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Original Article

EVALUATING THE PROTECTIVE EFFICACY OF COMBINATION AND *WITHANIA SOMNIFERA* AND VITAMIN E AGAINST CADMIUM INDUCED OXIDATIVE STRESS MEDIATED HEPATIC HISTOPATHOLOGY AND GENOTOXICITY IN MURINE MODEL

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ABSTRACT

Objective: Cadmium (Cd) exposure develops various serious pathological conditions because of its long-term retention in tissues. *Withania somnifera* (WS) is a known antioxidant due to its pharmacological properties, whereas vitamin E (VE) is a dietary antioxidant. The present study tries to study the combinational effect of WS and VE against Cd-induced toxicity.

Methods: Amelioration of Cd-induced toxicity by WS and VE was evaluated in mice liver by studying biochemical, morphological and genotoxic parameters. For this study mice (n=6) were given short-term Cd exposure of 5 mg/kg b. wt. intraperitoneally (ip) for 5 d. WS leaf extract was given orally (500 mg/kg b. wt.) and in combination with VE (100 mg/kg b. wt.) to study its preventive effects against Cd-induced oxidative stress-mediated histopathological and genotoxic alterations.

Results: Cd significantly enhanced lipid peroxidation (LPO ($p<0.0001$) and declined the levels of antioxidant viz. reduced glutathione (GSH ($p \leq 0.0001$), glutathione-S-Transferase (GST ($p \leq 0.0001$), superoxide dismutase (SOD ($p \leq 0.0001$) and catalase (CAT) ($p \leq 0.0001$) activity. Histopathological examination of Cd-treated mice liver exhibited marked structural damage. Metal generated oxidative stress and imbalance of pro- and antioxidants forms the basis for marked structural changes and genotoxicity. Individual treatments of WS and VE reduced the Cd-induced toxicity but when WS was given in combination with VE for the same period, levels of antioxidants were brought to near normal levels.

Conclusion: WS in combination with VE reduced Cd-induced oxidative burden and restored the levels of anti-oxidant enzymes resulting into improved histoarchitecture and reduced genotoxic insult.

Keywords: Cadmium, *Withania Somnifera*, Vitamin E, Liver, Oxidative stress

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INTRODUCTION

Essential heavy metals are required in very small amount for various metabolic processes in the cell, but at high concentration, they create physiological stress leading to the generation of free radicals. Cadmium (Cd) has been ranked 7thin the top 20 Hazardous Substances Priority List [1]. Acute Cd poisoning causes pulmonary edema, fulminate hepatitis, haemorrhage, testicular injury and lethality whereas prolonged exposure to Cd causes bio-accumulative damage to various organ systems [2, 3]. Liver is the main target organ for short-term Cd toxicity. Tolerance to acute Cd hepatotoxicity depends on the pre-synthesized metallothioneins (MT) in the liver, which functions to sequester Cd in the cytosol, with a reduction in the amount of available Cd for other critical organelles [4]. Soluble salts of Cd accumulate in the body, leading to toxicity in the liver, kidney, lungs, brain, testes, heart and central nervous system.

Withania somnifera (WS) is popularly known as ashwagandha is a palnt in the solanaceae family. It has active constituents like withaferin A and withanolide D which are responsible for its bio-preventive properties [5]. These constituents are known to elevate the levels of endogenous antioxidants such as superoxide dismutase (SOD), catalase (CAT), ascorbic acid and decrease lipid peroxidation (LPO) [6, 7]. Khare (2007) has demonstrated the hepato-protective efficacy of withaferin A [8]. Vitamin E (α-tocopherol) (VE) is an important component of the human diet and is the most effective lipid-soluble antioxidant in the biological system. It is one of the most efficient chain-breaking antioxidant [9] and allows free radicals to extract a hydrogen atom from its antioxidant molecules by rendering them inactive and breaks the chain of free radical reactions [10].

Thus the present study was designed to evaluate the preventive efficacy of the combination of WS and VE against Cd-induced hepatic cytotoxicity and genotoxicity.

MATERIALS AND METHODS

Selection of animals

Adult male BALB/c mice (n=6/group) weighing 25-30 gm were used throughout the studies. The mice were obtained from animal house of Panjab University after approval from the ethics committee of the institute (IAEC/282). All the animals were housed in polypropylene cages, fed standard rat pellet diet (Ashirwad Industries, Punjab, Hindustan Lever, India) and were given water and maintained on a 12 hour day and night regime. They were acclimatised for 10 d prior to experimental use. All animals used in the study were housed, cared and used experimentally in accordance with the 'Guide for the Care and Use of Experimental Animals'.

Plant used in the study

Leaves of WS Plant were collected from Chandigarh and were identified and voucher numbers were obtained from Botany department of Panjab University, Chandigarh (voucher no. 21250). The leaf of the plant was air dried and the extraction was done using Soxhlet extraction method and ethanol was used as a solvent.

Chemicals used in the study

$CdSO_4$ was obtained from Sigma Chem. co., St. Louis, Missouri, USA. VE was obtained from MERCK India Limited. All chemicals of analytical grade specifications were obtained from HIMEDIA Ltd., India.

Methods

Preparation of homogenate and post-mitochondrial supernatant from liver tissue

10% homogenates of liver tissue were prepared in 50 mmol Tris-HCl buffer (pH-7.4) using a homogenizer at 0-4 °C. The homogenates

were used for biochemical estimation of LPO, reduced glutathione **(**GSH) and protein content. Post mitochondrial supernatants (PMS) were prepared by centrifuging these homogenates at 10,000 rpm for 10 min at 4 °C and supernatants were used for the estimation of anti-oxidant enzymes (using Jenway 6305 UV/vis spectro-photometer).

Experimental design

S. No.	Groups	Treatment (for 5 d)
1	Control group	normal water and food pellet
2	Cd group	5 mg/kg b. wt.(ip)
3	WS group	500 mg/kg b. wt.(orally)
4	Cd+WS	5 mg/kg b. wt.(ip)+500 mg/kg b. wt.(orally)
5	VE group	100 mg/kg b. wt. (orally)
6	Cd+VE group	5 mg/kg b. wt.(ip)+100 mg/kg b. wt.(orally)
7	Cd+WS+VE	5 mg/kg b. wt.(ip)+500 mg/kg b. wt.(orally))+100 mg/kg b. wt.(orally)

Biochemical parameters

Homogenate and PMS of liver were prepared and various biochemical tests were performed by standard methods.

LPO

LPO was estimated by following the standard method of Beuge and Aust [11]. Homogenate (10%) was incubated in 150 mmol Tris-HCl (pH 7.4), 1.5 mmol ascorbic acids, 1.0 mmol ferrous sulphate and incubated for 15 min. Added 10% trichloroacetic acid and 0.375% thiobutyric acid (TBA) and kept in boiling water bath for 15 min. The absorbance of the clear supernatant was measured at 532 nm.

GSH

GSH was measured in the homogenate by the standard method of beutler et al. [12]. Homogenate and 0.2M di-sodium hydrogen phosphate buffer were mixed thoroughly. Then added 1.0 mmol Dithio-bisnitro benzoic acid (DTNB) prepared in 1% potassium citrate. The absorbance of the clear supernatant was taken at 412 nm.

Antioxidant enzymes

Glutathione-s-transferase (GST)

The activity of GST was measured in the PMS by the method of Habig et al. [13]. PMS was incubated in 0.2 M phosphate buffer, 20 mmol 1-chloro-2, 4-dinitrobenzene (cDNB). Kept this mixture at 37 °C for 5 min. and increase in absorbance/30 seconds was measured at 340 nm for 3 min.

Catalase (CAT)

CAT activity was measured in PMS by using the standard method of Luck [14]. H_2O_2 phosphate buffer is formed by mixing 0.067M phosphate buffer in 12.5 mmol H_2O_2 (pH 7). An assay mixture consisted of 2.9 ml of H_2O_2 phosphate buffer and 0.01 ml PMS. The decrease in absorbance at 240 nm was noted after 30 seconds for 3 min.

SOD

SOD was measured in PMS according to the method of Kono [15]. Solution A was prepared by 50 mmol Sodium carbonate in 0.1 mmol EDTA (pH 10.8), Solution B contained 96 µM Nitroblue tetrazolium (NBT) in solution A, Solution C was prepared by mixing 0.6% (v/v) Triton X-100 (w/v) in solution A and Solution D contained 20 mmol Hydroxylamine hydrochloride (pH 6.0). The rate of NBT reduction by SOD enzyme was recorded at 560 nm for 30 min.

Protein estimation

Protein content was determined by the method of Lowry [16].

Histopathological studies

The structural changes in the liver were evaluated by Hematoxylin and Eosin staining by the method of pearse [17]. Tissues were fixed into Bouin's fixative and cut at 5 microns in a microtome and stained with haematoxylin and eosin and observed under light microscope (Leica DC 100, PCI Interface Digital Camera).

Bone marrow chromosomal analysis

Bone marrow chromosomal analysis was done by the method of Das [18]. Prior to sacrifice, mice were injected with 4 mg/kg b. wt. of colchicine intraperitoneally (ip). Mice were sacrificed and bone marrow was flushed out of femur with hypotonic KCl (0.075M) solution, converted into a fine suspension of cells by repeated aspiration and pipetting. Then cell suspension was incubated at 37 °C and centrifuged to get a pellet. Cells were then fixed in Carnoy's fixative, slides were prepared, air dried and stained with 4% giemsa and observed under light microscope.

Statistical analysis

All values were expressed as mean±standard deviation (SD) of 6 animals per group. Statistical significance in all groups was analysed by applying ONE WAY ANOVA at 5% significant level using SPSS software.

RESULTS

Amelioration of oxidative stress by WS and VE

The oxidative stress was assessed by analysing LPO, levels of cellular non-enzymatic (GSH) and enzymatic anti-oxidants (SOD, CAT, GST). These parameters were evaluated spectrophotometrically in the liver tissues of mice exposed to Cd, as well as WS (500 mg/kg b. w.) and VE (100 mg/kg b. w.). Short term Cd exposure (5 mg/kg b. w.) caused statistically significant elevation ($p<0.0001$) in the levels of LPO (97%) in liver tissue as compared to the control mice. Individual treatments of WS and VE to Cd-treated mice significantly decreased the elevated levels of LPO (~24%, $p\leq 0.001$) but the concurrent treatment of WS and VE (6.96%) normalized the levels of LPO to control values (fig. 1 [A]).

A statistically significant decrease ($p \leq 0.0001$) was observed in the level of GSH (57%) and GST (80%) in liver tissues of Cd intoxicated mice as compared to control mice. WS and VE individual treatments restored the levels of GSH and GST in the hepatic tissue ($p\leq 0.001$). However, Concurrent treatment of WS with VE in the Cd-treated group more effectively preserved the anti-oxidant status of GSH (10.40%, $p \leq 0.05$) (fig. 1 [B]) and GST (17.79%, $p \leq 0.05$) (table 1).

Five days Cd exposure significantly decreased the levels of CAT (63.82%, $p\leq 0.0001$) and SOD (70.26%, $p \leq 0.0001$) in liver tissue as compared to the control. Individual treatments of WS and VE significantly ($p\leq 0.001$) increased the levels of these antioxidants as compared to the Cd-treated group but the combinational treatment of WS and VE to Cd intoxicated mice, worked in combination in elevating the levels of CAT and SOD ($p \leq 0.05$) (table 1).

Modulatory potential of WS and VE on Cd-induced histopathological alterations

Light microscopic examination of liver from control mice depicted the presence of cords of hepatocytes arranged around the central vein, occurrence of hepatic sinusoids in between the hepatic cords with the occasional distribution of Kupffer cell. (fig. 2 [A]). Similar histoarchitecture of the liver was also observed in WS (fig. 2 [B]) and VE treated mice (fig. 2 [C]). Cd intoxication induced marked structural alterations in liver including hepatocytic degeneration, multiple foci of Kupffer cells infiltration, Kupffer cells hyperplasia, sinusoidal widening and lobular inflammation. Multiple foci of the necrotic lesions and damaged portal triad were also observed (fig. 2 [D-F]).

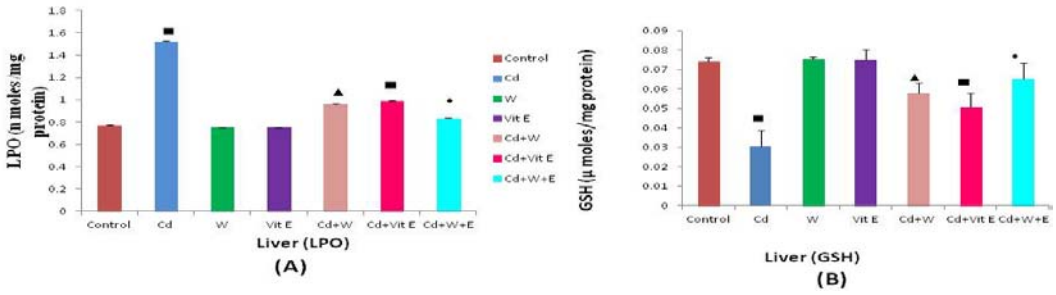

Fig. 1: Graphs showing levels of lipid peroxidation (LPO) and reduced glutathione (GSH) in liver tissue, (A) levels of LPO (n moles/mg protein) in livers of various treatment groups; (B) levels of GSH (µ moles/mg protein) in livers of various treatment groups. ■p ≤ 0.0001 (extremely statistically significant), ▲p ≤ 0.001 (very statistically significant), ●p<0.05 (statistically significant)

Table 1: Table showing protective effect of WS and VE on level of GST, CAT and SOD in mice with Cd-induced toxicity in liver

Liver	Control	Cd	WS	VE	Cd+WS	Cd+VE	Cd+WS+VE
GST	0.3033±0.056	0.0567±0.007■	0.3103±.026	0.3098±0.032	0.1933±0.015●	0.1730±0.015●	0.2633±0.035
CAT	50.51±1.486	16.940±1.525■	51.180±1.870	50.4067±1.158	40.980±1.537●	38.3167±2.53●	44.286±2.891
SOD	24.563±1.140	6.6400±1.630■	27.3833±2.265	25.4533±2.768	17.9067±1.743●	15.980±1.202●	20.653±2.065

Values of glutathione-S-transferase (GST), catalase (CAT) and superoxide dismutase (SOD) represented as mean±SD. The level of significance was considered as ●p<0.05 (statistically significant), ▲p ≤ 0.001 (very statistically significant), ■p ≤ 0.0001 (extremely statistically significant). Units: CAT-µmoles of H_2O_2 decomposed/min./mg protein; SOD–units/min./mg protein (1unit of the enzyme is defined as the amount of enzyme-inhibiting 50% nitroblue tetrazolium reduction);GST-µmoles GSH adduct formed/min./mg protein.

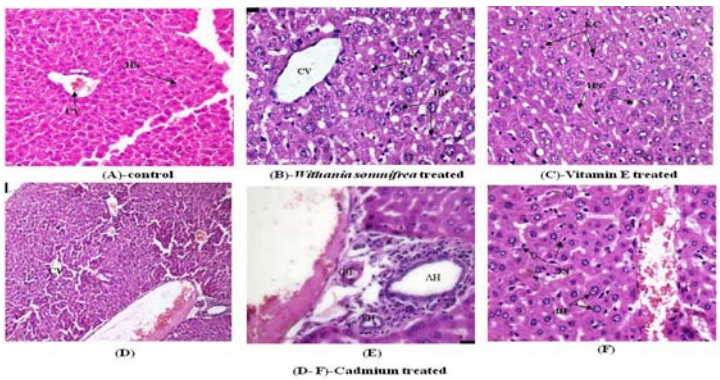

Fig. 2: Light micrographs of hepatic tissues from control and Cd-treated mice, (A) control mice showing normal histoarchitecture of liver with hepatocytes arranged in cords around central vein and well-defined sinusoids (X200); (B) WS and (C) VE treated mice liver showing normal hepatocytes arranged in cords around central vein. Well organized sinusoids separating the hepatocytes and few Kupffer cells present in the sinusoids (400X); (D-F) Cd-treated mice liver: (D) Large Kupffer cells infiltration, lobular inflammation, damaged hepatocytes (X100), (E) Widening of sinusoids and massive infiltration around portal triad (X400), (F) Appearance of binucleated and trinucleated cells in hepatocytes, damaged endothelial lining of central vein (X400)

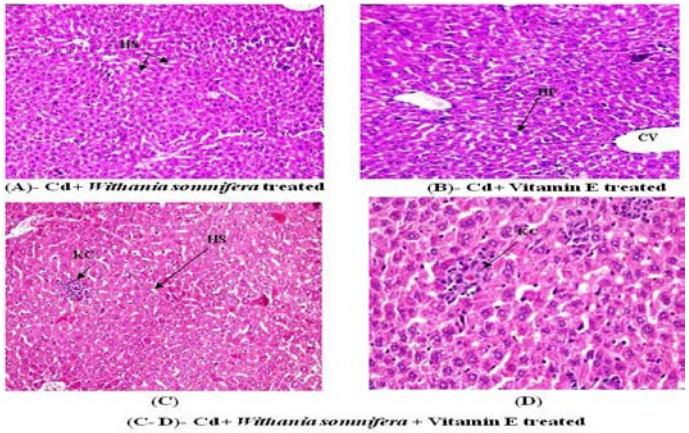

Fig. 3: Light micrographs of hepatic tissues from different groups getting various treatments in combination with Cd, (A) Cd+WS treated mice liver revealed normal hepatocytes and sinusoids with increased Kupffer cells infiltration (X100); (B) Cd+VE treated mice liver revealed normal hepatocytes and sinusoids, enhanced Kupffer cells infiltration and slight lobular inflammation (X100); (C-D) Cd+WS+VE treated mice liver revealed normal hepatocytes with well-organized sinusoids and very few foci of Kupffer cell infiltration (X100, X200)

Treatment with WS effectively prevented Cd mediated structural alterations in the liver (fig. 3 [A]). VE also modulated the structural alteration induced by Cd (fig. 3 [B]) but WS was found to be more effective than VE in individual treatments. However, the concurrent treatment of WS and VE prevented the structural alterations produced by Cd more promptly as compared to their individual treatments indicating their combinational efficacy (fig. 3 [C-D]).

Abbreviations for fig. 2 and 3: BD–bile duct, CV–Central vein, KC–Kupffer cells, HA–hepatic artery, HP–hepatocytes, HS–Hepatic sinusoids, HV–hepatic vein, PT–portal triad.

Genoprotective efficacy of WS and VE against Cd-induced chromosomal alterations

Metaphasic plates (100) were analysed from bone marrow chromosomes of all the groups. Metaphasic spread of control group revealed 40 chromosomes with normal contour and well-structured chromatids (fig. 4(A) and table 2). WS (fig. 4 [B] and table 2) and VE treatments also (fig. 4 [C] and table 2) revealed 40 chromosomes with normal contour and well-structured chromatids in light micrographs.

Five days Cd exposure induced genotoxic damage at the cellular and subcellular level. Bone marrow chromosomal analysis of Cd-treated mice revealed various chromosomal aberrations, which were then further classified into structural, physiological and numerical aberrations. Structural aberrations included centromeric fusion (18%), chromosomal breaks (2%), elongated chromosomes (4%), ring formation (10%) and Y chromatid separation (16%). Whereas physiological aberrations included the wooly (4%), sticky (4%) and condensed chromosomes (8%). Aneuploidy observed in 10% of the cells that signifies the numerical aberrations. (fig. 4 [D-I]) and table 2).

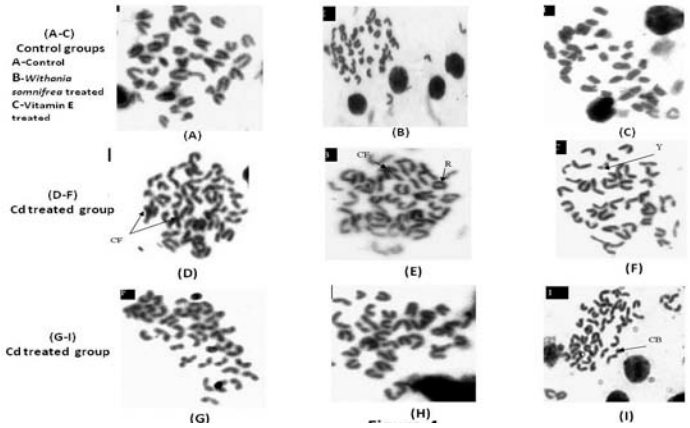

Fig. 4: Metaphasic spreads from bone marrow of various control groups (Saline, WS, VE) and Cd-treated mice, (A) control Group: normal metaphasic spreads with 40 acrocentric chromosomes; (B) WS Group: normal metaphasic plates with 40 chromosomes; (C) VE Group: Normal metaphasic spreads of 40 chromosomes; (D-I) Cd-treated mice showing chromosomal aberrations; (D) centromeric fusion; (E) ring formation and centromeric fusion; (F) Y chromatid break; (G) shortened and wooly chromosomes; (H) aneuploidy; (I) chromosome break

WS treatment ameliorated Cd-induced chromosomal defects like ring formation (2%), centromeric fusion (2 %), Y chromatid breaks (2%) and aneuploidy (4%) (fig. 5 [A-C] and table 2). Although VE was also found to be effective in reducing Cd mediated genotoxicity by decreasing abnormalities like ring formation (2%), condensed chromosomes (2%), Y chromatid breaks (2%), chromosomal breaks (2%), chromatid elongation (2%), but WS was found more effective in case of individual treatments (fig. 5 [D-F] and table 2).

Whereas, concurrent treatment of WS and VE was found to be most effective in attenuating Cd mediated chromosomal aberrations. A large number of normal metaphasic plates were seen in this group (90%) with very few cells revealing centromeric fusion (2%), Y chromatid breaks (2%), chromatid elongation (2%), sticky chromosomes (2%) and aneuploidy (2%) (fig. 5 [G-I] and table 2).

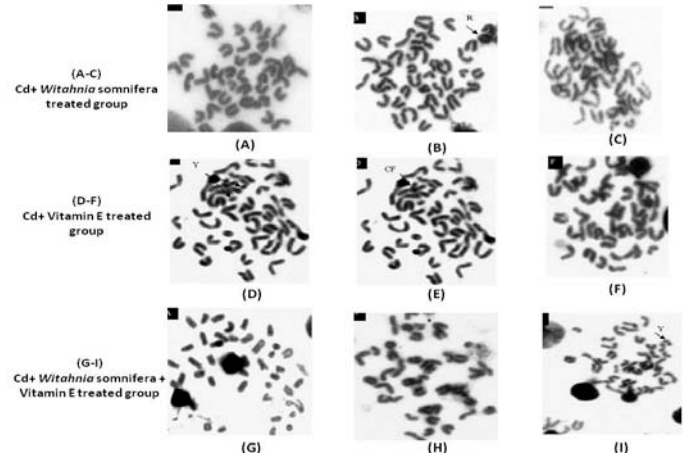

Fig. 5: Metaphasic spreads from bone marrow of different groups getting various treatments in combination with Cd (A-C) Cd+WS treated mice; (A)-normal extended 40 acrocentric chromosomes, (B)-ring formation, (C)-sticky and elongated chromosomes; (D-F) Cd+Vitamin E treated mice. (D)-Y chromatid break, (E)-centromeric fusion, (F)-aneuploidy; (G-I) Cd+WS+VE treated mice G-normal extended 40 acrocentric chromosomes, H-chromosomal break, I-Y chromatid breaks

Abbreviations for fig. 4 and 5: CB-chromosomal break, CF-centromeric fusion, R-Ring formation, Y-Y chromatid break.

Table 2: Effect of WS and VE on Cd-induced chromosomal aberrations in bone marrow cells of mice (5 d treatment) (% age of cells)

Chromosomal aberrations	Control	Cd	WS	VE	Cd+WS	Cd+VE	Cd+WS+VE
Structural aberrations							
Centromeric fusion	2	18			2	2	2
Chromosomal break		2				2	
Chromatid elongation		4			2	2	2
Y-chromatid break		16			2	2	2
Ring formation		10		2	2	2	
Physiological aberrations		8			2	2	
Condensed chromosomes							
Sticky chromosomes		4	2	2	2		2
Wooly chromosome		2				2	
Bridge formation		2				2	
Multiple chromatid breaks		4				2	
Numerical aberrations		10	2		4	2	2
Aneuploidy							
Normal metaphase plate	98	14	96	96	84	80	90

DISCUSSION

Cd exposure generates oxidative stress and contributes to the development of serious pathological conditions because of its long-term retention in tissues [19]. The exact mechanism of toxicity is not known but a number of mechanisms have been proposed including enhanced oxidative stress, the involvement of genotoxic mediators and failure of preventive mechanisms [20]. Thus present study tries to correlate the major prospective parameters to deduce the basis of Cd toxicity. Short term Cd exposure significantly increased the levels of hepatic LPO plausibly by both direct contribution and indirectly by activation of free radicals. The increased lipid peroxide levels might have contributed towards altered membrane functions [21]. Furthermore, enhanced LPO also contributes towards the piling up of the oxidative stress by adding more free radicals to the stock and by inhibiting the antioxidant enzymes activities. SOD is the first line of defence against free radicals which catalyzes the dismutation of superoxide anion radical ($O2^{\bullet-}$) into hydrogen peroxide (H_2O_2) by reduction, whereas CAT plays an important role in the elimination of H_2O_2 by initiating its hydrolysis into the water and prevents its accumulation in the body [22]. Thus increased MDA levels and decreased activities of SOD and CAT enzymes formed the basis of observed Cd-induced hepatic toxicity. These observations strongly correlate LPO mediated depression in hepatic levels of catalase and SOD. Casalino [23] also suggested Cd-induced displacement of Zn, Mn from the active sites of SOD, resulting in its decreased activity [23, 24].

Cd exposure caused more than 50% decline in the level of GSH and GST in liver. The decreased activity of GSH could be possibly due to competition between Cd-MT complex and GSH for sulphur containing amino acids. As Cd-MT complex has more affinity for sulphur containing amino acids thus masking the activity of GSH [20]. Furthermore, decreased level of GSH caused the decline in the level of GST and GR making the cell more susceptible to attack by free radicals [10].

In the present study, the histoarchitecture of Cd-exposed mice liver revealed many histopathological alterations like sinusoidal dilation, hepatocytic damage, kupffer cells infiltrations and multiple foci of lobular inflammation due to mononuclear cell and kupffer cell hyperplasia around the portal triad. This could appear due to inflammatory mediators released by kuppfer cells and generation of ROS which caused a subsequent elevation in LPO. In the present study, marked elevation in hepatic LPO and reduced antioxidants must have caused the observed histopathological alterations in the liver. These results are in accordance with the previous observations [25-26] who have also indicated that the imbalance of pro and antioxidant mediated LPO formed the basis of cellular and subcellular damage.

The genotoxicity of Cd is well established as Cd is regarded as a human carcinogen [27]. The observed Cd-induced structural, physiological and numerical chromosomal aberrations seemingly have developed due to the underlying stockpile of free radicals and by direct interaction of Cd with the DNA. Elevated levels of LPO enhanced the probability of interaction between Cd and DNA due to the leaky membranes [28]. Furthermore, the imbalance between oxidants and antioxidants adds fuel to the building up cellular oxidative stress and enhances the clastogenic potential of the metal. Most plausibly the duo of metal and oxidative stress could have played the significant role in the manifestation of Cd-induced genotoxicity.

In the present study, the anti-oxidant property of WS, VE and their combination has been exploited against Cd-induced toxicity. Treatment with WS markedly decreased the LPO and increased the levels of endogenous enzymes like CAT, GST, GSH and SOD. The observed antioxidant activity of WS could be attributed to its biologically active constituent's withaferin A and withanolides provide free phenolic hydrogen atom [30]. These phenolic hydrogen atoms are capable of scavenging Cd initiated free radical generation. Furthermore, WS instigated elevation in the levels of antioxidants and lowered metal induced biochemical and structural alterations. These observations are in conformity with Bhattacharya and his co-authors who have also documented WS mediated elevation in antioxidant enzymes [29-31]. As WS reduced the observed oxidative stress, so the genotoxic alterations were also seen to be reduced. Biologically active constituents of WS like steroidal lactones, alkaloids and flavonoids by reducing LPO might have prevented the free radicals to interact directly and indirectly with DNA and established the genoprotective potential of WS [32, 33].

VE treatment also reduced the Cd-induced oxidative stress by reducing lipid peroxidation and oxidative stress due to its high lipid solubility [34]. VE rapidly transfers its phenolic H-atom to a lipid peroxyl radical, converting it into a lipid hydroperoxide and a VE radical [35]. VE is the most potent lipid peroxyl radical scavenger that significantly lowers LPO and oxidative stress-mediated chromosomal damage by stabilizing leaky membranes [36]. This protective efficacy of VE could have inhibited Cd-induced free radical formation and its stockpiling and furthermore prevented histopathological and chromosomal alterations.

Peroxyl radical $\xrightarrow{\alpha\text{-Tocopherol}}$ Hydroperoxide radical (Tocopheroxyl)

CONCLUSION

WS and VE both being strong free radicals scavengers interfered well with the Cd instigated toxic reactions. Both these antioxidants reduced the Cd-induced hepatotoxicity but the combinational treatment of WS and VE most effectively attenuated Cd mediated alterations. Their reactive constituents acted in a duo that inhibited ROS and prevented biochemical, structural and genotoxic changes. VE by preventing the formation of peroxyl radical reduced LPO and inhibited all the chain reactions instigated by this radical and WS balanced the pro-and antioxidant status in the system by providing free phenolic hydrogen atoms to the free radicals and enhancing the levels of endogenous antioxidants. Thus this duo synergistically acted on the Cd generated ROS and prevented the building up oxidative stress and resulted in abrogation of Cd mediated toxicity.

Thus the present study proposes WS and VE as potential therapeutic drug supplements for ameliorating metal-induced toxicity.

AUTHORS CONTRIBUTION

Seema Rani, Vijay Lakshmi Sharma and Mani Chopra designed the research work; Seema Rani and Mani Chopra performed the experimental research work; Seema Rani, Vijay Lakshmi Sharma and Mani Chopra analysed the data; Seema Rani and Mani Chopra wrote the manuscript.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

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