

# A Biochemical Study of the Effect of Quercetin on Cisplatin Induced Rat Tissues Toxicity

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**Abstract** The present study was aimed to analyze the protective effect of quercetin against cisplatin-induced hepatotoxicity, nephrotoxicity and cardiotoxicity in male albino rats. The animals were divided into six groups of 6 animals in each group and treated as follow: a control group, quercetin (50mg/kg), cisplatin (4 and 8 mg/kg, I.P), and a groups that received both quercetin and cisplatin. Cisplatin induced a significant increase in ALT, AST, LDH, ALP, Cholesterol, Triglycerides, Urea and Creatinine with both serum and homogenize tissues. However, both Glucose and Albumin levels were significantly decreases. Conversely, quercetin significantly modulated hepatotoxicity, nephrotoxicity and cardiotoxicity by the effect with oxidative markers. The study showed that the Quercetin could be promising antioxidants for reducing the risk of cisplatin induced nephrotoxicity, cardiotoxicity and hepatotoxicity.

**Keywords** Cisplatin, Chemical analysis, Iraq, Quercetin, Toxicity

## 1. Introduction

Cisplatin (Cis-dichloro diammine platinum) CDDP, is a commonly used as chemotherapeutic drugs for treatment of several solid tumor [1]. However, cisplatin has serious side effects on normal a cell which is causes toxicity of many of organs [2, 3]. This toxicity is closely associated with an increase in lipid peroxidation and reactive oxygen species (ROS) in the tissues as a result of increase and decrease of endogenous enzymes in the body systems [4]. Excess ROS causes significant oxidative damage by attacking bimolecular such as membrane lipids, DNA, and proteins in cells [5]. The oxidative stress is associated with many disease states including neurological diseases such as Alzheimer's brains and Parkinson's disease, chronic heart disease, and kidney and liver diseases [6]. The most endogenous antioxidants and tissues enzymes are related to oxidative tissue as glutathione (GSH), glutathione peroxidase (GSH-PX), superoxide dismutase (SOD), catalase (CAT), alanine aminotransferase (ALT), blood urea nitrogen (BUN), albumin, creatinine and aspartate aminotransferase (AST) are compounds that act as free radical scavengers. Recently many of natural products, such as lycopene, grape seed extract, green tea caffeic acid

phenethyl ester and royal jelly are demonstrated to have a protective role against cisplatin-induced kidney and liver oxidative damages [7, 8, 6].

As well as Quercetin is a safe and common dietary flavonoid found in onions, apples and tea [4]. It is able to scavenge oxygen radicals, inhibit lipid peroxidation and exert as anti-carcinogenic activities [9]. Few studies were found dealing with the role of Quercetin on the biochemical alteration of renal and hepatic toxicity of cisplatin (19). Therefore, this work was designed to assess the productive effect of orally administered of Quercetin against cisplatin induced nephrotoxicity, cardiotoxicity and hepatotoxicity.

## 2. Materials and Methods

### 1- Animals and Experimental protocol

Thirty six Male Albino Wister rats, weighting 200- 300 gm were used in this study; they were obtained from and maintained in the college of veterinary medicine university of Duhok, they were housed at temperature 20-25°C and fed commercial pellets with tap water.

The Rats were randomly divided into 6 groups each groups (6 rats) and treated as follow:

Group I: received single dose I.P (intraperitoneal) of distilled water 2 ml as a control.

Group II: received single dose I.P of cisplatin (4mg/kg).

Group III: received single dose I.P of cisplatin (8mg/kg)

Group IV: received single dose I.P of cisplatin (4mg/kg) and Quercetin 50mg/kg once daily for 7 days before and 7 days after a single dose of cisplatin 4 mg/kg I.P.

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Published online at <http://journal.sapub.org/ajb>

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Group V: received single dose I.P of cisplatin (8mg/kg) and Quercetin 50mg/kg once daily for 7 days before and 7 days after a single dose of cisplatin 4 mg/kg I.P.

Group VII: received oral dose of Quercetin 50mg/kg daily respectively for 7 successive.

Animals were anesthetized by ether, the blood was collected directly from the retro-ocular vein [10]. After that rats were slathered and blood poured into plain tubes, the clot was dispersed with glass rod and then centrifuged at 3000 rpm for 15 minute and placed in  $-20^{\circ}\text{C}$ . The serum was used for the estimation of biochemical test. While internal organs liver and kidney keep in  $-20^{\circ}\text{C}$  for homogenizing.

## 2- Biochemical study

The Biochemical test used in this study included these enzymes alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline Phosphatase (ALP), Glucose, Albumin, Cholesterol, Triglycerides, Urea and Creatinine.

At the end of experiment blood samples were obtained from retro-ocular vein the blood was allowed to clots for 20 min and centrifuged at 3000 rpm for 10 min then the Serum samples were used from all groups for determination of different biochemical analysis, the colorimetric methods used with UV Visible Spectrophotometer width length 505

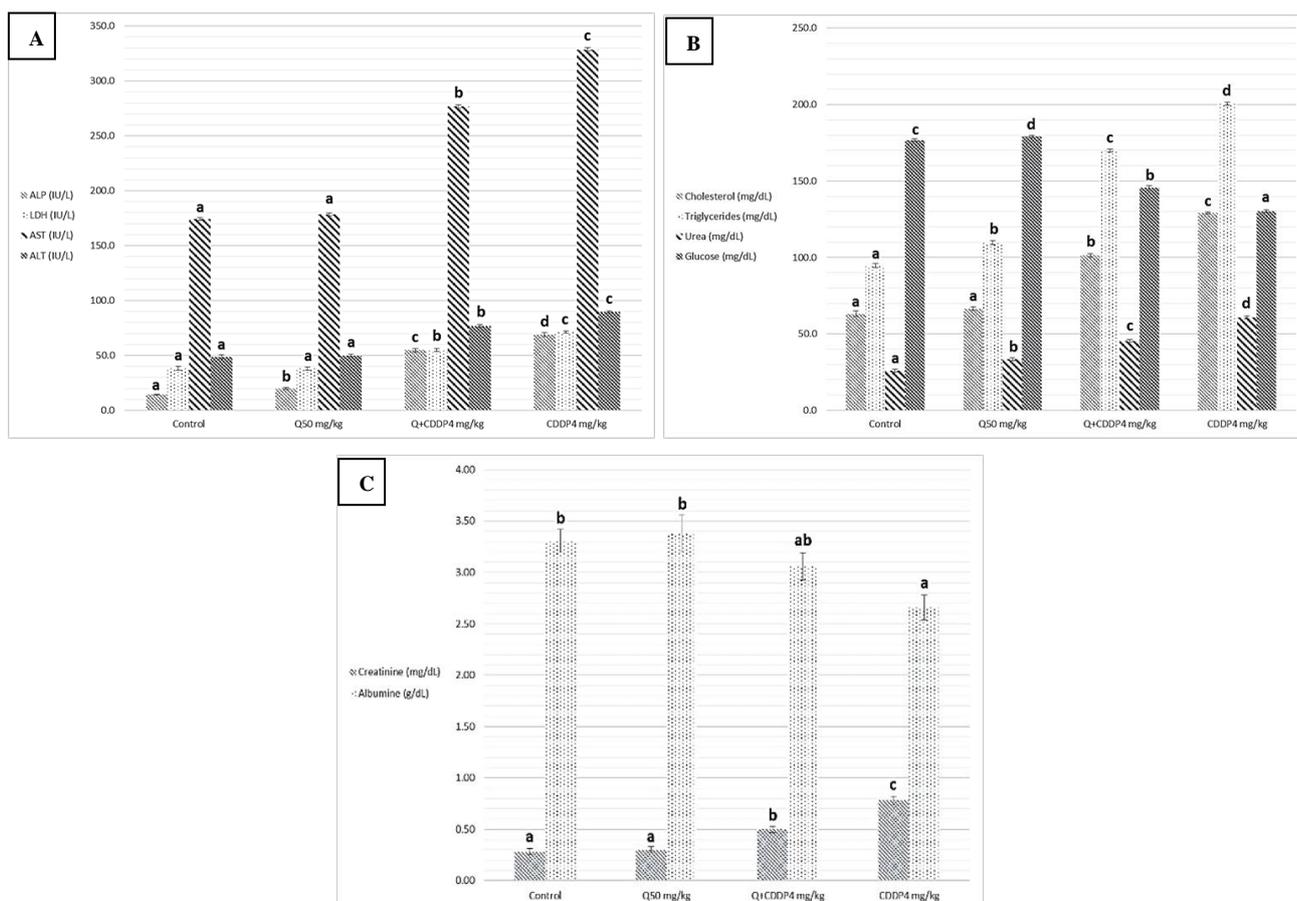
nanometer (Jenway Ltd, U.K) according to the different standardized commercially available kits each of one from different company.

Tissue homogenate from each organs ( kidney and liver) was prepared and storage at  $-20^{\circ}\text{C}$  then taken one gram from each organs after removed, cleared from adhering connective tissue; and homogenize using Ultrasonic Homogenize ( Sonic Rupture 400, U.S.A) with 10ml of 0.1M Tris- HCL buffer PH 7.4 according to [11]. The homogenates were Centrifuge  $-4^{\circ}\text{C}$  (D3024R with AS24-2 aluminum alloy rotor Kit, China) at 3000 rpm for 15 min and the supernatants obtained were transferred into eppendorf tubes, and preserved at  $-20^{\circ}\text{C}$  in a deep freezer until used for biochemical analysis.

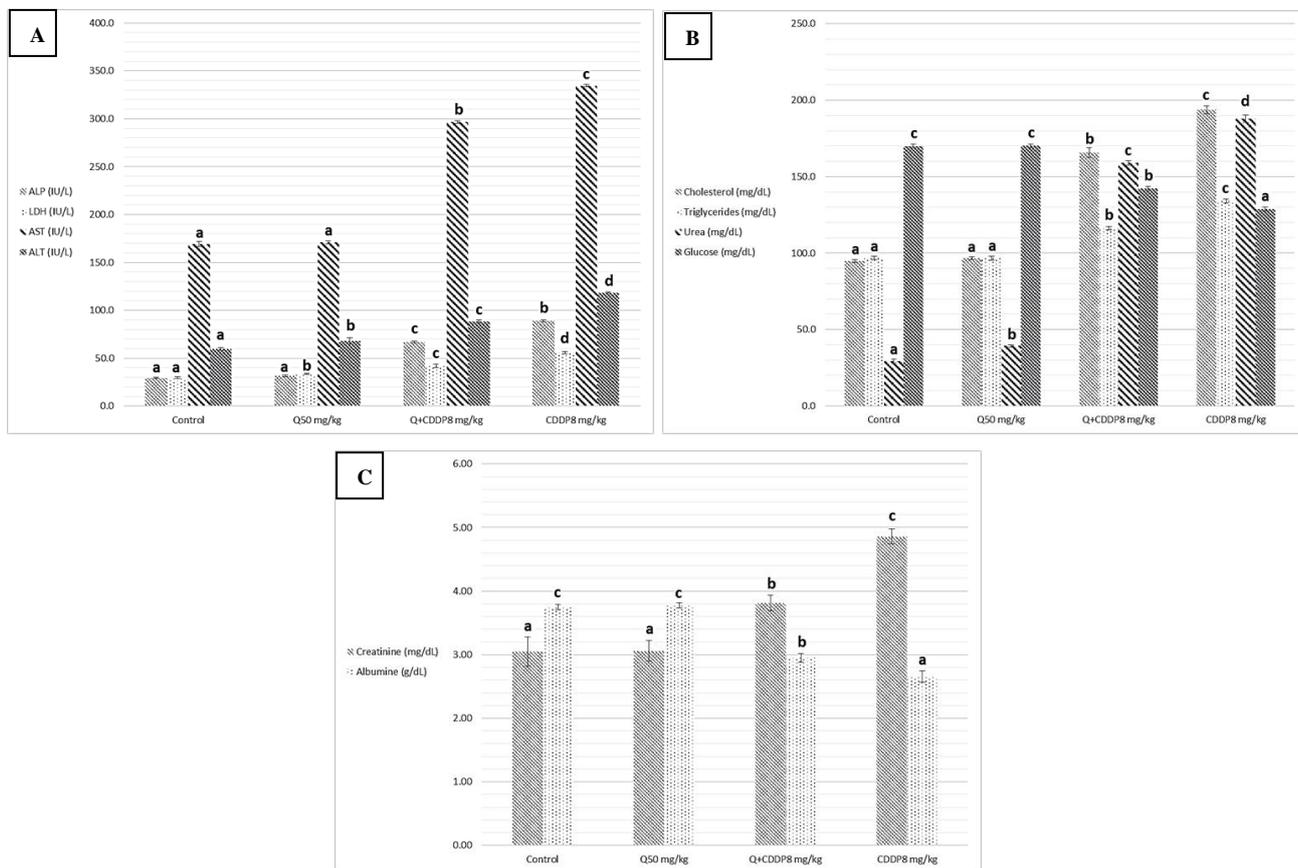
The study was analysis different of biochemical biomarkers by using specific kits for each marker and according to its manuscripts.

## Statistical Analysis

The data were analyzed using one-way analysis of variance (ANOVA) followed by post- hoc Duncan multiple range test (36). Comparison test for comparing means from different groups. The data were expressed as mean  $\pm$  SEM and a value of  $P < 0.05$  was considered statistically significant [12].



**Figure 1.** Effect of cisplatin 4 mg/kg on (A) ALP, LDH, AST, ALT. (B) Cholesterol, Triglycerides, Urea, Glucose. and (C) Creatinine and Albumin level Serum of albino rats. Data were expressed as the mean  $\pm$  SEM (n=6) ( $P \leq 0.05$ ). Different letters indicate a significant difference



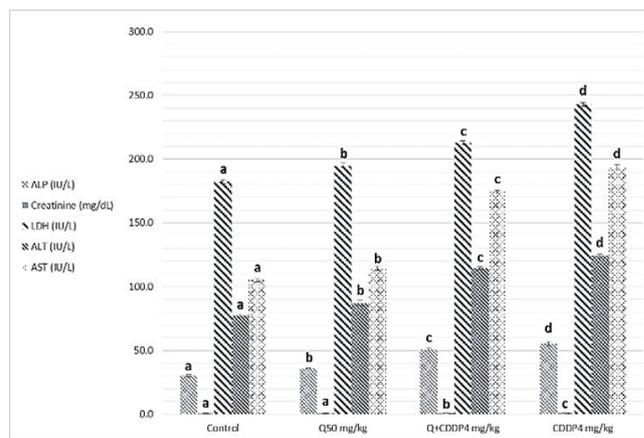
**Figure 2.** Effect of cisplatin 8 mg/kg on (A) ALP, LDH, AST, ALT. (B) Cholesterol, Triglycerides, Urea, Glucose. and (C) Creatinine and Albumin level Serum of albino rats. Data were expressed as the mean  $\pm$  SEM (n=6) ( $P \leq 0.05$ ). Different letters indicate a significant difference

### 3. Results

Results of biochemical analysis showed a significant increase levels of ALT, AST, ALP, LDH, urea, cholesterol, Triglycerides and creatinine in serum with cisplatin group at both doses four and eight mg/kg, compared to the control groups, Quercetin and Quercetin with cisplatin groups as showing in figure 1 and 2 (A, B and C), while analysis of biochemical parameters of homogenize tissue showed a significant increase levels of ALT, AST, ALP, LDH and creatinine concentrations only ( $p < 0.005$ ) as showing in figure (3, 4, 5 and 6). In other hand the level of both Glucose and albumin serum was significantly decreased ( $p < 0.005$ ) as showing in figures 1 and 2 (B and C).

The analysis of biochemical parameters in the group of rats received cisplatin with Quercetin at both doses, showed a significant decrease levels of ALT, AST, ALP, LDH, urea, cholesterol, Triglycerides and creatinine in serum as showing in figures 1 and 2 (A, B and C). Beside the level of ALT, AST, ALP, LDH and creatinine in homogenize tissue concentrations are significantly decreased ( $p < 0.005$ ), compared to Cisplatin group as showing in figures (3, 4, 5 and 6), but also these values were significantly increased comparing to the control and Quercetin group alone ( $p < 0.005$ ). Conversely the result showed that the level of both Glucose and albumin serum were significantly increased ( $p < 0.005$ ) compared to cisplatin group, while

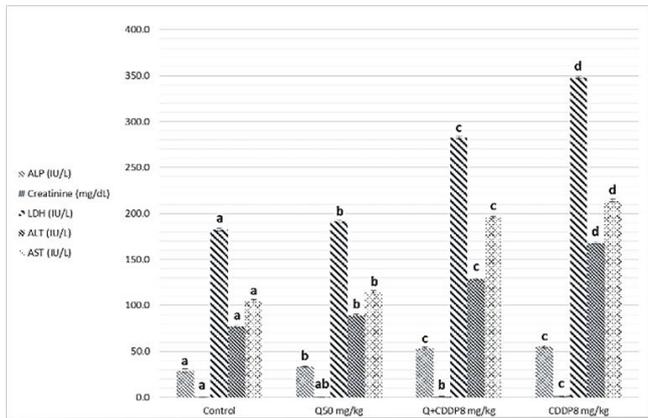
significantly decreased comparing to Quercetin and control group as showing in figures 1 and 2 (B and C).



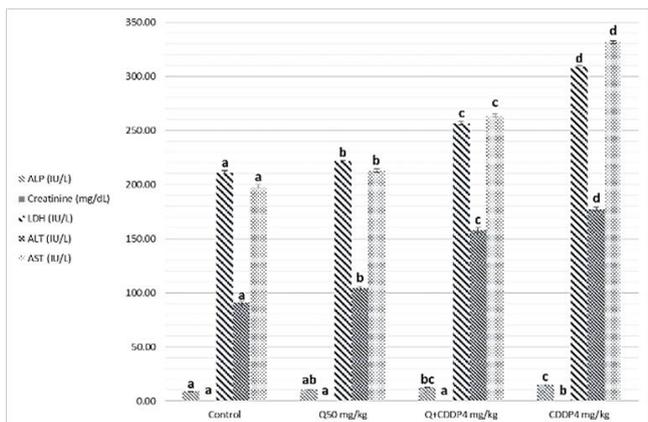
**Figure 3.** Effect of Cisplatin 4 mg/kg on ALP, Creatinine, LDH, AST and ALT level in kidney tissue of albino rats. Data were expressed as the mean  $\pm$  SEM (n=6) ( $P \leq 0.05$ ). Different letters indicate a significant difference

The result analysis of biochemical parameters in the group of rats received only Quercetin, showed a significant decrease of levels of ALT, AST, ALP, LDH, urea, cholesterol, Triglycerides and creatinine serum as showing in figures 1 and 2 (A, B and C) and levels of ALT, AST, ALP, LDH and creatinine in homogenize tissue concentrations ( $p < 0.005$ ) as showing in figures (3, 4, 5 and 6), compared to

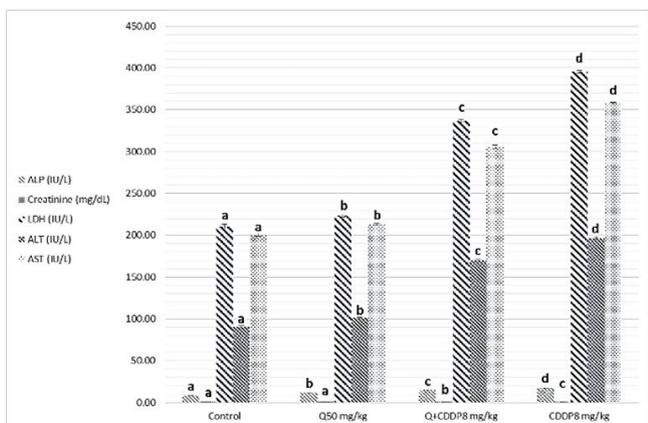
cisplatin, cisplatin with Quercetin and control groups ( $p < 0.005$ ), as showing in figures 1 and 2 (A, B and C), while the study showed that the levels of both Glucose and albumin serum were significantly increased ( $p < 0.005$ ) comparing to Cisplatin, Cisplatin with Quercetin and control group as showing in figures 1 and 2 (B and C).



**Figure 4.** Effect of cisplatin 8 mg/kg on ALP, Creatinine, LDH, AST and ALT. level in kidney tissue of albino rats. Data were expressed as the mean  $\pm$  SEM (n=6) ( $P \leq 0.05$ ). Different letters indicate a significant difference



**Figure 5.** Effect of Cisplatin 4 mg/kg on ALP, Creatinine, LDH, AST and ALT. level in liver tissue of albino rats. Data were expressed as the mean  $\pm$  SEM (n=6) ( $P \leq 0.05$ ). Different letters indicate a significant difference



**Figure 6.** Effect of Cisplatin 8 mg/kg on ALP, Creatinine, LDH, AST and ALT. level in liver tissue of albino rats. Data were expressed as the mean  $\pm$  SEM (n=6) ( $P \leq 0.05$ ) groups. Different letters indicate a significant difference

## 4. Discussion

Among the biochemical aspects, the increase level of many enzymes as AST, ALT, ALP, creatinine, urea, cholesterol, Triglycerides and LDH in serum and homogenized tissue of cisplatin treated rats group was found to be related with damage of the liver and kidney which are conversely associated to the functions and structure of organs especially with high dose compared to low dose of cisplatin as well as compared to groups of cisplatin treated with Quercetin, Quercetin alone and control groups. The elevation of such enzymes levels in both serum and homogenize tissue has been attributed to the damaged and revealed dysfunction of 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 as well as renal failures caused by the administration of cisplatin as a result of reduced activities of several enzymes that play a role in renal function, such as adenosine triphosphate production which serve as uncoupling of the mitochondrial oxidative phosphorylation [13-15]. In the same time the results were showed a decrease of level albumin and Glucose in serum, lower enzyme levels may be due to either lower release from the tissues to the serum, or decreased production of active enzymes and consequently lower activity in the serum [14, 16]. It has been postulated that the nephrotoxic mode of action of the drug cisplatin is similar to that of other heavy metals, and is related to the decrease in the intracellular concentrations of glutathione and protein-bound SH groups, which are required for normal cellular function as well as Mitochondrial damage might be attributed to inhibition of DNA synthesis and glutathione depletion induced oxidative stress and those finding are similar to our results [17-19]. The increase levels of urea and creatinine its another indication of the damage of the renal glomeruli and these data similar to other previous studies [20], have mentioned that cisplatin causes elevation the levels of the kidney function biochemical markers such as serum urea and creatinine. In opposite side the concentrations of creatinine and urea were decreased in rats that were administered cisplatin with Quercetin. These findings may be in concord with many other studies, which found Quercetin overcame the elevation of creatinine and urea levels caused by cisplatin [21]. The elevated of serum urea and creatinine concentrations in cisplatin injected rats could be attributed to the elevated of ROS [22]. In other hand the decrease levels of these biomarkers with administration of Quercetin was thought to be their antioxidant properties and improve of both the hepatic and renal dysfunctions as well as damage that induced by hepatotoxicity and nephrotoxicity which is confirmed by many of other reports which showed that antioxidant reduced damage of cells and had protective role [19, 23, 8]. In the present study showed that administration of Quercetin before or after cisplatin produced a significant decrease of levels biomarkers both in serum and homogenize tissue in comparison with rats treated with cisplatin and control group. These results suggested the

possible role of Quercetin which mediated a protection against cisplatin induced depletion of renal and hepatic glutathione could be ascribed to its antioxidant properties [24]. Furthermore, it has been reported that the main metabolites of Quercetin which are glucuronic acid conjugates and glutathione conjugates have antioxidant activity [20].

In the present work was found that cisplatin induces structural and functional changes in both kidney and liver as proved in result of this study, the significant increases of enzymes level in serum and homogenize tissue as creatinine and urea, in addition to the reduction of albumin and glucose level is a good marker that is a Cisplatin induced kidney damage, while significant increase in serum and homogenize tissue of AST, ALT, ALP and LDH levels indicated the liver and heart damage beside the elevation the level of both cholesterol and triglycerides.

Our conclusion showed that the Quercetin could be promising as antioxidants for reducing the risk of cisplatin induced nephrotoxicity, hepatotoxicity and cardiotoxicity So, the supplementation of this antioxidant could maintain the integrity of lipid in kidney and liver tissue under oxidative stress during chemotherapy.

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