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The Role of Quercetin on some Cardio-Vascular Parameters in Rats with Insulin Resistance Syndrome

Magda SH Afifi*.

Zoology Department, Women College, Ain Shams University.

ABSTRACT

The current work was designed to evaluate the effect of quercetin on carbohydrate and lipid disturbances associated with myocardial dysfunction as well as the changes in proinflammatory cytokines impairment induced by fructose intake in rats inducing insulin resistance (IR). To induce myocardial dysfunction as a result of insulin resistance (IR), rats were fed on diet richened with fructose and lard. A considerable changes were recorded in the carbohydrate profile (Glucose, insulin and HOMA), lipid profile (Cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol), cardiac profile (Aspartate aminotransferase [AST], lactate dehydrogenase [LDH], Creatine kinase [CK] and endotheline-1) and cytokines profile (TNF-α, IL-1ß and IL-6). Administration of quercetin significantly ameliorated all the previous mentioned parameters. The results indicated that quercetin possesses activity against IR associated with myocardial dysfunction, and that the mechanism of pharmacological and pharmakinetic actions were related at least in part to the antioxidant activity of quercetin.

Keywords: Insulin resistance (IR) - Myocardial dysfunction – Quercetin – Rats.

Corresponding author

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INTRODUCTION

Metabolic syndrome, a clinical condition which comprises various specific abnormalities including abdominal obesity, insulin resistance, dyslipidemia, hypertension and cardiovascular diseases[1], has become a major and increasingly prevalent [2] that parallels the dramatic worldwide epidemic of type 2 diabetes and obesity. It is directly associated with an increased risk of developing cardiovascular diseases [3], which are the major cause of premature mortality in type 2 diabetes patients. Managing the disorders clustered in this syndrome and in the mechanisms involved in its development is of great interest to prevent or reduce the risk posed by the pathologies implicated.

The etiopathology of the metabolic syndrome has not yet been fully elucidated. It seems to be the result of a complex combination of several etiologic factors that accompany central obesity and insulin resistance [4]. Recent studies have highlighted the involvement of a proinflammatory state that induces insulin resistance and leads to clinical and biochemical manifestations of the metabolic syndrome. The pathway leading to this pathology would involve an abnormal production of hormones and cytokines from the adipose tissue, namely an excessive production of proinflammatory mediators such as IL-6 and tumor necrosis factor-α (TNF-α) together with a lower secretion of the antiinflammatory adipocytokine adiponectin [5].

The general increases in consumption of calories, and specifically of refined carbohydrates and fructose, is clear and correlates positively with an alarming increases in metabolic syndrome [6]. There is considerable evidence supporting the ability of high fructose diets to upregulate the lipogenesis pathway, leading to increase of triglyceride production. Insulin and glucose are known to directly regulate lipid synthesis and secretion

Dietary patterns which include a high intake of plant food content, such as vegetables, legumes, and fruits, have been directly associated with the management and prevention of obesity, type II-diabetes and other cardiovascular risk factors. Plant foods contain flavonoids, polyphenolic compounds reported to have protective effects against chronic pathologies such as coronary events, cardiovascular disease mortality and diabetes [7].

Among flavonoids, the flavonol quercetin (3,3',4',5,7-pentahydroxyflavone) is one of the most widely distributed in human dietary sources [8]. In animal models and cellular lines, this molecule has been reported to have cardioprotective and antiinflammatory effects [9],[10]. These studies have engendered interest in the development of dietary supplements or drugs that would allow for more convenient and higher dose administration of quercetin, which might prove useful for the prevention or treatment of functional alterations clustered in the metabolic syndrome[11].

It has been reported that quercetin exhibits a wide range of biological functions, including antioxidant, anticarcinogenic and anti-inflammatory activities [12],

[18]. More recently, beneficial effects on blood pressure and heart disease have been described [13].

The main aim of this study was to examine the enhancement effects of quercetin administration on the disturbances present in the insulin resistance (metabolic syndrome) associated with cardiovascular diseases (CVDs) due to the corrections in pro-inflammatory cytokines profile (TNF-α, IL-1ß and IL-6) and lipid profile (Cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol) associated with modulation in the cardiac profile (AST, LDH, CK and endotheline-1) due to different mechanisms which were discussed in this work according to available recent researches.

MATERIALS & METHODS

Fifty adult male albino rats (*Rattus rattus*) with age 12±1 week old and their weight 200±10g were employed in this study. They were housed in a well ventilated Vivarum at Zoology Department, Women College, Ain Shams University, Egypt. The animals were caged in wire bottom galvanized metal wall boxes

under controlled environmental and nutritional conditions (25°C and 55-60 % relative humidity). The animals fed on a standard diet. Food and tap water were served *ad libitum* with fresh supplies presented daily.

EXPERIMENTAL DESIGN:-

This study was included two experiments: In the first experiment, the rats were randomly divided into two main groups according to the type of diet. In the first group (normal control rats group), twenty rats were fed on a control diet, containing 50% corn starch, 20% soybean, 10% sucrose 5% corn oil, 10% cellulose, 5% ash and served as control. While, the second group [Insulin resistance rats group], thirty rats were fed on the same previous diet except that corn starch and sucrose were replaced with an equal amount of fructose (30%) and lard (30%) according to [14].

After one month, ten rats from each previous group were taken to compare the alterations in serum carbohydrate, lipid, cardiac and cytokines profiles due to induction of insulin resistance (IR) with pronouncing of cardiovascular diseases (CVDs) in rats.

In the second experiment (30 rats), three comparisons were made between normal control rats (10 rats) and two equal subgroups of rats with experimentally insulin resistance (IR) (20 rats). The first experimentally insulin resistance (IR) subgroup was served as recovery group. The second insulin resistance (IR) subgroup rats were injected intraprotenially with quercetin (Sigma Chem. Co., St Louis, Mo. USA) at a dose of 100 mg / kg body weight for 2 months [10] as served as quercetin . All animal subgroups were divided into two intervals (One and two months; five rats in each interval).

At the end of each experimental period, blood was collected by cardiac puncture under light ether anesthesia and allowed to clot for 30min at room temperature. The serum was separated by centrifugation at 3,000 rpm at 30°C for 15min and used for the estimation of carbohydrate profile (Glucose, insulin and HOMA), lipid profile (Cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol), cardiac profile (Aspartate aminotransferase [AST], lactate dehydrogenase [LDH], Creatine kinase [CK] and endotheline-1) and cytokines profile (TNF-α, IL-1ß and IL-6).

Serum concentrations of glucose, total cholesterol, triglycerides and HDL-cholesterol were determined colorimetrically using commercial kits from Human, Germany. Serum activities of AST, LDH and CK were measured kinetically using commercial kits from ProLabo, France. The serum levels of insulin, endotheline-1, TNF-α, IL-1ß and IL-6 were assayed using commercial ELISA (Sandwich Immunoassay Technique) specific kit for rats BioVision Research Products Co. (USA).

The homeostatic model assessment (HOMA) value as a measure of IR was calculated using the following formula: fasting insulin (μU/L) × fasting glucose (mmol/L)/22.5 [15].

Data were statistically analyzed using Student “t” test in the first experimental. Moreover, two way analysis of variance (ANOVA) followed by Duncan’s multiple range test was used in the second experimental according to [16].

RESULTS & DISCUSSION

The metabolic syndrome is increasingly recognized as a strong predictor of patient risk for developing coronary artery disease. It is associated with an atherogenic dyslipidemia characterized by elevated levels of triglycerides (TGs), reduced levels of HDL-cholesterol and a preponderance of small dense low-density lipoprotein (LDL) particles [17]. An atherogenic dyslipidemia is an integral component of metabolic syndrome and a major contributor to the cardiovascular risks in patients. These alarming situations increase the priority for developing new methods and technologies to investigate and to fight the metabolic syndrome and its related comorbidities. Translational physiology offers us specific animal models for investigating these conditions to help support biomedical research efforts towards finding the necessary cures [18].

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One of the major underlying cause and/or outcome of metabolic syndrome is dyslipidemia, which contribute greatly to the cardiovascular problems associated with the syndrome. The animal models have a vital role to play in extending our understanding of metabolic syndrome and its related comorbidities. Conventional laboratory animals such as mice [19] and rats [20] have been examined to gain a better perceptive of the relationship between disorders of lipid metabolism and their clinical correlations. High-fat diets frequently used to induce different aspects of metabolic syndrome in rodent models.

In the current study, insulin resistance associated with cardiovascular diseases (IR-CVDs) rats group recorded a significant ($p<0.001$) elevation in the levels of serum glucose and insulin associated with a significant increment in the value of insulin resistance (HOMA-IR) as compared to their corresponding control group (Table 1). These results may be due to a defect in insulin binding caused by decreasing receptor number or their affinity or/and defects at the level of effect molecules such as glucose transporters and activities of their enzymes involved in glucose metabolism. These data are parallel with those obtained by [3], [2], [14].

Table (1): Comparison between normal and cardiovascular diseases (CVDs) rat groups on serum carbohydrate profile related to insulin resistance.

Groups / Parameters	Control	Obesity	%
Glucose (mmol/L)	6.278 ± 0.74	13.867 ± 0.94*	
Insulin (µU/L)	27.011 ± 1.06	46.334 ± 1.73*	
IR (HOMA)	7.524 ± 0.83	29.573 ± 1.64*	

- Values are expressed as mean ± SE - n= number of rats. - * significant at (P< 0.001).

Hepatic metabolism of fructose leads to alterations in the activities of key enzymes of glucose metabolism and activation of stress sensitive pathway that may desensitize insulin signaling [21]. Fructose is phosphorylated in the liver by adenosine triphosphate to form fructose-1-phosphate [22].

The pronouncing of CVDs associated with IR may be attributed to the fructose supplementation which led to excess formation of malonyl-CoA which inhibits β-oxidation, increase hepatic lipid droplet formation [23].

Judging from the data in table (2), IR-CVDs rats group showed a significant ($p<0.001$) elevation in serum total cholesterol, triglyceride and LDL-cholesterol levels as compared to their corresponding normal fed rats. These results may be due to the elevation in the lipid oxidation, the disturbance in the hypothalamus-pituitary-thyroid axis, changes in de novo lipogenesis pathway, alteration in the leptin receptors, disturbance in the over-expression of resistin gene, suppression in adiponectin production or/and the decrease of β-oxidation of lipid in the matrix of mitochondria. These results are in agreement with the viewpoint that mitochondrial dysfunction participates in the pathogenesis of IR rats at different levels, mainly including lipid oxidation impairment and the induction of peroxidative production[23].

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Table (2):Comparison between normal and cardiovascular diseases (CVDs) rat groups on serum lipid profile related to insulin resistance.

Groups	Control	Obesity	%
Parameters			
Cholesterol (mg/dL)	62.38±1.24	114.71±1.95*	
TG (mg/dL)	69.57±1.32	140.24±2.06*	
HDL (mg/dL)	19.26±0.52	24.13±0.59*	
LDL (mg/dL)	29.18±0.62	62.53±0.89*	

- Values are expressed as mean ± SE - n= number of rats. - * significant at (P< 0.001).

Myocardial cell contains marker enzymes such as AST, LDH and CK. Myocardial ischemia induces cell membrane to permeate or rupture, which results in the leakage of the AST, LDH and CK into blood. Hence, the AST, LDH and CK activities in serum reflect the alterations of membrane integrity and the degree of myocardial injury [(24)]. The current results showed that fed high fructose/fat diet caused a significant elevation in AST, LDH and CK activities associated with a considerable increment in the level of endotheline-1 (Table 3). These results are in harmony with those previous recorded by [(3), (2)]. They attributed these results to the elevation in the free radicals production, deficiency in the immune system, disturbance in the citrellin cycle and alteration in the expression gene of nitric oxide synthase activity (NOS).

Table (3): Comparison between normal and cardiovascular diseases (CVDs) rat groups on serum cardiac profile related to insulin resistance.

Groups	Control	Obesity	%
Parameters			
AST (U/L)	48.35± 0.92	70.14 ±1.13*	
CK (U/L)	102.73± 1.16	213.82 ±1.87*	
LDH (U/L)	229.61± 2.89	341.65± 3.77*	
Endotheline-1 (pg/ml)	0.52± 0.011	1.27± 0.024*	

- Values are expressed as mean ± SE - n= number of rats. - * significant at (P< 0.001).

One factor that plays a role in the development of insulin resistance is the tumor necrosis factor alpha (TNF-α). TNF-α is a pleiotropic cytokine which occurs in many pathological processes such as inflammation, allergy, congestive heart failure, etc.[(25)].Moreover, myocardium destruction is associated with a dramatic inflammatory response leading to release of TNF-α, IL-1ß and IL-6 as markers to cytotoxic injury (26) , (25).

In this study, the levels of TNF-α, IL-1ß and IL-6 were significantly (p<0.001) increased in fructose/fat fed rats when compared to control ones (Table 4). The tumor necrosis factor (TNF- α), is thought to represent the first step towards the subsequent development of heart fibrosis. Damaged heart cells generate ROS which stimulate the release of proinflammatory cytokines like TNF- α from myocardial cells. Increased TNF-α production led to suppress insulin receptor signal transduction in fructose-fed rats. [(27)] also noted that plasma concentrations of IL-1ß and IL-6 were higher in high fructose-fed. Quercetin is a plant-derived flavonoid commonly used as a nutritional supplement and is found in foods such as onions, apples, red wine, broccoli

and tea [28].Laboratory studies show that quercetin may have anti-inflammatory and antioxidant properties, and it is currently being investigated for a wide range of potential health benefits [29]. There is also research that has been done that suggests that quercetin reduces blood pressure in hypertensive subjects [30].

Table (4): Comparison between normal and cardiovascular diseases (CVDs) rat groups on serum cytokines profile related to insulin resistance.

Groups / Parameters	Control	Obesity	%
TNF-α (pg/ml)	6.097 ± 0.049	10.112 ± 0.087*	
IL-1ß (pg/ml)	4.352 ± 0.039	6.867 ± 0.061*	
Il-6 (pg/ml)	10. 338 ± 0.077	21.556 ± 0.134*	

- Values are expressed as mean ± SE - n= number of rats. - * significant at (P< 0.001).

Table (5): Amelioration effects of quercetin on serum carbohydrate profile in CVDs rats groups related to IR.

	Parameters	Control	CVD associated with IR	
			Recovery	Quercetin
Glucose	1 month (n=5)	6.301 ± 0.76	11.008 ± 0.88	8.024 ± 0.81
(mmol/L)	2 months (n=5)	6.313 ± 0.77	9.531 ± 0.87	7.019 ± 0.79
Insulin	1 month (n=5)	26.941 ± 1.06	35.104 ± 1.58	32.331 ± 1.49
(µU/L)	2 months (n=5)	27.027 ± 1.09	30.195 ± 1.36	28.462 ± 1.27
IR	1 month (n=5)	7.569 ± 0.85	23.573 ± 1.52	14.027 ± 1.18
(HOMA)	2 months (n=5)	7.576 ± 0.88	17.897 ± 1.36	10.134 ± 0.93

- $^{A, B, C}$ Means with a common superscript within a row are significantly different at (P<0.05).
- $_{a, b}$ Means with a common subscript within a column are significantly different at (P<0.05).

Table (6): Amelioration effects of quercetin on serum lipid profile in CVDs rats groups related to IR.

	Parameters	Control	CVD associated with IR	
			Recovery	Quercetin
Cholesterol	1 month (n=5)	62.41±1.27 Aa	98.71±1.79 Ba	83.97±1.63Ca
(mg/dL)	2 months (n=5)	62.39±1.25 Aa	84.65±1.65 Bb	70.52±1.48 Cb
TG	1 month (n=5)	69.38±1.31 Aa	114.56±1.87 Ba	90.55±1.79 Ca
(mg/dL)	2 months (n=5)	69.47±1.33 Aa	86.24±1.74 Bb	78.81±1.56 Cb
HDL	1 month (n=5)	19.29±0.53 Aa	22.66±0.58 Ba	20.44±0.55 Ca
(mg/dL)	2 months (n=5)	19.32±0.55 Aa	21.53±0.56 Bb	19.51±0.56 Cb
LDL	1 month (n=5)	30.24±0.68 Aa	53.14±1.02 Ba	45.42±0.86 Ca
(mg/dL)	2 months (n=5)	29.18±0.66 Aa	45.87±0.89 Bb	35.25±0.74 Cb

- $^{A, B, C}$ Means with a common superscript within a row are significantly different at (P<0.05).
- $_{a, b}$ Means with a common subscript within a column are significantly different at (P<0.05).

In the current work, supplementation of quercetin after induction of CVDs related to IR in rats led to a significant (p<0.05) decrease in the serum carbohydrate profile (Glucose, insulin and HOMA) as well as lipid profile (cholesterol, triglycerides and LDL- cholesterol) associated with a remarkable decline (p<0.05) in the serum HDL- cholesterol level through the whole experiment periods (1 & 2 months) (Tables 5 & 6). These data may be attributed to the hypoglycemic and hypolipidemic effects of quercetin which acts as an antioxidant agent (Free radical scavenger, elevates autoimmune system, decreases the absorbance of fat from the intestine, modulates leptin receptor, enhancement of adiponectin expression, corrects the β-oxidation of lipid

in the matrix of mitochondria and adjustment the hypothalamus-pituitary-thyroid axis (HPTA). These data are in agreement with those obtained by (31) , (32) , (33)

The current results are in good accordance with other published studies carried out in animal models showing insulin resistance or type II diabetes [(2), (34)]. Nevertheless, there is no general agreement concerning the effect of quercetin on glycemic control. For example, [(29)], whose experiment induced insulin resistance in rats by feeding animals on a high-fat diet observed that quercetin, at a dose of 0.8 % in the diet exacerbated diet-induced insulin resistance at 3 weeks. The authors of those studies which found positive effects of quercetin on glycemic control. Also, these results may be due the anti-oxidative protective action on the pancreatic islets, the increase in adiponectin circulating concentration, the inhibition of small intestine glucosidase activity, the increase in glucokinase activity and the increase in GLUT4 transporters in skeletal muscle [(35), (34) ,(36)].

Quercetin and its glycoside have been shown to have hypocholesterolemic actions in other experimental models [(37),(2), (38)]. The hypocholesterolemic mechanisms of polyphenols have been attributed to their antioxidant action resulting in the inhibition of LDL oxidation, but there is increasing evidence that these compounds act by other mechanisms, including the alteration of hepatic cholesterol absorption, triglyceride assembly and secretion or by beneficial effects on inflammation [(6)]. Be that as it may, the current results suggest that the antiinflammatory effects of quercetin are independent of the hypocholesterolemic action *in vivo*. Moreover, the quercetin produces hypocholesterolemic and hypotriglyceridemic effect dependent on the time of treatment. Also, flavonoids have also been described as modulators of lipid homeostasis in the adipose tissue and liver through the inhibition of phosphodiesterases [(2), (38)].

Table (7): Amelioration effects of quercetin on serum cardiac profile in CVDs rats groups related to IR.

	Parameters	Control	CVD associated with IR	
			Recovery	Quercetin
AST	1 month (n=5)	48.55± 0.91 Aa	66.45 ±1.09Ba	60.54 ±1.03Ca
(U/L)	2 months (n=5)	48.47± 0.92 Aa	60.23 ±1.02 Bb	51.18 ±0.97 Cb
LDH	1 month (n=5)	229.61± 2.89Aa	306.32± 3.49 Ba	269.77± 3.34 Ca
(U/L)	2 months (n=5)	231.04± 2.92Aa	271.21± 3.37Bb	238.33± 3.11Cb
CK	1 month (n=5)	102.88± 1.17Aa	189.44 ±1.79 Ba	170.17 ±1.67 Ca
(U/L)	2 months (n=5)	103.19± 1.19Aa	167.56 ±1.66Bb	148.39 ±1.45Cb
Endotheline-1	1 month (n=5)	0.52± 0.012 Aa	0.96± 0.021 Ba	0.83± 0.019 Ca
(pg/ml)	2 months (n=5)	0.54± 0.01 Aa 1	0.78± 0.016 Bb	0.65± 0.014 Cb

- $^{A, B, C}$ Means with a common superscript within a row are significantly different at (P<0.05).
- $_{a, b}$ Means with a common subscript within a column are significantly different at (P<0.05).

Quercetin has been proposed to have anti-atherogenic, anti-inflammatory and anti-hypertensive properties, leading to the beneficial effects of this compond against cardiovascular disease [(39)]. From table (7), the supplementation of quercetin to rats groups which fed on high fructose/fat diet caused a significant depression in the activities of AST, LDH and CK as well as a remarkable decrease in the level of endotheline-1 dependent on the time of treatment (One and two months). These data may be due to the antioxidant effects of quercetin. However, quercetin appears to improve endothelial function in a NOS independent pathway [(40)]. Quercetin has anti-clotting abilities due to its ability to competitively bind plasminogen and also modulates plasmin concentration via urokinase plasminogen activator modulation [(41)]. Furthermore, the decrease in cholesterol levels was associated with reduced formation of atherosclerotic plaques in the aorta and carotid artery [(42)]. Also in male Wistar rats, a diet supplemented with 0.5-percent quercetin for two weeks decreased LDL and increased high-density lipoprotein (HDL) levels [(43)].

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Table (8): Amelioration effects of quercetin on serum cytokines profile in CVDs rats groups related to IR.

	Parameters	Control	CVD associated with IR	
			Recovery	Quercetin
TNF-α	1 month (n=5)	6.112± 0.052Aa	8.657 ± 0.081Ba	7.389±0.071Ca
(pg/ml)	2 months(n=5)	6.108±0.050Aa	7.593±0.074Bb	6.425±0.063Cb
IL-1ß	1 month (n=5)	4.395 ± 0.041 Aa	6.015±0.058 Ba	5.552±0.049 Ca
(pg/ml)	2 months (n=5)	4.355±0.039 Aa	5.492 ± 0.054 Bb	4.793±0.045 Cb
IL-6	1 month (n=5)	10. 67±0.079Aa	17.898±0.126 Ba	14.016±0.115 Ca
(pg/ml)	2 months (n=5)	10.359±0.076Aa	13.974±0.112Bb	11.745±0.093Cb

- $^{A, B, C}$ Means with a common superscript within a row are significantly different at (P<0.05).
- $_{a, b}$ Means with a common subscript within a column are significantly different at (P<0.05).

The anti-inflammatory effects of quercetin are clearly seen in *in vitro* studies[(2),(3)]. This property was different in human and animal studies, which may

result from the different physiology of species and also different levels of oxidative stress and inflammatory status [(44)]. In the current study, quercetin intake significantly decreased the serum concentration of IL-1ß; IL-6 and TNF-α. This finding was consistent with the results of [(2)], which showed that a higher dosage of quercetin (10 mg/kg) in obese rats that had higher levels of cytokines could reduce the visceral production of TNF-α and increase adiponectin.

Quercetin supplementation could cause a significant reduction on the disturbances presents in the insulin resistance (metabolic syndrome) associated with cardiovascular diseases (CVDs) due to the corrections in pro-inflammatory cytokines profile (TNF-α, IL-1ß and IL-6) and lipid profile (Cholesterol, triglycerides, HDL-cholesterol and LDL-cholesterol) associated with modulation in the cardiac profile (AST, LDH, CK and endotheline-1) due to different mechanisms with considering the biological effects of quercetin *in vitro*. So, more studies are needed with a stronger design and sample size with different doses of quercetin to reach the optimal dietary (quercetin) approaches to stop cardiovascular diseases (CVDs) associated with insulin resistance (metabolic syndrome) diet includes high amounts of fruits and vegetables, it is a good source of antioxidants such as quercetin.

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