EFFECT OF QUERCETIN ON THE BIOCHEMICAL PARAMETERS OF THE ALLOXAN INDUCED DIABETES IN MALE RATS

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ABSTRACT

Oxidative stress constructions is directly concerned with diabetes mellitus. For that reason, this study was performed to look into the biochemical variation occurred by oxidative anxiety and to estimate the antioxidant effect of quercetin in alloxan formed diabetes in male albino rats. The rats were thirty six male albino rats, they were divided into six groups 6 rats per each were treated as follows: control group, quercetin group (50mg/kg), Diabetic control group, Diabetic with quercetin group, Diabetic with insulin group, Diabetic with quercetin plus insulin group. The result illustrated that diabetes caused a significant increased in glucose, alanine aminotransferase, alkaline Phosphatase, aspartate aminotransferase, triglycerides, cholesterol, urea and creatinine level in serum of rats. Whereas the albumin level was decreased considerably. In opposition, quercetin significantly changed the level of biochemical enzymes and oxidative markers used in this study with or without insulin. It has been concluded that the quercetin could be shown as a potential antioxidants in reducing the risk of oxidation induced by diabetes that lead to damage of the pancreas, liver and kidney.

INTRODUCTION

The condition of inadequate or lack of insulin production and/or action from β-cells, builds up a metabolic confusion that called diabetes mellitus (DM) or hyperglycemia (1). Persistent hyperglycemia contributes to several complications, for instance cardiomyopathy, vascular injury, retinopathy, renal damage and neuropathy (2).

An essential task in the etiology of diabetes is oxidative anxiety (3). Diabetes and experimental animal forms reveal high oxidative stress indicators and reactive oxygen species in the pancreatic islets because of constant and chronic high sugar level, in that way, they reduce the action of the anti-oxidative security system and thus elevates free radical production (4, 5). Structural and functional irregularity in the liver initiates by affecting oxidative stress on the glycogen and lipid metabolism (6).

In case of diabetes, free radicals are excessively produced by oxidation of the sugar, glycation of protein non-enzymaticaly, and the consequent oxidative degradation of proteins glycation (7, 8). Abnormally, high levels of free radicals and the concurrent decline of antioxidant defense mechanisms may lead to the harm of cellular organelles and enzymes (9, 10), development of insulin resistance and increased peroxidation of lipid.

Alloxan which is an organic complex, chemically known as 5, 5-dihydroxyl pyrimidine-2, 4, 6-trione, is derived from the urea, a carcinogen and cytotoxic glucose analog (11). The compound has the molecular formulae, C4H2N2O4 and a relative molecular mass of 142.06. It is a well- known mediator for creating diabetes generally used to induce Type II diabetes in animals (12).

Alloxan is urea imitative, which produce selective death of β-cells of the pancreatic islets. Alloxan is used for making experimental hyperglycemia in animals like mice, rabbits, rats and dogs. (13). Severe diabetes that produced by alloxan makes the blood sugar levels the same to a completely pancreatic remove, for that reason a test plant extract producing a significant hypoglycemia must be worked through a different mechanism, therefore, it is suggested that drugs used for producing non insulin dependent diabetes mellitus are used on moderate diabetic animals (14).

Flavonoids normally present, and it is common in the plant kingdom. Their purpose as plant pigments, and is dependable for the colors in plants and fruits (15). The compound of quercetin is one of the major clusters of polyphenolic material of natural flavonoid, it has an antioxidant and it does not allow to develop inflammation. It is observed that as a member of the flavonoids' family quercetin (3,5,7,3 ', 4'-pentahydroxyflavo's) which is obtained 50- 500 mg in a normal daily food has a lot of tasks such as antioxidant for metabolism, anti-carcinogenic, antiviral, anti-thrombosis, anti-inflammatory, anti-ischemic, and anti-histamine feature (16,17).

At this time, quercetin supplements are usually presented throughout commercial sources in doses changeable from 250 to 1500 mg. One or more of the different

isoforms of these compounds of quercetin are commonly used for example quercetin aglycone, rutin, and other glycoside versions. Generally, quercetin supplements are promoted to the public as alternative therapy for treating allergies, asthma, bacterial infections, gout, arthritis, eye disorders, diabetes, hypertension, and neurodegenerative confusions. The most usual form of quercetin that is used is the quercetin aglycone supplement. Although rutin is a products of quercetin that has a small quantity of a glycoside as well. (18)

The aim of this study is to estimate the effect of the quercetin on the alloxan-created diabetes mellitus in addition to evaluate some of biochemical variation that related to diabetic disease.

MATERIALS AND METHODS

Induction of Diabetes

The animals were fasted during the night and diabetes was generated by a single subcutaneous (S.C.) injection of a recently prepared solution of alloxan (140mg/kg body weight) in 0.9% NaCl saline solution into all the animals without quercetin and control groups in order to make diabetes.

However 72 hours later in the development of diabetes, rats with moderate diabetes, had glucosuria and increased blood glucose level (ranged above 250 mg/dl) (17). The induction of diabetes was ensured by urine strips.

Animals and Experimental design

In this study, 36 male albino rats were used, weighting 200- 300 gm; they were obtained from the animal house and kept in the college of the veterinary medicine university of Duhok, the temperature of the rats was under controlled. They were administered with a commercial pellets and tap water.

The rats were haphazardly separated into 6 groups, each group (6 rats) and were treated as follows:

Group I (C): Normal control rats were administered standard pellets and water for 30 days.

Group II (Q): Quercetin control rats. In which, the rats received quercetin (50 mg/kg dissolved in distill water) then administered according to weight, 2 ml for each rat by oral gavage once daily until the last of the experiment.

Group III (D): Diabetic control rats. They were induced diabetes with 140 mg/kg S.C. Injection of Alloxan with feed and water for 30 days.

Group IV: Diabetic with quercetin (DQ): The rats after production of diabetes and they were treated with quercetin in a daily dose.

Group V: Diabetes with Insulin (DI): in which diabetes was induced in the rats and received insulin in a daily dose of 3 IU/rat S.C.

Group VII: Diabetes with quercetin plus Insulin (DQI): (in which diabetes was induced and treated with both insulin and quercetin with the same above dose.

Treatment was started after 48-72 hours of induction of diabetes and confirmation of the occurrence of persistent hyperglycemia.

Biochemical study

The Biochemical tests were used in this study. They consisted of alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST), glucose, albumin, cholesterol, triglycerides, urea and creatinine.

After 30 days of the experiment, the blood samples were collected from the retro-orbital plexus, following fasted of the rats overnight and under light ether anesthesia using a heparinized glass capillary and collected in tubes. Blood was allowed clotting and the serum separated by centrifugation at 4000 RPM for 10 minutes, to measure serum biochemical tests. The calorimetric methods were used with a UV visible spectrophotometer with length 505 nanometers according to the different standardized commercially available kits, each of them is from a different company. Determination of different biochemical biomarker using specific kits for each marker and according to its manuscripts.

Statistical Analysis

Study of variance (ANOVA) throughout the general linear model method of SPSS (10.0) software considering replicates as experimental units, and the values were expressed as means ± standard error. The significance of difference between mean by considering the differences significant was done by using Duncan's multiple range test at ($P < 0.05$) (19).

RESULT

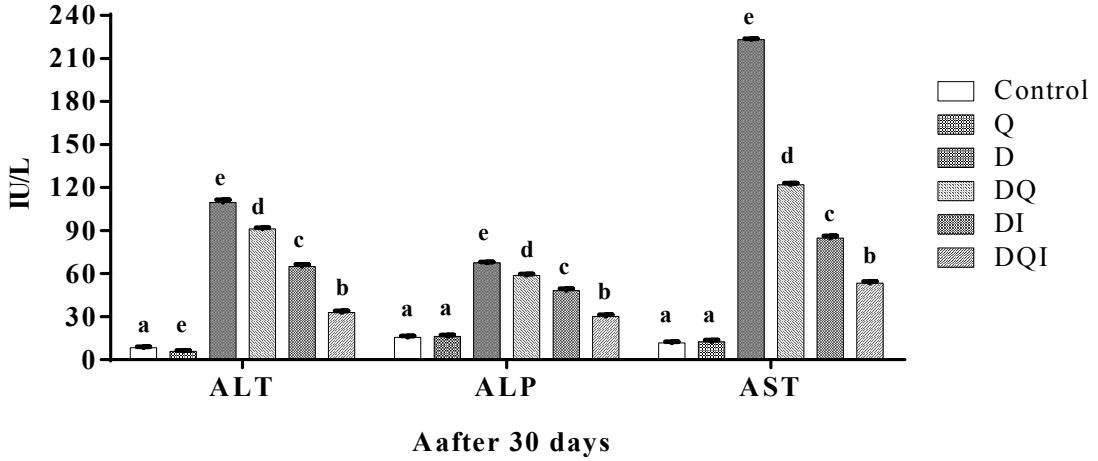
The biochemical analysis results in each of diabetic, diabetic with quercetin, diabetic with insulin, diabetic with insulin plus quercetin when compared to a normal control &quercetin group, analysis of biochemical parameters showed a significant increase of ALT, ALP, AST, Glucose, urea, triglycerides, and cholesterol levels in

serum while decreased the albumin serum level at the same time as showed in figures (A, B, C, D)

In the group of diabetic rats which received only quercetin , the analysis of biochemical parameters showed a limited decrease of ALT, ALP, AST, glucose, creatinine, urea, cholesterol, and triglycerides serum levels as observed in figures (A, B, C) ($p < 0.005$) compared to diabetic control, but also significantly increased when compared to quercetin and control groups ($p < 0.005$), as showed in figures (A, B, C), while the albumin level was extensively improved ($p < 0.005$) when compared to diabetic control (D)

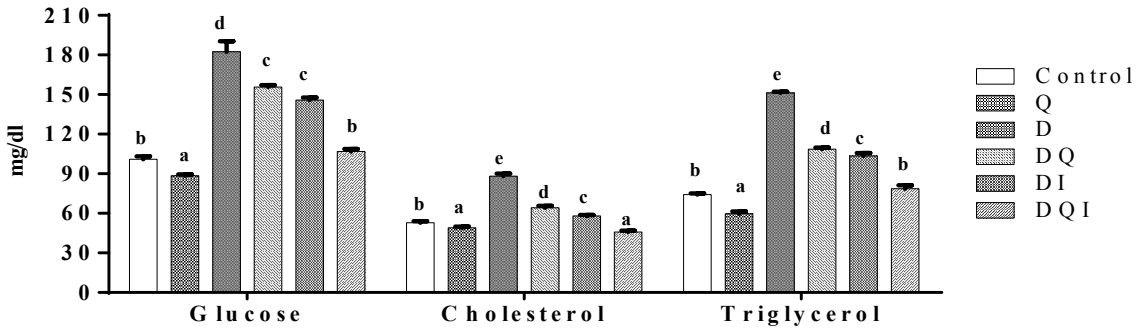
In the group of diabetic rats that received only insulin, the analysis of biochemical parameters showed a significant decreased of ALT, ALP, AST, glucose, creatinine, urea, triglycerides and cholesterol serum levels ($p < 0.005$), when compared to diabetic control as showed in figures (A, B, C), however these values were significantly increased when compared to the control and quercetin group ($p<0.005$). Whereas the albumin serum level was significantly elevated ($p < 0.005$) when compared to the diabetic group and diabetic with quercetin but significantly decreased as compared to quercetin and control groups as showed in the figure (D).

In the group of rats that received quercetin with insulin, the analysis of biochemical parameters showed a sharp decrease the in the levels of ALT, ALP, AST, glucose, creatinine, urea, triglycerides, and cholesterol serum levels as viewed in figures (A, B, C) ($p < 0.005$), when compared to diabetic, diabetic with insulin and diabetic with quercetin so these values were the same or near to the values of the control and quercetin group ($p<0.005$). On the other hand, when the albumin level compared to diabetes, diabetic with insulin and diabetes with quercetin showed significantly increased ($p < 0.005$) and the level was near to the values of quercetin and control groups as showed in figure (D).



Aafter 30 days

Figure (A): The effect of quercetin 50 mg/kg on ALT, ALP and AST in Diabetic rats. Data were expressed as the mean ± SEM (n=6) (P≤0. 05) When compared with quercetin and Control groups. Different letters indicated a significant difference and similar letters indicated no significant difference.



Aafter 30 days

Figure (B): The effect of quercetin 50 mg/kg on Glucose, Cholesterol and Triglyceride in Diabetic rats. Data were expressed as the mean ± SEM (n=6) (P≤0. 05) When compared with quercetin and Control groups. Different letters indicated a significant difference and similar letters indicated no significant difference.

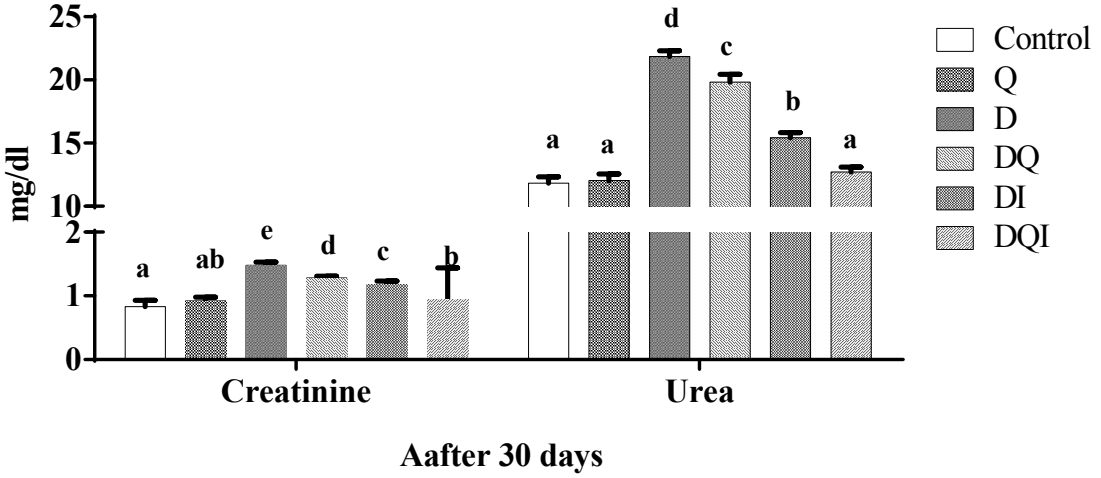


Figure (C): The effect of quercetin 50 mg/kg on Creatininea and Urea in Diabetic rats. Data were expressed as the mean ± SEM (n=6) (P≤0. 05) When compared with quercetin and Control groups. Different letters indicated a significant difference and similar letters indicated a no significant difference.

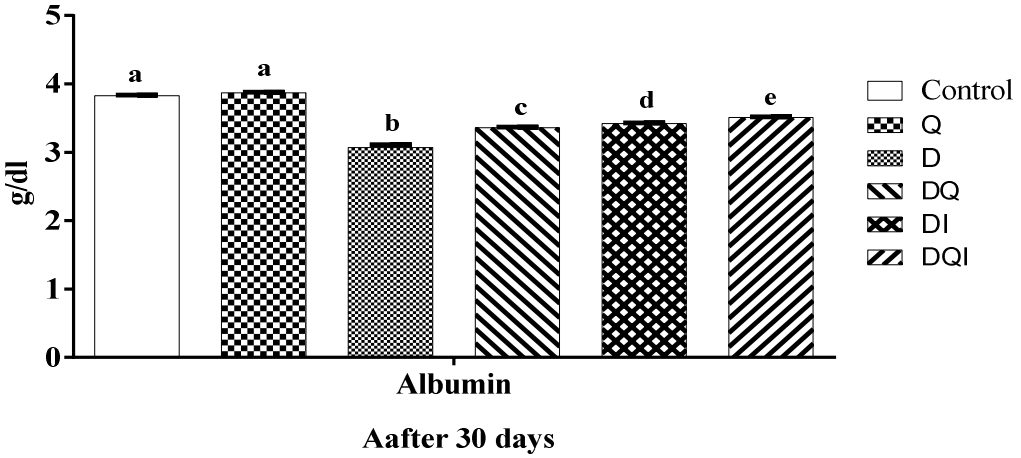


Figure (D): The Effect of quercetin 50 mg/kg on Albumin in Diabetic rats. Data were expressed as the mean ± SEM (n=6) (P≤0. 05) When compared with quercetin and Control groups. Different letters indicate a significant difference and similar letters indicated a no significant difference.

DISCUSSION

Studies on diabetes mellitus observed that the incidence of oxidative stress ascends as a result of enlarge in the stage of free radicals construction and reduce cell antioxidant capacity, which all collectively can cause oxidative tension and harm the tissue in diabetic patients (20, 21).

Among the biochemical aspects, the increased level of serum oxidative markers AST, ALP, ALT, glucose, creatinine, urea, cholesterol, and triglycerides, whereas there was decrease in albumin serum level of all alloxan induced diabetic groups compared to the control and the quercetin group was found to be related to damage of the liver, pancreas and kidney and also showed differentiation among all above groups in alteration in mentioned biochemical tests (21). The elevation in the serum levels of these various biomarkers has been recognized to the damaged and exposed dysfunction of the renal and hepatic cells because these enzymes are normally found in the cytoplasm of these cells and are released into the circulation when damage of cells occur (22). The current work focused on the improvement prospective of quercetin, against alloxan-produced oxidative injury in rats. Numerous studies have promoted qurcetin and its analogues as exceptional antioxidants in vivo (23, 24, 25).In the current study, oral quercetin administration (50 mg/kg/day) for 4 weeks after the induction of diabetes was found to have a beneficial effect on glycemic control in alloxan generated diabetic rats. Any significant decrease did not occur in glucose levels after daily quercetin treatment in a normal control rats and this in agreement with studies of (26). In diabetic rats the hypoglycemic consequence of quercetin is well documented (27). The urea and creatinine are metabolic yields cleaned liberally from the blood by the kidney and when alteration occurred in the kidney it results in an increase in plasma levels of these substances in the circulation is which agreement with (28). The present work found that diabetes induces structural and functional changes in kidney, pancreas and liver, which was proved by the significant increase in serum creatinine, AST, ALP, ALT, glucose and urea levels of diabetic rats, suggesting their leakage from damaged liver due to extensive oxidative damage beside the elevation the level of both cholesterol and triglycerides (29). In addition to the reduction in the albumin level is a good marker that is diabetes induced kidney damage, Increase in serum ALP was identified to be related to destruction of intra

hepatic and extra hepatic bile flow, hepatobiliary injury, erythrocyte damage or changed bilirubin metabolism (30, 31).

However, as was the case with the other parameters, complete normalization occurred only with combined treatment with quercetin and insulin. Our results confirmed this defensive effect of quercetin experimented by other researchers in diabetic animal models of liver insult (32, 33).

At the conclusion of the present study, we recognized that quercetin had the ability to keep against diabetes-generated oxidative damage in rat tissues. This study recommended a possible role of the quercetin as a supplement during an extended period of diabetes.

تاثير الكيورسيتين على المعاير الكيموحيوية لداء السكرى المستحدث بالالوكسان في ذكور الجرذان

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الخلاصة

الاجهاد التأكسدي له علاقة مباشرة مع داء السكر، ولهذا السبب صممت هذه الدراسة لتقييم التغيرات الكيميائية الحياتية التي يحدثها الاجهاد التأكسدي بالإضافة الى دراسة تأثير الكيورسيتين كمضاد للأكسدة في الجرذان المصابة بداء السكر المستحدث بالالوكسان. استخدمت ٣٦ من ذكور الجرذان من نوع Albino قسمت عشوائيا الى ستة مجاميع (كل مجموعة ستة جرذان) وتم معاملتها كالأتي: مجموعة سيطرة سليمة، مجموعة أعطي الكيورسيتين ٥٠ ملغم/كغم من وزن الجسم، مجموعة استحدث فيها داء السكر بحقنها بمادة الالوكسان واعتبرت مجموعة سيطرة موجبة، مجموعة مصابة بداء السكر أعطيت الكيورسيتين ، مجموعة مصابة بداء السكر اعطي الأنسولين، وأخيرا مجموعة مصابة بداء السكر مع الانسولين إضافة الكيورسيتين. أظهرت النتائج في الجرذان التي استحداث داء السكر زيادة معنوية في تركيز الكلوكوز، الالنين ناقل الأمين، الاسبارتيت ناقل الأمين، الفوسفاتيز القاعدي، الشحوم الثلاثية، الكولسترول، الكرياتينين، وحامض اليوريك. مع انخفاض في تركيز الالبومين. وعند المعاملة بلكيورسيتين أظهرت النتائج تغيير معنوي في جميع تركيز الانزيمات ومعايير الاكسدة مع او بدون الانسولين.

تشير نتائج الدراسة الحالية ان الكيورسيتين يمكن ان يعتبر مضاد اكسدة قوي من خلال خفض مخاطر الاكسدة الناجمة عن داء السكر والتي تؤدي الى تلف في البنكرياس، الكبد، والكلية.

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