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Aloe vera (L.) Burm.f. renders protection against chromium-induced damage in mice: A preliminary study on renal and testicular tissues

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The present study was designed to assess the modulatory effects of *Aloe vera* on chromium-induced alterations in renal and testicular tissues of mice. For this study, male Balb/c were divided into four groups: Group I (control), Group II (PD) [orally administered with potassium dichromate], group III (AV) [orally administered with *Aloe vera*] and group IV (AV+PD) [administered with *Aloe vera* and potassium dichromate]. Histopathological alterations and levels of renal (urea, blood urea nitrogen, uric acid, creatinine) and testicular (sperm count and motility) organ function markers indicated damage to these organs. This was accompanied by increased serum lactate dehydrogenase activity. Chromium appeared to weaken the DNA repair machinery of the cells, as observed from the decrease in mRNA expression of DNA-PK and MGMT genes in renal tissue. Improvement in histoarchitecture and functional markers of organs, decrease in cell damage marker, and increased expression of DNA repair-associated genes are indicative of protection rendered by *A. vera* against chromium-induced harmful effects. This preliminary study indicates the beneficial effects of *A. vera* against heavy metal toxicity. However, it is imperative to exhaustively study the possible modulatory effects of *A. vera* and decipher the underlying mechanisms at play during the amelioration of chromium-induced toxicity. Such studies would warrant its use against managing heavy metal toxicity.

Keywords: *Aloe vera*, Chromium, Heavy metal, Renal, Testicular, Toxicity

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Introduction

The natural presence of heavy metals and their widespread use in industry, technology and medicine has made them an imminent threat to the environment and all forms of life. Most of the environmental contamination and human exposure to heavy metals results from anthropogenic activities such as mining and smelting operations, industrial production and use of metals and metal-containing compounds[1]. Chromium (Cr) is one of the most dangerous heavy metals found naturally in volcanic dust, minerals and soil. Its abundant use in industrial operations makes it a common environmental pollutant[2]. This element has a high redox potential, and its oxidation states range from (0) to (VI) and have varying stability. Trivalent Cr [Cr (III)] and hexavalent Cr [Cr (VI)] are its most stable forms. In comparison to Cr(III), Cr(VI) is more water soluble and toxic, thus harming animals and humans more severely[3].

Cr(VI) and its metabolites, particularly chromates, enter the human body through dermal exposure, inhalation and ingestion. The International Agency for Research on Cancer has classified Cr(VI) as a Group I occupational carcinogen[4]. Cr(VI) compounds can cause various health problems such as chronic skin and nasal irritation, emphysema, contact dermatitis, ulceration, cancer, etc[4]. Some ways by which chromium exerts its damaging effects include enhancing oxidative stress, mitochondrial damage, genomic instability, mutations, epigenetic modifications, adduct formation with various macromolecules, etc[5]. The reactions between Cr(VI) and biological reductants like thiols and ascorbate result in the overwhelming production of reactive oxygen species (ROS), culminating in oxidative stress in cells[6]. Cr(VI) can interfere with cofactor fixation sites in enzymes, inhibit vital enzyme-mediated reactions, weaken the immune system, alter cellular architecture, etc., all of which have deleterious consequences[4]. Observations in humans and experimental animals have revealed that exposure to chromium and its compounds leads to systemic harmful effects in the body. Impairment in kidney function and detrimental changes in kidney tissue architecture have

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been observed in rats administered with Cr(VI) compounds[7-9]. Chromium exposure has been known to cause functional and morphological effects in male reproductive organs in monkeys, rats, mice, and rabbits[10]. Studies have revealed abnormal levels of genotoxic and oxidative stress markers in workers from several Cr(VI) related industrial activities[11,12].

Although chelation therapy is commonly used for managing heavy metal toxicity, it has been suggested to use combination therapy involving antioxidants to mitigate adverse effects and overcome the limitations of synthetic chelating agents[13,14]. Plants have been attracting attention for ameliorating metal toxicity because of their fewer side effects and being economically viable[15,16]. The presence of phytochelatins, metallothioneins and numerous antioxidants in plants facilitate metal detoxification and mitigate oxidative stress emanating from heavy metal exposure[17,18].

Aloe barbadensis Mill., a synonym of *Aloe vera* (L.) Burm.f., is a member of the Liliaceae family. This plant is a treasure trove of various phytochemicals, minerals, carbohydrates, enzymes, amino acids, vitamins, etc., all of which contribute to its many medicinal benefits such as immunomodulatory, anti-inflammatory, antifungal, hypoglycemic, antiulcer, radiomodulatory[19,20]. A literature survey has revealed that *A. vera* has the potential to mitigate the harmful effects of metal exposure. In rodents, protective effects of this plant have been observed against metals like arsenic, cadmium and aluminum[16,21-23]. This was evident from the effects observed in hepatic, renal and testicular tissues. To the best of our knowledge, no reports are available on its modulatory effects on rodents in response to chromium exposure.

Since chromium has widespread use, its exposure and consequential harmful effects are imminent. It thus becomes necessary to study and develop safe ways to manage its toxic effects. Upon noting the positive effects of *A. vera* in mitigating damage caused by other heavy metals, the present study was designed to assess the modulatory effects of *A. vera* on chromium-induced effects in mice. The results of this preliminary study indicate its beneficial effects in managing chromium-induced toxicity in renal and testicular tissues of mice.

Materials and Methods

Chemicals

Potassium Dichromate (K_2CrO_7) was purchased from Qualigens (India). Diethyl pyrocarbonate (DEPC) and tri-reagent were procured from Sigma Chemical Co. (St Louis, MO, USA). Molecular biology-grade chemicals for RNA isolation were purchased from Amresco, Ohio (USA). One-step Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) kit was obtained from Thermo Fisher Scientific (USA). Primer pairs were synthesised by Sigma-Aldrich (India). Nicotinamide adenine dinucleotide hydrogen (NADH), agarose, bovine serum albumin (BSA), ethidium bromide (EtBr) and Folin-ciocalteu reagent were purchased from reputed Indian manufacturers (Sisco Research Laboratory Pvt. Ltd, Central Drug House Pvt. Ltd., Himedia Pvt. Ltd.). Diagnostic kits for renal function markers were obtained from Reckon Diagnostics Pvt. Ltd (ENZOPAK, India). Other chemicals used for reagent preparation were purchased from reputed Indian manufacturers and were of the highest purity/ analytical grade.

Plant sample collection and preparation of *A. vera* gel extract

Fresh leaves of *A. vera* (Herbarium PAN no. 21953) were collected from the botanical garden of Panjab University, Chandigarh during the months of October-November (2021). *Aloe vera* gel extract was prepared in our laboratory following the method reported by us previously[19,24]. After washing the leaves, the colourless gel was separated from the thick outer green cuticle. The gel was put in an oven at 60°C for 72 hours until it dried into a powder. This powder was reconstituted in distilled water, vortexed, and then centrifuged for 10 minutes at 5000Xg. The clear supernatant was collected and filtered through Whatman filter paper (0.45 mm) to get a filtrate, which was then dried at 60°C oven for 72 hours. The powdered extract obtained was reconstituted in distilled water before its oral administration to the animals. We have reported previously that phytochemical analysis of this extract indicated the presence of carbohydrates, tannins and anthraquinones. 2-azino-bis-3-ethylbenzothiazoline-6-suphonic acid (ABTS) and 1,1-Diphenyl-2-picrylhydrazyl (DPPH) radical scavenging assays were used to demonstrate the free radical scavenging potential of *A. vera* gel extract[19]. The extract exhibited concentration-dependent enhancement in radical scavenging activity with IC_{50} values of 20 and 50 µg/uL in ABTS and DPPH assays, respectively.

Treatment of animals and study design

The experimental protocols were approved (approval no: PU/45/99/CPCSEA/IAEC/2021/581) by

the Institutional Ethics Committee of Panjab University, Chandigarh, India, and were then conducted according to the Indian National Science Academy guidelines for the use of experimental animals. Healthy male Balb/c mice weighing (30-35 g) were obtained from Central Animal House, Panjab University, Chandigarh, India. Mice were housed in the animal house at the Department of Biophysics, Panjab University, Chandigarh, India. The animals were kept in polypropylene cages bedded with sterilised rice husk and provided with a standard pellet diet and water *ad libitum* throughout the treatment period. After one week of acclimatisation to the experimental conditions, animals were randomly divided into four groups (n=6-8) based on their treatment.

Group I (control) animals were given a normal diet and water *ad libitum*. Group II (PD) animals were orally administered with potassium dichromate daily for 30 days at a dose of 10 mg/kg b.w.[25]. Group III (AV) animals were orally administered with *A. vera* gel extract at a dose of 50 mg/kg b.w. on alternate days for 45 days. Group IV (AV+PD) animals were orally administered with *A. vera* gel extract and potassium dichromate, as explained for Group II and Group III, respectively. In group IV, *A. vera* gel extract administration was started 15 days prior to potassium dichromate. *A. vera* gel extract and potassium dichromate were dissolved in distilled water for oral administration. The dose of *A. vera* gel extract was standardised in our laboratory, considering the reports available in the literature. *A. vera* gel extract at a dose of 50 mg/kg b.w did not cause any detrimental effects, as evidenced by normal histoarchitecture and biochemical parameters of various organs [26]. Blood was withdrawn from the retro-orbital plexus on the 30th and 45th day to assess tissue injury and renal function markers in serum. Kidneys and testes were excised on the 45th day, and various assessments were carried out in appropriate samples.

Histopathological studies

Haematoxylin and Eosin (H&E) staining was carried out using the method described in the literature[27]. After the completion of the treatment regimen, animals were dissected, and tissues were excised and immediately fixed in neutral 10% formalin for 12 hours. After fixation, the tissues were dehydrated in increasing ethanol concentrations (30, 50, 70, 90, and 100%) for 1 hour each. After dehydration, the tissues were immersed in benzene for 30 minutes before being successively moved into 1:1 benzene: paraffin wax (melting point 58–60°C) for 1 hour, followed by two 3-hour changes in pure melted wax. This was followed by the final embedding in paraffin wax. These samples were manually sectioned with a rotary microtome to obtain 4–5 µm-thick paraffin sections and then transferred to glass slides. The slides were dewaxed in xylene for staining and rehydrated for 2 minutes in descending alcohol concentration (100, 90, 70, 50, and 30%). After this, the slides were immersed in water for 1 minute before being dipped in hematoxylin for 15-20 seconds. If the tissue got overstained, it was dipped in acid water, and if the staining was appropriate, it was dipped in ammonia water for stain fixing. Tissues were then dehydrated in increasing concentrations of alcohol (30, 50, and 70%) for 2 minutes each. They were stained with eosin for 15-20 seconds before being dipped in 90 and 100% alcohol for 1 minute each. After that, the slides were immersed in xylene for 20 minutes. The stained sections were mounted using distyrene polystyrene xylene and examined with a light microscope (Leica DM 3000).

Lactate dehydrogenase (LDH)

The activity of LDH in serum was calculated using the method described in the literature[28]. For the reaction mixture, 800 µL of phosphate buffer (50 mM, pH 7.5), 50 µL of sodium pyruvate (0.5 mM), 50 µL of NADH (0.1 mM), and 10 µL of serum sample were added to 1 mL cuvette. The enzyme activity was measured at 340 nm by monitoring the oxidation of NADH as the decrease in OD/min for a minimum of 3 minutes. The results were expressed as nanomoles of NADH consumed/min/mg protein using an extinction coefficient of 6.22 $mM^{-1} cm^{-1}$.

Renal function markers

Renal function markers were estimated in serum using kits [creatinine: CC3-CRK.008; urea: CC2-UAB.019; uric acid: CC2-URK-20N (MFG: 04/2021-ED: 03/2023)] obtained from Reckon Diagnostics, India. The procedure described by the kit manufacturer was followed to carry out the tests. The absorbance of the coloured complex produced was recorded using a Shimadzu UV spectrophotometer (UV-1800).

Testicular function markers

Sperm count and sperm motility were calculated according to the method described in literature[29]. The

cauda epididymis (free of fat, vas deferens and other tissue) of mice was removed from each side and squeezed out in 1 mL of saline solution (0.9% NaCl). The number of spermatozoa was counted using a haemocytometer and expressed as sperm counts per mm^3. For sperm motility, the spermatozoa from the vas deferens were removed and flushed out into 0.2 mL normal saline. Motility was assessed by determining the motile and non-motile spermatozoa counted under a light microscope. Motility was expressed as a percentage of motile spermatozoa.

mRNA expression of genes

mRNA expression of DNA damage repair genes was assessed in renal tissue by using endpoint assay, i.e. reverse transcriptase polymerase chain reaction (RT-PCR). Total RNA was isolated from the kidney using a tri-reagent and used for PCR analysis. The reaction mixture was prepared according to the kit manufacturer's instructions, and the RT-PCR reaction was performed in a thermal cycler using the following conditions: the RT reaction was performed at 50°C for 50 minutes and activation at 95°C for 1 minute. PCR was followed by 35 cycles of 94°C (denaturation) for 45 seconds, 56°C (annealing, variable) for 45 seconds, and 72°C (extension) for 1 minute. Finally, the products were incubated at 72°C for 10 minutes to extend any incomplete single strand. The final PCR products were analysed using 1.5% agarose gel electrophoresis, and densitometric analysis of the bands was done using Image J software (NIH, USA). *β*-actin was used as a loading control. The following primers were used: MGMT [forward primer: 5-CAGTGAGGCTGTGTTTGA GACC-3; reverse primer: 5-GCCAGGGCTGCTAA TTGCT-3] (NM_001377037); DNA-PK [forward primer: 5-TCACCACAACCCTGCTCATGC-3; reverse primer: 5-AGACTGCAGGCACTGCCTTTCC-3] (NM011159) and β-actin [forward primer:5-ATCCGTAAAGACCTCTAT-3; reverse primer:5-AACGCAGCTCAGTAACAG-3][30].

Protein estimation

Protein in serum samples was estimated by the method described in the literature[31]. The test is based on the formation of a coloured cupric protein complex when the protein sample is treated with alkaline copper tartarate. The intense blue colour produced with the Folin-Ciocalteau reagent is a result of the reduction of phosphomolybdic acid and phosphotungstic acid by aromatic amino acids, which can be read at 620 nm. BSA was used as the standard to estimate the protein content.

Statistical analysis

Data is expressed as Mean±S.D. The statistical evaluation of data was performed using SPSS software (version 22.0). A one-way analysis of variance (ANOVA), followed by Boniferroni's post hoc test, was used to assess statistical significance. $P \leq 0.05$ was considered statistically significant.

Results

Assessment of alterations in renal tissue architecture

Kidneys from control and AV groups revealed normal histoarchitecture with regular glomeruli in the cortex and corticomedullary region. PD-exposed animals exhibited a decrease in the number and increased size of renal corpuscles, enlarged glomerulus, glomeruli showing large Bowman's space, vacuolation, degeneration of cells of the renal tubules and necrosis in epithelial cells of the proximal tubules. In the AV+PD group, histoarchitectural alterations were evidenced mainly by shrinkage of the glomerular tuft. The thick and thin parts of the loop of Henle and collecting ducts present in the inner medulla revealed normal histoarchitecture in all the treatment groups (Fig. 1).

Assessment of renal function markers

Urea

At 30 and 45 days of treatment, a significant increase in serum urea level was observed in the PD group compared to the control and AV groups. A significant decrease was observed in serum urea level in the AV+PD group compared to the PD group, and a significant increase was observed compared to the control group. At 30 days, the serum of the AV+PD group exhibited an increase in urea level compared to the AV group; however, no alterations were observed at 45 days. No change in serum urea level was observed in the AV group compared to the control group (Fig. 2a and 3a).

Blood urea nitrogen (BUN)

At 30 and 45 days of treatment, a significant increase in BUN level was observed in the PD group compared to the control and AV groups. A significant decrease was observed in BUN level in the AV+PD group compared to the PD group, and a significant increase was observed compared to the control group. At 30 days, the serum of the AV+PD group exhibited

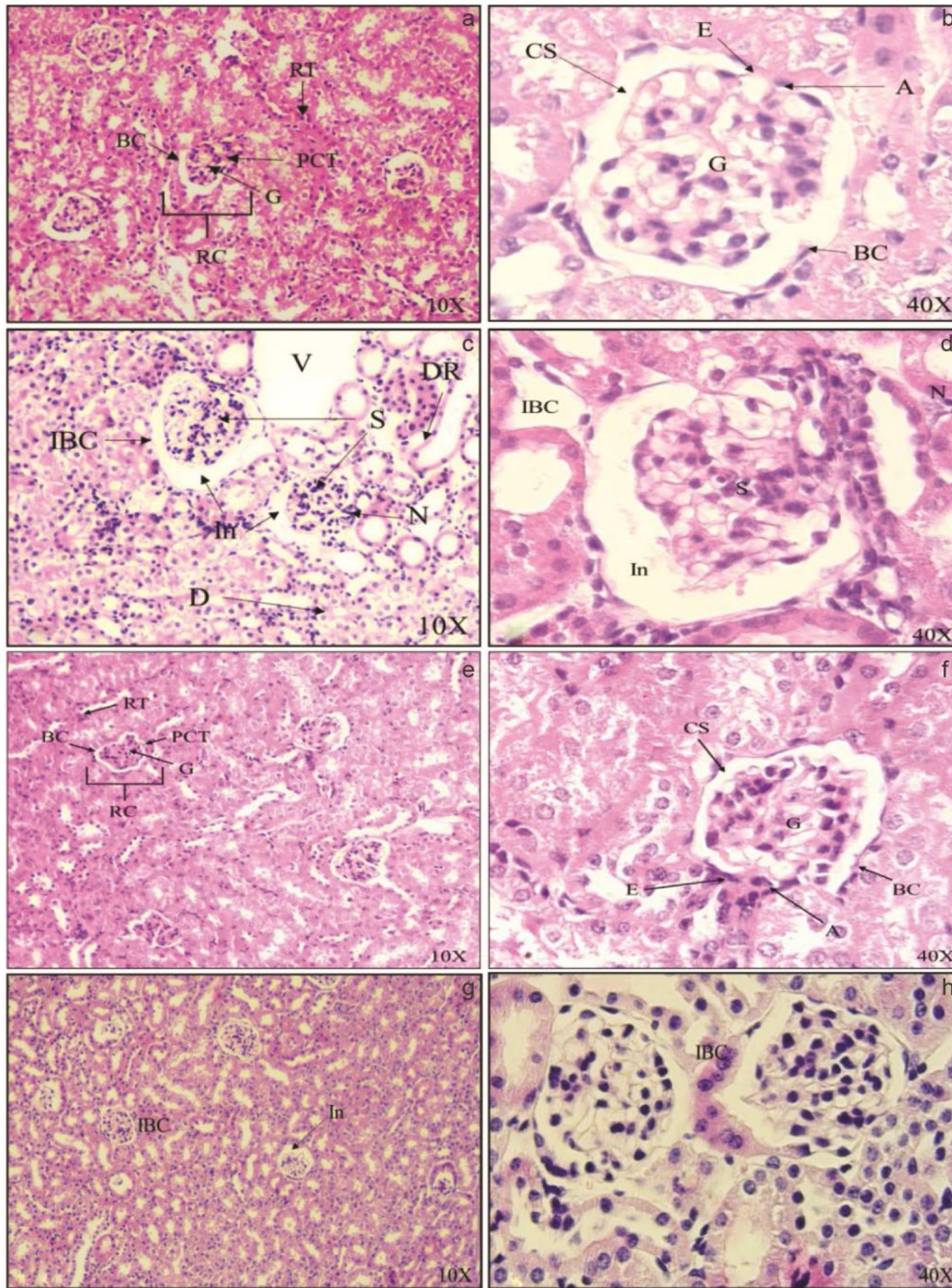

Fig. 1 — Hematoxylin and Eosin stained sections of kidney (cortex), a,b) Control [10x, 40x]; c,d) PD [10x, 40x]; e,f) AV [10x, 40x]; and g,h) AV+PD [10x, 40x].
[Bowman's capsule (BC), Glomerulus (G), Proximal convoluted tubule (PCT), Renal corpuscle (RC), Capsular space (CS), Renal tubules (RT), Afferent arteriole (A), Efferent arteriole (E), decrease in number of renal corpuscles (D), enlarged glomerulus (EG), shrinkage of glomerular tuft (S), glomeruli showing large Bowman's space (In), increased renal corpuscle size (IRC) vacuolation (V), degeneration of cells of the renal tubles (DR) and necrosis in epithelial cells of the proximal tubules (N)].

an increase in BUN level compared to the AV group; however, no alterations were observed at 45 days. No change in BUN level was observed in the AV group compared to the control group (Fig. 2b and 3b).

Uric acid

At 30 and 45 days of treatment, serum uric acid level was observed to increase in the PD group compared to the control group and remain unaltered compared to the AV group. In the AV+PD group,

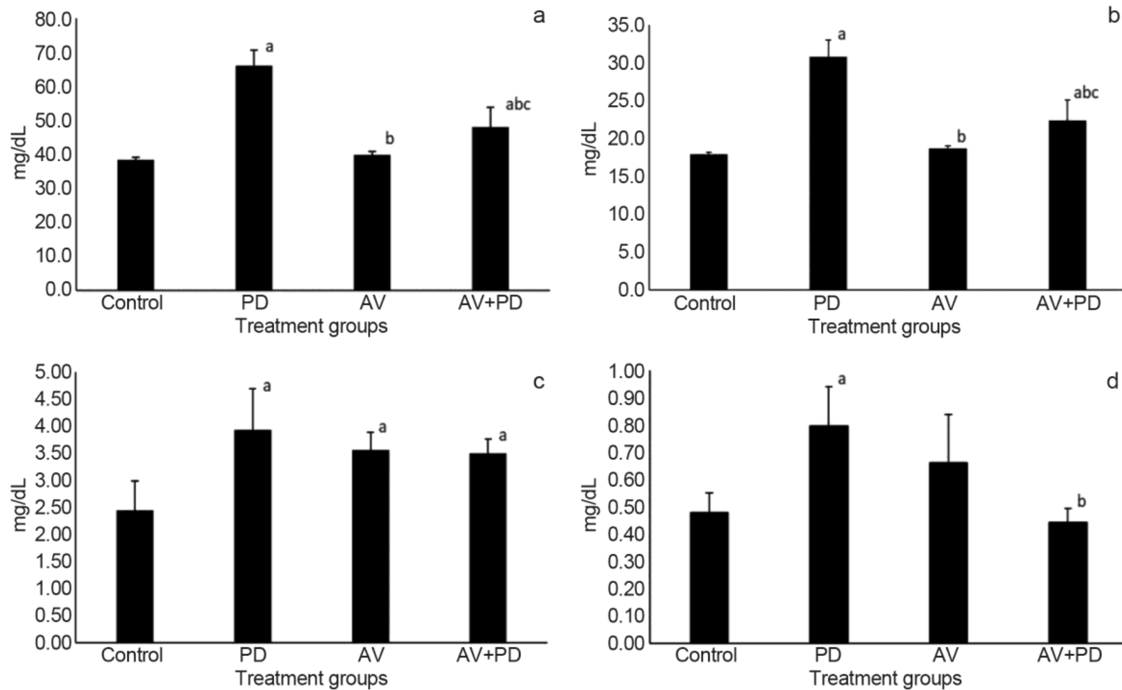

Fig. 2 — Modulatory effect of *A. vera* on renal function markers during potassium dichromate administration in mice (at 30 days). a) Urea; b) BUN; c) Uric acid; and d) Creatinine.
Data is represented as Mean±SD (n=6-8) and analysed by One Way ANOVA followed by Bonferroni post hoc test [a]$P \leq 0.05$ significant wrt control group; [b]$P \leq 0.05$ significant wrt PD group; [c]$P \leq 0.05$ significant wrt AV group.

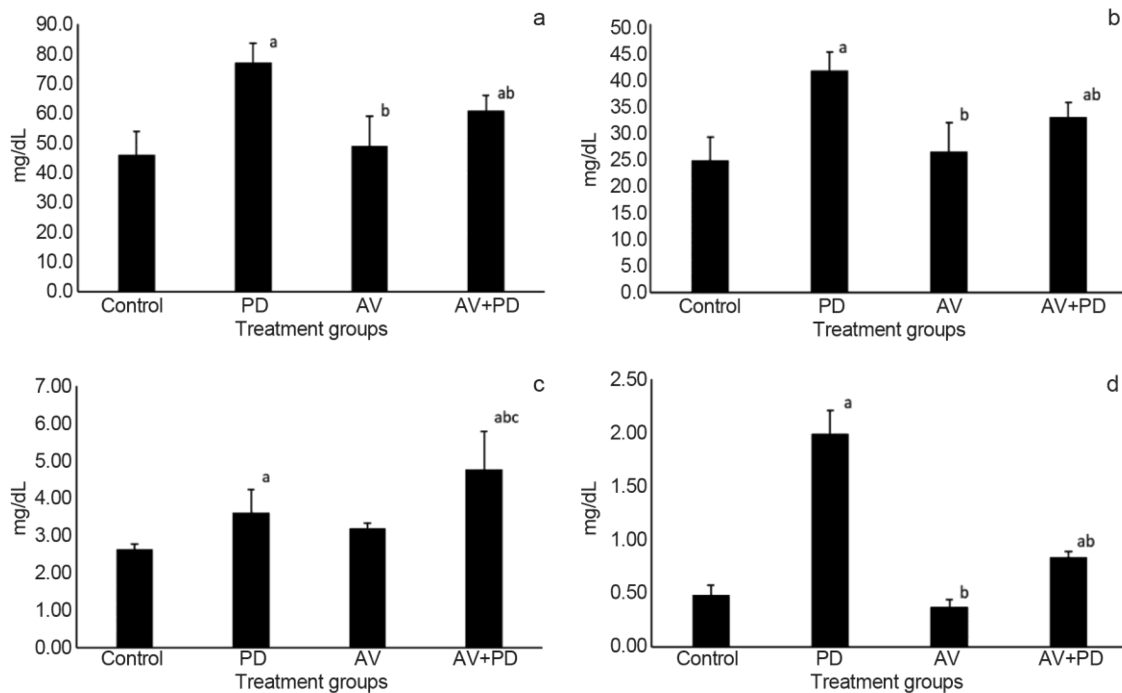

Fig. 3 — Modulatory effect of *A. vera* on renal function markers during potassium dichromate administration in mice (at 45 days). a) Urea; b) BUN; c) Uric acid; and d) Creatinine.
Data is represented as Mean±SD (n=6-8) and analysed by One Way ANOVA followed by Bonferroni post hoc test [a]$P \leq 0.05$ significant wrt control group; [b]$P \leq 0.05$ significant wrt PD group; [c]$P \leq 0.05$ significant wrt AV group.

differential changes were observed. At 30 days, no significant change was observed in serum uric acid level in the AV+PD group compared to the PD and AV groups. However, a significant increase was observed when compared to the control group. A significant increase was observed in serum uric acid levels in the AV group compared to the control group. At 45 days, a significant increase was observed in serum uric acid level in the AV+PD group compared to PD, control, and AV groups. No change in serum uric acid level was observed in the AV group compared to the control group (Fig. 2c and 3c).

Creatinine

At 30 and 45 days of treatment, a significant increase in serum creatinine level was observed in the PD group compared to the control group. A significant decrease was observed in the AV+PD group compared to the PD group. In the AV+PD group, at 30 days, no change was observed compared to the control and AV groups and at 45 days, a decrease was observed in comparison to the control group, and no change was observed in comparison to the AV group. No change was observed in the AV group compared to the control group (Fig. 2d and 3d).

Assessment of DNA damage repair associated genes

A significant decrease in the mRNA expression of MGMT was observed in the PD group compared to the control group. The expression in the AV+PD group increased compared to the PD and AV groups. No change was observed in the AV+PD group compared to the control group. The AV group showed a significant decrease in expression compared to the control group (Fig. 4a).

A significant decrease in the mRNA expression of DNA-PK was observed in the PD group compared to the control group. The expression in the AV+PD group increased compared to the PD and control groups. No change was observed in the AV+PD group compared to the AV group. No change was observed in the AV group compared to the control group (Fig. 4b).

Assessment of alterations in testicular tissue architecture

Testes from control and AV groups showed normal histoarchitecture, which consisted of well-organized seminiferous tubules with all types of spermatogonial cells and normal interstitial connective tissue. Testes of PD-administered animals revealed an increase in interstitial space, shrinkage of seminiferous tubules and Leydig cell damage. Animals of the AV+PD group revealed organised histoarchitecture of

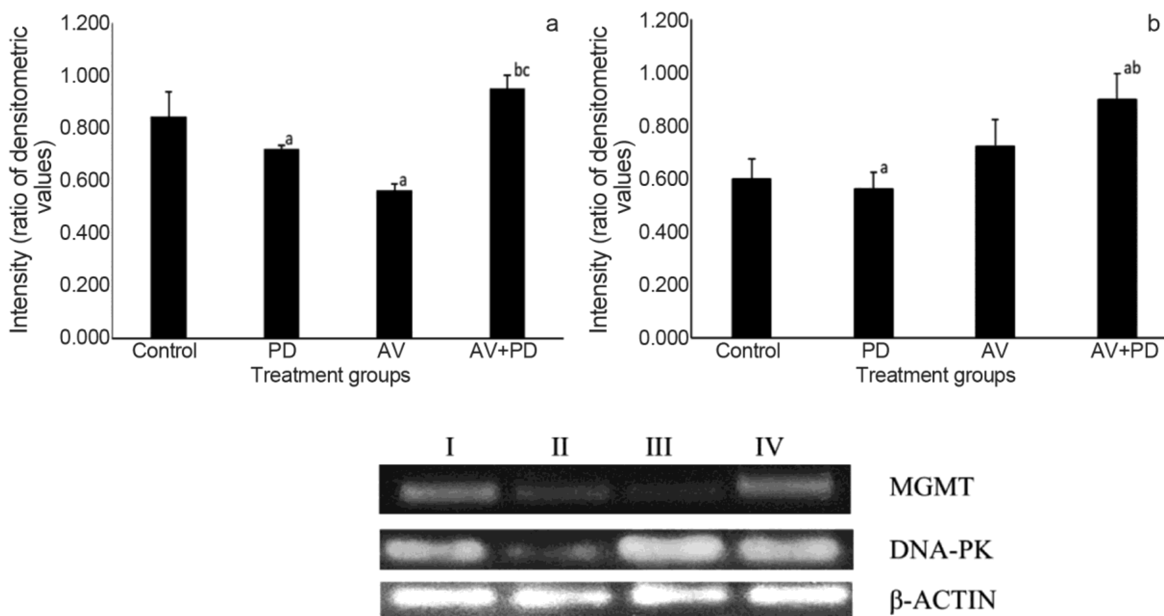

Fig. 4 — Modulatory effect of *A. vera* on mRNA expression of DNA damage repair associated genes during potassium dichromate administration in mice.
(Lane I: Control; Lane II: PD; Lane III: AV; Lane IV: AV+PD). a) O-6-methylguanine-DNA methyltransferase (MGMT); and b) DNA-dependent protein kinase (DNA-PK).
Data is represented as Mean±SD (n=3) and analysed by One Way ANOVA followed by Bonferroni post hoc test [a]$P \leq 0.05$ significant wrt control group; [b]$P \leq 0.05$ significant wrt PD group; [c]$P \leq 0.05$ significant wrt AV group.

seminiferous tubules but exhibited debris in the lumen of the testes (Fig. 5).

Assessment of testicular function markers

Sperm count was observed to decrease in the PD group when compared to the control and AV groups. A significant increase was observed in sperm count in the AV+PD group compared to the PD group. No change in sperm count was observed in the AV and AV+PD groups compared to the control group (Fig. 6a).

Sperm motility was observed to decrease in the PD group when compared to the control and AV groups. A significant increase was observed in sperm motility in the AV+PD group compared to the PD group. No change in sperm motility was observed in the AV and AV+PD groups compared to the control group (Fig. 6b).

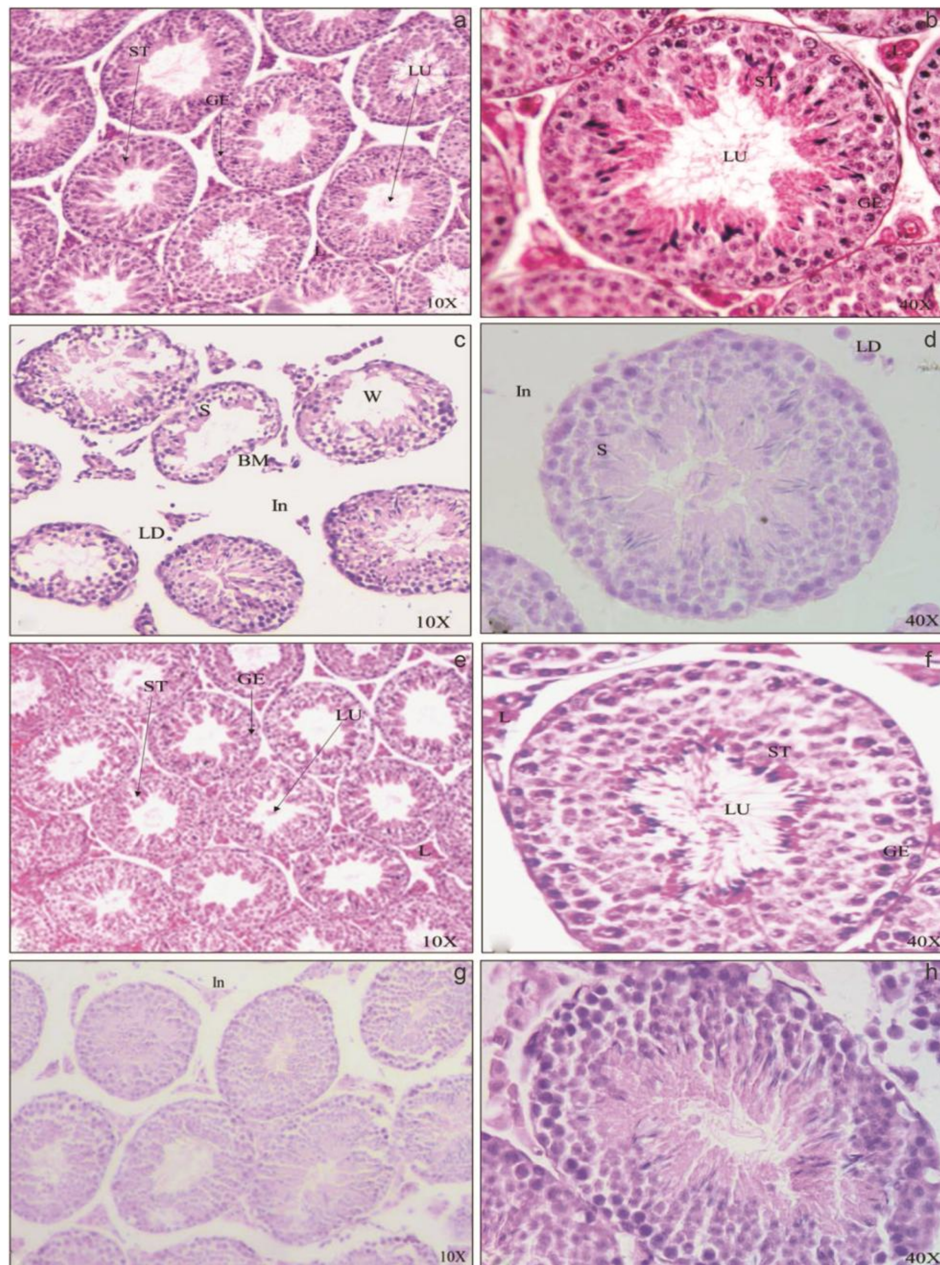

Fig. 5 — Hematoxylin and Eosin stained section of testis. (a,b) Control [10x, 40x]; (c,d) PD [10x, 40x]; (e,f) AV [10x, 40x]; (g,h) AV+PD [10x, 40x].
[Seminiferous tubules (ST), Lumen (LU), Germinal epithelium (GE), Leydig cells (L), Increased interstitial space (In), Small and distorted seminiferous tubules (S), Leydig cell damage (LD), Lumen decreased (W), Basement membrane damage (BM)].

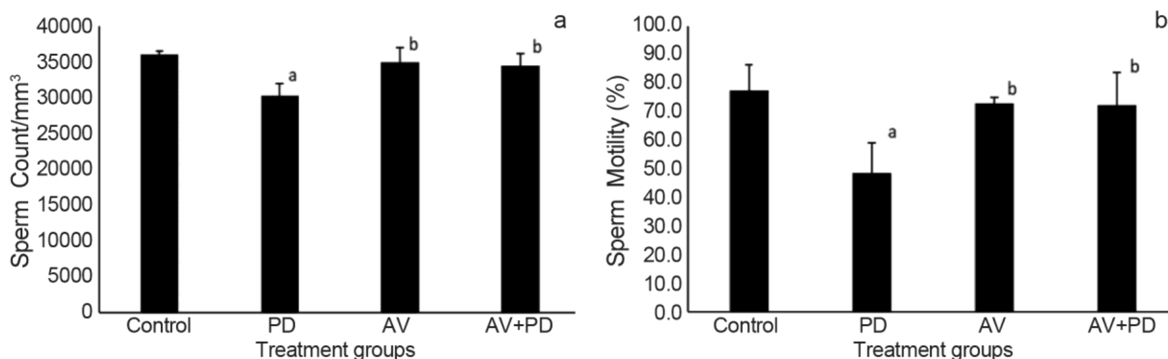

Fig. 6 — Modulatory effect of *A. vera* on testicular function markers during potassium dichromate administration in mice (at 45 days). a) Sperm count, and b) Sperm motility.
Data is represented as Mean±SD (n=3) and analysed by One Way ANOVA followed by Bonferroni post hoc test [a]$P \leq 0.05$ significant wrt control group; [b]$P \leq 0.05$ significant wrt PD group.

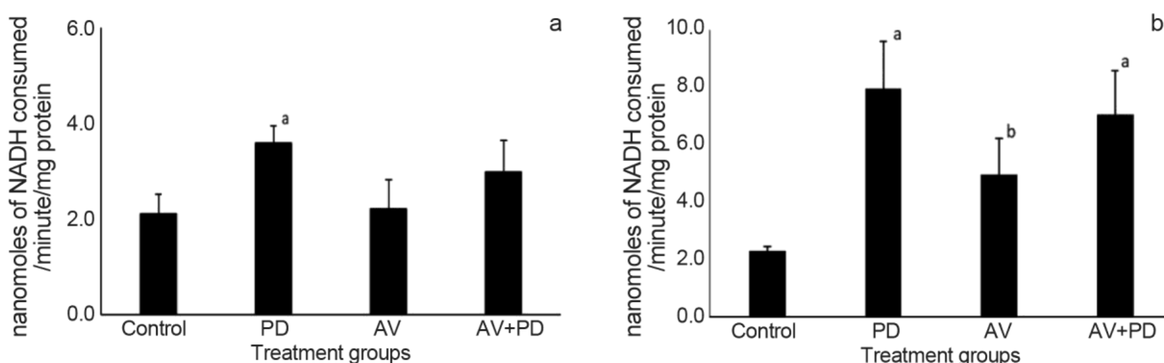

Fig. 7 — Modulatory effect of *A. vera* on serum LDH activity during potassium dichromate administration in mice. a) 30 Days; and b) 45 Days.
Data is represented as Mean±SD (n=4-6) and analysed by One Way ANOVA followed by Bonferroni post hoc test [a]$P \leq 0.05$ significant wrt control group; [b]$P \leq 0.05$ significant wrt PD group.

Assessment of tissue injury marker

At 30 and 45 days of treatment, a significant increase in serum LDH activity was observed in the PD group compared to the control group. At both these time points, serum LDH activity remained unaltered in the AV+PD group compared to the PD group, increased compared to the control group (at 45 days) and remained unaffected compared to the control group (at 30 days). No significant change was observed in serum LDH activity between the control and AV groups (Fig. 7a and 7b).

Discussion

Globalisation and exhaustive industrialisation have increased heavy metal pollution worldwide. The non-biodegradability of chromium leads to its bioaccumulation and its consequential harmful effects[32]. Studies on animal models and humans make it amply clear that safe and effective agents must be continuously explored to help ameliorate chromium-induced toxic effects. Although experimental reports are available that demonstrate the beneficial effects of *A. vera* against many heavy metals, to the best of our knowledge, no reports are available indicating its potential to counteract the effects of chromium exposure in rodents. Keeping this in mind, the present study was designed to determine the modulatory effects of *A. vera* against potassium dichromate-induced damage in renal and testicular tissues of mice.

Urea, BUN, uric acid, and creatinine are some of the routine clinical markers that are closely monitored to keep track of the progress of renal pathologies. In the present study, abnormally high levels of these markers in the PD group serum indicate aberrant kidney function. Several reports with similar observations suggested chromium administration to rodents disrupted their kidney functioning, evidenced by the deranged serum markers[33-37]. In the AV+PD group, animals showed a significant improvement in

these markers when compared to PD-exposed animals. Several plants and plant-derived products (*Olea europaea*, *Spirulina platensis, Syzygium cumini*, *Fagonia indica*, *Rosmarinus officinalis*) have been observed to exhibit nephroprotective effects in animals in response to chromium as exhibited from the improved renal function markers[34,36-38]. Histoarchitectural alterations indicating damage in renal and testicular tissues were evident in chromium-administered animals. These results were consistent with the previously observed changes caused by chromium exposure in rodents[33-40]. *A. vera* was able to mitigate these changes, which is evident.

Epididymal sperm count and motility are widely accepted simple and sensitive biological markers for assessing the effects of toxicants on the male reproductive system. Some investigational studies in humans have suggested a decrease in sperm count and motility after exposure to Cr(VI)[40]. The abundant presence of highly unsaturated fatty acids, strong ROS-generating networks, and high mitotic and metabolic activity in testicular tissue makes it susceptible to oxidative stress-mediated pathologies[39]. Cr(VI) disturbs normal spermatogenic metabolism, proliferation and differentiation, which may result in low sperm counts and defective sperm function[39]. In consonance with reports available in the literature, chromium exposure caused a decrease in sperm count and motility in the PD group. As evident, *A. vera* was able to improve these parameters. We have previously reported the radioprotective potential of *A. vera* against X-ray-induced damage in mice, as evidenced by improved histoarchitecture and organ function markers of renal and testicular tissue in mice[19,24].

Elevated LDH levels have been observed during various pathological conditions and exposure to toxicants. Abnormally raised levels of LDH because of changes in the permeability of cells followed by leakage of soluble enzymes into circulation have been reported in various instances of pathologies and exposure to toxicants[41]. In the present study, an increase in serum LDH activity was observed in chromium-administered mice. These results are consistent with previously reported studies, which showed a significant elevation in LDH activity after chromium exposure in rats[42,43]. Histopathological alterations, aberrant organ function markers, and raised serum LDH activity indicate tissue damage in PD-exposed animals. Although no modulation in LDH activity was observed in the AV+PD group when compared with the PD group, the results of the other parameters investigated certainly point towards amelioration of chromium-induced harmful effects.

The o-6-methylguanine-DNA methyltransferase (MGMT) gene encodes a DNA repair protein involved in defence against mutagens, carcinogens and toxicants. MGMT protein mends the DNA lesions by catalysing the transfer of methyl groups from various abnormally methylated moieties of DNA, including O(6)-alkylguanine, to its own molecule[44]. In the present study, renal tissue of PD group animals showed a significant decrease in mRNA expression of the MGMT gene compared to the control group. It had been previously observed that cadmium and nickel exposure caused downregulation of MGMT in human bronchial epithelial cells[45,46]. In the AV+PD group, animals showed an increase in MGMT mRNA expression compared to the PD group. It has been reported that phytochemicals like genistein could reactivate methylation-silenced genes such as retinoic acid receptor beta (RARβ) and MGMT in oesophagal squamous carcinoma cells and prostate cancer cells, indicating its protective potential[47].

Double-stranded breaks (DSBs) are one of the most harmful types of DNA damage since unrepaired or mis-repaired DSBs lead to genomic instability or chromosomal aberrations with far-reaching negative consequences. A major repair mechanism for rejoining DSBs involves DNA-dependent protein kinase (DNA-PK)[48]. In the present study, renal tissue exhibited decreased expression of DNA-PK in the PD group. There are reports suggesting dysregulation of DNA repair machinery during heavy metal toxicity. It has been reported that cadmium exposure is associated with hampered DNA repair mechanisms, such as nucleotide excision repair, base excision repair, mismatch repair, and DSBs repair[48]. In the AV+PD group, animals exhibited an increase in DNA-PK gene expression compared to the PD group. This enhanced expression of DNA-PK hints towards the protective potential of *A. vera* against probable chromium-induced DNA damage. It has been previously reported that botanical extracts (*Curcuma longa* extract, *Styphnolobium japonicum*, and *Rosmarinus officinalis*) have been associated with the promotion of DNA repair and upregulation of associated genes in men[49].

As previously mentioned, we reported that *A. vera* gel extract had the potential to protect against X-ray-induced deleterious effects in various tissues of mice

and this radioprotective potential was explained by the observed antioxidant potential of *A. vera*[19,24,50]. *A. vera* provided remarkable protection against various metals such as arsenic, aluminium, and cadmium[16,21-24,51]. *A. vera* has also been used for environmental phytoremediation in instances of air, water, and soil contamination with heavy metals such as arsenic, cadmium, lead, mercury, nickel, and chromium. Aloin and aloe-emodin present in *A. vera* are known to impart metal chelation and antioxidant properties[52,53]. Antioxidant and metal chelating abilities of *A. vera* have been reported and reviewed earlier[54].

Conclusion

Chromium exposure to mice caused damage to excretory and reproductive organs, as evidenced by their altered tissue function markers, histoarchitecture, and serum LDH activity. It appeared that chromium exposure may have the potential to weaken the DNA damage repair machinery of the cells, which may prove conducive to genotoxicity. *A. vera* provided some degree of protection against the harmful effects of chromium exposure. However, it is emphasised that thorough studies should be carried out exploring the possible modulatory effects of *A. vera* on other organs. Mechanistic studies unravelling the protective mechanisms will strengthen and warrant its use against ameliorating heavy metal toxicity.

Conflict of interest

The authors declare that there are no conflicts of interest.

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