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Biochemical Changes in the Kidney and Liver of Albino Mice Following Acute Administration of Cisplatin

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Abstract:

The present study was planned to assay the effects of Cisplatin on biochemical variables in serum, liver and kidney. Experiments were carried out on 20 mice which divided into two groups (10 animals of each one). The first group was control, Group II: received a single dose of cisplatin (7 mg/kg) intra peritoneal. Evaluation of functional alterations in the kidney and liver were performed by biochemical analyses. CP-induced reductions in weights of body ,kidney and liver, Analysis of biochemical parameters showed significantly higher ALT and AST serum concentrations in Cisplatin group in comparison with control group ($p<0.05$), while there was no noticed a significant differences between levels of serum total protein in two groups of study. Also, a significant ($P<0.05$) increase in urea and creatinine levels were determined in CP-treated mice compared to control group. oxidative stress markers expressed by evaluating the serum levels of glutathione (GSH) and malondialdehyde (MDA).cisplatin at 7mg/kg ip caused a significant decrease in serum GSH and a significant increase in serum MDA. Finally, the study concluded that the biochemical alterations observed in kidney and liver of mice by Cisplatin may be mediated by free radicals.

Key words: Cisplatin, oxidative stress, Lipid peroxidation, liver and kidney.

Introduction:

Cisplatin is a platinum-based drug [1] , which is one of the most effective antineoplastic agents used for treatment of testicular, ovarian, bladder, cervical, lung, and neck cancers [2]. The cytotoxic action of the drug is often thought to be associated with its ability to bind DNA to form cisplatin–DNA adducts .The major dose-limiting side effect of cisplatin is its nephrotoxicity and hepatotoxicity, and nephrotoxicity can result in severe nephropathy leading to acute renal failure [3] .Cisplatin-related hepatotoxicity rarely occurs at standard doses. However, at higher doses, hepatotoxicity is

frequently observed and can alter the clinical situation of cancer patients [4 and 5]. Little is known about cisplatin-induced liver injury and the mechanism of its hepatotoxicity. The alterations in the kidney and liver functions induced by cisplatin are closely associated with an increase in lipid peroxidation and reactive oxygen species (ROS) in the tissues [6 and 7]. In addition, cisplatin may have some mechanisms of liver injury such as functional and structural mitochondrial damage, apoptosis, and perturbation in Ca2+ homeostasis [8 and 9]. This study attempts to investigate the effect of its repeated administration on some biochemical parameters of the liver and kidney**.**

Materials and methods

Experimental Animals

In the present study, 20 adult male mice((28-32g) were maintained under controlled room temperature of 22±3 ^{0}C with 12 hours light/dark cycles and the humidity level of 50- 60%. All animals had access to laboratory chow and tap water.

Experimental design:

The mice were divided into two equal groups, 10 animals for each as following:

Group 1 (Control): Administered physiological saline ip as a single dose.

Group 2 (Control + ve): Given (Cisplatin) intraperitoneally (at 7mg/Kg) as a single dose [5 and 40]

Drugs and chemicals

Cisplatin (CDDP) **Company** Ebewe, Austrla, and all other chemicals included were purchased from Sigma Aldrich.

Methods:

Body Weight and Organ Weight.

The body weight of animals recorded at the end of the experiment was nearest to gram. The absolute weight of organ (liver and kidney) for different groups of experimental animals was recorded to the nearest milligram on a digital balance.

The Relative organ weight or organo-somatic index (OSI) was calculated as the following formula:

Organ ratio (%) = organ weight (g) ×100/body weight (g) [7]

Kidney and liver function assessment

Mice of the group 2 were slaughtered slaughter72 hours after the injection of Cisplatin [10 and 11]. Blood samples were then centrifuged at 2000 rpm for 10 min in a refrigerated centrifuge to separate serum samples from the cells Serum AST, ALT, urea, creatinine, total protein, MDA, GSH were measured.

Assay of MDA and GSH

Malondialdehyde (MDA) and Glutathione Reducced (GSH) were assayed using the method described.

The concentration of MDA and GSH was calculated and expressed as nmol /ml analysis was done using spectrophotometer

Statistical analysis:

The data was analyzed using SPSS version 17. 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. Fisher Exact Test was applied to observe association between qualitative variables. Differences between groups were considered to be statistically significant, if $p<0.05$.

Results:

Effects of Cisplatin on body and organs weights:

The induced body weight changes by CDDP are recorded in Table (1) .The mean final body weight and gain body weight significantly reduced in CDDP group compared with control. On the other hand, results showed that administration of Cisplatin significantly decreased the absolute and the relative liver and kidney weights compared with those of control group ($p<0.05$).

Effect of Cisplatin on GSH and MDA levels

The changes in serum MDA and GSH levels are shown in (Table 2) when compared to the control groups, the MDA levels were significantly ($p < 0.05$) higher in groups administered with CDDP. On the other hand, The CDDP - treated rats showed significantly ($p<0.05$) reduced GSH levels when compared with the control groups.

Effect of Cisplatin on kidney and liver biomarkers

A single dose of Cisplatin (7mg/kg BW) resulted in prominent nephrotoxicity as evidenced by significant increase in serum creatinine and urea levels in Cisplatin group compared to control. (Table 3). (Table 4) showed many

biochemical abnormalities in the liver of CDDP-injected animals with single dose (7mg/kg bwt), Hepatotoxicity was monitored quantitative analysis of the serum AST and ALT activities, which were used as the biochemical markers of liver damages. There were statistically significant increases in AST and ALT in CDDP-treated group compared to control group. But no significant difference of serum total protein level was noticed in CDDP-treated group when it was compared to the control group.

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

Groups	Initial weight(g)	Final weight(g)	Final-initial weight
Control	30± 1.023	31±0.024	1 ± 0.135
Cisplatin	30±1.006	28±0.22*	-2 ± 0.321*

Data are presented as mean ± SE (n=10) *: significant difference between control and treated group at P< 0.05.

Table (2): Mean weight and Relative weight of Kidney and liver in different groups of mice**.**

Groups	Kidney (g)	Relative weight 100g of body weight\g	Liver(g)	Relative weight 100g of body weight\g
Control	0.58±0.0127	2.29 ±0.119	1.78±0.018	6.76±0.272
Cisplatin	0.39±0.031*	1.40±0.097*	1.09±0.157*	4.73 ±0.312*

Data are presented as mean ± SE (n=10) *: significant difference between control and treated group at P< 0.05.

Table (3): Biochemical parameters of kidney function tests in different group of mice.

Groups	Urea (mg %)	Creatinine (mg %)
Control	28.4 ± 0.740	0.61 ± 0.01
Cisplatin	60.5 ± 0.924*	2.87 ±0.012*

Data are presented as mean ± SE (n=10) *: significant difference between control and treated group at P< 0.05.

Table (4): Biochemical parameters of liver function tests in different groups of mice.

Groups	AST(U/L)	ALT(U/L)	Total Protein(g\dl%)
Control	1.099±1.034	26 ± 0.810	6.56 ± 0.009
Cisplatin	111 ± 1.021*	88 ± 1.004*	6.470± 0.012
Groups	Serum GSH level(nmol/ml)	Serum MDA level(nmol/ml)	
Control	3.47±0.14	10.48 ±0.43	
Cisplatin	0. 91±0.037*	25.3± 1.51*	

Data are presented as mean ± SE (n=10) *: significant difference between control and treated group at P< 0.05.

Discussion

Cisplatin is one of the most active cytotoxic agents in the treatment of cancer. Liver and kidney toxicity are major complications, which is a dose-limiting factor for cisplatin therapy [8]. These findings agree with other investigators who reported that the nephrotoxic and hepatotoxic effect of Cisplatin produced marked reduction of the Rat body weight [9]. Such reduction of the body weight in Cisplatin-treated Mice might be in part due to gastrointestinal toxicity and concomitant loss of the animal appetite with subsequent reduction of food ingestion [10]. The decrease of body weight of cisplatin-treated rats might be due to gastrointes tinaltoxicity and dysfunction, or because of the anorexic effect of the drug, which considered side effects of the chemotherapy. 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 [11].

Various reports are available showing that Cisplatin affects weight and serum creatinine, urea and significant decrease in kidney weight [3 and 12].
Cisplatin is a major antineoplastic drug used for the treatment of solid tumors. Its chief dose limiting side effect is nephrotoxicity; 20% of patients receiving high-dose Cisplatin have severe renal dysfunction. The kidney accumulates cisplatin to a greater degree than other organs and is the major route for its excretion. The Cisplatin concentration in proximal tubular epithelial cells is about 5 times the serum concentration [13].The disproportionate accumulation

of cisplatin in kidney tissue contributes to cisplatin-induced nephrotoxicity [14].

In most of the clinical studies concerning Cisplatin-induced nephrotoxicity, only plasma levels of creatinine and/or clearance of creatinine or blood urea nitrogen are used to evaluate the glomerular filtration rate [7], Unbound cisplatin is filtered at the glomerulus (80% of a dose is excreted in 24 hours). Renal blood flow can decrease within 3 hours after cisplatin infusion, and glomerular filtration rate (GFR) falls after the decrease in renal blood flow. The changes in GFR and renal blood flow probably reflect increased renal vascular resistance secondary to tubular-glomerular feedback from increased sodium chloride delivery to the macula dense [15].

The proximal tubular dysfunction observed in cisplatin nephrotoxicity precedes alterations in renal hemodynamics. Forty-eight to 72 hours after cisplatin administration, there is impaired proximal and distal tubular reabsorption and increased vascular resistance [16].

The expression patterns of outer medullary water channels aquaphorin 1 and 2, of sodium transporters [13 and 17]. The effect on sodium and water transport represents an early change in cisplatin toxicity since the inhibition of the transporters occurs in rats without elevation of BUN and creatinine [13 and 18].

Determination of the activity of hepatic enzymes released into the blood by the damaged liver is one of the most useful tools in the study of hepatotoxicity [19]. The specific and nonspecific biochemical parameters which were known to be altered by hepato-toxins were measured as markers for evaluating the hepato-protective activity of many drugs [19], the study of serum enzyme activity has been found to be of great importance in the assessment of liver damage [19 and 20].

The increase in AST and ALT activities in the CDDP group was found to be related to damage in the liver and change in hepatic functions. The rise in levels of serum AST and ALT has been attributed to the damaged structural integrity of the liver, because these are normally located in the cytoplasm and are released into the circulation after hepatic damage [21].

Cisplatin acts on cancer cells by releasing free radicals such as superoxide radicals, hydroxy radicals, peroxyl radicals, and singlet oxygen, which at the same time damage liver and kidney cells [21 and 22]. Reactive oxygen species are generated under normal cellular conditions and are immediately detoxified by major scavenger enzymatic and non-enzymatic molecules [23 and 24]. However, excessive ROS production by CDDP causes antioxidant imbalance and leads to lipid peroxidation and antioxidant depletion [22].

In this investigation, Cisplatin significantly increased serum MDA, and decrees GSH levels in the CDDP treated group compared with their levels in the controls. These results are in agreement with [25]. Increased MDA levels indicated that lipid peroxidation, mediated by ROS [23 and 25].

Lipid peroxidation is a key process in many pathological events and it is induced by oxidative stress. Lipid peroxidation is regarded as one of the fundamental mechanisms of cellular damage caused by free radicals having reacted with lipids causing peroxidation that eventually results in the release of products such as malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and hydroxyl radicals [18 and 24].

GSH is the most important molecule for maintaining cell integrity and participation in cell metabolism [14]. The role of GSH, which are non-protein thiols in the cells, in the formation of conjugates with electrophilic drug metabolites (most often formed by cytochrome P450-linked monooxygenase) is well established [25]. Furthermore, cisplatin treatment resulted in a significant reduction in, the first line of defense, enzymatic antioxidants like GSH protects against cellular injury caused by oxidative stress either by converting the toxic radicals to nontoxic end products or by scavenging free radicals. Cisplatin was found to cause depletion of both GSH and protein thiols [26-28]. The significant reduction in GSH levels promoted by CDDP resulted in the accumulation of these highly reactive free radicals and eventually generate reactive oxygen species that lead to detrimental effects in different tissues. This damage occurs due to imbalance between reactive oxygen species generation and antioxidant system [29 and 30].

Based on the present research it is, concluded that the potent free radicals scavenger and antioxidant agent's such as some natural products, grape seed extract, caffeic acid and royal jelly seem to be highly promising agents for protecting liver and kidney from oxidative damage and preventing organs dysfunction as a result of exposure to cisplatin [31-34] . This study may moreover, be useful to write down that antihypertensive and antianginal drugs may use against various drugs induced toxicity.

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