ISSN (Print 0974-2115) (Online 2349-8552)
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

Preventive effect of Curcumin against Cisplatin-related hepatotoxicity in Swiss Albino mice (*Mus musculus* L.)

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ABSTRACT

Chemotherapy is a type of cancer treatment that kills rapidly proliferating cells. Being a non-specific mode of treatment, it can cause damage to healthy cells like bone marrow cells, epithelial cells, hair follicles etc., resulting in various side effects. Cisplatin, a platinum coordination complex, is a common chemotherapeutic drug that is used in the treatment of several types of cancers, including testicular, ovarian and bladder cancer. Cisplatin interferes with DNA replication by forming cross-linking of DNA in the form of 1, 2-intrastrand crosslinks with purine bases. It can also cause missense mutations and breaks in DNA. The DNA injury triggers cell death and inhibits RNA and protein synthesis, especially in rapidly dividing cells. However, it has been observed to have profound participation in neuropathy, ototoxicity and nephrotoxicity, few among many other side effects. Changes in the activity of certain serum enzymes have been implicated to cisplatin-induced hepatotoxicity. In this study, we investigated the effect of cisplatin on Swiss Albino mice (*Mus musculus* L.) animal model. Mice were intraperitoneally administered with cisplatin at varying sublethal doses and exposure periods. They were tested for hepatotoxicity by estimating several biochemical parameters. The mid-toxic dose was co-treated with curcumin supplementation since curcumin has prominent anti-radical, anti-inflammatory and anti-mutagenic activities. The investigation was further extended to study the effect of cisplatin on hepatic cell viability and inspect presence of chromosomal aberrations. The present study shall assist in better comprehension of cisplatin-induced hepatotoxicity and effects of curcumin to prevent related side effects.

KEY WORDS: Cisplatin, Curcumin, Hepatotoxicity, Centromeric Fusion, Pulverization.

1. INTRODUCTION

Chemotherapy is a type of cancer treatment, which involves administration of anti-neoplastic drug or in combination with other drugs following a specific treatment regimen. It acts by killing rapidly proliferating cells. Being a non-specific mode of treatment, it can cause damage to healthy cells like bone marrow cells, epithelial cells, hair follicles etc., resulting in various side effects. Common side effects include fatigue, hair loss, anemia, nausea, vomiting, appetite changes and many more. Cisplatin (CAS No. 15663-27-1, MF-Cl2H6N2Pt; NCF-119875), cisplatinum or *cis*-diamine dichloro platinum (II), a platinum coordination complex, is a common chemotherapeutic drug that is used in the treatment of several types of human cancers, including testicular, ovarian, bladder, lung, head and neck cancer. It is efficacious in treating various types of cancers, including carcinomas, germ cell tumors, lymphomas, and sarcomas (Desoize and Madoul, 2002). It is a water-soluble inorganic complex containing a central platinum atom surrounded by two chlorine atoms and ammonia moieties at the cis position in the horizontal plane with a square planar geometry.

Copper transporter Ctr1 mediates the uptake of cisplatin in yeast and mammals (Ishida, 2002). In most cases, increased CTR1 expression increases platinum accumulation in cells leading to heightened sensitivity to cisplatin. Cisplatin activation occurs with its entry into the cell and subsequently, the water molecules in the cytoplasm displace chloride atoms on cisplatin. This renders the hydrolyzed product as a strong electrophile that has the potential to react with nucleophiles such as nitrogen donor atoms on nucleic acids and sulfhydryl groups on proteins. Cisplatin can induce blockage in cell division and concomitant apoptotic cell death by causing DNA damage in cancer cells when it binds to the N7 reactive center on purine residues. The 1, 2-intrastrand cross-links of purine bases with cisplatin includes the 1, 2-intrastrand d(GpG) adducts and 1, 2-intrastrand d(ApG) adducts representing about 90% and 10% of adducts, respectively. Other adducts like 1, 3-intrastrand d(GpXpG) adducts, inter-strand crosslinks and nonfunctional adducts have also been reported to contribute to cisplatin-related toxicity (Dasari and Tchounwou, 2014). Cisplatin can also induce generation of reactive oxygen species (ROS) depending on the length of exposure and its concentration (Brozovic, 2010), thereby triggering cell death from oxidation of essential macromolecules and DNA damage.

Curcumin (1,7-bis(4-hydroxy-3methoxyphenyl)-1,6-heptadien-3,5dione), also known diferuloyl methane, is a yellowish polyphenol that is obtained by solvent extraction of the rhizome of *Curcuma longa* L (turmeric). It has been used as a medicinal herb in several Asian countries' traditional system for innumerable pathologies due to its anti-radical, anti-inflammatory, anti-mutagenic, anti-cancer and anti-microbial activities (Pulido-Moran, 2016). It down regulates the nitric oxide synthase activity in macrophages, consequently reducing the production of ROS formed in response to oxidative stress. Additional studies in microglial cells (brain macrophage analogs) demonstrated reduced nitrogen oxide generation and protection of neural cells from oxidative stress following

curcumin treatment. Curcumin exhibits its antioxidant property (Sharma, 1976) by chelating free oxygen radicals (Subramanian, 1994) and thus protects hemoglobin from oxidation (Unnikrishnan and Rao, 1995). *In vitro*, it has the ability to block the production of ROS like superoxide anions, hydrogen peroxide and nitrite radicals (Joe, 1994).

Therefore, in this study, we investigated the hepatotoxic effects of cisplatin at different dosage and treatment periods. We optimized the dosage based on biochemical assay of an enzyme and subsequently monitored the effect of cisplatin at that dosage on other enzymes. Furthermore, the protective effect of curcumin treatment was studied based on the same set of enzymes. The investigation was then extended to cytological and chromosomal study.

2. MATERIALS AND METHODS

Animal model: Healthy female Swiss Albino mice (*Mus musculus* L.), about 3 to 4 months old and weighing between 17g to 23g, were procured from M/s Scientific Concern, Kolkata, India and reared in animal cages. The mice were randomly divided into different experimental groups, with four (4) mice per cage for each control and tested series. The animals were maintained under standard conditions of temperature (25 ± 1°C) and humidity with a repetitive 12 hours light/dark cycles. They were fed with normal diet (ground gram pulse) and drinking water *ad libitum*. All animals were acclimatized to the laboratory conditions for 1 week before the start of the study. The Guide for the Care and Use of Laboratory (Institute of Medical Animal Laboratory Resources, National Academy Press, USA) was obeyed throughout the experimental duration. The experimental protocol also conformed to the National Guidelines on the Proper Care and Use of Animals in Laboratory Research (Indian Sciences Academy, New Delhi, India).

Treatment Protocol:

Vehicle control: This batch of mice was injected with distilled water @ 1 ml/100 g body weight (b.w.).

Cisplatin treatment group: Each treatment batch consisted of 4 mice. 3 separate groups were administered (i.p.) with Cisplatin (Merck, USA) @ 12.5 mg/kg b.w. (Dose 1), 18.75 mg/kg b.w. (Dose 2) and 25 mg/kg b.w. (Dose 3). Each dose was observed for 7 and 14 days. The mid-toxic dose (18.75 mg/kg b.w.) was counter-treated with curcumin (Sigma Aldrich, USA) supplementation (gavage) @ 375 mg/kg b.w. daily for 7 days treatment.

Methodology: Female Swiss Albino mice (*Mus musculus* L.) of same age and body weight were treated with aforementioned sublethal doses of cisplatin. Mice were not fed for 1 light/dark cycle after required exposure period as per treatment protocol and then sacrificed. Liver was dissected out and homogenized in Tissue homogenizer. Homogenates were centrifuged and supernatant was subjected to enzymatic assays. Specific activity of acid and alkaline phosphatase were estimated using the method of Bergmeyer and Bernt (1974), with minor modifications and expressed in micromole of Para nitro phenol produced per mg of protein per minute following the Lowry protocol (Lowry, 1951). Enzyme activity of Glutamate Oxaloacetate Transaminase (GOT) and Glutamate Pyruvate Transaminase (GPT) were quantified using the assay kit protocol by Robonik, India. Glutathione (GSH) level was quantified following Ellman's test protocol (Ellman, 1958) and expressed in mg GSH per litre protein. Peroxidase enzyme activity was evaluated following Sigma Aldrich, USA protocol. Hepatic cell viability was estimated using 3-(4, 5 dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reagent (Robonik, India) following the protocol by Faedmaleki (2014), with certain minor modifications. Structural chromosomal aberrations were studied by flame and dry technique using protocol by Adler (1984).

Data Analysis: Mean ± standard error of mean (SEM) was calculated for each dose for all parameters. These values were compared between control and treated doses using unpaired Student's t-test by calculating the P value. $P < 0.05$ was considered statistically significant.

3. RESULTS

Effect of cisplatin and curcumin on acid phosphatase: Specific activity of acid phosphatase initially increased and then gradually decreased with increasing dose of cisplatin (Fig.1A), all changes being statistically significant at 5% level of significance. Dose 2 @ 18.75 mg/kg b.w. for 7 days showed considerable extent of toxicity being neither too high nor too low. This mid-toxic dose was co-treated with curcumin supplementation, which helped to regain the specific activity to non-significant changes when compared with vehicle control.

Effect of cisplatin and curcumin on alkaline phosphatase: Specific activity of alkaline phosphatase lowered with increasing dose of cisplatin (Fig.1B) but all the changes observed were statistically non-significant. The shift in specific activity stayed non-significant even when dose 2 for 7 days was co-treated with curcumin supplementation.

Effect of cisplatin and curcumin on GOT and GPT: Enzyme activity of both GOT and GPT showed pronounced increment when cisplatin was administered at dose 2 for 7 days (Fig.1C and Fig.1D) showing the severity of toxicity induced by cisplatin. The activity of both GOT and GPT lowered, being comparable to the vehicle control (statistically non-significant), when this dose was co-treated with curcumin.

Effect of cisplatin and curcumin on glutathione: The level of glutathione increased prominently with the administration of cisplatin at dose 2 for 7 days (Fig.1E) but co-treatment with curcumin supplementation dropped

the level to statistically non-significant difference when compared with the vehicle control. This conspicuously shows the extent of oxidative stress induced by cisplatin.

Effect of cisplatin and curcumin on peroxidase: Irrespective of the administration of cisplatin at dose 2 for 7 days or co-treatment with curcumin, the enzyme activity remained statistically non-significant (Fig.1F), thereby concluding that this enzyme is inherently protected from the toxic effect induced by cisplatin.

Effect of cisplatin and curcumin on hepatic cell viability: Administration of cisplatin at dose 2 for 7 days minimally lowered the cell viability (statistically non-significant) but there was prominent increment in cell viability when this dose was co-treated with curcumin supplementation (Fig.1G), the change being extremely statistically significant. This reflects the proliferative effect of curcumin on the hepatic cells, thereby insinuating towards a track of cure from the toxic effect induced by cisplatin.

Effect of cisplatin and curcumin on chromosomes: Apparently, there were no major structural chromosome abnormalities for the vehicle control (Fig.2A) and curcumin control (Fig.2B) specimen. However, conspicuous structural chromosome aberrations were noticed throughout the field of the slide for the 7 days batch receiving dose 2 and for the 7 days batch that received additional co-treatment of curcumin supplementation. The batch deprived of curcumin co-treatment showed structural abnormalities like centromeric fusion and numerous cases of pulverization (Fig.2C). Again, the batch that received both cisplatin dosage and curcumin co-treatment showed pronounced cases of only centromeric fusion but reduced number of pulverization (Fig.2D).

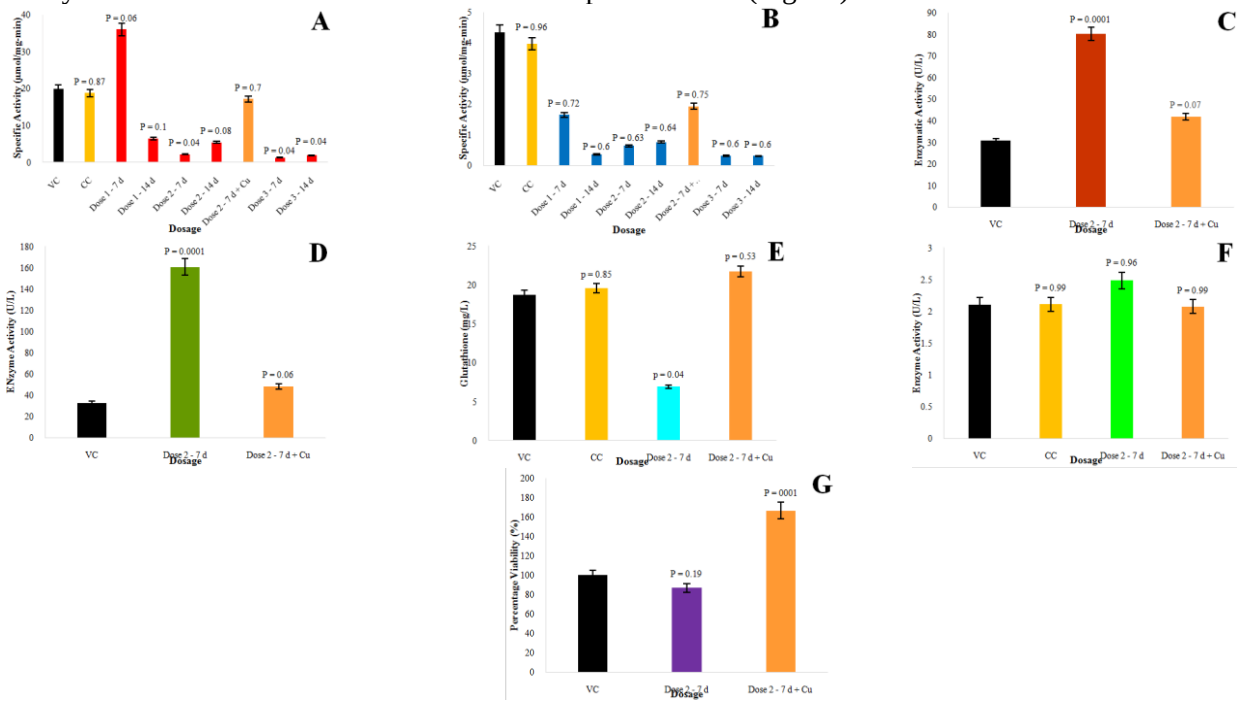

Figure.1. Comparative study of (A) acid phosphatase, (B) alkaline phosphatase, (C) GOT, (D) GPT, (E) reduced glutathione, (F) peroxidase, and (G) cell viability. *Bars* indicate average ± standard error of mean. Here, VC = Vehicle Control, CC = Curcumin Control, d = days and Cu = Curcumin

Statistical difference was evaluated using unpaired Student's t-test and $P < 0.05$ was considered significant

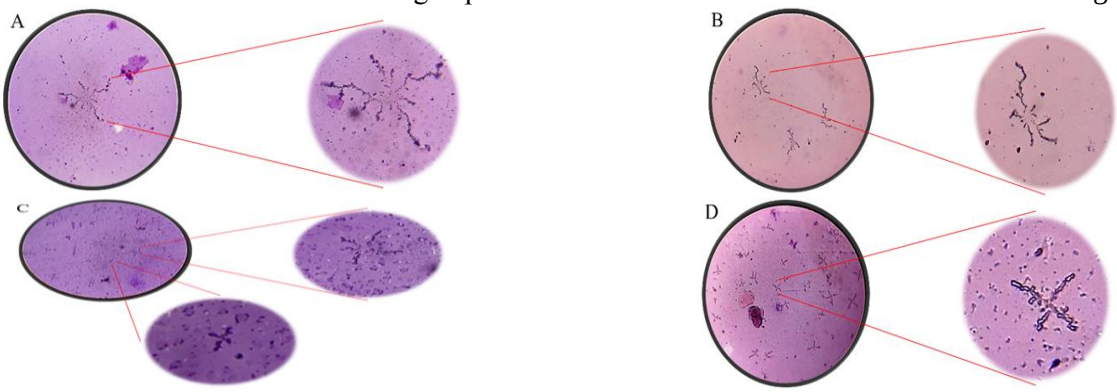

Figure.2. 40x magnified view of mice chromosomes procured from bone marrow.(A) Curcumin control, (B) Vehicle control, (C) Dose 2 for 7 days showing cases of structural aberrations like centromeric fusion and pulverization, and (D) Dose 2 for 7 days + Curcumin showing cases of centromeric fusion but reduced cases of pulverization

DISCUSSIONS

Cisplatin administration to mice led to prominent hepatotoxicity, as have been noticed in the activity of several enzymes. Phosphatases are enzymes that catalyze the removal of phosphoric acids from different mono phosphoric esters, an important reaction in several body processes including neoplastic growth. At the biochemical level, cisplatin treatment resulted in the decrease in the activities of acid (significant change) and alkaline phosphatases in the liver. Investigators also linked reduction in low molecular weight acid phosphatase to aberrant protein tyrosine phosphorylation found in Alzheimer brains (Shimohama, 1993).

Transaminases are the most sensitive biomarkers directly concerned in causing cellular damage and toxicity because of their presence in cytoplasm and its release into the circulation post cellular damage. Elevation of the hepatic enzymes level in serum and bilirubin are the indicators for impaired liver functions (Iseri, 2007). Enzyme activities of transaminases (GOT and GPT) procured from liver homogenate were significantly escalated with the administration of cisplatin at dose 2 for 7 days, clearly insinuating towards cisplatin-induced hepatotoxicity. Similar observations have been recorded by other scientists (Cao, 2018; Singh, 2017; Cagin, 2015).

Under normal physiological conditions, cells govern ROS levels by balancing the generation of ROS with their elimination by scavenging system (reduced glutathione-GSH, superoxide dismutase-SOD, and catalase-CAT). However, oxidative stress conditions can induce excessive ROS generation that can damage cellular proteins, lipids and DNA, leading to fatal lesions in cells. Depletion in the level of reduced glutathione is a clear indication of cisplatin-induced oxidative stress and the same has been reported by other investigators (Silva, 2001; Weijl, 1998).

Cytotoxicity of cisplatin is predominantly attributed to its interaction with nucleophilic N7-sites of purine bases in DNA (Eastman, 1987) and evidence strongly favors intrastrand adducts to cause severe lesions (Pinto and Lippard, 1985). Cisplatin is a highly mutagenic drug, inducing chromosome aberrations in rat bone marrow cells and in peripheral blood lymphocytes of patients (Osanto, 1991; Antunes, 1999; Antunes, 2000). Prominent structural chromosome aberrations like centromeric fusion and pulverizations were noted owing to the toxic effects of cisplatin.

Curcumin co-treatment at 375 mg/kg b.w. greatly ameliorated the toxic effects induced by cisplatin @18.75 mg/kg b.w. for 7 days. Repeatedly the enzymatic activity of curcumin control was found to be comparable (statistically non-significant) to vehicle control, which establishes that curcumin does not induce any form of additional toxicity. Previous workers (Mukherjee, 2007) investigated that curcumin could significantly reduce arsenic-induced DNA injury owing to ROS generation and increased lipid peroxidation and the effect was more prominent when the blood cells were pre-incubated with Curcumin prior to arsenic treatment. Curcumin checked the damage by quenching ROS, limiting lipid peroxidation level and increasing the level of detoxification enzymes (phase II) like catalase, superoxide dismutase and glutathione peroxidase. Curcumin has been used in India for several novel properties since centuries. Therefore, it is time tested with no reported side effects and hence, it can be used safely as food supplements in chemotherapy patients. In our present study, curcumin co-treatment was found to significantly ameliorate the exacerbating effects induced by cisplatin as in cases of enzymes like phosphatase, transaminase and reinstated the level of reduced glutathione. This is highly promising in treating the hepatotoxic effects induced by cisplatin. Again, curcumin exhibited noteworthy proliferative effect on the hepatic cells by remarkably escalating the cell viability. Though curcumin failed to repair centromeric fusion induced by cisplatin, yet it substantially reduced the cases of pulverization. In our study, curcumin stood as a potential protective substance to counter the effects of cisplatin-related hepatotoxicity on mice.

4. CONCLUSION

It can be concluded that cisplatin induces severe side effects when it is administered at high dose for a long duration. Hence, special care should be taken while administering this drug as a chemotherapeutic regimen. Curcumin stood as a promising food supplement that can ameliorate the adverse effects induced by cisplatin. This study can pave way for considering curcumin as a prospective therapeutic agent in the treatment of cisplatin-induced hepatotoxicity.

5. ACKNOWLEDGEMENT

The authors wish to thank the authorities of Heritage Institute of Technology, Kolkata for providing necessary infrastructure and amenities, and M/s Scientific Concern, Kolkata for providing the experimental specimen.

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