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RESEARCH

Cyto and Genoprotective Potential of Tannic Acid Against Cadmium and Nickel Co-exposure Induced Hepato-Renal Toxicity in BALB/c Mice

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Abstract

Tannic acid (TA) is a metal chelating polyphenol that plays a crucial role in metal detoxification, but its modulatory role in co-exposure of these heavy metals' exposure needs to be explored. Cadmium (Cd) and nickel (Ni) are inorganic hazardous chemicals in the environment. Humans are prone to be exposed to the co-exposure of Cd and Ni, but the toxicological interactions of these metals are poorly defined. Present study was undertaken to study the preventive role of TA in Cd–Ni co-exposure-evoked hepato-renal toxicity in BALB/c mice. In the current investigation, increased oxidative stress in metal intoxicated groups was confirmed by elevated peroxidation of the lipids and significant lowering of endogenous antioxidant enzymes. Altered hepato-renal serum markers, DNA fragmentation, and histological alterations were also detected in the metal-treated groups. Present study revealed that Cd is a stronger toxicant than Ni and when co-exposure was administered, additive, sub-additive, and detrimental effects were observed. Prophylactic treatment with TA significantly reinstated the levels of lipid peroxidation (LPO), non-enzymatic, and enzymatic antioxidants. Moreover, it also restored the serum biomarker levels, DNA damage, and histoarchitecture of the given tissues. TA due to its metal chelating and anti-oxidative properties exhibited cyto- and genoprotective potential against Cd–Ni co-exposure-induced hepatic and renal injury.

Keywords DNA damage · Heavy metals · Oxidative stress · Genotoxicity

Introduction

Heavy metals are defined as naturally occurring elements that have a high atomic weight and a density at least five times higher than that of water [1]. While few heavy metals are essential for biological processes in trace amounts, their excessive exposure can lead to adverse health effects in humans. Humans are exposed to these metals through anthropogenic activities and occupational exposure and can bioaccumulate via air, water, and soil [2–5]. The exposure of heavy metals extends their critical concerns to the environment owing to their complex nature that often involves co-exposure of these metals [6]. Their concurrent presence can lead to unpredictable and potential supra-additive, additive, or sub-additive effects on human health, which may exacerbate the adverse outcomes associated with individual metal exposures [5, 7].

Understanding the toxicity of co-exposure to Cd and Ni is crucial due to their widespread environmental presence. Cadmium (Cd) is an industrial and environmental pollutant, which is poorly excreted due to its long biological half-life of 10–35 years [8]. The main routes of its exposure are tobacco smoking, drinking contaminated water, metal plating, batteries, and air pollution silently wreaking havoc on human health [9]. Likewise, nickel (Ni) is a well-known essential trace element, but at the same time is hazardous due to its wide use in electroplating, alloy production, jewelry, stainless steel, and electrical batteries [10]. Electrical and electronic waste (e-waste), which is the largest source of Ni–Cd contamination, poses a tremendous threat and concern to human health [11]. According to the Global E-waste Monitor, ~ 53.6 million metrics tons (mMt) of e-waste were produced in 2019 globally, and Asia was the largest generator of e-waste in 2019 (24.9 mMt) [12].

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Both Ni and Cd can bioaccumulate in different organs and tissues of the body causing hepatorenal dysfunction, carcinogenicity, neurotoxicity, reproductive toxicity, and developmental and gastrointestinal abnormalities [9, 10, 13]. These observed toxicities are most plausibly caused by heavy metal-induced oxidative stress and imbalance of pro- and anti-oxidant system [8, 10]. Therefore, examination of toxicant interaction is important to understand co-exposure-mediated complex mechanisms underlying their toxicity. Moreover, the hepato-renal system, necessary for the metabolism and excretion of xenobiotics, necessitates the examination of co-exposure in this system [14].

To date, no effective approach exists to counteract the adverse consequences of individual or co-exposure of heavy metals, making it essential to uncover the potential of nutraceuticals in this field [13, 15]. Plant-based polyphenols, flavonoids, and alkaloids exhibit antioxidant potential due to their hydrogen-donating and metal-chelating properties [16–18]. Tannic acid (TA) is a polyphenolic plant compound, typically found in tea, wood barks, walnut, berries, and Chinese galls with excellent free radical scavenging, antioxidant, metal-chelating, and quenching properties [19]. The antioxidant property of TA is attributed to its hydroxyl groups, which allow them to act as reducing agent, hydrogen donor, and quencher of singlet oxygen making it a suitable candidate to be explored against metal toxicity [20]. Thus, we hypothesized that TA could be a potent therapeutic agent against Cd–Ni co-exposure-induced biochemical and histological alterations and genotoxicity in hepato-renal tissues. Therefore, this research was aimed to investigate the antioxidant and cyto/genoprotective effects of TA against hepato-renal toxicity induced by individual and co-exposure of cadmium and nickel in BALB/c mice.

Materials and Methods

Chemicals

Cadmium sulfate ($CdSO_4$, CAT #7790–84-3) with 98% purity was obtained from Loba Chemie, Mumbai and nickel chloride ($NiCl_2$, CAT #7791–20-0) with 98% purity was purchased from Central drug house (P) Ltd., New Delhi. The tannic acid (TA, CAT #151013) of analytical research grade was used for the present study was supplied by Thomas baker (Chemicals) Pvt. Ltd., Mumbai. All other chemicals of analytical grade specifications were obtained from Himedia Ltd., SRL, CDH, and Merck.

Experimental Animals

Forty-eight adult female BALB/c mice of 2–3 months age weighing ~ 25–40 g were procured from the central animal house of Panjab University, Chandigarh (approval number PU/45/99/CPCSEA/IAEC/2015/678). Animals were housed under a standard controlled temperature of 25 ± 3 °C, 12 h light–dark cycle, and fed a standard rodent pellet diet (Ashirwad Industries, Punjab, Hindustan Lever, India; Cat #23,099,010) and water ad libitum. All the animals were acclimatized for 7 days prior to experimentation and used as per the "Guide for the Care and Use of Experimental Animals" approved by the Institutional Animal Ethics Committee, Panjab University.

Effective Dose Selection and Experimental Design

Cadmium was administered intraperitoneally (i.p.) with 0.7 mg/kg b.wt. (low dose) which is 1/10th of LD_{50} in mice models for 30 days [21]. To obtain the toxicologically effective nickel dose, LD_{50} of the nickel chloride was calculated by employing probit analysis, which came out to be 22.98 mg/kg b.wt. Based on that observation, the sub-chronic dose of 7.06 mg/kg b.wt. of Ni (1/3rd of LD_{50}) was given intraperitoneally for 30 days to induce observable toxicity in mice. Pre-treatment of 100 mg/kg b.wt. of tannic acid was given through gavage for 15 days, and this dose was selected from the data available in the literature [20]. The oral route was chosen to mimic the commonly used mode of administration of TA to humans.

The adult mice were randomly divided into the following eight groups following acclimatization for 1 week. One control group and seven experimental groups comprised six mice in each group (Table 1).

All mice were euthanized 24 h after the last exposure, and liver and kidney tissues from experimental animals were collected for biochemical analysis and histopathological studies. Bone marrow from the femur bone of mice was flushed and collected for the micronuclei assay, and the comet assay was performed on the blood lymphocytes.

Oxidative Stress Biomarkers

Homogenates (10%) of liver and kidney tissues were prepared in 50 mM Tris–HCl buffer (pH 7.4, 4 °C) using the homogenizer. The homogenates and further the supernatants obtained were then used for spectrophotometric determination of lipid peroxidation (LPO), reduced glutathione (GSH), total protein concentration by method of Lowry et al. (1951) with slight modifications, catalase (CAT) activity, superoxide dismutase (SOD), glutathione-S-transferase (GST), and glutathione reductase (GR) [22–29].

Liver and Kidney Function Markers

For liver function, serum glutamate pyruvate transaminase (SGPT, CAT #CC2-ALT.17N), serum glutamate

Table 1 Experimental design for Cd–Ni induced hepato-renal toxicity in BALB/c mice

Groups		Dose	Duration
1	Control (CON)	Saline	30 days (i.p.)
2	Cadmium (Cd)	0.7 mg/kg b.wt	30 days (i.p.)
3	Nickel (Ni)	7.06 mg/kg b.wt	30 days (i.p.)
4	Tannic acid (TA)	100 mg/kg b.wt	15 days (oral)
5	Tannic acid + Cd (TA + Cd)	TA, 100 mg/kg b.wt Cd, 0.7 mg/kg b.wt. of Cd	TA, 15 days (oral) Cd, next 30 days (i.p.)
6	Tannic acid + Ni (TA + Ni)	TA, 100 mg/kg b.wt Ni, 7.06 mg/kg b.wt	TA, 15 days (oral) Cd, next 30 days (i.p.)
7	Cd + Ni co-exposure (Cd + Ni)	Cd, 0.7 mg/kg b.wt Ni, 7.06 mg/kg b.wt. (after 8 h exposure of Cd)	Co-exposure for 30 days (i.p.)
8	Tannic acid + Cd-Ni co-exposure (TA + Cd + Ni)	TA, 100 mg/kg b.wt Cd, 0.7 mg/kg b.wt. Cd Ni, 7.06 mg/kg b.wt. (after 8 h exposure of Cd)	TA, 15 days (oral) Co-exposure of Cd and Ni for next 30 days (i.p.)

Abbreviations: *Cd*, cadmium; *i.p.*, intraperitoneal; *mg/kg b.wt.*, milligram per kilogram body weight; *Ni*, nickel; *TA*, tannic acid

oxaloacetate transaminase (SGOT, CAT #CC3-AST.16N), alkaline phosphatase (ALP; CAT #CC2-ALK.02U), and total bilirubin (CAT #CC3-BIG.004) were estimated. Urea (CAT #CC2-UAB.019) and creatinine (CAT #CC3-CEN.024) concentrations were analyzed for kidney function. These serum assays were done by using commercially available kits from Reckon Diagnostics Pvt. Ltd., Gujarat (India).

Histological Studies

For histological assessment, double staining was done with hematoxylin and eosin (H&E) [30]. Briefly, the mice liver and kidney were isolated, fixed in 10% formaldehyde, dehydrated using different grades of alcohol, and embedded in paraffin. After that 5-µM-thick sections were cut, dewaxed. These sections were then hydrated with alcohol gradient and stained with hematoxylin for 1 min. The samples were then washed with phosphate buffered saline (PBS) and stained with eosin for 15 s before dehydration with an alcohol gradient. The sections were treated with xylene, mounted in DPX, and viewed under a light microscope. Then, tissue samples were evaluated with a light microscope to observe cellular damage. To determine an appropriate scoring system for liver and kidney tissue alterations, the scores were derived semi-quantitatively using light microscopy [31].

Genotoxic Parameters

Micronuclei Assay

Post-sacrifice the femur was cleared, and bone marrow was flushed out of the femur using a syringe filled with 1 mL of fetal calf serum into centrifuge tubes. Cells were dispersed by repeated aspiration and pipetting and collected by centrifugation at 1200 rpm for 10 min at 4 °C. A 5 µL of bone marrow cell suspension was placed on the centre of the acridine orange (10 µL of 1 mg/mL) coated slide. This protocol was done according to the method of Hayashi et al. (2000) [32]. Stained cells were examined by fluorescent microscope, and micronuclei frequency in polychromatic erythrocytes (PCE) was evaluated by scoring 1000 PCE and normo-chromatic erythrocytes (NCE) per animal ($n = 6$) per group.

Comet Assay

The extent of DNA damage was quantified in lymphocytes by studying DNA migration patterns through single gel electrophoresis [33]. Briefly, ~ 1.5 mL blood from the jugular vein was collected and centrifuged (1400 rpm for 30 min) in histopaque to separate lymphocytes. These lymphocytes were lysed, electrophoresed, neutralized, and fixed on slides in different buffers. Slides were stained with EtBr and viewed under the fluorescence microscope. A total number of 150 cells per animal ($n = 6$) were analyzed using CometScoreTM (version 1.5) software. Three replicates were prepared per slides and three slides were produced per animal in each group. Fifty randomly selected cells were analyzed per replicate per sample. The parameters investigated to determine the DNA damage level were % DNA in the comet tail and tail moment.

DNA Fragmentation

For genomic DNA fragmentation, agarose gel electrophoresis was performed using standard phenol:chloroform:isoamyl alcohol method. Briefly, liver and kidney tissues were homogenized in Tris-EDTA (TE) buffer and after centrifugation, the pellet was dissolved in lysis buffer. After addition of 5% sodium dodecyl sulfate (SDS) and proteinase K, the

phenol:chloroform:isoamyl alcohol (25:24:1) mixture was added and centrifuged. Further, isopropanol was added to the obtained aqueous layer and incubated for 2–3 h. After centrifugation, the pellet was washed with 70% ethanol and the final pellet was dissolved in 20 µL of TE buffer and stored at 20 °C until further use. DNA integrity was checked on 0.8% agarose gel.

Interaction Index for Cadmium-Nickel Co-exposure

The combined effect of Cd and Ni was measured quantitatively using Bliss independence method or also called as relative-effect multiplicative model. Using this method, the relative levels (θ_X) of a parameter X were calculated and further, the interaction index (γ) value was determined using the equation

$$\gamma_X = log(\theta_{AB}) - log(\theta_A) - log(\theta_B)$$

where θ_A = normalized level of parameter X in Cd exposed group, θ_B = normalized level of parameter X in Ni exposed group, and θ_{AB} = normalized level of parameter X in Cd + Ni co-exposed group.

The calculated value of $\gamma < 0$, $\gamma = 0$, or $\gamma > 0$ indicates a sub-additive, additive, or supra-additive interaction of Cd and Ni co-exposure, respectively [34, 35]. These calculated interaction index values (γ) for combinational exposure of Cd and Ni are given in Table 2.

Table 2 Calculated interaction index values (γ) for combinational exposure of Cd and Ni

	Liver	Kidney
Oxidative stress markers		
LPO	−0.06	−0.38
GSH	0.11	0.26
CAT	0.13	0.06
SOD	0.07	0.14
GST	0.16	0.09
GR	0.13	0.20
Liver function markers		
SGPT	−0.47	
SGOT	−0.49	
ALP	−0.45	
Bilirubin	0.14	
Kidney function markers		
Urea	−0.08	
Creatinine	−0.08	
Micronuclei assay		
−1.14		
Comet assay		
% DNA in tail	−0.43	
Tail moment	−0.34	

Statistical Analysis

The data was expressed as mean ± standard deviation. The comparison of control, experimental, and TA-treated groups was statistically analyzed by one-way ANOVA (analysis of variance) followed by Tukey's post hoc test. Values with $p < 0.05$ were considered statistically significant.

Results

Oxidative Stress Biomarkers

Thirty days of cadmium (0.7 mg/kg b.wt.) and nickel (7.07 mg/kg b.wt.) exposures (individual and co-exposure) instigated statistically significant ($p < 0.05$) intensification in the levels of LPO in hepatic and renal tissues of mice in comparison with the control group. Co-exposure of these inorganic metals caused ~ 1.4-fold and threefold increase in LPO in hepato-renal tissues, respectively. The oral pre-treatment of TA for 15 days lowered the LPO levels by ~ 1.1-fold in hepatic and twofold in renal tissue, respectively, compared to Cd- and Ni-exposed groups, respectively ($p < 0.05$). While in the co-exposure groups, TA caused significant reduction of LPO levels by 1.6-fold and twofold in liver and kidney tissues, respectively ($p < 0.05$; Fig. 1a). Cd and Ni intoxication significantly decreased the reduced glutathione levels (GSH) in the liver and kidney ($p < 0.05$). While TA pre-treatment efficiently restored the GSH levels in both the organs to near control values in the case of Cd, Ni, and co-exposed groups ($p < 0.05$) (Fig. 1b).

Administration of Cd and Ni significantly declined the levels of CAT, SOD, GST, and GR in liver and kidneys, respectively ($p < 0.05$). Cd alone was found to be more toxic; it caused more reduction of antioxidants than Ni alone. The co-exposure of both the metals exhibited sub-additive effect in enhancing LPO ($\gamma = -0.06, -0.38$) and supra-additive interaction in reducing GSH levels ($\gamma = 0.11, 0.26$) in liver and kidney tissues, respectively. Furthermore, supra-additive effect was observed in all other oxidative stress markers of liver and kidney tissues. TA treatment significantly elevated the levels of CAT, SOD, GST, and GR antioxidants in all the Cd-, Ni-, and co-exposure-treated groups (γ values are given in Table 2) ($p < 0.05$) (Fig. 1c–f).

Liver and Kidney Function Markers

Thirty days of cadmium and nickel (individual and co-exposure) intoxication caused a statistically significant ($p < 0.05$) increase in the levels of SGOT, SGPT, and ALP, whereas there was a significant decline in the levels of

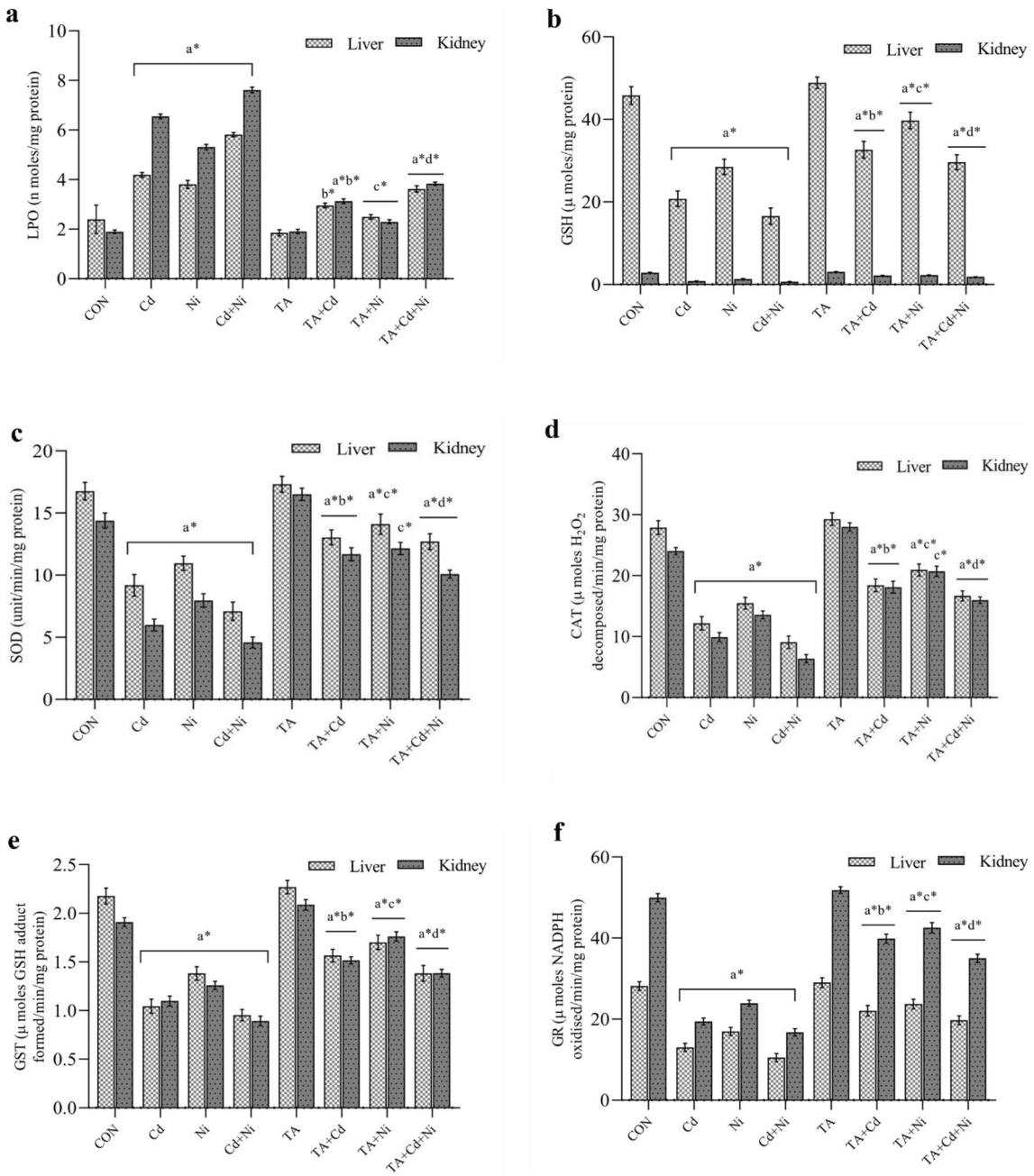

Fig. 1 **a** Lipid peroxidation (LPO) (n moles/mg protein). **b** Reduced glutathione (GSH)-μ moles/mg protein. **c**–**f** Levels of endogenous antioxidant enzymes SOD (units/min/mg protein), CAT (μ moles of H2O2 decomposed/min/mg protein), GST (μ moles GST adduct formed/min/mg protein), and GR (moles NADPH oxidised/min/mg protein) in the liver and kidney tissues of rats in CON-, Cd-, Ni-, Cd + Ni-, TA-, TA + Cd-, TA + Ni-, and TA + Cd + Ni-treated groups, respectively. Values are shown as mean ± S.D. ($n = 6$); levels of significance, * $p < 0.05$ (statistically significant); a, comparison with control group; b, comparison with cadmium treated group; c, comparison with nickel treated group; d, comparison with Cd + Ni co-exposure treated group. ANOVA followed by Tukey's honestly significant difference test

serum bilirubin ($p < 0.05$). Pre-treatment of tannic acid effectively restored the levels of these hepatic markers ($p < 0.05$) (Fig. 2a). Principle kidney function markers, i.e., urea and creatinine, were elevated in blood serum after sub-chronic exposure to Cd, Ni, and their co-administration ($p < 0.05$). However, TA decreased the levels of urea by 1.3-fold and of creatinine by 1.1-fold in blood serum in co-exposure group ($p < 0.05$) (Fig. 2b).

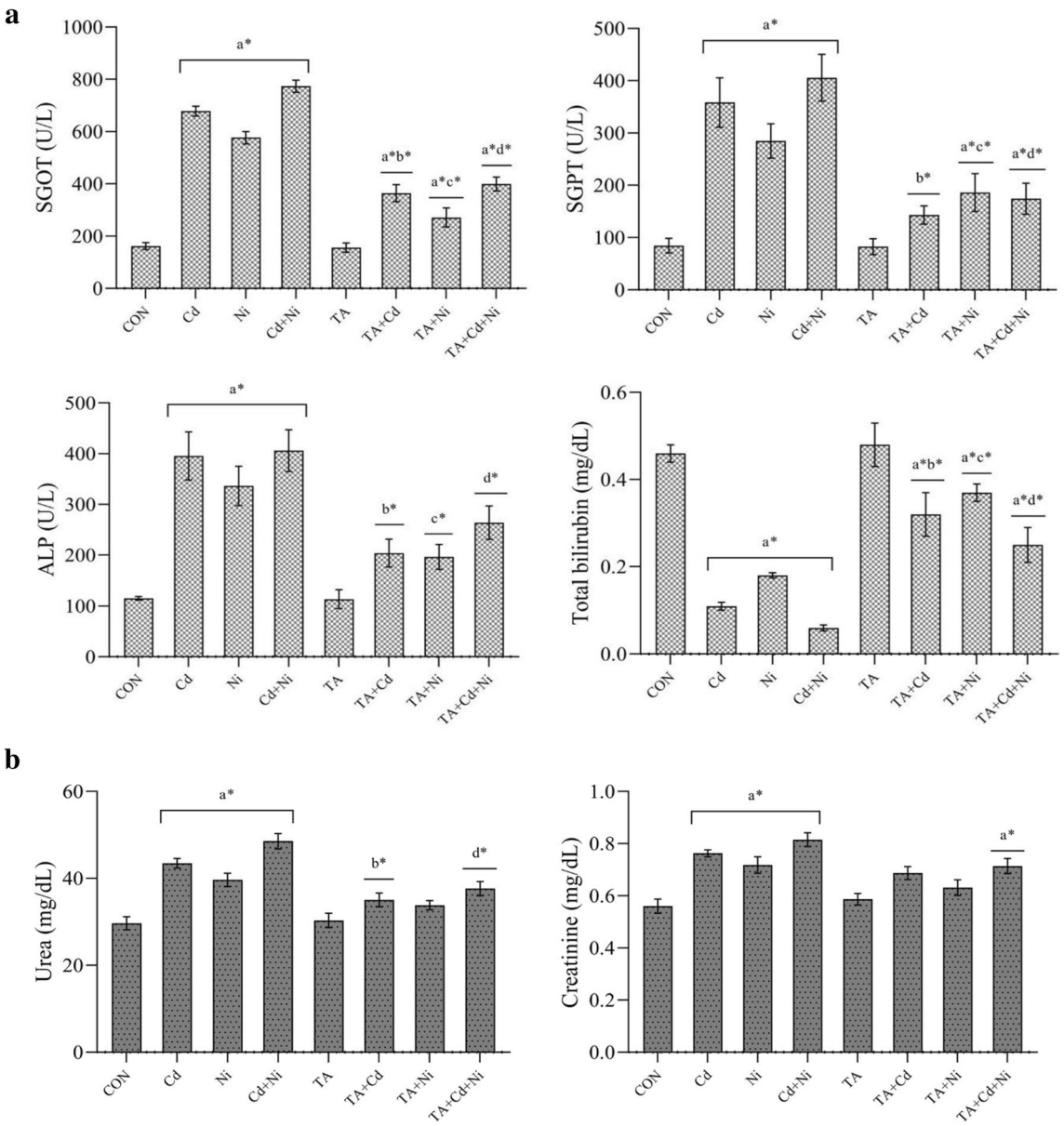

Fig. 2 **a** Levels of liver function markers SGPT (U/L), SGOT (U/L), ALP (U/L), and total bilirubin (mg/dL) in control, Cd, Ni, Cd + Ni, TA, TA + Cd, TA + Ni, and TA + co-exposure treated groups. **b** Levels of kidney function markers urea (mg/dL) and creatinine (mg/dL) in CON-, Cd-, Ni-, Cd + Ni-, TA-, TA + Cd-, TA + Ni-, and TA + Cd + Ni-treated groups. Values are shown as mean ± S.D. ($n = 6$); a, = comparison with control group; b, comparison with cadmium treated group; c, comparison with nickel treated group; d, comparison with cadmium + nickel treated group. ANOVA followed by Tukey's honestly significant difference test

Histopathology

Individual exposures of Cd and Ni for 30 days caused histopathological alterations in liver like vacuolization (25%, 18%), few foci of Kupffer cell infiltration (26%, 19%), widening of sinusoids (18%, 16%), damaged hepatocytes (13%, 10%), and vascular congestion around the central vein (Fig. 3a). Thirty-day co-exposure of Cd and Ni exhibited pronounced histoarchitectural alterations in liver revealing evident vacuolization (30%), Kupffer cell infiltration (32%), sinusoidal widening (25%), and highly damaged hepatocytes (14%) indicative of sub-additive effect of co-exposure. Pre-treatment of mice with TA effectively attenuated the individual cadmium and nickel exposure-induced histopathological alterations in the liver as indicated by normal hepatocytes, reduction in sinusoidal widening, decreased vacuolization, and reduced Kupffer cell infiltration ($p < 0.05$), whereas in the co-exposure group, moderate ameliorative efficacy of TA was observed (Fig. 3a, b).

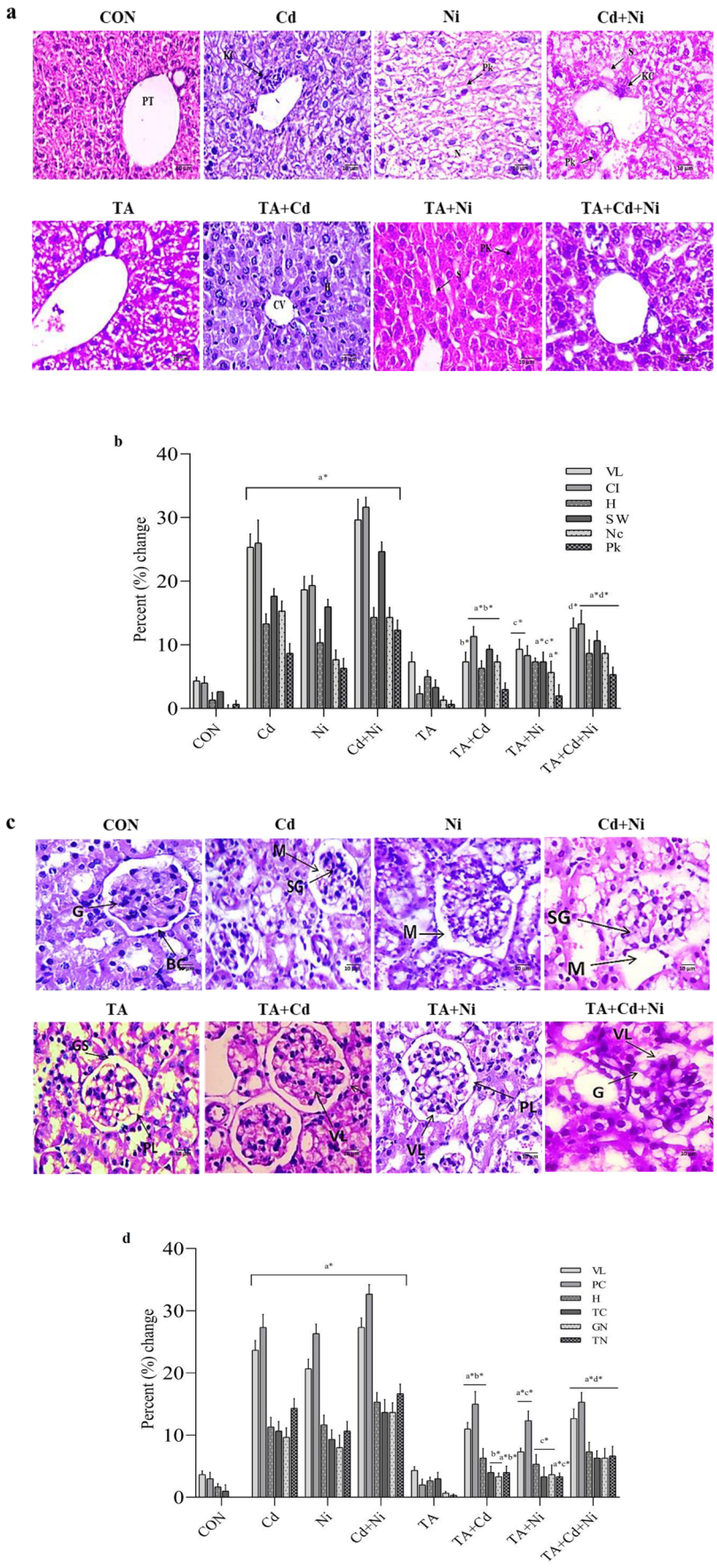

Fig. 3 **a** Comparative light micrographs of normal and metal treated liver of CON, Cd, Ni, Cd + Ni, TA, TA + Cd, TA + Ni, and TA + Cd + Ni groups of mice (×400). **b** Graphical representation of the percent of histological alterations in liver tissue in each group. Abbreviations: CV, central vein; PT, portal triad; H, hepatocytes; S, sinusoids; Nc, necrosis; Pk, pyknosis; KC, Kupffer cell; VL, vacuolization; CI, cellular infiltration; Hm, hemorrhage; SW, sinusoidal widening. **c** Comparative light micrographs of normal and metal treated kidney of CON, Cd, Ni, Cd + Ni, TA, TA + Cd, TA + Ni, and TA + Cd + Ni groups of mice (×400). **d** Graphical representation of percent histological alterations in kidney of each group. Abbreviations: BC, Bowman's capsule; G, glomerulus; M, mesangiolysis; PC, protein cast; C, congestion; SG, shrinked glomerulus; VL, vacuolization; HM, hemorrhage; TC, tubular congestion; GN, glomerular necrosis; TN, tubular necrosis. Values are shown as mean ± S.D. ($n = 3$); a, comparison with control group; b, comparison with cadmium treated group; c, comparison with nickel treated group; d, comparison with cadmium + nickel treated group. ANOVA followed by Tukey's honestly significant difference test

After 30 days of Cd and Ni individual exposures, kidneys demonstrated significant histoarchitectural changes like enlargement of mesangial space (24%, 21%), congestion in proximal and distal convoluted tubules (11%, 9%) due to protein casts (27%, 26%), glomerular shrinkage (10%, 8%), and tubular degeneration (14%, 11%), revealing the nephrotoxic nature of Cd and Ni, respectively. Cd exposure was found more toxic than Ni individual exposure as mesangiolysis and infiltration of mononuclear cells were visible at multiple foci in Cd treated mice kidneys. Cd–Ni co-exposure has more pronounced toxic effects in the renal tissue exhibiting sub-additive effect. A large number of shrunken glomeruli (14%), mesangiolysis, enlargement in mesangial space (27%), interstitial inflammation, and necrotic lesions in tubular cells (17%) along with dense protein cast in PCT and DCT (33%) were observed due to co-exposure of metals. Pre-treatment with TA significantly restored the histoarchitecture of individual Cd and Ni exposure groups indicated by normal glomeruli, PCT, and DCT ($p < 0.05$). Pre-treatment with TA in the co-exposure group decreased the impact of heavy metals, but mononuclear cell infiltration and few foci of tubular congestion were visible, indicating moderate amelioration of TA pre-treatment in this case (Fig. 3c, d).

Micronuclei Induction

The Cd and Ni exposures enhanced the levels of micronucleation in bone marrow cells by approximately 26- and 22-fold, respectively, as compared to the control ($p < 0.05$). The combinational exposure of Cd and Ni showed sub-additive effects ($\gamma = -1.14$), i.e., a 44-fold increase in the micronucleation in bone marrow cells was observed (Fig. 4a, b). Pre-administration of TA significantly ($p < 0.05$) reduced micronucleation as compared to toxicant groups. TA more efficiently reduced the micronucleation in bone marrow cells in Cd and Ni alone treated groups as compared to the co-exposure due to heavy load of genetic damage.

Comet Assay

A significant ($p < 0.05$) increase in comet assay parameters, such as % DNA in tail (8.58 ± 0.92, 7.27 ± 0.89) and tail moment (0.85 ± 0.12, 0.80 ± 0.11), was observed in blood lymphocytes of mice treated with Cd and Ni, respectively, when compared with normal control mice (Fig. 5a, b). After co-exposure of these heavy metals, sub-additive effect in DNA damage, i.e., % DNA in tail (10.41 ± 0.99; $\gamma = -0.43$) and tail moment (0.93 ± 0.13; $\gamma = -0.34$), was observed. Pre-treatment

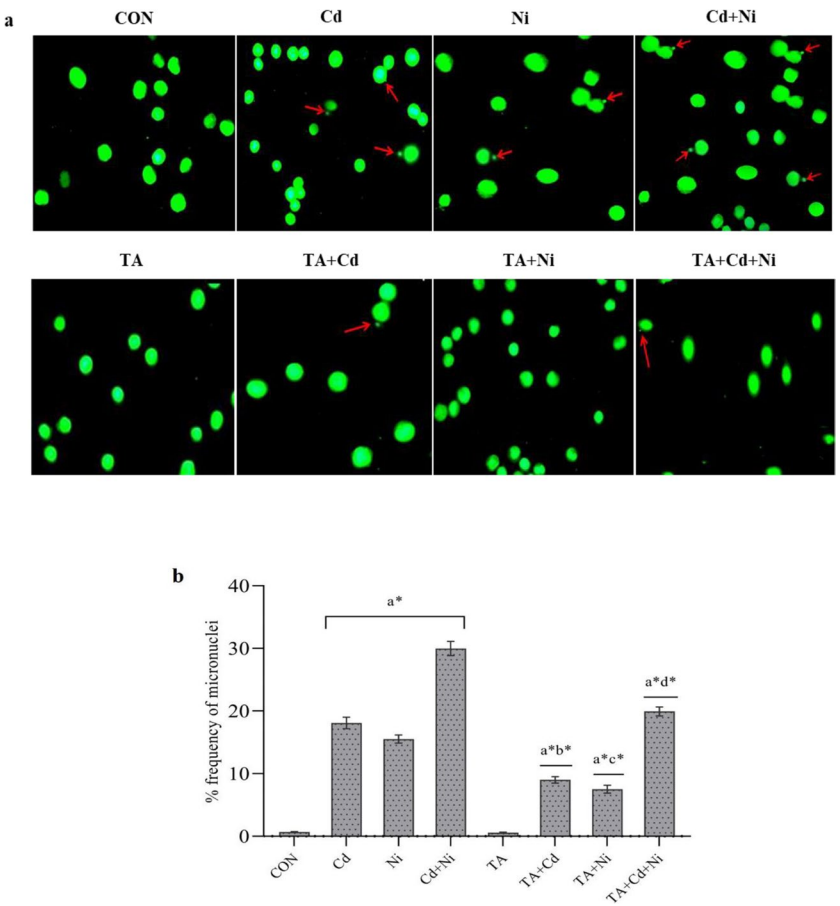

Fig. 4 **a** Photomicrographs illustrating micronuclei formation in bone marrow cells of **a** CON-, Cd-, Ni-, Cd + Ni-, TA-, TA + Cd-, TA + Ni-, and TA + Cd + Ni-treated mice. **b** Percentage frequency of micronuclei/1000 cells in bone marrow cells of CON-, Cd-, Ni-, Cd + Ni-, TA-, TA + Cd-, TA + Ni-, and TA + Cd + Ni-treated mice. Values are shown as mean ± S.D. ($n = 6$); a, comparison with control group; b, comparison with cadmium treated group; c, comparison with nickel treated group; d, comparison with cadmium + nickel-treated group. ANOVA followed by Tukey's honestly significant difference test

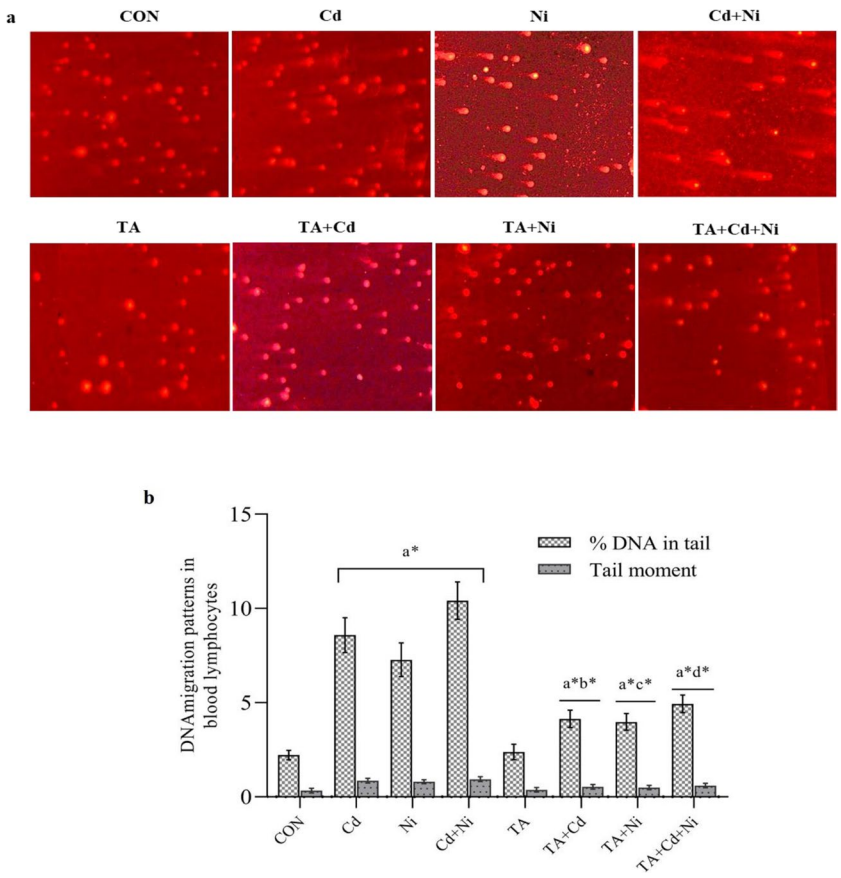

Fig. 5 **a** Photomicrographs of comet assay in peripheral blood lymphocytes of CON-, Cd-, Ni-, Cd + Ni-, TA-, TA + Cd-, TA + Ni-, and TA + Cd + Ni-treated groups. **b** Graphical representation of DNA migration patterns, i.e., percent DNA in tail and tail moment in lymphocytes of mice. Values are shown as mean ± S.D. ($n = 6$); a, comparison with control group; b, comparison with cadmium treated group; c, comparison with nickel-treated group; d, comparison with cadmium + nickel-treated group. ANOVA followed by Tukey's honestly significant difference test

of TA for 15 days to Cd and Ni individual as well as co-exposed mice demonstrated a substantial DNA protective efficacy by significantly reducing % DNA damage and tail moment (4.93 ± 0.47, 0.60 ± 0.11) (Fig. 5a, b).

DNA Fragmentation

Agarose gel electrophoretograms revealed intact DNA bands in control and TA groups (lanes 1 and 5) of hepato-renal tissues. While, 30 days Cd and Ni exposure revealed shearing of DNA in liver and kidney tissues (lanes 2 and 3). DNA shearing was more evident in co-exposure group revealing maximum DNA fragmentation in both the tissues as indicated in lane 4. Pretreatment of TA to Cd, Ni, and co-exposed groups markedly protected DNA damage as demonstrated in lanes 6, 7, and 8, respectively, of electrophoretograms revealing genoprotective efficacy of TA. Moderate DNA preventive efficacy of TA was seen in Cd–Ni co-exposure group (Fig. 6a, b).

Discussion

Co-exposure to Cd and Ni may produce additive or supra- or sub-additive interactions that could enhance their toxic effects. Evaluating these interactions is essential for risk assessment and management of toxicity related to their co-exposure [36]. In the present study, 30-day cadmium and nickel exposures exhibited a marked elevation in lipid peroxidation in hepatic and renal tissues. These observations comply with the observations of other studies, who have documented the cytotoxic effects of cadmium and nickel in rats [37–39]. Similarly, the co-exposure of Cd and Ni caused an additive effect in LPO in hepato-renal organs. Moreover, a significant decline of GSH levels was also recorded in the liver and kidney of Cd, Ni individual and co-treated groups. These results are validated by the studies of Micali et al. (2018) and Yu et al. (2018), who observed decreased levels of GSH after co-exposure intoxication [40, 41]. The most probable reason for augmented LPO and decreased GSH could be the enhanced generation of free radical species, like superoxide anions, hydroxyl radicals, and hydrogen peroxide by nickel and high affinity of cadmium toward the thiol group of GSH [38, 42]. Enhanced levels of free radicals cause oxidation of cellular macromolecules, which formed the basis of redox imbalance, genotoxicity, and histological alterations [43].

Oxidative stress-mediated suppression of cellular antioxidant defense was confirmed by declined levels of CAT, SOD, GST, and GR, in individual and co-exposed groups. Most plausibly, augmented consumption of these enzymatic

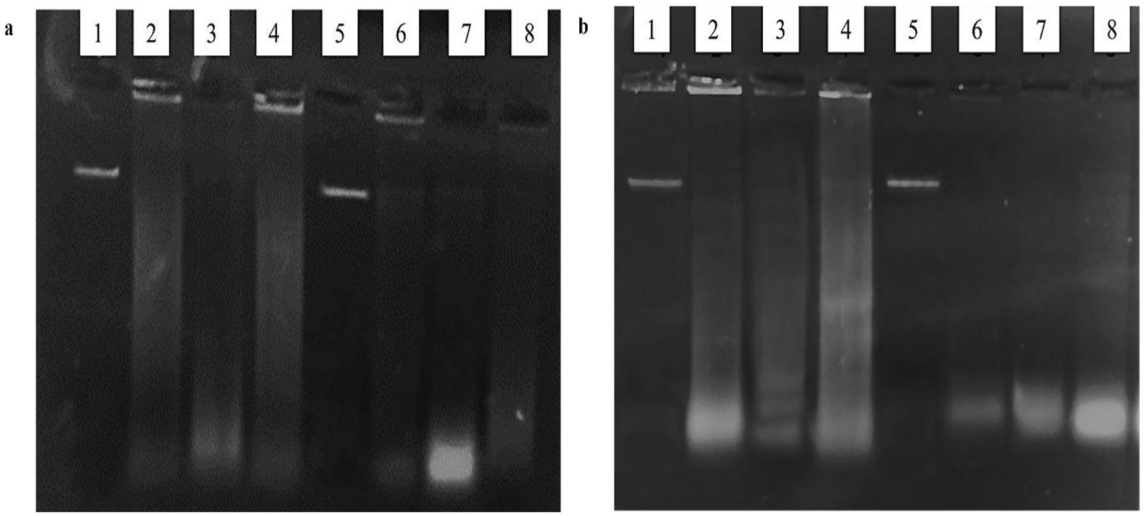

Fig. 6 Representative agarose-gel electrophoretograms of extracted DNA from **a** liver and **b** kidney of BALB/c mice. Control (lane 1), cadmium (lane 2), nickel (lane 3), Cd + Ni (lane 4), TA (lane 5), TA + Cd (lane 6), TA + Ni (lane 7), and TA + Cd + Ni (lane 8) treated mice

antioxidants in scavenging free radicals instigated unalterable inhibition in their activities [8, 10, 14]. Many authors have documented similar observations of heavy metal-induced suppression in antioxidant enzymes and support the present study [44–46]. Moreover, present study has evidently exhibited that combinational exposure of Cd and Ni has supra-additive effect on cellular antioxidant suppression. In current study, TA-mediated reduction in LPO could be due to free radical scavenging or electron transferring ability to electrophilic radicals. Phenolic groups of TA might chelate the metal ions and stop the progression of free radical formation via complexing ferrous ions and inhibiting steps of the Fenton reaction [19, 47]. These properties of TA allow it to act as a reducing agent by converting Fe (II) to Fe (III), hydrogen donor, and quencher of singlet oxygen [19, 21]. TA scavenges reactive metabolites due to the presence of the galloyl moiety, allowing efficient $H^{\bullet}$-atom transfer to the free radicals and converting them to less reactive metabolites [48]. In the present study, tannic acid reduced LPO and restored the levels of antioxidants in hepato-renal tissues and re-established the balance of pro- and antioxidants.

Oxidative stress and subsequent lipid peroxidation might have formed the basis for observed histoarchitectural alterations in hepatic and renal tissues following Cd–Ni individual and co-exposures. These observations are in accordance with the study of Yu et al. (2018) and Zou et al. (2020) who have documented cadmium- and nickel-induced histological changes in hepatic tissue [41, 49]. Furthermore, observed increase in levels of AST and ALT frequently utilized indicators of hepatocellular necrosis, correlates and validates Cd–Ni co-exposure-induced hepatocellular damage. Multiple reports support these observations and highlight the adverse effects of heavy metal co-exposure in liver [14, 31, 39, 50]. Kidneys being the principle excretory organ exhibits evident metal co-exposure-induced tissue damage. Plausibly, these metals get attached to the lipid membranes of the glomerulus and result in the accumulation of lipid droplets in the voids of the glomerular membrane, which results in decreased glomerular filtration rate and increase in nitrogenous waste products (urea and creatinine) in the serum in intoxicated metal groups [9, 44]. Observed elevation in renal LPO, kidney function markers, and histological alterations are in consensus with above mentioned reports. However, TA treatment significantly lowered the levels of serum hepato-renal function biomarkers in Cd–Ni individual and co-exposure groups, indicating its redox balancing and ameliorative potential. This study is in close agreement with the observations of Akomolafe et al. (2014), who have studied the modulatory potential of TA against cisplatin-induced nephrotoxicity in rats [51].

Moreover, the present study revealed Cd–Ni co-exposure-induced sub-additive effect in causing genotoxicity. Genotoxic potential of these metals is confirmed by DNA fragmentation, and enhanced micronucleation and comet formation in bone marrow cells. Cd and Ni exposures lead to increased free radicals, which can induce DNA cross-links, DNA strand breaks, and modification of DNA bases. The present observations of enhanced comet tailing and micronucleation in Cd–Ni co-exposure are in accordance with studies of Kaushal et al. (2019) and El-Habit and Moneim (2014), who have also documented heavy metal-induced genotoxicity [52, 53]. Cd–Ni individual and co-exposure demonstrate genotoxic potential of these moieties plausibly by inhibiting DNA repair enzymes [44, 49, 52]. Co-exposure of these metals can cause DNA modifications such as fragmentation, micronuclei formation,

chromosomal aberrations, and aneuploidogenicity by direct binding to DNA and/or enhancing the effects of other mutagens to generate genetic lesions [14, 54–57]. In the present study, TA has exerted its genoprotective potential possibly by two mechanisms: first, by preventing the oxidative damage of DNA by directly quenching metal ions and harmful free radical species, and second, by binding to DNA and reducing its susceptibility to damaging effects of heavy metals [58, 59]. However, its moderate efficacy against Cd–Ni co-exposure is plausibly due to competition among heavy metals for binding sites on TA, potentially hindering metal ion sequestration. Overall, this study suggests that free radical scavenging and metal chelating properties of TA have helped in restoring cellular redox imbalance, integrity of DNA, and normal histoarchitecture of hepato-renal tissues in Cd–Ni individual and co-exposure groups. Thus, it is noteworthy to add that use of TA containing drinks is an effective, economical, and convenient source of antioxidants, if added in daily routine.

Taken together, present observations suggest that Cd is a more potent toxicant than Ni metal in individual exposures, while their combinational exposure exhibits either supra-additive, additive, or sub-additive effects. Owing to antioxidant and genoprotective properties, TA exhibits remarkable ameliorative efficacy against individual metal exposure and moderate efficacy in co-exposure group. For optimizing TA efficacy in co-exposure scenario, exploring various TA dosages, potential structural modifications to enhance its selectivity or capacity, and understanding its interactions with metal ions at molecular level are pivotal. Although TA-based nano-formulations are worthy of further investigation.

Author Contribution All authors contributed to the study's conception and design. Material preparation, data collection, and analysis were performed by M.S., P.D., S.K., A.u.A., S.M., M.B., and M.C. The first draft of the manuscript (along with figures) was prepared by M.S., and all the authors commented on all versions of the manuscript. All authors read and approved the final manuscript.

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Data Availability The data that support the findings of this study are available from the corresponding author, Chopra M, upon reasonable request.

Declarations

Ethics Approval This study was performed as per the "Guide for the Care and Use of Experimental Animals" approved by the Institutional Animal Ethics Committee (IAEC), Panjab University (PU/45/99/CPCSEA/IAEC/2015/678).

Competing Interests The authors declare no competing interests.

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