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Acute nephrotoxicity of platinum in albino mice

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Abstract:

platinum (CDDP) is a potent anticancer agents used for the treatment of solid tumors. However, its clinical use is often limited by its adverse effects including nephrotoxicity. The present study was designed to estimate if Royal Jelly can inhibit or at least ameliorate the alteration in some renal **function and** structures induced by Cisplatin in mice or not. Four equal groups of mice: control, RJ-treated (300mg/kg, once oral dose/ 10 days), CP-treated (7 mg/kg once i.p. on day 11th.), combined RJ and CP-treated were used. Body and kidneys weights of each mouse were calculated. Serum levels of kidney biomarkers were assessed. Urea and Creatine levels. Renal samples from each mouse were prepared for light and electron microscopic examinations. Hemorrhage, glomerular atrophy, inflammatory cell infiltration, tubular necrosis and degeneration were observed in CP-treated mice. Also, a significant ($P<0.05$) increase in Urea &Creatine levels were determined in CP-treated mice compared to control group. However, most of CP-induced histomorphological, ultrastructural and biochemical changes were improved in animals pretreated with RJ. These results suggested that the effects of Cisplatin on renal function and structures could be totally or to a great extent inhibited by Royal Jelly.

Keywords: platinum, Royal Jelly, kidney, Biochemical, histological, mice.

Introduction

Platinum-based chemotherapeutic agents are well known to exhibit powerful anti-cancer activity in the treatment of solid tumours**(Pabla and Dong, 2008).** Cisplatin (cis-diamminedichloroplatinum(II), CDDP), produced a mar-ked decrease in renal blood flow and glomerular filtration rate. The alteration in the kidney functions induced by CDDP are closely assocated with an increase in lipid peroxidation and reactive oxygen species (ROS) in the tissues **(Antunes et al., 2000)**. CDDP may have some mechanisms of Renal cell apoptosis, oxidative stres-s, and inflammation have been recognized as the mechanisms for CDDP-induced nephrotoxicity. **(Liu et al. 1998)**. Endogenous antioxidants such as reduced glutathione (GSH), glutathione peroxidase (GSH- PX), superoxide dismutase (SOD), catalase (CAT) are compounds that act as free r-adical scavengers. Some natural products, such as grape seed extract **Yousef et al. (2009)**, green tea **Yapar et al. (2009)**, and caffeic acid phenethyl ester **Iraz et al. (2006)**, are demonstrated to have a protective role against CDDP-induced kidney oxidative damages. Royal Jelly (RJ) has received particular attention because it is a highly efficient antioxidant and has free radical scavenging capacity **(Silici et al., 2011).** The present study was performed to investigate the possible role of royal jelly in the prevention of CDDP-induced nephrotoxicity in mice.

Materials And Methods:

Materials:

Animals : Animals In present study, adult female mice (25-30 g) The mice were maintained under controlled room temperature of 22±3oC with 12 hours light/dark cycles and the humidity level of 50- 60%, All animals had accessed to laboratory chow and tap water.

Chemicals and drugs

CDDP (CDDP) .
From BMC .
Molecular Formula: $Cl_2H_6N_2Pt$ or $(NH_3)_2PtCl$
Formulation: 50 mg CDDP /50 ml aqueous solution.
Company: Ebewe , Austrla
Administration: given intraperitoneally (ip) (at 7mg/Kg, b.w) as a single dose.
Administration : It was administered daily by oral intubation at (300 mg/kg, b.w) were dissolve in distilled water daily for ten days.

Method

Experimental design:

The females were divided into four equal groups, 10 animals for each as following:

Group 1: (Control - ve): Administered distilled water orally for ten days and given normal saline ip at tenth day of the experiment.

Group 2: (Control + ve): Given CDDP ip at (7mg/Kg, b.w) as a single dose **(Al-Majed, 2007; Karadeniz et al., 2011 and Silici et al., 2011) .**

Group 3: Administered Royal Jelly oral intubation at (300 mg/kg, b.w) **(Nomura et al. 2007 and Ashry and Elkady, 2014)** daily for ten days.

Group 4: Administered Royal Jelly daily by oral intubation at (300 mg/kg, b. w) for10 days and given CDDP ip at (7mg/Kg, w.b) as a single dose.

Body Weight

The body weight of animals recorded at the end of the experiment was nearest to gram. After 72 hours CDDP administration **(Gheissar et al., 2012; and Moghim et al., 2014),** all mice were killed by the slaughter.

Methods Were used for Biochemical and Histopatholgical:

Biochemical:

Blood samples were collected in test tubes through the neck blood vessels and allowed to stand for 2 hours at room temperature then centrifuged at 2000 rpm for 10min. the clear supernatant was used for the estimation of kidney functions. The Serum urea and creatinine, were colorimeter measured analysis was done using selectra M.

Histopathological:

The kidney fix in 10% neutral formalin and prepar for examination according to **(Bancroft and Stevens, 1996).**

Methods used for Statistical analysis:

The data was analysed using Mini tap version 16. Mean±SE is given for quantitative variables. One way ANOVA was used to compare the groups and Tukey post hoc test was used for detail analysis

Results:

Morphological findings:

Body weightfindings

Animals of all groups showed no exhibit any case of mortality or death.

The induced body weight changes by CDDP are recorded in Table (1) and presented in (Fig.1) The mean final body weight and gain body weight significantly reduced in CDDP group compared with control (22 ± 0.943)versus (28±0.919). Also, the administration of RJ in concomitant exposure to CDDP showed increased body weight but result was not significant.

Table(1):The mean body weight (g) in different groups of mice.

Data are presented as mean ± SE (n=10)a: significant difference between control and treated group at P ≥0.05. cant as compared to Cisplatin

Groups	Initial weight (g)	Final weight (g)	Weight difference= Final weight-initial weight
Control	26±1.349	28 ±0.919	2 ± 0.527
Cisplatin	26±1.337	0.94322 ±	0.527[a]-4 ±
Royal jelly	26±1.349	29±0.948	3± 0.738
Cisplatin + Royal jelly	26±1.175	0.99423 ±	1.033 ±3-

Fig.(1-a): The mean body initial weight (g) in different group of mice. Results are presented as mean ± SE *P ≥ 0.05.

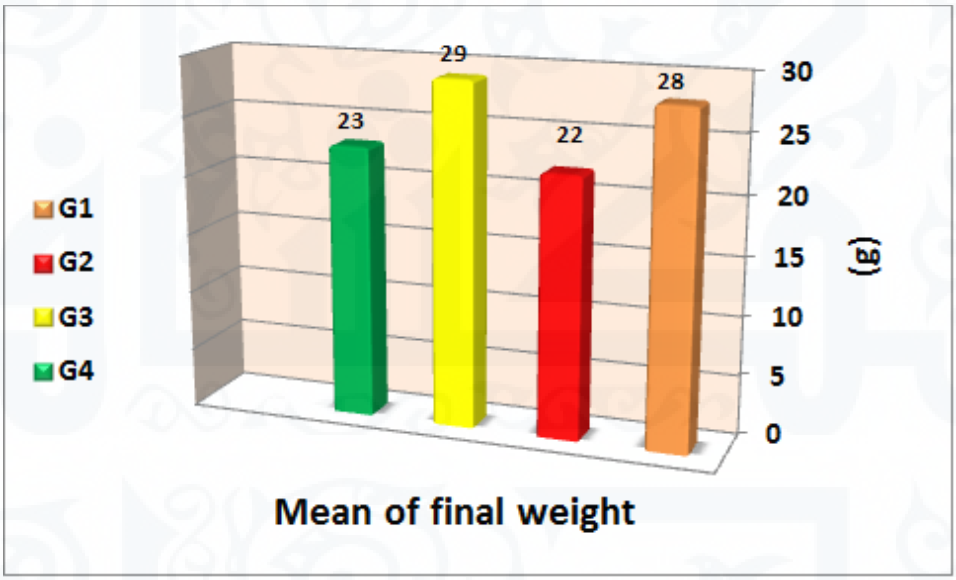

Fig.(1-b): The mean body final weight (g) in different group of mice.Results are presented as mean ± SE *P ≥ 0.05.

kidney function tests findings

Blood serum urea and creatinine obtained from experimental mice are tabulated in Table (2) and (Fig.2).Animals treated with Royal jelly alone showed no significant biochemical and histopathological effects in kidney .CDDP caused a marked reduction in renal functions, as characterized by a significant increase in both urea and creatinine level compared to control levels (P≥0.05) .Values of urea and creatininesignificantly decreased (P<0.05)in the mice treated with CDDP and RJ as compared to CDDP- treated mice.

Table (2): Biochemical parameters of kidney function tests in different group of mice.

Groups	Urea (mg%)	Creatinine (mg%)
Control	**29.1 ± 0.943**	**0.69 ± 0.014**
Cisplatin	**64.7 ± 0.949[a]**	**[a]2.493 ±0.016**
Royal jelly	**29.2 ± 1.033**	**0.69 ± 0.011**
Cisplatin + Royal jelly	**46 ± 1.155[b]**	**1.48±0.017[b]**

Data are presented as mean ± SE(n=10) a: significant difference between control and t-reated group at P ≥0.05. **b**: significant difference between Cisplatin and protector-treated group at P ≥0.05.

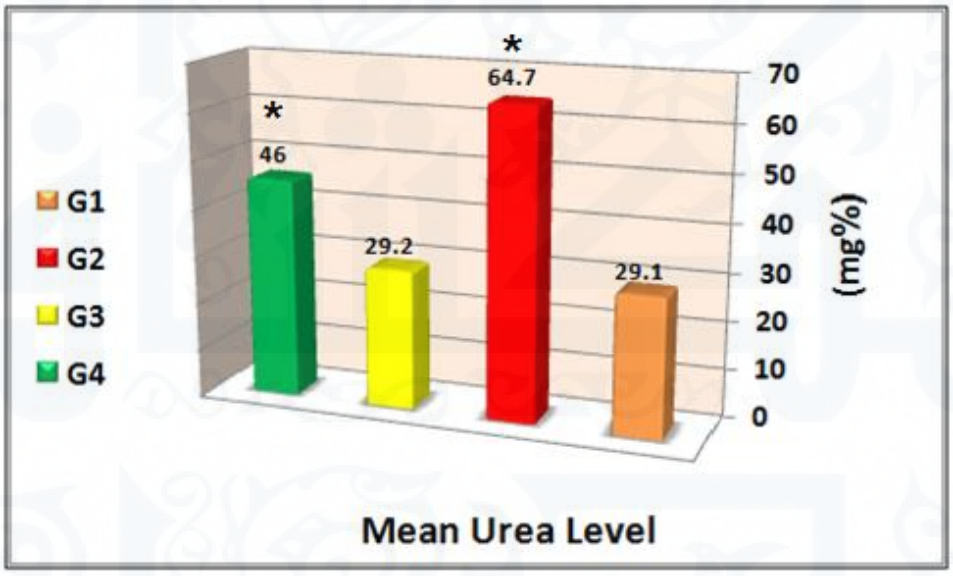

Fig.(3-a): The mean level Urea (mg%) of different groups. Results are presented as mean ± SE *P ≥ 0.05.

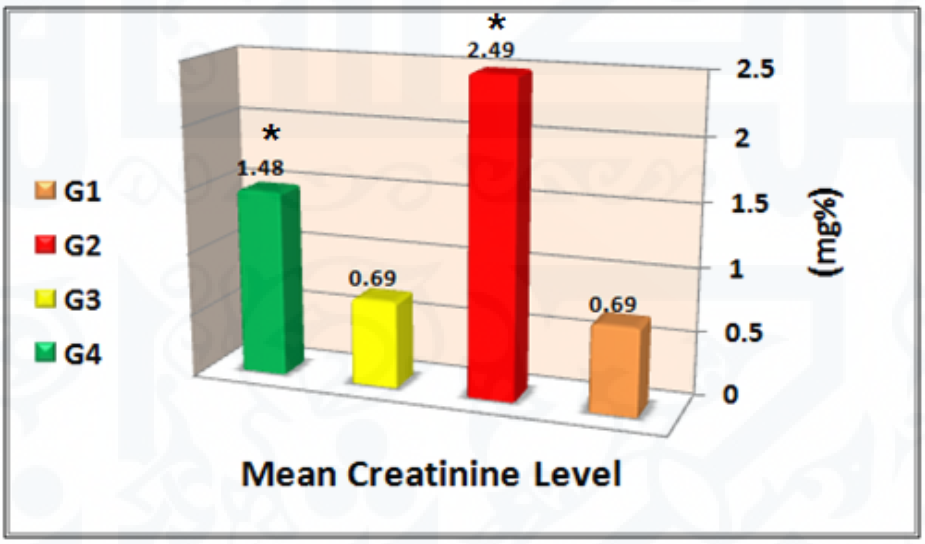

Fig.(3-b): The mean level Creatinine (mg%) of different groups. Results are presented as mean ± SE *P ≥ 0.05.

Histopathological findings :

In control group and RJ group, the kidney was no histopathological alter-ation and the normal histological structure of the glomeruli and tubules at the cortex were recorded in (Fig.4,5).In CDDP group, the kidney necrosis was detected in the tubular lining epithelium (Fig.6,7), associated with focal inflammatory cells infiltration in between the tubules at the cortex (Fig.8). There was focal haemorrhage in between the tubules and glomeruli (Fig.9). In CDDP plus RJ group, in the kidney There were focal few inflammatory cells infiltration in between the tubules and congestion in the blood vessels (Fig.10).

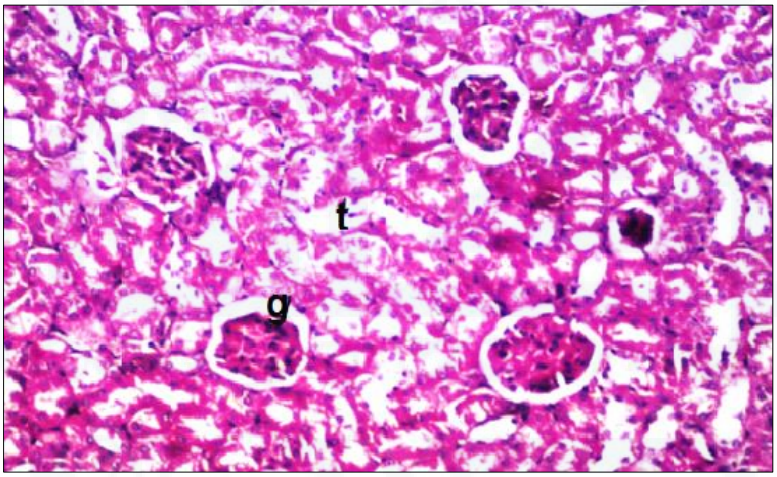

Fig.(4): Histological section of kidney from control mice. Showing normal histological structure of the glomeruli (g) and tubules (t) at the cortex. (H&E, X 40)

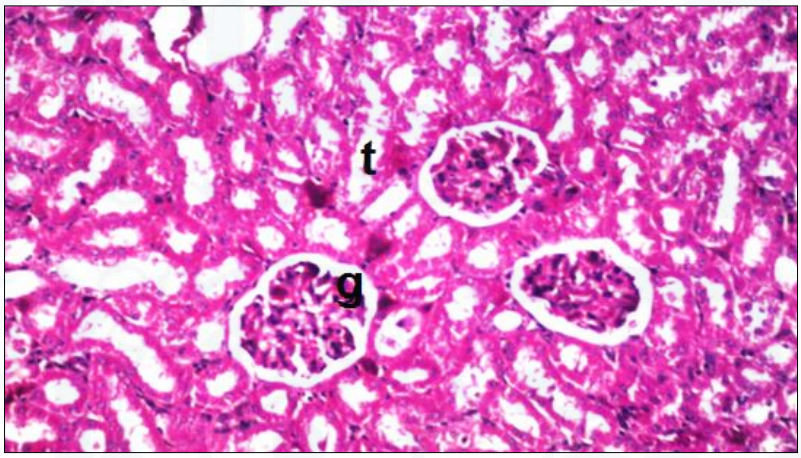

Fig.(5): Histological section of kidney from mice treated with RJ at daily oral dose of (300 mg/kg, b. w) for 10 days. Showing normal histological structure. (H&E, X 40)

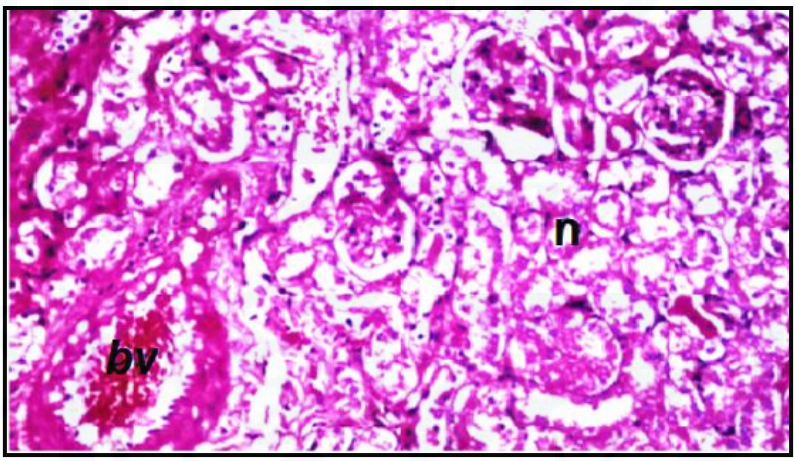

Fig.(6): Histological section of kidney from mice treated with CDDP at (7mg/Kg, b. w) Showing necrosis (n) in the tubular lining epithelium with Congestion blood vessels (bv) at the cortex. (H&E, X 40)

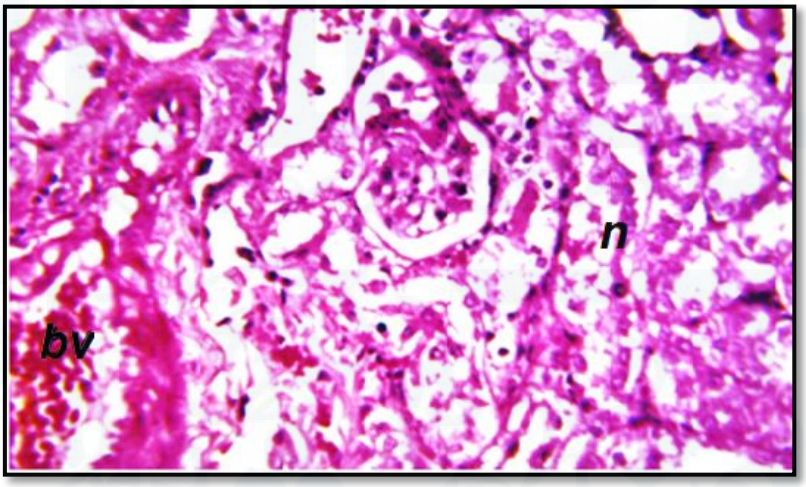

Fig.(7): Histological section of kidney from mice treated with CDDP at (7 mg /Kg, b. w). Showing the magnification of (Fg.10) toidentify the neccrosis (n) in lining epithelium. (H&E, X 40)

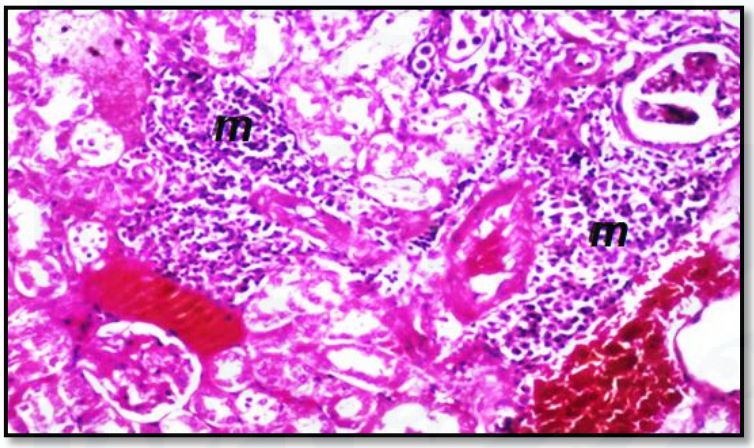

Fig.(8): Histological section of kidney from mice treated with CDDP at (7 mg/Kg, b. w) Showing focal inflammatory cells infiltration (m) in between the tubules at the cortex . (H&E, X 40)

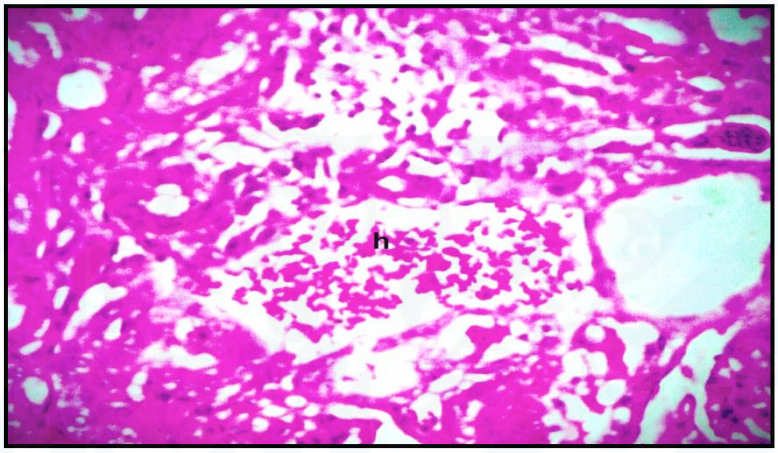

Fig.(9): Histological section of kidney from mice treated with CD-DP at (7mg/Kg, b. w). Showing focal hemorrhage (h) in between the tubules. (H&E, X 40)

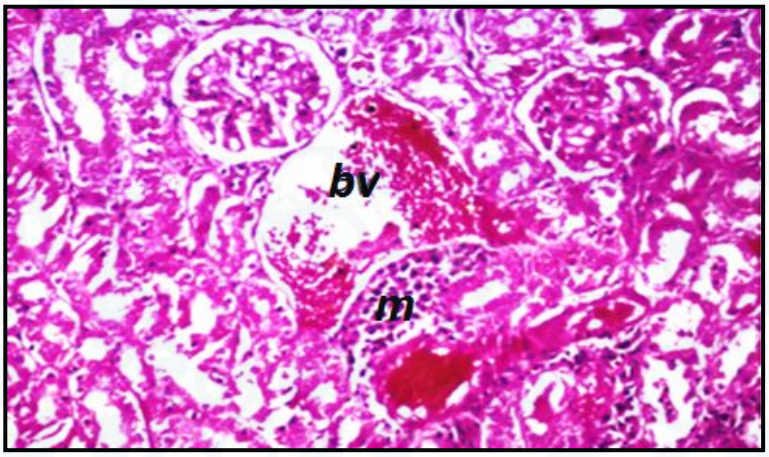

Fig.(10): Histological section of kidney from mice treated with CDDP at (7mg/ Kg, b. w) plus RJ at a daily oral dose of (300 mg /kg, b. w) for 10 days before administration of CDDP. Showing focal few inflammatory cells infiltration (m) in between the tubules an-d congestion in the blood vessels (bv). (H&E, X 40)

Dissection

In this study, CDDP caused statistically significant reduction of the bo-dy, were recorded in CDDP-treated mice compared to control Mice. These findings agree with other investigators who reported that the nephrotoxic effect of CDDP produced marked reduction of the mice body weight (**Pandir and Kara, 2013 and Abdelhamid et al. 2014)**, The decrease of body weight of CDDP-treated mice might be due to gastrointestinal toxicity and dysfunction **Mora et al. (2003)**, or because of the anorexic effect of the drug, which were considered side effects of the chemotherapy **(El-Sayyad et al., 2006)**.Nausea and vomiting are considered the foremost unpleasant side effects of chemotherapy from the patients viewpoint and experienced by 20-90% of cancer patients during chemotherapy **(Schnell, 2003 and Kovac, 2006).**but RJ treatment for 10 consecutive days, before CDDP administration mice, efficiently increase in the weight comparedC- DDP-treated MiceRJ is contains many important compounds with biological activity such as free amino acids, proteins, sugars, fatty acids, minerals, and vitamins**Karaali et al. (1988)**, This finding is in accordance with the previous study of **(El-Nekeety et al., 2007; Abdelhamidet al., 2014 and Galgamuwa et al., 2016).**

The anticancer drug Cisplatin is a very effective compound in the treatment of several cancers. Its clinical use, however, is associated with severe side effects. Main side effect which limits its use in treatment of cancers is nephrotoxicity (Mansour et al.,2002 and Pabla and Dong ,2008).Experimental evidence has suggested that CP deteriorates renal function (Arany and Safirstein, 2003) and glomerular filtration rate (GFR) in a dose-dependent manner (Yao et al., 2007). Tubular damage manifests through impaired reabsorption which is characterized by reduced glomerular filtration rate, increased serum creatinine and blood urea concentrations, hypokalemia. In 20–30% of patients treatment with Cisplatin induces acute kidney injury (Miller,2010 and Hanigan and Devarajan, 2003). The mechanism underlying Cisplatin nephrotoxicity is incompletely defined. The pathophysiological mechanism of Cisplatin-induced tubular damage is complex and involves a number of interconnected factors such as accumulation of Cisplatin mediated by membrane transportation, conversion into nephrotoxins, DNA damage, mitochondrial dysfunction oxidative stress, inflammatory response, activation of signal transducers and intracellular messengers and activation of apoptotic pathways (Peres and Cunha,2013).

In our study, serum BUN and creatinine levels were significantly increased after cDDP administration. Histological results showed that severe degeneration of corticThis result agreed with previous studies that have demonstrated the involvement of BUN and creatinine levels in cDDP-induced nephrotoxicity. (**Antunes et al., 2000 ; Parlakpinar et al.,2002** and **Mora et al. 2003**) tubular cells also accompanied with cDDP administration**.**suggested that underlying mechanism in Cisplatin-induced nephrotoxicity is oxidative stress through elevation of ROS and reduction of the antioxidant defense system.

Cisplatin generates ROS such as superoxide anion and hydroxyl radicals and stimulates renal lipid peroxidation (**Halliwell and Gutteridge,1999 and Taguchi,2005**). It has been shown in various studies that Cisplatin administrations are associated with increased formation of free radicals, and with heavy oxidative stress (**Antunes , etal.,2000** and**Antunes et al., 2001**). Treatment with RJ of CDDP-treated mice resulted in beneficial effects against nephrotoxicity induced by CDDP in mice. It w-as shown that RJ has a protective effect on the serum and Histological results. This protection was evidenced by significantly reduced levels of urea and creatinine in RJ group in relation to the group of animals that received only Cisplatin. Moreover, histological findings showed that RJ administration caused less degenerative alterations There were focal few inflammatory cells infiltration in between the tubules and congestion in the blood vessels in th-e kidneys as in previous studies**(Yapar et al., 2009; Karadeniz et al., 20-11 and Hassan et al., 2014).**Several studies in experimental animals have demonstrated that RJ has antioxidant and therapeutic activities. Also, there are a few reports about its antioxidative role connected to the anti-aging effects of RJ. (**Inoue, 2003).Nagai et al., 2001** examined the antioxidative effect ofRJ and other bee products by measuring scavenging abilities of the superoxide radical. In addition, it has been demonstrated that RJ has an anticancerogenic activity. (**Bincolettoa,2005**) . (**Fujii et al.,1990**) reported the anti-inflammatory properties of RJ in streptozotocininduced diabetic rats. Recently, studies in the literature have demonstrated that RJ also has antihypercholesterolemic activity, insulin-like activity, hypoglycemic activity, antifatigue effect, and wound-healing properties. (**Salazar-Olivo and Paz-Gonza´lez,2005)**

conclusion

In conclusion, the result of the present study clearly demonstrated that cDDP-induced nephrotoxicity induces oxidative damage in kidneys. However, supplementation with RJ can protect against cDDP-induced toxicity, by reduction of the effects of free radicals and preventing lipid peroxidative .Therefore, the antioxidant action of RJ may be used as a ‘‘nephrotoxicitylimiting agent’’ for reducing effects of chemical agents.

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