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CISPLATIN INDCUED OXIDATIVE STRESS IN THE LIVER OF WISTAR ALBINO RATS

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Abstract: The present study was conducted to assess cisplatin (CP) induced toxicity in Wistar Albino rats. The study included control group (Group I) and cisplatin group (Group II) with 12 rats in each. Cisplatin was used at 7.5mg/kg b.w. as single dose to induce toxicity. In CP control group the levels of endogenous antioxidant enzymes such as SOD, CAT and GPx were significantly ($p<0.05$) reduced and MDA levels were significantly ($p<0.05$) increased compared to those of normal control. The increased MDA level caused by lipid peroxidation was as a result of oxidant injury to liver by CP. In conclusion CP induces hepatotoxicity in rats through oxidative stress by decreasing levels of endogenous antioxidant enzymes and increasing levels of MDA.

Keywords: Cisplatin, hepatotoxicity, oxidative stress, antioxidants

1. INTRODUCTION

Cisplatin (CP) is one of the chemotherapeutic agents used against wide range of cancers including ovarian, testicular, head and neck, bladder and lung cancers (Saad *et al.*, 2004). Cisplatin interferes with replication and transcription in proliferative cells by the formation of covalent cross-linked interstrand and intrastrand adducts between DNA bases. Since it does not distinguish between malignant and normal fast-growing cells, the treatment with cisplatin in cancer patients is associated with several side effects which decrease the survival rate in the patients (Barbas *et al.,* 2008).

Hepatotoxicity is one of the major side effects of cisplatin produced at high dose exposure. The mechanism of cisplatin induced toxicity is not well understood. However, cisplatin is preferentially taken up and accumulated in the liver second only to kidney and induces oxidative stress (Abdelmeguid *et al.,* 2010). Cisplatin induces oxidative stress in liver by enhancing the production of reactive oxygen species and nitrogen reactive species. The

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imbalance between formation of ROS and RNS and antioxidants leads to pathological consequences in the liver (Yilmaz *et al.,* 2005).

2. MATERIALS AND METHODS

2.1 Experimental animals

Twenty-four male Wistar Albino rats of the age group 8 to 10 weeks with an average live weight of 180- 200g were procured from a commercial animal facility, Bangalore. They were housed in cages and allowed to acclimatize under ambient temperature and standard light and dark cycle. They were given a nutritionally adequate specified rat's diet and water *ad libitum* throughout the experimental period. The experimental procedures were carried out according to the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and approved by the Institutional Animal Ethics Committee.

Cisplatin, as Kemoplat (1mg/ml) injection was procured from Fresenius Kabi India Pvt. Ltd. Pune, India. All other chemicals and reagents used for the study were procured from local sources and were of analytical grade.

2.2 Experimental design

After procurement, the rats were maintained under standard laboratory conditions for a period of 15 days for acclimatization in the experimental animal house. The rats were divided, based on the body weight, into two groups with twelve rats in each group. Group I was normal control, administered with PBS intraperitoneally on the day of injection and observed till 45 days. Group II rats were administered with single dose of cisplatin at the rate of 7.5 mg/kg b.w. intraperitoneally with overnight fasting and observed for 45 days.

2.3 Collection of liver samples

From both the groups two rats were sacrificed on 7^{th}, 14^{th}, 28^{th} and six animals on 45^{th} day post induction of cisplatin toxicity, using over dose of Ketamine hydrochloride intraperitoneally. Liver of both group animals were collected in chilled normal saline and then transferred to -80˚C for further analysis.

2.4 Analysis Antioxidant parameters and lipid peroxidation

Endogenous antioxidant parameters such as catalase (CAT), glutathione peroxidase (GPx) and superoxide dismutase (SOD) were assessed from the liver samples by the method described by Caliborne (1985), Rotruck *et al.,* (1973) and Marklund and Marklund (1974) respectively. To assess oxidative injury induced lipid peroxidation, liver was subjected for estimation of malondialdehyde (MDA) by the method of Yagi (1976).

3. RESULTS AND DISCUSSION

The results our study showed a significant (P<0.05) elevation in the MDA levels (Table 4 and Figure 4) and levels of CAT, GPx and SOD (Table 1, 2 and 3) (Figure 1, 2 and 3) significantly (P<0.05) decreased at all the intervals of observation with comparison with normal control (Group 1) animals whose values remained in the normal range throughout the experiment.

Table-1. The mean (±SE) values of catalase (µmol/min/mg protein) in liver of rats in different groups at different time intervals

Groups	Days post treatment			
	7th day	14th day	28th day	45th day
Group I (Control)	50.79 ± 0.139^{ax}	49.525 ± 0.22^{ay}	52.81 ± 0.17^{az}	52.37 ± 0.30^{az}
Group II (CP)	26.575 ± 0.115^{bx}	26.35 ± 0.1^{bx}	27.84 ± 0.07^{by}	30.19 ± 0.50^{bz}

Values with different superscripts in a row and column vary significantly at p<0.05

Figure-1. The mean (±SE) values of catalase (µmol/min/mg protein) in liver of rats in different groups at different time intervals.

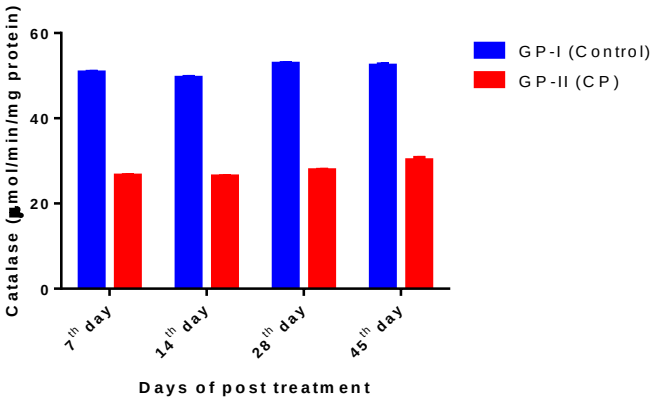


Table-2. The mean (±SE) values of glutathione peroxidase (U/mg protein) in liver of rats in different groups at different time intervals

Groups	Days of post treatment			
	07th day	14th day	28th day	45th day
Group I (Control)	32.155 ± 0.25^{ax}	30.895 ± 0.08^{ay}	32.915 ± 0.16^{ax}	33.96 ± 0.06^{ax}
Group II (CP)	10.78 ± 0.06^{bx}	12.93 ± 0.03^{by}	15.5 ± 0.1^{bz}	15.88 ± 0.16^{bz}

Values with different superscripts in a row and column vary significantly at p<0.05

Figure-2. The mean (±SE) values of glutathione peroxidase (U/mg protein) in liver of rats in different groups at different time intervals

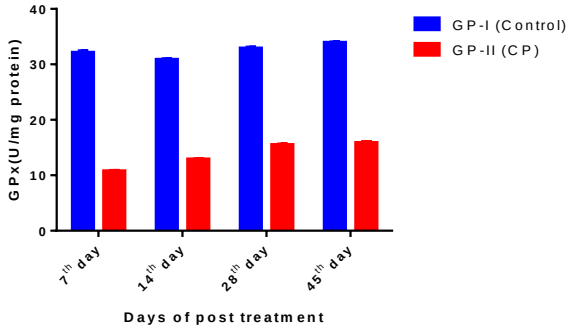


Table-3. The mean (±SE) values of superoxide dismutase (U/min/mg protein) in liver of rats in different groups at different time intervals

Groups	Days post treatment			
	07th day	14th day	28th day	45th day
Group I (Control)	28.32±0.05^{ax}	29.355±0.04^{ax}	30.705±0.15^{ay}	31.605±0.42^{az}
Group II (CP)	9.57±0.13^{bx}	13.57±0.13^{by}	14.745±0.04^{bz}	16.03±0.23^{bw}

Values with different superscripts in a row and column vary significantly at $p<0.05$

Figure-3. The mean (±SE) values of superoxide dismutase (U/min/mg protein) in liver of rats in different groups at different time intervals.

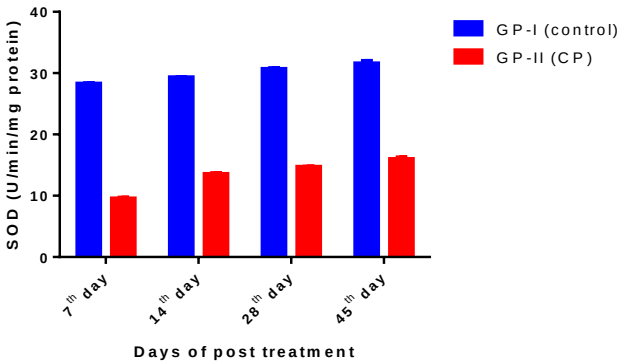
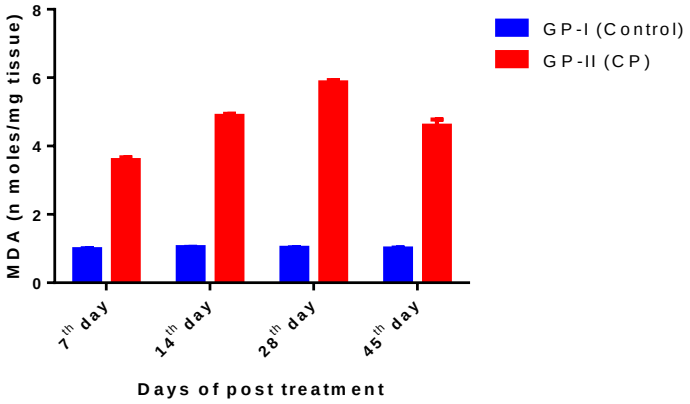


Table-4. The mean (±SE) values of malondialdehyde (n moles/mg tissue) in liver of rats in different groups at different time intervals.

Groups	Days post treatment			
	07th day	14th day	28th day	45th day
Group I (Control)	0.98±0.03^{ax}	1.045±0.04^{ax}	1.025±0.014^{ax}	1.00±0.02^{ax}
Group II (CP)	3.595±0.07^{bx}	4.885±0.06^{by}	5.86±0.06^{bz}	4.6±0.17^{bw}

Values with different superscripts in a row and column vary significantly at $p<0.05$

Figure-4. The mean (±SE) values of malondialdehyde (n moles/mg tissue) in liver of rats in different groups at different time intervals



These findings indicated that cisplatin treatment can induce liver damage through oxidative stress. Under normal physiological conditions, cells control reactive oxygen species levels by balancing the generation of reactive oxygen species with their elimination by scavenging system (reduced glutathione-GSH, superoxide dismutase-SOD, and catalase-CAT). But under oxidative stress conditions, excessive reactive oxygen species generated can damage cellular proteins, lipids and DNA, leading to fatal lesions in cells.

Oxidative stress is one important mechanism involved in the cisplatin hepatotoxicity. The mitochondria are the primary target in cisplatin toxicity with loss of mitochondrial protein sulfhydryl group and reduction in the mitochondrial membrane potential (Saad *et al.*, 2004). Mitochondrial glutathione (GSH) is essential in the regulation of inner mitochondrial permeability and enzyme function by keeping SH in the reduced state. In cisplatin induced toxicity, there is an increase in the intracellular calcium level which activates NADPH oxidase and ROS production by damaged mitochondria (Yao *et al.*, 2007). GSH is one of the most important molecules in the cellular defense against chemically reactive toxic compounds. The reduced form of GSH is necessary for detoxification of xenobiotics. The reduction in GSH levels induced by cisplatin causes suppression of antioxidant enzyme defense system sensitizing the cells to ROS, thus causing hepatic injury (Mansour *et al.*, 2006; Divya *et al.*, 2016; Nasr, 2014 and Ciftci *et al.*, 2017).

Nicotinamide adenine dinucleotide (NADH), which helps to maintain SH groups, declines with cisplatin treatment resulting in the inhibition of some dehydrogenases, which cause uncoupling of oxidative phosphorylation with formation hydroxyl radical and oxidative

stress. These free radicals attack polyunsaturated lipids and proteins and initiate lipid peroxidation. (Aggarwal, 1998 and Yilmaz, *et al.*, 2005).

The drug is also involved in the alteration of the thiol status of tissue resulting in the alteration in the enzymatic antioxidants. The intracellular redox homeostasis is maintained by the thiol group (-SH) containing molecules. Under certain conditions, thiol group may lead to formation of thiol radicals that in turn interacts with molecular oxygen, generating reactive oxygen species (Desoize, 2002).

The antioxidants play an important role in protection against damage caused by reactive oxygen species (ROS). Reduction in the antioxidant enzyme levels in the current study could be attributed to their utilization in elimination of excess of reactive oxygen species generated during cisplatin toxicity. Bilgic *et al.,* (2018) indicated that in CP induced hepatotoxicity, liver cells encounter large quantities of ROS which overwhelm their detoxification capacity and succumb to toxic effects with depletion of antioxidants. Under normal conditions, protection against ROS is by utilization of NADPH by glutathione reductase to maintain the reduced state of cellular glutathione which is an important cytosolic antioxidant.

The observations of the study concurred with previous studies which have demonstrated involvement of oxidative stress, lipid peroxidation and mitochondrial dysfunction in cisplatin induced hepatotoxicity (Mansour *et al.*, 2006; Fasihi *et al.*, 2012; Karale and Kamath, 2016 and Ciftci *et al.*, 2017).

CONCLUSIONS

Single dose of Cisplatin at 7.5 mg / kg b.w. intraperitoneally induces oxidative stress in Wistar Albino rats by increasing MDA and decreasing the cellular endogenous antioxidants enzymes such as CAT, GPx and SOD.

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