290(33):20348-59.
45. **Obi BC, Okoye TC, Okpashi VE, Igwe CN, Alumanah EO.** Comparative Study of the Antioxidant Effects of Metformin, Glibenclamide, and Repaglinide in Alloxan-Induced Diabetic Rats. *Journal of Diabetes Research* 2016;2016:5 pages.
46. **Bulatova N, Kasabri V, Qotineh A, Al-Athami T, Yousef AM, AbuRuz S. et al.** Effect of metformin combined with lifestyle modification versus lifestyle modification alone on proinflammatory-oxidative status in drug-naïve pre-diabetic and diabetic patients: A randomized controlled study. *Diabetes Metab Syndr.* 2018;12(3):257-267.
47. **Pereira BC, Momentti AC, Barbosa PF, De FFBR, dos Santos FA, Fava FH, et al.** Influence of treatment with quercetin on lipid

- parameters and oxidative stress of pregnant diabetic rats, *Revue Canadienne De Physiologie Et Pharmacologie* 2013;91:171–177.
48. **Galati G, Moridani MY, Chan TS, O'Brien PJ.** Peroxidative Metabolism of Apigenin and Naringenin versus Luteolin and Quercetin: Glutathione Oxidation and Conjugation. *Free Radical Biology & Medicine* 2001;30:370–382.
49. **Grisouard J, Timper K, Radimerski TM, Frey DM, Peterli R, Kola B, et al.** Mechanisms of metformin action on glucose transport and metabolism in human adipocytes. *Biochem Pharmacol.* 2010 ;80(11):1736-45.
50. **Dhanya R, Arya AD, Nisha P, Jayamurthy P.** Quercetin, a lead compound against Type 2 diabetes ameliorates glucose uptake via AMPK pathway in skeletal muscle cell line, *Front. Pharmacol* 2017;8:336.
51. **Alam MM, Meerza D, Naseem I.** Protective effect of quercetin on hyperglycemia, oxidative stress and DNA damage in alloxan induced type 2 diabetic mice. *Life Sci* 2014;109: 8-14.
52. **Lee J, Lee S, Zhang H, Hill MA, Zhang C, Park Y.** Interaction of IL-6 and TNF- α contributes to endothelial dysfunction in type 2 diabetic mouse hearts. *PLoS ONE* 2017;12(11):e0187189.
53. **Hyun B, Shin S, Lee A, Lee S, Song Y, Ha NJ, et al.** Metformin down-regulates TNF- α secretion via suppression of scavenger receptors in macrophages. *Immune Netw* 2013;13:123–132.
54. **Cameron AR, Morrison VL, Levin D, Mohan M, Forteach C, Beall C, et al.** Anti-inflammatory effects of metformin irrespective of diabetes status. *Circ Res* 2016;119:652–665.
55. **Chen S, Jiang H, Wu X, Fang J.** Therapeutic Effects of Quercetin on Inflammation, Obesity, and Type 2 Diabetes. *Mediators of Inflammation* 2016;2016:5 pages.
56. **Dokumacioglu E, Iskender H, Sen TM, Ince I, Dokumacioglu A.** The Effects of Hesperidin and Quercetin on Serum Tumor Necrosis Factor-Alpha and Interleukin-6 Levels in Streptozotocin-induced Diabetes Mode. *Pharmacogn Mag.* 2018;14(54):167–173.