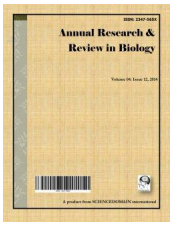

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Cadmium Induced Toxicity in Human Peripheral Blood Lymphocytes Culture and Its Amelioration by Vitamin C

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Authors' contributions

This work was carried out in collaboration between all authors. Author AP collected samples, carried out the experiments and wrote the first draft of the manuscript. Authors AN and NS helped in sample analysis and in proof reading of manuscript. Authors AP, SC and DJ were involved in study designed and statistical analysis. Author DJ was also involved in overall management of the study. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Cadmium is a ubiquitous and toxic environmental pollutant increasing worldwide due to high consumption in various industries. At the cellular level, cadmium affects cell proliferation, differentiation, apoptosis and other cellular activities. In contrast to cadmium, vitamin C (ascorbic acid) is a powerful reducing agent known to capable of rapidly scavenging several reactive oxygen species (ROS).

Aim: Hence, the present work aimed to investigate the cadmium chloride (Low dose-10^{-6} M; Mid dose-10^{-5} M; High dose-10^{-4} M) induced cytotoxic and genotoxic effects in human peripheral blood lymphocyte culture (PBLC) of healthy individuals (n=10) and its amelioration by vitamin C (10^{-5} M). The study was divided in various groups consisting of control, vitamin C, cadmium alone, cadmium

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with vitamin C and ethyl methanesulfonate as positive control.
Results: After genotoxicity indices and free radical toxicity parameters evaluated, results showed alteration in free radical toxicity indices and genotoxic indices in cadmium exposed cultures. Although vitamin C co-exposure revealed protective effects against genotoxicity by scavenging free radicals and balancing the activity of antioxidant defense system with decreasing in level of lipid peroxidation.
Conclusion: Vitamin C led to significant decrease in cadmium induced toxicity and substantial amelioration was observed in all studied parameters.

Keywords: Cadmium; free radical toxicity; genotoxicity; PBLC; vitamin C.

1. INTRODUCTION

Cadmium (Cd) is a non-essential heavy metal belonging to group IIB of the periodic table of elements [1] which is a wide spread environmental pollutant that has gained greater public prominence due to its increased use in industrial processes like smelting, battery, plastic manufacturing and due to world-wide increase in discard of electronic–waste such as cell phones and computers containing this toxic metal [2,3]. Unlike essential trace elements such as copper, iron, selenium, zinc and others, cadmium largely has no known biological function [4]. As a chemical element, cadmium cannot be degraded, and its concentration in the environment increases steadily and largely because of human activities [5]. Recovery and safe removal of cadmium from habitats is unrealistic in view of its ubiquitous occurrence in various forms in the environment. The potential for cadmium to cause toxicity has been demonstrated by the results of numerous experimental and epidemiological studies [6-9]. A toxic peculiarity of cadmium is that exposure of cells to low, micromolar concentrations results in significant toxicity [10]. Furthermore, cadmium is considered a carcinogen and exposure to it has already been linked to cancer of the prostate, liver and lung [11].

Cadmium is known to deplete glutathione and protein-bound sulfhydryl groups, which results in enhanced production of reactive oxygen species (ROS) such as superoxide ion, hydroxyl radicals and hydrogen peroxide [12]. Also, cadmium exerts its toxic effects via oxidative damage to cellular organelles by inducing the generation of ROS. Reactions of these ROS with cellular biomolecules have been shown to lead to lipid peroxidation, membrane protein damage, altered anti-oxidant system, DNA damage, altered gene expression and apoptosis [13]. If these ROS-mediated stress events are not balanced by repair processes, affected cells undergo apoptosis or necrosis [14].

Besides the oxidative stress induced by cadmium exposure, the damage to genetic material is also major concern. The genotoxic effect of cadmium in human lymphocyte and alteration in different genotoxic parameters were demonstrated by various scientist [15,16]. So, it can be hypothesized that cadmium may induced the genotoxicity possibly via the production of free radicals and causes oxidative stress.

As oxidative stress and genotoxicity are one of the important mechanisms of cadmium-induced damages, it can be possible to treat cadmium induce toxicity by administration of some antioxidants and, it may be an important therapeutic approach in cadmium related anomalies [17]. There are many herbal home-based remedies which can reduce the toxicity, through their antioxidant properties, like vitamin C, garlic, amla, and curcumin. Among them, vitamin C (ascorbic acid) is a water soluble dietary antioxidant that plays an important role in controlling oxidative stress [17-19]. It is an excellent source of electron and thus donates electron to free radicals and quenches their reactivity [20]. In addition to scavenging ROS vitamin C can regenerate other small molecule antioxidants, such as α-tocopherol, glutathione (GSH), urate, and β-carotene, from their respective radical species [21]. It can protect DNA damage induced by reactive oxygen species [22]. It can participate in the redox mechanism of the cell and thereby neutralize ROS. Vitamin C offers effective protection against lipid peroxidation and genotoxicity which is probably due to its strong antioxidant and nucleophilic nature [23,24].

2. MATERIALS AND METHODS

2.1 Chemical Reagents

All chemicals utilized in biochemical parameters were procured from Merck (AR Grade), while media and culture reagent were procured

from HiMedia and Sigma Aldrich (Culture Grade).

2.2 Treatment and Experimental Groups

The treatment and experimental protocol is described in Table 1.

Table 1. Treatments of experimental groups

Groups	Treatments
Group I	Control (Con.)
Group II	Vitamin C (Vit.C) (10^{-5} M)
Group III	Low Dose of Cadmium Chloride (LD) (10^{-6} M)
Group IV	Mid Dose of Cadmium Chloride (MD) (10^{-5} M)
Group V	High Dose of Cadmium Chloride (HD) (10^{-4} M)
Group VI	High Dose of Cadmium Chloride (10^{-4} M) + Vitamin C (10^{-5} M)
Group VII	Ethyl Methanesulfonate (EMS) (1.93 X 10^{-3} M)

All test chemicals were added at the time of culture setup. Cadmium chloride:0.01 M stock of $CdCl_2$ were prepared by dissolving it in sterilized millipore water and filtered through 0.22 µm filter paper. The dilutions were obtained to get 10^{-4}, 10^{-5} and 10^{-6} M concentrations for high, mid and low doses of cadmium respectively. Vitamin C (VC):1 mg/ml aqueous solution of Vitamin C was prepared, filtered by 0.22 µm filter paper. Cultures were exposed to 10^{-5} M of vitamin C alone and in combination with high dose of cadmium chloride. Ethyl methanesulfonate (EMS) treatment: Lymphocytes treated with EMS at the dose of 1.93 X 10^{-3} M as a positive control.

2.3 Sample Collection

All the experimental protocols were approved by the Institutional Human Ethical Committee of Department of Zoology, Gujarat University. Each voluntary participant got the detailed information regarding the research study and its significance. After that, their informed written consent was availed and sample collection was done.

2.4 Cell Culture

To culture the lymphocytes, blood samples from healthy donors (age 18-25 years) were collected in sterile condition by vein puncture in heparinized vacutainers. While selecting the donors care was taken that subjects were free from any drug, alcohol or tobacco insult and had not been knowingly exposed to environmental or occupational hazards. Lymphocyte cultures were setup according to the standard protocol of Hungerford [25] with slight modifications by adding 0.5 ml whole blood to 7 ml of RPMI-1640 media (pH- 7.4) supplemented with 10 % heat-inactivated fetal calf serum along with 100 µg/ml streptomycin and 100 units/ml penicillin. Lymphocytes were stimulated by 100 µl phytohemagglutinin (PHA;1 mg / 1 ml) and incubated for 72 hours at 37°C. For sister chromatid exchanges (SCEs) study, 80 µl bromodeoxyuridine (BrdU; 1 mg / 1 ml) was added at 0 hour. At 69th hours, 20 µl of colchicine (1 mg / 5 ml) was added to the cultures to arrest cell division at metaphase stage. The cultures were harvested after 72 hours of incubation by centrifugation at 2000 rpm for 15 min. and then supernatant is discarded, the pellet treated with hypotonic solution for 15 min. in 0.075 M potassium chloride at 37°C. After that cells were fixed in 1:3 (v/v) Acetic acid:Methanol solution. The fixation step was repeated twice. The cells pellet was resuspended in fixative and dropped onto clean chilled slides.

2.5 Fluorescence-Plus-Giemsa (FPG) Staining for Sister Chromatid Exchanges (SCEs)

The staining method was done according to standard protocol of Perry and Wolf [26]. The slides were stained with fluorescent dye Hoechst 33258 (stock: 1 mg/1 ml Sorenson's buffer, pH 6.8; this solution was diluted 10 times to prepare working solution) for 20 minutes in the dark. Slides were then kept in 2X saline sodium citrate (1.753 gm NaCl and 0.852 gm trisodium citrate in 100 ml distilled water) and then exposed in UV light for 50-55 min. After exposure, slides were washed in distilled water, stained immediately in 2% Giemsa prepared in distilled water for 3-5 min and SCEs were scored. Metaphases in their second *in vitro* division were selected for scoring on basis of the spreading of chromosomes and differentiation of chromatids. Hundred metaphases from each culture were analyzed for SCEs.

2.6 Analysis of Cell Cycle Proliferative Index (CCPI)

From the slides prepared for FPG staining, cells dividing for the first (M1), second (M2) and third (M3) divisions in the culture were identified by the differential staining pattern of sister chromatids. Total 100 metaphases were analyzed for each group of each individual

classifying them as first (M1), second (M2) or third (M3) generation cells.

The SCEs/Cell, SCEs/Chromosome, CCPI, AGT and PDT for each individual were calculated according to the following formulas:

$$\text{SCEs/Cell} = \frac{\text{Total SCE scored}}{\text{Total M2 Metaphase Plates Observed}}$$

$$\text{SCEs/Chromosome} = \frac{\text{SCEs/Cell}}{46\ (\text{Total number of chromosome in metaphase plate})}$$

$$\text{CCPI} = \frac{1(\text{M1 Plates}) + 2(\text{M2 Plates}) + 3(\text{M3 Plates})}{\text{Total Plates Metaphase Scored}}$$

$$\text{AGT (Hour)} = \frac{72\ \text{Hour (BrdU Time)}}{\text{CCPI}}$$

$$\text{PDT (Hour)} = \frac{24\ \text{Hour}}{\text{CCPI}}$$

2.7 *In Vitro* Free Radical Toxicity Study

For free radical toxicity analysis 7 ml blood was collected by vein puncture from healthy and non-smoking volunteers (n=10) in heparinized vacutainers with prior written consent. 7 ml HiSep (HiMedia Pvt. Ltd., Mumbai) and 7 ml of freshly collected blood was centrifuged at 1000 rpm for 15 minutes. WBCs were separated and after counting it immediately cultures were set up by inoculating 10^6 cells / 1.5 ml culture media supplemented with 10% fetal calf serum along with 100 µg/ml Streptomycin and 100 units/ml Penicillin and 20 µl of phytohemagglutinin (PHA;1 mg / 1 ml) according to the standard protocol with slight modifications [25]. All test chemicals (vitamin C, cadmium chloride and EMS) were added at the time of culture setup. The cultures were incubated at 37°C for 72 hours in an incubator. After 72 hours of incubation culture were used as samples to perform assay like Total protein, Lipid peroxidation, Superoxide dismutase, Catalase, Total Glutathione, Glutathione S-transferase, Glutathione peroxidase and Glutathione reductase [27-34].

2.8 Statistical Analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) using Graph Pad Prism 5. The values are mentioned as Mean ± S.E. The individual comparison was achieved by Tukey's Multiple comparison test. Statistical decision was made with a significance level of $P<.05$. According to the formula, which is given below the percentage amelioration was calculated.

$$\text{Percentage Amelioration} = \frac{\text{Prooxidant Group} - \text{Respective Antioxidant Group}}{\text{Prooxidant Group} - \text{Control}} \text{X}100$$

3. RESULTS

3.1 Genotoxicity Indices

3.1.1 Sister chromatid exchanges (SCEs)

Cadmium chloride revealed considerable dose dependent increase ($P<.05$; $P<.01$ and $P<.001$ for; Group III, IV and V respectively) in SCEs/Cell and SCEs/Chromosome as compared to control (Table 2). Any alteration in Vitamin C alone (Group II) was not observed as compared to control, where as its co-supplemented cultures together with high dose of cadmium (Group VI) showed a significant ($P<.001$) reduction in SCEs/Cell and SCEs/Chromosome as compared to the high dose of cadmium treated cultures (Group V) and percent amelioration for SCEs/Cell and SCEs/Chromosome were 84.83% and 91.66% respectively (Table 2) While the positive control ethyl methanesulfonate (Group VII) showed high significant ($P<.001$) results in all genotoxic indices as compared to control.

3.1.2 Cell cycle proliferative index (CCPI)

Cell cycle proliferative index was declined as dose of cadmium increases in the cultures (Table 2). At low dose (Group III) the value was not significant, while mid and high dose cultures showed significant decreased (Group IV- $P<.01$ and Group V- $P<.001$) in CCPI as compared to control (Group I). Vitamin C co-supplemented cultures with high dose of cadmium (Groups VI) showed highly significant ($P<.001$) recovery as compared to high dose of cadmium treated culture (Group V) and showed 87.95% amelioration.

3.1.3 Average generation time (agt) and population doubling time (PDT)

Both AGT and PDT were non-significantly elongated at low dose of cadmium, while the mid and high doses showed significant result ($P<.001$) as compared to control (Table 2).

Table 2. Frequency of genotoxicity parameters of control and all treated groups

Groups	SCEs/Cell	SCEs/ chromosome	CCPI	AGT	PDT
I-Con.	5.11±0.35	0.12±0.007	2.47±0.04	29.07±0.46	9.69±0.15
II-Vit.C (10^{-5} M)	5.02±1.04^{NS}	0.10±0.022^{NS}	2.53±0.03^{NS}	28.44±0.27^{NS}	9.48±0.09^{NS}
III-LD (10^{-6} M)	8.1±0.34*	0.20±0.03*	2.11±0.23^{NS}	36.15±3.08^{NS}	12.05±1.02^{NS}
IV-MD (10^{-5} M)	8.6±0.98**	0.21±0.02**	1.89±0.23**	40.52±3.15***	13.50±1.05***
V-HD (10^{-4} M)	12.1±0.40***	0.24±0.01***	1.61±0.10***	45.36±2.08***	15.12±0.69***
VI-HD + Vit.C (10^{-4} M + 10^{-5} M)	6.17±1.24$^{\#\#}$ (84.83%)	0.13±0.027$^{\#\#}$ (91.66%)	2.34±0.06$^{\#\#}$ (87.95%)	30.81±0.064$^{\#\#}$ (89.48%)	10.27±0.21$^{\#\#}$ (89.31%)
VII-EMS (1.93 X 10^{-3} M)	14.47±0.52***	0.26±0.01***	1.38±0.01***	51.86±0.39***	17.28±0.13***

*Values are mean±SE; Values in parenthesis indicate percentage amelioration by vitamin C. Con.=Control; Vit.C=Vitamin C; EMS=Ethyl Methanesulfonate. LD= Low dose; MD=Mid dose; HD=High dose. *P<.05; **P<.01; ***P<.001; NS= nonsignificant, when groups II to V and VII were compared to group I. #P<.01; ##P<.001, when group VI was compared to group V.*

Alone vitamin C treated culture (Group II) showed nonsignificant effect when compared to control. Co-supplemented culture of high dose of cadmium with vitamin C (Group VI) revealed significant decreased (*P*<.001) in mean frequency of AGT and PDT when compared with high dose of cadmium culture (Group V). Mitigation for AGT and PDT with vitamin C were 89.48% and 89.31% respectively.

3.2 Free Radical Toxicity Indices

3.2.1 Total protein and lipid peroxidation (LPO)

Dose dependent significant decrease (low dose- *P*<.05; mid dose- *P*<.01; high dose- *P*<.001) was observed in protein levels for cadmium treated cultures as compared to control. While the quantification of thiobarbituric acid reactive species (TBARS) was done as biochemical evidence for oxidative stress induced lipid peroxidation which increased as dose increased in cultures (low dose- *P*<.05; mid dose- *P* <.01; high dose- *P*<.001). On the other hand, vitamin C treated cultures along with high dose of cadmium (Group VI) showed significant amelioration for protein (*P*<0.01) and LPO (*P*<.001) respectively when compared to cadmium treated group (Group V). Ethyl methanesulfonate treated culture (Group VII) showed highly significant alteration (*P*<.001) in all studied parameters. Percent amelioration for protein and LPO were 81.25% and 87.69% respectively for Group VI (Table 3).

3.2.2 Superoxide dismutase (SOD) and catalase (CAT)

Superoxide dismutase and catalase are one of the important key enzymes in defense against oxidative stress of cell. A dose dependent depletion was observed in cadmium treated culture in both the parameters. At low dose, a significant (*P*<.01) decrease was seen in SOD and CAT whereas, mid and high doses showed a highly significant (*P*<.001for both Groups IV and V) decrease as compared to control. On supplementation of vitamin C to cadmium treated culture (Groups VI), a highly significant (*P*<.001) recovery was noted as compared to cadmium treated group (Group V) and percent amelioration were 95.81% and 57.5% for SOD and CAT respectively (Table 3).

3.2.3 Glutathione reductase (GR) and glutathione S-transferase (GST)

GR and GST showed significant decrease (*P*<.01) in groups treated with low dose of cadmium (Group III) as compared to control (Group I) while mid and high dose showed highly significant decrease (*P*<.001 for both Groups IV and V). While the cultures supplemented with vitamin C in combination with high dose showed significant amelioration (*P*<.01 for GR and *P*<.001 for GST) when compared to high dose of cadmium (Group V). Percent amelioration by vitamin C (Group VI) were 53.16% and 91.05% respectively for GR and GST (Table 3).

3.2.4 Glutathione peroxidase (GPx) and glutathione (GSH)

Glutathione peroxidase activity and Glutathione level were significantly decreased after cadmium treatment at various doses (Group III, IV and V). At low dose both the GPx enzymatic activity and GSH level were significantly decrease (*P*<.01) but at the mid and high dose the steep demotion in the activity of GPx and level of GSH were

Tables 3. Frequency of free radical toxicity parameters of control and all treated groups

Groups	Total protein	LPO	SOD	CAT	GR	GST	GPx	GSH
I-Con.	0.28±0.02	6.52 ±0.46	6.42±0.26	60.30±5.91	84.54±7.84	24.84±1.25	1.62±0.08	3.04±0.20
II- Vit.C (10^{-5} M)	0.30±0.01^{NS}	6.06±0.40^{NS}	7.01±0.19^{NS}	66.0±3.63^{NS}	86.26±6.90^{NS}	26.03±1.3^{NS}	1.81±0.14^{NS}	3.55±0.06^{NS}
III-LD (10^{-6} M)	0.17±0.0*	10.5±1.02*	5.28±0.36**	42.18±2.19**	60.12±2.12**	16.0±0.78**	1.22±0.04**	2.29±0.21**
IV-MD (10^{-5} M)	0.16±0.04**	11.4±1.21**	3.32±0.12***	30.36±1.36***	47.93±1.80***	15.36±0.73***	1.06±0.05***	1.69±0.21***
V-HD (10^{-4} M)	0.12±0.05***	12.29±0.67***	2.84±0.10***	22.58±0.92***	40.0±1.17***	10.75±0.50***	0.74±0.03***	1.57±0.08***
VI-HD+Vit.C (10^{-4} M+10^{-5} M)	0.25±0.04$^{\#}$ (81.25%)	7.23±0.48$^{\#\#}$ (87.69%)	6.27±0.24$^{\#\#}$ (95.81%)	44.27±2.07$^{\#\#}$ (57.50%)	63.68±2.74$^{\#}$ (53.16%)	23.58±1.13$^{\#\#}$ (91.05%)	1.54±0.08$^{\#\#}$ (90.90%)	2.86±0.18$^{\#\#}$ (87.75%)
VII-EMS (1.93X10^{-3} M)	0.12±0.01***	12.58±0.83***	2.68±0.0***	11.66±0.42***	25.99±0.5***	4.68±0.21***	0.32±0.01***	0.46±0.03***

Values are mean±SE; Values in parenthesis indicate percentage amelioration by Vitamin C.

*Con.=Control; Vit.C=Vitamin C; EMS=Ethyl Methanesulfonate. LD= Low dose; MD=Mid dose; HD=High dose; Total protein (mg protein/10^6 cells); LPO=lipid peroxidation (mM MDA formed/60 min/mg protein); SOD=superoxide dismutase (unit superoxide dismutase/mg protein); CAT=catalase (nM of H_2O_2 consumed/minute/mg protein); GR=glutathione reductase (nM of NADPH oxidized/minute/mg protein); GST=glutathione-S-transferase (µm of CDNB-GSH conjugates/min/mg protein); GPx= glutathione peroxidase (mM total glutathione consumed/minute/mg protein); GSH=total glutathione (mM total glutathione/mg protein). *P< .05; **P< .01; ***P< .001; NS= nonsignificant, when groups II to V and VII were compared to group I. #P< .01; ##P< .001, when group VI was compared to group V*

observed (*P*<.001). Vitamin C administrated along with high dose of cadmium showed highly significant increase (*P*<.001) in activity of GPx and GSH level as compared to high dose of cadmium (Group VI). Percent amelioration for GPx activity and GSH level were 90.90% and 87.75% after vitamin C administration (Table 3).

4. DISCUSSION

The aim of present study was to evaluate the oxidative stress and adverse genotoxic effects of various doses of cadmium chloride upon PBLC and its amelioration if any by antioxidant vitamin C. Cadmium induces oxidative stress which increases the LPO and disturbs the antioxidant enzymes (GSH, GST, GR, GPx, SOD and Catalase) that leads to free radical generation which ultimately is responsible for genotoxicity in the form of increase in SCEs, it also alter the cell cycle by declining the CCPI and elongation of AGT and PDT. Sister chromatid exchange analysis in human PBLC has often been applied as a cytogenetic testing of potentially mutagenic and carcinogenic chemicals [35]. An increased rate of SCEs could be a sign of persistent DNA damage [36], in present study rise in SCEs was observed after cadmium exposure to PBLC. Similarly, other studies also indicate increased rates of SCEs due to cadmium in human, rat and mice [37-39]. Cadmium is known to affect the cell proliferation, differentiation and progression leading to alteration in DNA synthesis and causes apoptosis [40,41]. In this study, cadmium also affects the cell proliferation by declining the CCPI and cell progression by elongation of AGT and PDT. At cellular level, cadmium damage DNA repair processes in which the cellular redox status plays a crucial role [42,43]. The basic mechanism involved in cadmium toxicity are gene regulation [44], oxidative stress [12,45], disruption of cadherin, inhibition of DNA repair and interference with apoptosis [46].

The actual mechanism of cadmium induced genotoxicity is found to be quite complex but oxidative stress is assumed to be the major molecular basis underlying cyto-genotoxicity caused by cadmium [7]. It has been shown that cadmium enters in the cells through calcium channels, either by mimicking them or replacing them [47]. Incongruent to other heavy metals cadmium is not able to produce free radicals by itself, however reports have indicated superoxide radicals could be generated indirectly [48]. Reports from Patra and co-workers suggested participation of reactive oxygen species (ROS) in cadmium-induced testicular damage in mice [18]. Cadmium is also shown to generate superoxide anion, hydrogen peroxide and hydroxyl radicals *in vivo* and have been detected by the electron spin resonance spectra in rats [12]. Moreover, cadmium is potent cell poison and known to cause oxidative stress by increasing lipid peroxidation and/or changing intracellular glutathione levels [48]. Similarly, in this study also there was alteration in LPO and GSH level as well as antioxidant defense system and it may be one of the cause for highly significant genotoxicity at higher dose of cadmium.

Cadmium has been shown to be exogenous source which produce indirect production of ROS in various cell line [41,49]. Excessive production of ROS disturbs the balance between ROS and antioxidant agents (enzyme and antioxidant substances) in the cells. When the cells are under oxidative stress, Catalase, GR and GPx respond by altering their activities. In this study, we have observed a decrease in the activities of SOD, Catalase, GPx, GST and GR in cadmium chloride treated lymphocyte culture. The decrease might be due to cadmium induced indirect production of free radicals which in turn can impair the antioxidant defense mechanism, leading to an increased membrane LPO and decreased in GSH content [39,50].

Anti-oxidants are agents that significantly inhibit the rate of oxidative activity [51]. Vitamin C is the best known preventive antioxidant [19,20]. Hence, in our study we aimed to assess the protective effect of vitamin C against cadmium induced free radical toxicity and genotoxicity. Vitamin C is an important and potent, water soluble and dietary antioxidant capable of scavenging or neutralizing an array of reactive oxygen species. It is an essential cofactor for many enzymes involved in diverse metabolic pathways [52]. It inhibits oxidation by an effect on calcium metabolism, protein kinase C inhibition and catalysis of the reduction of hydrogen peroxide which protect biological membranes from oxidative degradation [53]. It scavenges the aqueous ROS by very rapid electron transfer that inhibit lipid peroxidation [54]. Tezcan et al. also found the protective effect of vitamin C on cadmium induced reactive oxygen species on human erythrocytes [55]. Ji and co-workers showed the ameliorative effects of it against cadmium in testis of mice [56]. Other studies [23, 57,58] also showed ameliorative effects of vitamin C due to its antioxidant properties against various toxicants like aluminum, arsenic and fluoride. In this study also, vitamin C is found to be effective against cadmium treated human

lymphocyte by protecting against the cytogenetic damage and reduces the ROS level by balancing the activity of antioxidant defense systems. In support to our results Kini and co-workers had also found vitamin C reduces the cadmium induced oxidative damage by decreasing the LPO level and increases the GSH and SOD content in testis of mice [59]. Hence, it can be postulated that there may be a correlation between the oxidative stress and cytogenetic damage caused by cadmium and vitamin C which in turn can be used as a potent antidote against cadmium induced toxic effects.

5. CONCLUSION

It can be concluded that cadmium chloride induced oxidative stress and elevated the level of LPO. It affects the antioxidant defense system by distressing the activities of enzymes like SOD, CAT, GST, GR, GPx and declining the level of GSH. It also showed genotoxicity in the form of increased SCEs and it also declined CCPI with elongation of AGT and PDT.

Supplementation of vitamin C ameliorated cadmium induced free radical toxicity and genotoxicity due to antioxidant properties and by quenching of ROS. Upon co-supplementation with antioxidant like vitamin C genotoxic parameters of cadmium exposure were also ameliorated. This suggests that one of the cause to induce DNA damage and cell cycle inhibition is primarily by free radical generation. Vitamin C which can be easily available by consumption of natural products like vegetables and many citrus fruits, can be helpful to those individuals who are environmentally or occupationally exposed to cadmium.

6. RECOMMENDATION

Occupationally cadmium exposed worker and those who exposed environmentally should be supervised at regular intervals and provided with safety and precautionary measures such as masks and their working hours should be managed. They should also be counselled and advice to have the antioxidant such as vitamin C in their daily diet which may reduce the harmful effects of long term cadmium exposure.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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