

Effect of Quercetin on Diabetic Rat

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Abstract

Background: Quercetin (QR) is one of the major constituents of the methanolic extract of the leaves of *Psidium guajava* (Guava leaves).

Objectives: The work's aim is to understand the Quercetin's mechanism in improving insulin resistance, use the homeostasis model assessment for insulin resistance (HOMA-IR) to determine the influence of quercetin on glycemic control, Look at how quercetin affects diabetes-related lipid metabolism and lipid profile measures. Analyze the impact of QR on oxidative stress in diabetic rats and contrast its antidiabetic effects whether administered as a nutrient or supplement.

Materials and methods: Sixty adult male Wister rats that were matched in age and had starting body weights between 150 and 200 g were used in this study. One normoglycemic control group and 3 diabetic control groups (15 rats per group) were used. The diabetic control rats received the vehicle orally as saline daily, the normoglycemic control group received quercetin orally in a dose of 50 mg/kg per day, and the diabetic rats received quercetin orally in a dose of 100 mg/kg per day.

Results: After six weeks of therapy, the rats with diabetes receiving isolated quercetin at doses of 50 and 100 mg/kg had reduced blood glucose levels, and their triglyceride, low-density lipoprotein, and total cholesterol profiles all significantly improved. Also, there was significant decrease homeostatic model assessment for insulin resistance, serum transaminases, hepatic malondialdehyde, and HMG CoA expression in liver and a substantial rise in levels of insulin, hepatic GSH, and insulin receptor substrate (IRS2) expression & Phosphoinositide 3-kinases in liver as compared to those of control group, but non-significance changes in high-density lipoprotein, AKT expression in liver were observed.

Conclusions: Quercetin could be considered as a potential hypoglycemic medication with possible mechanisms controlling the hyperglycemic state and cholesterol, triglycerides and LDL levels.

Keywords: Quercetin; Rat with diabetes; lipid profile; HOMA-IR.

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Introduction

Diabetes mellitus (DM), one of the most common illnesses in the world, occurs by defects in insulin production and/or action, resulting in impairments in lipid, carbohydrate, and protein metabolism. Over the recent decade, there has been an increase in DM frequency due to aging population and lifestyle changes that encourage obesity. (Urrutia et al., 2021). Every area and demography in worldwide is affected by diabetes, including those living in low-income and middle-income nations. Globally, diabetes had pandemic proportions reaching to 9% prevalence (463 million adults) in 2019 according to 9th edition of International Diabetes Federation (IDF). There is an exponential increase in the number of people living with diabetes. Additionally, according to the IDF, by 2045, at least 629 million individuals would have diabetes. Genetics, inactivity, smoking, alcohol use, dyslipidemia, deficient β -cell sensitivity, high insulin levels, and increased glucagon function are the main risk factors for prediabetes and diabetes, according to (Alam et al., 2021).

Type-2 diabetes affects between 90 and 95 percent of diabetics. Present treatments may successfully lower hyperglycemia, but they are often unable to control fluctuations in glycemic parameters throughout the day and keep them at a safe level, which is a major pharmacovigilance issue. This sets off a chain of pathological conditions, including increased glycation and inactivity of the body's antioxidant defense cause oxidative stress and lipid free radical oxidation, which in turn cause diabetic angiopathies to form and progress. Furthermore, a lipid profile violation, evidenced by

dyslipidemia, hypercholesterolemia, and/or hypertriglyceridemia, is a common trigger for the progression of diabetes problems (Budniak et al., 2021).

Phytotherapy offers several advantages over standard therapy with oral drugs including its low toxicity, minimal pharmacological action, and ability to be taken for extended periods of time without experiencing any severe side effects. Quercetin (QR) is one of a rising number of flavonoids that have been shown to have anti-hyperglycemic properties. It is a flavonol-type flavonoid, which is found in a variety of plants. *Psidium guajava* is a vital source of vitamin C and flavonoids, making it one of the most important crops. The methanolic extract of *P. guajava* leaves revealed Quercetin as the major flavonoid. Also, it was reported that Quercetin may exert different activities of medical benefits like boosting secretion of insulin, decreasing insulin resistance, preserving glucose homeostasis, and reducing oxidative damage, apoptosis, and inflammation (Bhattacharya et al., 2014).

The work's aim is to understand Quercetin's mechanism in improving glycemic control, insulin resistance, by using the homeostasis model evaluation for insulin resistance (HOMA-IR) estimation, investigate quercetin's effect on lipid metabolism & lipid profile parameters during diabetes, study the effect of QR on oxidative stress in rats with diabetes and compare between antidiabetic effect of QR in nutrient dose and supplemental dose.

Materials and Methods

Plant material: The herbarium of the Pharmacognosy department, Faculty of Pharmacy, Assiut University, Assiut, Egypt, verified the *Psidium guajava* leaves

after they were collected from the guava trees growing in the fields of the Assiut area.

Extract preparation: The collected plant was air dried at room temperature (26 °C) for two weeks and ground to get about 500 g of uniform powdered leaves. The obtained powdered plant was extracted using methanol (MeOH) by maceration for five days at room temperature (4×2L) till exhaustion. A dark brown viscous residue (101 g) was produced when the methanolic extract was concentrated at decreased pressure using a rotary evaporator. The sticky residue was dissolved in 120 mL of distilled water and fractionated using liquid-liquid partition with different solvents; *n*-hexane, dichloromethane (DCM) and ethyl acetate (EtOAc), to produce four distinct fractions—*n*-hexane fraction (51.6 g), DCM fraction (10.2 g), ethyl acetate (EtOAc) fraction (15.7 g), and aqueous fraction—in increasing polarity (19.2 g). Each of obtained fractions was subjected to thin layer chromatographic studies (TLC) and co-chromatography with quercetin authentic sample which revealed the presence of quercetin in appreciable amount in the EtOAc fraction (Dmitrienko et al., 2012)

Chromatographic isolations of EtOAc fraction: About 7.5 g of the fraction of EtOAc was subjected to column chromatography (CC) using normal silica gel as adsorbent [200 g, 4 cm (ID) × 160 cm (L)] and mixtures of DCM and MeOH as mobile phase in the order of increasing polarities (100% DCM, 95% DCM, 90% DCM, 85% DCM, 75% DCM, and 100% MeOH). Fractions of 150 mL were collected. The fractions eluted with DCM-MeOH (85:15) were concentrated under reduced pressure and monitored by

using silica gel TLC and system DCM-MeOH (80:20), then collected to afford fraction E-1 (2.3 g). About 500 mg of fraction E-1 were further subjected to CC using sephadex LH-20 as stationary phase [100 g, 2 cm (ID) × 150 cm (L)] and MeOH as fluent. The fractions eluted from this column were monitored by using silica gel TLC and system DCM-MeOH (80:20) to afford pure compound Qu (200 mg) (Dmitrienko et al., 2012)

Spectroscopic data of compound

Qu: Yellow amorphous powder; IR ν_{max} cm^{-1} : 3290, 1668, 1429 and 1240 cm^{-1} ; UV λ_{max} (MeOH) 370 & 254 nm; HRESIMS negative mode spectrum displayed a molecular ion peak at m/z 301.0352 [M-H]⁻ (calcd.: 301.0348); ¹H-NMR data (400 MHz, DMSO-*d*₆): δ H 6.20 (1H, d, *J*= 2.0 Hz, H-8), δ H 6.20 (1H, d, *J*= 2.0 Hz, H-6), 6.83 (1H, d, *J*= 8.4 Hz, H-5'), 7.63 (1H, dd, *J*= 2.4, 8.4 Hz, H-6') and δ H 7.78 (1H, d, *J*= 2.4 Hz, H-2') (Dmitrienko et al., 2012).

Experimental animals: Male adult, sixty age-matched Wister rats with initially body weights ranging from 150-200 g were selected for this investigation. They were produced and raised at the rat's house, Faculty of Medicine, Assiut University, Assiut, Egypt. Until the trial began, rats were kept on a well-balanced diet of bread with a supply of water. Throughout the course of the experiment, animals were maintained at a temperature of 22.3 °C, with a 12-hour light/dark cycle, and a constant relative humidity. The Institutional Animal Ethics Committee's rules for the use and care of laboratory animals were followed throughout the experiment.

Induction of diabetes: A single intraperitoneal (i.p.) injection of streptozotocin (STZ), newly dissolution in

0.9% saline, at a dosage of 30 mg/kg, was used to cause type 2 diabetes, after two weeks of acclimatization (Asadi et al., 2021). All groups' blood glucose levels were assessed on the third day after receiving STZ injections using strips of reagent and glucometer in samples taken from the tail vein. Animals with blood glucose levels of 350 mg/dL or greater on three separate occasions were classified as diabetic. Regarding the rats with blood glucose level below 350 mg/dL, they were all given another dose of STZ 30 mg/kg (Asadi et al., 2021). The next experiments were performed on both normoglycemic and streptozocin treated diabetic rats.

Study design: There were four groups of 15 rats each, one of normal blood sugar and three with diabetes: (1) oral administration of daily saline to a normoglycemic control group; (2) Diabetic control rats receiving the vehicle orally as saline daily; (3) rats with diabetes treated for 6 weeks by quercetin orally in a dose 50 mg/kg per day; (4) Diabetic rats treated for 6 weeks with quercetin orally in a dose 100 mg/kg per day. For six weeks, the used dosage of quercetin was taken orally after being dissolved in saline (Zhao et al., 2021). Rats fasting for the previous night had their blood sugar levels checked every day using a glucometer. The animals were fasted overnight at the conclusion of the experiment, and blood samples were taken for biochemical analyses.

Blood sampling and biochemical analysis: After an overnight fast, 3-5ml of venous blood was taken from the retro-orbital sinus using glass capillaries while the animals were given mild anesthesia with diethyl ether to calm them down (Stone, 1954). The obtained sera were centrifuged at 3000 rpm for 10 minutes after the samples had been collected in

Wassermann tubes, kept at -20 C until analysis, and then the samples were recovered, allowed to clot at room temperature for 30 minutes. To evaluate biochemical parameters such as serum fasting blood glucose levels, triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and total cholesterol (TC), serum samples were examined using several enzymatic colorimetric tests.

Routine Biochemical assays: The fasting blood glucose level was estimated through an enzymatic colorimetric method (GOD/PAP) reported by (Trinder, 1969) using Glucose Enzymax kit, LOT: 118257 supplied by Vito Scient, Cairo, Egypt.

The serum alanine aminotransferase (ALT) level was determined by ALT colorimetric method, (catalog no. 246001,246002 supplied by Egyptian Biotechnology (S.A.E), Egypt). The serum aspartate aminotransferase (AST) level was determined by AST_ colorimetric method, (catalog no. 260001,260002 supplied by Biotechnology (S.A.E), Egypt).

The serum TC level was estimated by an enzymatic colorimetric method reported by Ellefson using CHOLESTEROLLQ kit (Hodgson et al., 1976), catalog no. 230001 supplied by Egyptian Company for Biotechnology (S.A. E). The serum HDL-C level was estimated by an enzymatic colorimetric method precipitating reagent reported by Friedewald et al. (1972) using HDLC-P kit, catalog.no. 266001 supplied by Egyptian Company for Biotechnology (S.A. E). The serum TG level was estimated by an enzymatic colorimetric method reported by (Bucolo and David, 1973) using TRIGLYCERIDES- kit,

catalog no.314001 supplied by Egyptian Company for Biotechnology (S.A.E).

Colorimetric assay: Malondialdehyde (MDA) and reduced glutathione (GSH) levels were measured in blood samples using commercial colorimetric kits (catalog no. TA 2511, provided by Biodiagnostics, Egypt). and, correspondingly, (catalog no. MD 25 29 provided by Biodiagnostics, Egypt), in accordance with the manufacturer's specifications (**Kei, 1978**).

Tissue samples: For the purpose of collecting tissue, rats were sacrificed via cervical dislocation. The liver samples were collected, weighed, and then split into two portions: one was preserved in an RNA later solution at a concentration 10 times its weight and maintained at 80°C until the total RNA extraction, while the other was fixed in 10% formalin for histological investigation.

Liver tissue samples and RNA Extraction and Real-Time PCR: According to the manufacturer's instructions, total RNA was isolated from frozen tissue (liver tissue samples) using

the Gene JET RNA Purification Kit (catalog no ID: 73404 QIAGEN, USA). Reverse transcription of 80 g of RNA into complementary DNA (cDNA) was carried out using the High-Capacity cDNA Reverse Transcription kit (catalog no. 4375575 Rev.E, Applied Biosystems, USA) after quantification using a Nano Drop spectrophotometer. cDNA was then amplified using the Maxima SYBR Green qPCR Master Mix kit from Applied Biosystems in the United States (catalog no. 4310251 Rev.D). AKT The primer sets listed in (**Table.1**) were used for the amplification. Using the Applied Biosystems 7500 Fast Real-time PCR equipment (Applied Biosystems, Germany), a two-step reaction procedure was carried out. The first phase included a denaturation cycle at 95 °C for 10 min, followed by 40 amplification cycles at 95 °C for 15 sec and 60 °C for 1 min. Using the 2CTmethod and GAPDH as a reference gene, the gene expression levels of UFs samples were represented as fold-change vs normal liver sample (**Livak and Schmittgen, 2001**).

Table 1: The sequences of the PCR primers

Gene	Primer sequence
HMG-CoA Reductase (Hmgco)	Forward:5'-ACCGTGGGTGGTGGGAC-3' Reverse:5'-GCCCTTGAACACCTAGCATC-3'
Irs2	Forward: 5'-CTGGCTGCGATGACAACACTAC-3' Reverse:5'-CGGTATACACCGGTCAGGTT-3'
PI3K	Forward: 5'-CGCCGTTCCCTGTGAATGATA-3' Reverse: 5'-TTGCTTTGACCTGACCCATCT-3'
Akt	Forward: 5'-CTGCCCTTCTACAACCAGGA-3' Reverse: 5'-GTGCTGCATGATCTCCTTGG-3'
GAPDH	Forward: 5'-GTTACCAGGGCTGCCTTCTC-3' Reverse: 5'-CCCGTTGATGACCAGCTTC-3'

Protein assay and insulin assay by Enzyme-Linked Immunosorbent Assay (ELISA): Using a Glass-Col Homogenizer, tissue was physically

homogenized in an ice water bath. Total Protein Colorimetric Assay Kit (catalog no. E-BC-K165-S Elabscience Biotechnology Inc, USA) was utilized to

conduct the protein assay. According to the manufacturer's specifications, the concentrations of insulin were measured using a commercial ELISA kit (catalog no. E-EL-R3034 provided by Elabscience Biotechnology, USA).

Histological and Immunohistochemical Analyses: Liver samples were cleaned in tap water before serving the night in 70% ethyl alcohol. The specimens were dehydrated by immersing them for 30 minutes each in increasing concentrations of ethyl alcohol (70, 80, 90, and 100%). Xylene was used to clean the tissue samples before they were embedded in paraffin wax and sealed with freshly melted paraffin. Haematoxylin and eosin stain (H&E) was used to stain serial 5- μ m sections. For light microscopy histopathological investigation. Moreover, Periodic Acid-Schiff (PAS) stain was carried out on hepatic sections representing different experimental groups for demonstrating the glycogen content within hepatocytes (Chen et al., 2018).

Statistical analysis

Statistical Package for Social Science (SPSS) version 24 (SPSS Chicago, IL,

USA) was used for data input and analysis. Numbers, percentages, means, and standard deviations were used to show the data. Data represented as mean \pm SD. In order to compare quantitative variables across groups, an independent sample T-test was utilized. Also, ANOVA was used to compare variables across many groups. When ($P < 0.05$), a P-value is deemed statistically significant.

Results

Characterization of the isolated compound Qu

A) IR characterization of compound Qu

Qu: The IR absorption spectrum of the Qu showed broad absorption peak at 3290 cm^{-1} is characteristic for the OH stretching vibration in the ring that reveals hydroxyl group presence. The sharp band at 1668 cm^{-1} was assigned for carbonyl aryl ketonic stretching vibrations. The absorption band positioned at 1429 cm^{-1} is common with the C=C aromatic stretching vibrations. Vibrations of C-O stretching of aryl ether were determined at 1240 cm^{-1} (Fig.1).

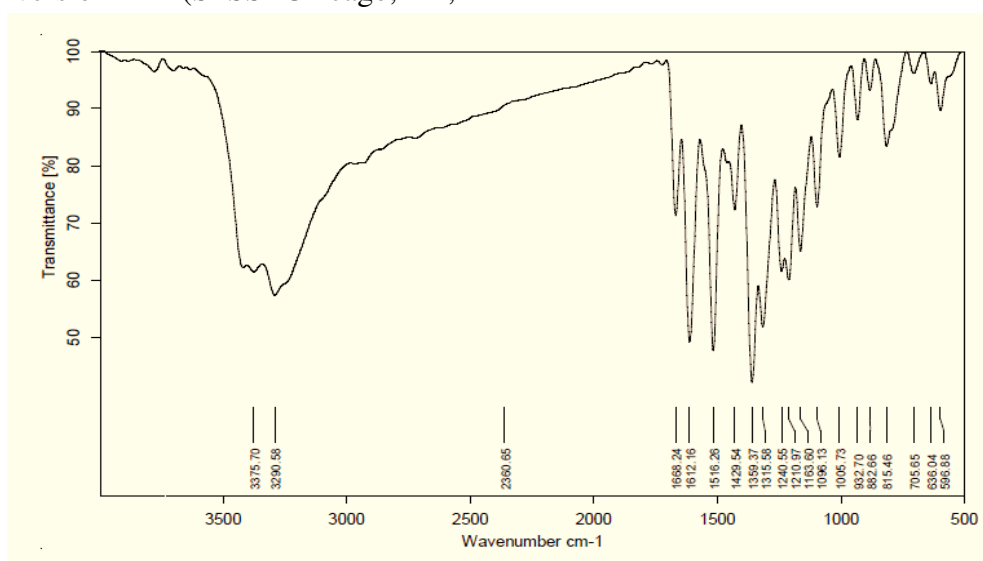


Fig.1. FT-IR spectrum of isolated Compound Qu

B) ¹H-NMR spectral data of compound

Qu: The isolated chemical Qu's ¹H-NMR spectra (400 MHz, DMSO-d₆) showed resonances brought on by aromatic systems present in its structure. Due to a 3', 4' Di substitution of ring B and a typical meta-coupled

pattern for H-6 and H-8 protons (H 6.20 and 6.40, d, J = 2.0 Hz), the aromatic region showed an ABX system corresponding to the catechol protons in ring B at H 7.78 (1H, d, J = 2.4 Hz, H-2'), 7.63 (1H, dd, J = 2.4, 8.4 Hz (**Fig.2**).

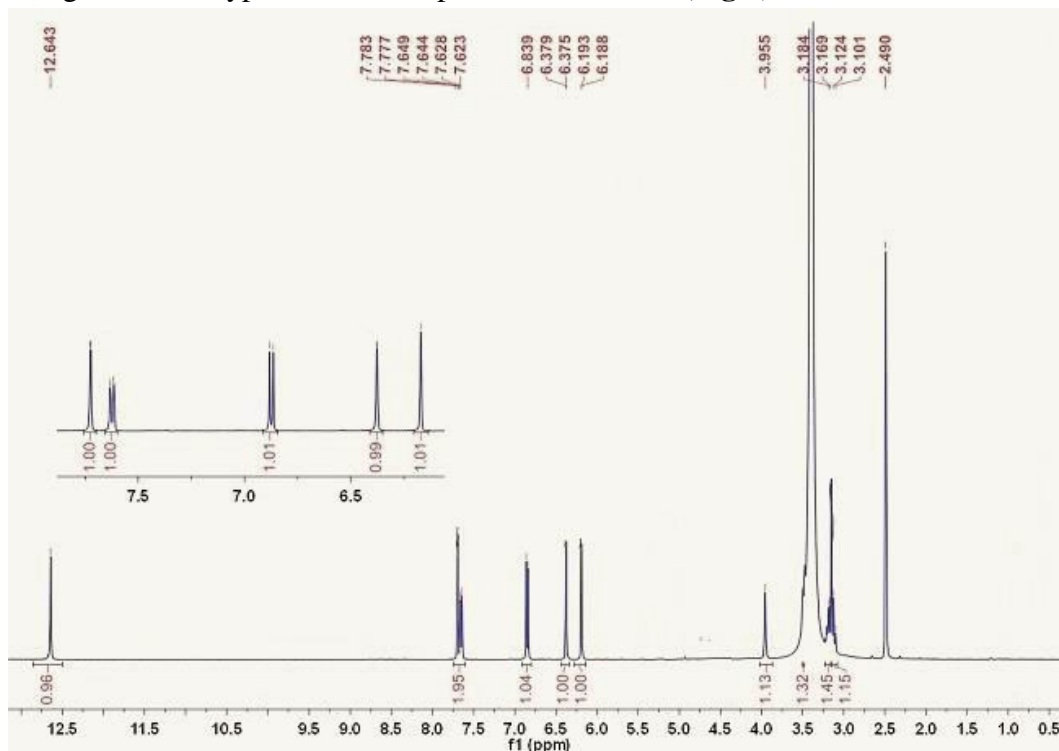


Fig.2. ¹H NMR Spectrum of the compound Qu (DMSO-d₆, 400 MHz)

C) Mass spectrophotometric analysis of compound Qu:

The HRESIMS negative mode spectrum displayed a The chemical formula C₁₅H₁₀O₇ is consistent with the molecular ion peak at m/z 301.0352 [M-H]⁻ (calcd.: 301.0348) which is known to be the molecular formula of quercetin.

Quercetin's effect on blood glucose

There was an extremely substantial variation (P 0.000) in the groups' blood glucose levels. There was a really

important issue. (P<0.000) among quercetin (50mg/kg) groups & control group and each of diabetic, there was moderate significant difference (p<0.005) between control & quercetin (100mg/kg) group. There is highly substantial variation (p<0.000) between diabetic group and each of quercetin (50mg/kg), quercetin (100mg/kg) groups. There was moderate substantial variation (p<0.007) among quercetin (50mg/kg) group and quercetin (100mg/kg) group, (**Fig.3**).

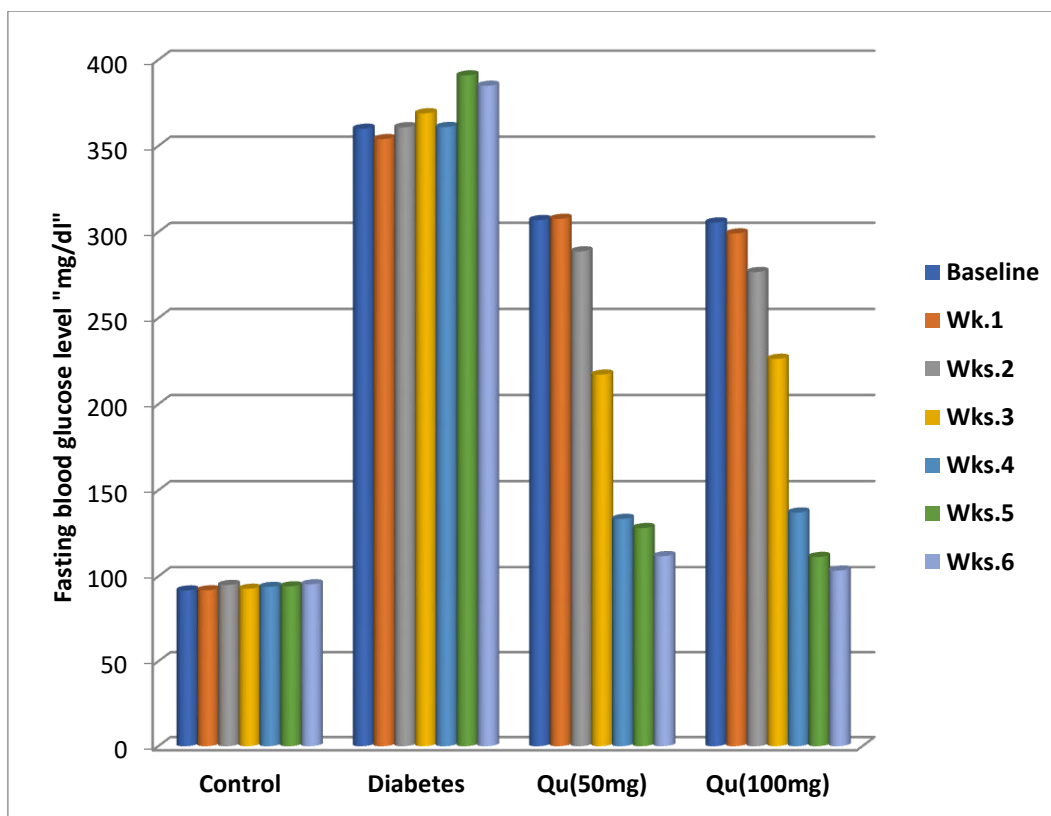


Fig.3. Effect of oral daily administration of (50, 100 mg/kg) Quercetin extract on fasting blood glucose level in diabetic male rat

Effect of quercetin on plasma lipid profiles and enzymes

Regarding cholesterol there is moderate substantial variation ($P < 0.001$) among different groups, there was highly significant different between control & diabetic group ($P < 0.000$), also there was moderate significant difference ($p < 0.009$) between diabetic group & each of Quercetin (50mg/kg) and Quercetin (100mg/kg) groups. Regarding TG there was highly significant difference ($p < 0.000$) between different groups. There was highly significant different ($P < 0.000$) between control & diabetic group. There was non-significant difference between control group & each of quercetin (50mg/kg) and quercetin (100mg/kg) groups. There was moderate substantial

difference ($p < 0.009$) between group with diabetes & each of quercetin (50mg/kg) and quercetin (100mg/kg) groups. Regarding LDL There was highly substantial variation ($P < 0.000$) between different groups, there was highly substantial variation ($p < 0.000$) between control & diabetic, quercetin (50mg/kg) and quercetin (100mg/kg) groups. Also, there was highly substantial variation ($p < 0.000$) between group with diabetes & both of quercetin (50mg/kg) and quercetin (100mg/kg) groups. But there is non-substantial variation ($p > 0.05$) between quercetin (50mg/kg) and quercetin (100mg/kg) groups. Regarding HDL There was no significant difference ($P > 0.05$) between different groups (**Table .2**).

Table 2. Effect of oral daily administration of (50, 100 mg/kg) Quercetin extract on different parameters of lipid profile in diabetic male rat

Item	Control group “n=15”	Diabetes group “n=15”	Qu (50mg) “n=15”	Qu (100mg) “n=15”	p-value
Cholesterol mg/dl	19.91±3.16	24.90±3.48	20.77±4.57	19.31±4.57	P<0.001** P1<0.000*** P2=0.591n.s P3=0.687n.s P4<0.009** P5<0.001** P6=0.420n.s
TG mg/dl	0.69±0.13	4.61±1.33	1.03±0.63	0.98±0.43	P<0.000*** P1<0.000*** P2=0.591n.s P3=0.687n.s P4<0.009** P5<0.001** P6=0.420n.s
LDL mg/dl	0.31±0.08	5.35±1.68	0.98±0.37	0.84±0.14	P<0.000*** P1<0.000*** P2<0.000*** P3<0.000*** P4<0.000*** P5<0.000*** P6=0.181n.s
HDL mg/dl	0.62±0.12	0.59±0.20	0.70±.19	0.66±0.22	P=0.372n.s P1=0.651n.s P2=0.166n.s P3=0.449n.s P4=0.135n.s P5=0.324n.s P6=0.645n.s

Data represented as mean ±SD.TG:triglycerides.LDL: low-density lipoprotein.HDL:high-density lipoprotein.* p<0.05, **p<0.01, ***p<0.001. P-value: comparison between different groups, P1: comparison between control & diabetic group, P2: comparison between control & Qu50 group, P3: comparison between control & Qu100 group, P4: comparison between diabetic group & Qu50, P5: comparison between diabetic group & Qu100, P6: comparison between Qu50 & Qu100.

Regarding ALT and AST, there was decrease in Quercetin groups than diabetic group with substantial variation

among groups (P<0.04). There is moderate substantial variation between control group with diabetic group (P<0.007). There was a substantial variation between control

group and Quercetin (50mg). Regarding HOMA-IR there was moderate significant between different groups ($P < 0.008$). There was highly significant difference ($P < 0.000$) between of diabetic group and control group. There was significant difference ($P < 0.03$) between Quercetin

(50mg) group & with control group. There was moderate substantial variation ($p < 0.001$) between diabetic & quercetin (50,100mg/kg) groups. There was substantial variation ($p < 0.04$) between quercetin 50mg& quercetin 100mg groups (Table 3).

Table 3. Effect of oral daily administration of (50, 100 mg/kg) quercetin extract on liver enzyme and different parameters of lipid enzymes in diabetic male rat.

Item	Control group "n=15"	Diabetes group "n=15"	Qu (50mg) "n=15"	Qu (100mg) "n=15"	p-value
ALT U/L	24.5±6.61	31.40±6.25	29.60±7.19	27.90±6.47	P<0.04* P1<0.007** P2<0.04* P3=0.176n.s P4=0.476n.s P5=0.144n.s P6=0.496n.s
AST U/L	27.10±8.24	32.37±2.25	28.16±7.19	27.37±3.79	P<0.05* P1<0.02* P2=0.683n.s P3=0.937n.s P4<0.04* P5<0.000*** P6=0.648n.s
HOMA- IR	1.78±0.253	4.314±0.37	1.645±0.43	1.756±0.65	P<0.008** P1<0.000*** P2<0.03* P3=0.483n.s P4<0.001** P5<0.001** P6<0.04*

Data represented as mean±SD. ALT: alanine transaminase, AST: aspartate aminotransferase. HOMA-IR: homeostatic model assessment for insulin resistance. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. P-value: comparison between different groups, P1: comparison between control & diabetic group, P2: comparison between control & Qu50 group, P3: comparison between control & Qu100 group, P4: comparison between diabetic group & Qu50, P5: comparison between diabetic group & Qu100, P6: comparison between Qu50 & Qu100.

Qu activated IRS-2/ PI3K / AKT signaling in the Hepatic tissue

Regarding PIK3 There was great substantial variation ($P < 0.000$) among

various groups. A substantial variation ($P < 0.04$) was existed between control group & diabetic group, a substantial variation ($p < 0.01$) was existed between

control & quercetin (100mg/kg) groups. A moderate substantial variation ($p < 0.001$) was existed between diabetic group & quercetin (50mg/kg), a highly substantial variation ($p < 0.000$) was existed between diabetic group & quercetin (100mg/kg) group. A highly substantial variation ($P < 0.000$) was existed between various groups.

Regarding AKT, A highly substantial variation ($P < 0.000$) was existed among control group & (diabetic, qu 100mg/kg groups). Also, a substantial variation ($p < 0.04$) was existed among diabetic & qu (50mg/kg) groups. a moderate substantial variation ($p < 0.003$) was existed among diabetic & qu (100mg/kg) groups. A substantial variation ($p < 0.02$) was among qu (50mg/kg) & qu (100mg/kg) groups.

Regarding IRS, A highly substantial variation ($P < 0.000$) was existed among various groups. Also, a highly substantial

variation ($P < 0.000$) was existed among control group & each of diabetic, quercetin (50mg/kg) groups there was non-substantial difference ($p > 0.05$) among control group and quercetin (100mg/kg) group, a substantial variation ($p < 0.01$) was existed among diabetic group & quercetin (50mg/kg) group and a moderate substantial variation ($p < 0.001$) was existed among diabetic group & quercetin (100mg/kg) group. Also, there was moderate significant difference between quercetin (50mg/kg) & quercetin (100mg/kg) groups.

Regarding HMG CoA reductase enzyme there was non-significant difference in liver diabetic male rat, between diabetic group and quercetin (50mg/kg) group and between quercetin (50mg) & quercetin (100mg) ($P > 0.05$). A highly substantial variation was existed among control group and other groups ($p < 0.000$) (Table. 4).

Table 4. Effect of oral daily administration of (50, 100 mg/kg) Quercetin extract on expression PIK3, AKT, IRS and HMG CoA in liver diabetic male rat

Item	Control group "n=15"	Diabetes group "n=15"	Qu (50mg) "n=15"	Qu (100mg) "n=15"	p-value
PIK3 (fold change)	1.094±0.52	0.52±0.48	1.041±0.43	1.44±0.139	P<0.000*** P1<0.04* P2=0.465n.s P3<0.01* P4<0.001** P5<0.000*** P6<0.002**
AKT (fold change)	1.862±0.025	1.05±1.05	1.137±0.39	1.52±1.11	P<0.000*** P1<0.000*** P2<0.000*** P3<0.04*

					P4=0.464n.s P5<0.003** P6<0.02*
IRS (fold change)	1.38±0.39	0.59±0.07	0.73±0.18	1.15±0.58	P<0.000*** P1<0.000*** P2<0.000*** P3=0.230n.s P4<0.01* P5<0.001** P6<0.009**
HMG CoA (fold change)	0.32±0.078	1.248±0.39	0.93±0.56	0.707±0.17	P=0.221n.s P1<0.000*** P2<0.000*** P3<0.000*** P4=0.082n.s P5<0.000*** P6=0.157n.s

Data represented as mean ±SD. PIK3: Phosphoinositide 3-kinases. HMG CoA: 3-hydroxy-3-methylglutaryl coenzyme A. IRS:insulin receptor substrate. * p<0.05, **p<0.01, ***p<0.001. P-value: comparison between different groups,0.01, parison between control & diabetic group, P2: comparison between control & Qu50 group, P3: comparison between control & Qu100 group, P4: comparison between diabetic group & Qu50, P5: comparison between diabetic group & Qu100, P6: comparison between Qu50 & Qu100.

Effect of Quercetin extract on fasting insulin levels in diabetic male rat by ELISA

As regard insulin, a moderate substantial variation among different groups (P<0.001). A moderate substantial variation (P<0.001) among diabetic group with control and a substantial variation (P<0.001) was existed among diabetic group with Quercetin (50mg/kg &100mg/kg) groups, as regard, a substantial variation (P<0.04) was existed among Quercetin (50mg/kg) group with control group. Regarding malondialdehyde “MDA”, A highly substantial variation was existed among different groups (P<0.000). A moderate substantial variation (P<0.001) was existed among

each of control group &diabeteic group, quercetin (50, 100 mg/kg) groups and between diabetic group& Quercetin (50,100mg/kg) groups (**Fig.4**).

Oxidant and antioxidant results:

Regarding GSH, a highly substantial variation was existed among different groups (P<0.000). A moderate substantial variation (P<0.001) was existed among diabetic group with control group. a substantial variation (p<0.02) was existed among control group & qu (50mg/kg &100mg/kg) groups. A substantial variation (P<0.01) was existed among diabetic &qu (50mg/kg) groups. And a moderate substantial variation (P<0.001) was existed among diabetic & QR (100mg/kg) groups (**Table .5**).

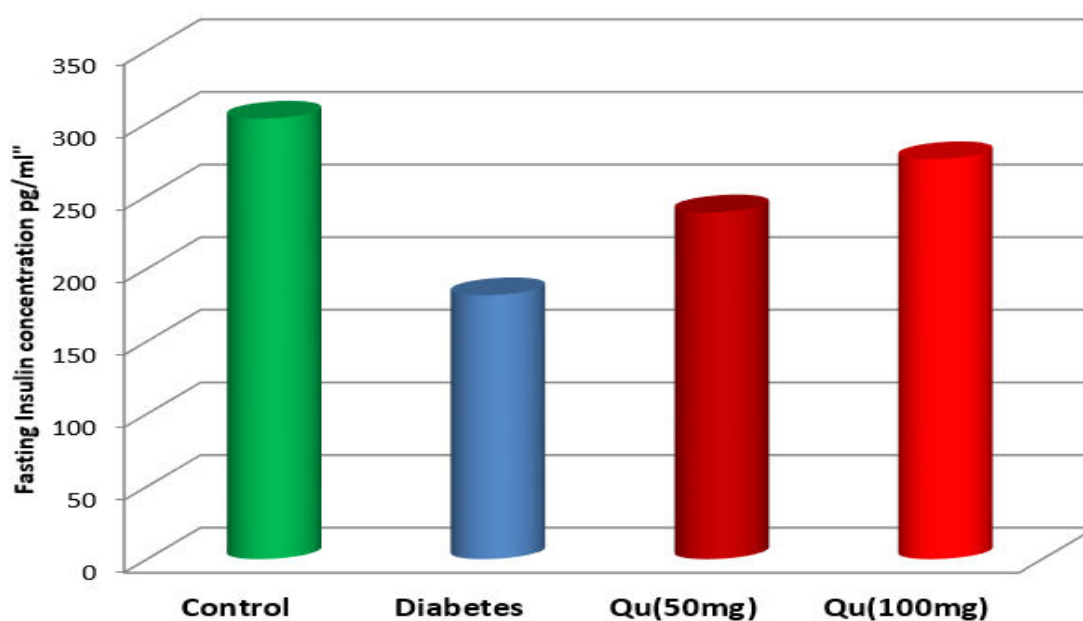


Fig.4. Effect of oral daily administration of (50, 100 mg/kg) Quercetin extract fasting insulin levels in diabetic male rat

Table 5. Effect of oral daily administration of (50, 100 mg/kg) Quercetin extract on serum levels of GSH peroxidase enzyme and malondialdehyde in diabetic male rat

Item	Control group "n=15"	Diabetes group "n=15"	QR (50mg) "n=15"	QR (100mg) "n=15"	p-value
GSH mU/ml	38.75±4.76	26.34±3.46	31.65±2.65	33.43±2.31	P<0.000*** P1<0.001** P2<0.02* P3<0.02* P4<0.01* P5<0.001** P6= 0.338n.s
MDA nmol/ml	1.41±0.26	2.81±0.23	1.87±0.21	1.46±0.56	P<0.000*** P1<0.001** P2<0.001** P3<0.001** P4<0.001** P5<0.001** P6<0.01*

Data represented as mean ±SD. GSH: reduced glutathione. MDA: malondialdehyde. * p<0.05, **p<0.01, ***p<0.001. P-value: comparison between different groups, P1: comparison between control & diabetic group, P2: comparison between control & QR 50 group, P3: comparison between control & QR 100 group, P4: comparison between diabetic group & QR 50, P5: comparison between diabetic group & QR 100, P6: comparison between QR 50 & QR 100.

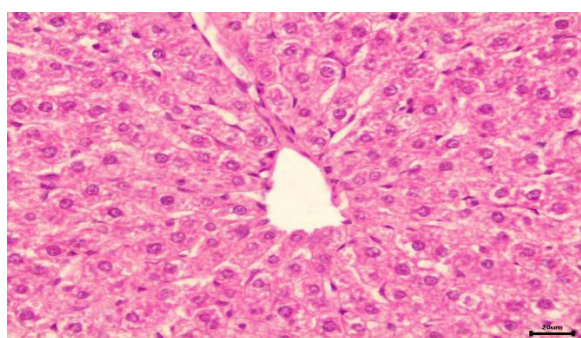
Histological Results

Microscopic examination of hepatic sections from normal control rats stained by H&E showed classical hepatic lobules with central veins and peripheral portal areas. Each lobule revealed normal hepatic architectural and cytological criteria. Hepatocytes arranged in plates separated by blood sinusoids were seen radiating from central veins. They

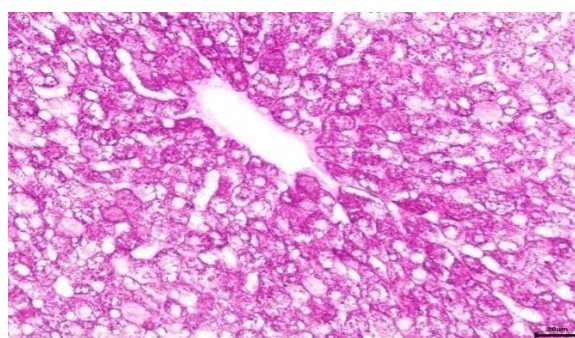
appeared polyhedral in shape with homogenous acidophilic cytoplasm and small uniform vesicular nuclei.

Normally appeared portal areas contained portal veins, hepatic arteries and bile ductules were also detected. PAS-stained sections demonstrated normal distribution of purple-colored glycogen granules within cytoplasm of hepatocytes (Fig.5)

A) First group (Normal control)



(A)



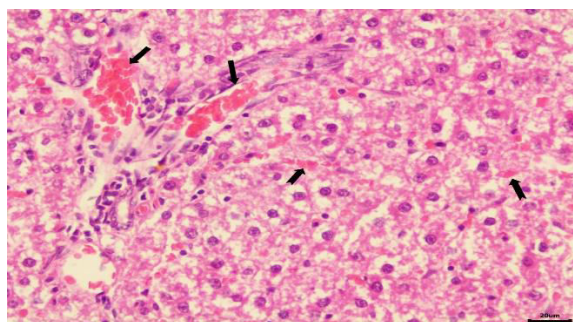
(B)

Fig.5. Liver of normal control rats showing (A): normal hepatocytes with normal radial arrangements of hepatic cords around central vein (H&E, bar=20um), (B): normal distribution of purple-colored glycogen in cytoplasm of hepatocytes (arrow) (PAS, bar=20um)

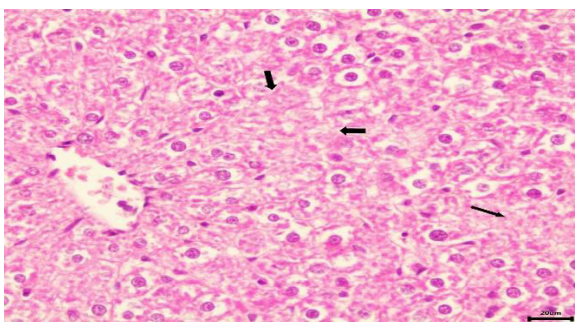
Histopathological examination of H&E-stained sections from different hepatic lobes of diabetic control rats revealed obvious histomorphological changes. These changes were indicated by

appearance of diffuse sinusoidal congestion as well as congestion of central veins and portal blood vessels and focal areas of coagulative necrosis were detected (Fig.6)

B) Second group (Diabetic control)



(A)



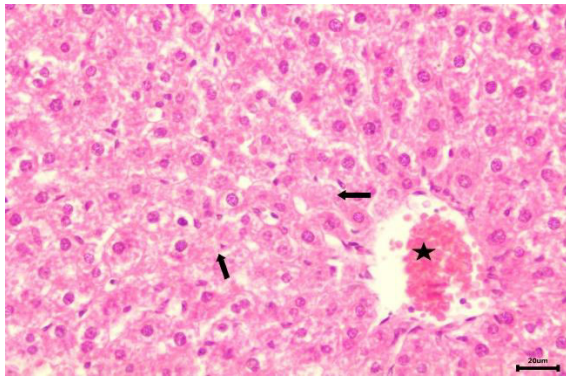
(B)

Fig.6. Liver of diabetic control rats by (H&E, bar=20um) showing (A): diffuse sinusoidal congestion (notched arrows) and congestion of portal blood vessels (arrows), (B): focal areas of hepatocytes coagulative necrosis (arrows)

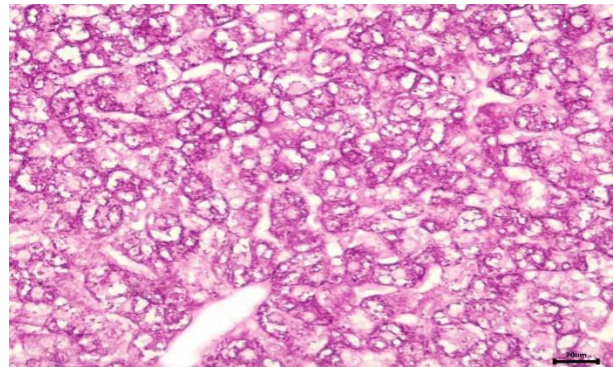
Quercetin treatment at dose 50mg/kg/day obviously improved the histomorphological appearance of the hepatic tissue sections and preserved hepatic architecture in all lobules. Congestion of central veins and

coagulative necrosis of sporadic hepatocytes were observed only in some hepatic lobules. PAS stain demonstrated that quercetin restored the level of glycogen within hepatocytes (**Fig.7**)

C) Third group (QR treated, 50mg/kg)



(A)



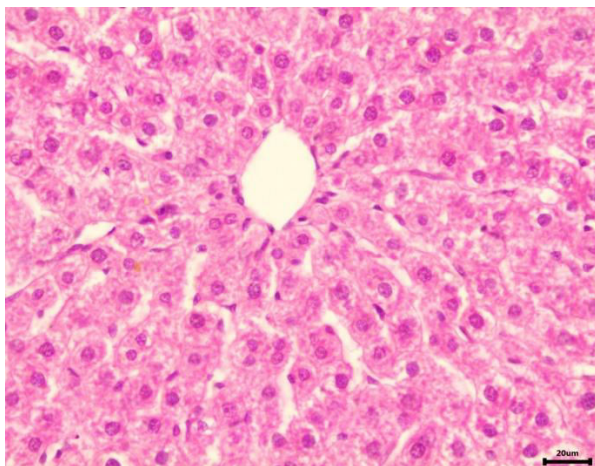
(B)

Fig.7. Liver of diabetic rats treated with QR (50mg/kg) showing (A): congested central vein (star) and coagulative necrosis of sporadic hepatocytes (arrows) (H&E, bar=20um), (B): wide distribution of purple-colored glycogen granules in cytoplasm of hepatocytes (PAS, bar=20um)

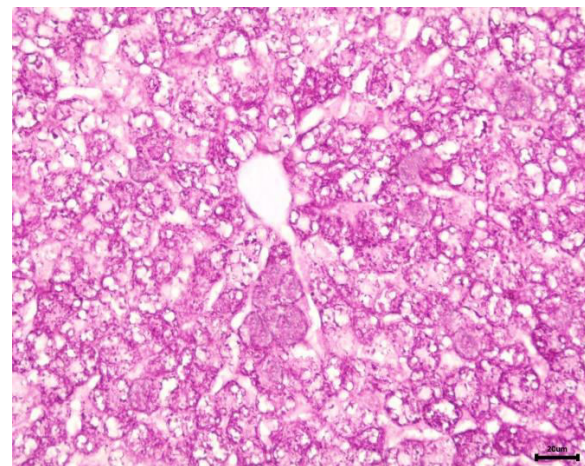
Hepatic sections from rat treated with QR at dose of 100mg/kg displayed normal histomorphological appearance of

hepatocytes. PAS stain highlighted restored levels of glycogen within cytoplasm of hepatocytes (**Fig.8**)

D) Fourth group (QR treated, 100mg/kg)



(A)



(B)

Fig.8. Liver of diabetic rats treated with QR (100mg/kg) showing (A): normal hepatocytes and normal histological architecture (H&E, bar=20um), (B): wide distribution of purple-colored glycogen granules in cytoplasm of hepatocytes (PAS, bar=20um)

Discussion

An estimated 382 million individuals globally currently suffer from diabetes mellitus (DM), and that number is projected to rise to 592 million by the year 2035, placing a significant strain on global public health (Zheng et al., 2018). The flavonoid family includes QE, which may be derived from a variety of fruits and vegetables, including apple, onion, berries, numerous seeds, nuts, barks, tea, flowers and brassica vegetables (Vitale et al., 2018).

In the current work AST was substantially decreased in Quercetin groups than diabetic group. This agrees with Yang et al., 2018 who stated substantial reduction in the mean value of both AST & ALT levels in the QR -treated group. Also, another study reported that diabetes induced ALT, AST rise can be decreased by treatment with QR to its normal values (Chen et al., 2018).

The result illustrated that diabetes caused significant damage of hepatocytes as indicated by increased levels of ALT&AST and indicated by liver histopathological examination. Contrarily, quercetin improved the levels of oxidative indicators utilized in this investigation without insulin and markedly lowered the biochemical enzymes level with conclusion that Quercetin may be a useful antioxidant in lowering the danger of oxidation brought on by diabetes, which may result in liver damage (Nabi and Abdullah, 2019).

In the current study HMG-CoA reductase enzyme expression in the liver decreased in quercetin groups compared with diabetic male rat group. This agrees a study reported that QR significantly inhibited HMG CoA reductase activity compared with the diabetic group (Shao et

al., 2019). Another research established that activation of AMPK inhibited the expression of the HMG-CoA reductase enzyme, which controlled the production of cholesterol (Kabirifar et al., 2018). As a result, the quercetin-treated groups' cholesterol levels returned to normal. HMG-CoA reductase levels in groups that received QR were also found to be lower. (Prabu et al., 2010).

In our study, a substantial rise in IRS2 expression in the liver tissue existed in QR (50,100 mg/kg) treated groups compared with diabetic groups. This is in line with (Cheng et al., 2021) who suggested that the phosphorylation of IRS-2 was upregulated in QR treated groups. On the other hand, IRS-2 may control the catalytic and gene expression of the liver (Nagy and Einwallner, 2018).

In the current work, a substantial rise existed in PI3K with oral daily administration of Quercetin extract than in diabetic male rat group with significant difference between (50&100 mg/kg) Quercetin groups. This agrees with a study which suggested that QR alleviated hepatic insulin resistance (Zhu et al., 2021). On the other hand, QR increased PI3K activity to reduce hepatic resistance to insulin. (Mohamed et al., 2018).

In the current work, a substantial rise existed in AKt with oral daily administration of (50, 100 mg/kg) Quercetin extract than in diabetic male rat group. This agrees with a study reported In a STZ-induced diabetes rat model, quercetin significantly improved the disordered lipid and glucose metabolism. This finding was associated with diabetic rats' stimulation of the hepatic Akt pathway (Peng et al., 2017). Also, QR enhanced lipid metabolism through controlling Akt-regulated SREBPs and the

expression of proteins (**Iskender et al., 2017**). All these findings collectively may shed light about mechanism of quercetin improving effect on glucose and lipid and lipid metabolism.

In our study, there was a significant rise in fasting insulin level in diabetic rats with oral daily administration of (50, 100 mg/kg) Quercetin extract groups compared with diabetic male rat group with significant difference between (50&100 mg/kg) QR groups. This agrees with a study reported that reduced serum insulin levels in diabetic rats is improved by quercetin administration. (**Li et al., 2018**).

In the current work, a substantial decrease existed in blood glucose levels in QR treated groups compared with diabetic groups. This agrees with a study which observed that in diabetes model mice and rats, quercetin is additionally displayed to lower blood glucose levels while maintaining islet function, sensitivity to insulin, and β -cell counts. Also, compared to the normal control group, the glucose level progressively climbed in the STZ-treated group, but it considerably dropped in the QE group. Quercetin is demonstrated to significantly lower blood glucose levels starting on day 7 when compared to the group that received STZ (**Chen et al., 2016**).

In present study, The HOMA-IR, which in rats provides a decent assessment of glucose tolerance evaluation, entire-body sensitivity to insulin, and concentration of serum fructosamine, was enhanced by both the 50mg/kg and 100mg/kg dosages of QR. This agrees with a research on HOMA-IR index which found that quercetin enhanced glycemic control (**López-Martínez et al., 2013**). A different investigation demonstrated that QR increases insulin action independently

of the fat deposited in adipose tissues. (**Polakof et al., 2011**).

In the current study, oral daily administration of (50, 100 mg/kg) Quercetin extract significantly decreased levels of serum malondialdehyde “MDA” in diabetic male rat. This agrees with the study that reported Quercetin therapy substantially lower the elevated MDA level and rised the reduced GSH-Px enzyme activity in hepatic tissues. Moreover, in both their& our studies Quercetin –treated diabetic rats showed an improved histological appearance (**Senyigit et al., 2019**). In addition a study found that Quercetin treatment decreased blood MDA and serum NO in an experimental animal study (**Adewole et al., 2006**).

In our study, glutathione peroxidase enzyme was substantially elevated in both groups of QR treated diabetic rats contrasted to diabetic group. Another study agrees with our results in their study, when compared to the controls group, the levels of reduced glutathione (GSH) and the GSH/GSSG ratios were considerably lower in the diabetics carotid artery homogenates. Furthermore, diabetic rats' carotid arteries showed a rise in MDA levels and decreases in GSH peroxidase levels and SOD activity (**Ferlita et al., 2019**). The substantial reduction in the level of MDA and the substantially elevated levels of GSH peroxidase and antioxidant SOD found in the carotid artery of diabetic rats, however, indicate that quercetin administration, whether in conjunction with or without moderate exercise training, protects against free radical-induced harm and the lipid peroxidation process. (**Sena et al., 2013**).

In our study there was significant improvement of lipid profile with oral

daily administration of (50, 100 mg/kg) Quercetin extract in diabetic male rat compared with diabetic group. This agrees with study reported marked increase levels of TG, TC, LDL, and VLDL in the STZ-treated group contrasted with those in normal control group (Yang and Kang, 2018). In contrast, they also observed that QR significantly decreased these parameters in diabetic male rats. A study founded that QR may be able to lower elevated levels of cholesterol in the blood by particularly blocking intestinal cholesterol absorption by decreasing the expression of the epithelial cholesterol transporter Niemann Pick C1-like 1 (NPC1L1) (Asgharian et al., 2022). A study demonstrated that QR's ability to improve lipid profiles may be linked to the fact that there is evidence that flavonoids may combine with chylomicrons and lipoproteins in the liver and intestine and then be transported into these particles. (Ali et al., 2020).

Conclusion

Quercetin was isolated from the leaves of *Psidium guajava* showed remarkable hypoglycemic effect, and anti-atherosclerotic potentials (improved lipid profile) by significant reduction of serum glucose blood level, serum TC, serum TG, serum LDL-C, and increased HDL-C. Quercetin might have mediated their activity by interfering with lipid biosynthesis. A 100 mg dosage of Quercetine has been demonstrated to provide the greatest anti-hyperglycemic effect in all animals treated with it. Finally, we reach the conclusion that quercetin is an exceptional antihyperglycemic substance.

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Conflict of Interest: Nil

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