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DANDELION (*TARAXACUM OFFICINALE*) LEAVES PROTECTS AGAINST CHROMIUM-INDUCED NEPHROTOXICITY AND GENOTOXICITY IN RATS

Mbarka Hfaiedh[a*], Dalel Brahmi[a] and Lazhar Zourgui[a,b]

[a]Research Unit of Active Biomolecules Valorisation, Higher Institute of Applied Biology of Medenine, University of Gabes, 4119 Medenine, Tunisia.

[b]Higher Institute of Applied Biology ISBAM Medenine 4119, University of Gabes, Tunisia.

***Corresponding Author: Mbarka Hfaiedh**

Research Unit of Active Biomolecules Valorisation, Higher Institute of Applied Biology of Medenine, University of Gabes, 4119 Medenine, Tunisia.

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ABSTRACT

Chromium is a toxic and carcinogenic compound widely distributed in environment. In this study dandelion (*Taraxacum officinale*) leaves extract (TOE) was evaluated for its antioxidant and antigenotoxicity efficacy against sodium dichromate-induced kidney injury. The modulatory effects of TOE upon sodium dichromate-induced renal dysfunction and genotoxicity in Wistar albino rat model were investigated by assaying oxidative stress biomarkers, serum kidney toxicity markers, DNA fragmentation and chromosomal aberrations in bone marrow cells. Our results clearly showed that sodium dichromate induced significant alterations in all tested oxidative stress markers. In addition, it induced DNA damage as indicated by DNA fragmentation and chromosomal aberrations. On the other hand pre-treatment with TOE was found to provide significant protection against sodium dichromate-induced nephrotoxicity by inhibiting lipid peroxidation, preserving normal antioxidant activities and protecting the renal tissues from lesions and DNA damage. Present findings suggest a prominent role of TOE against sodium dichromate-induced kidney dysfunction and genotoxicity.

KEYWORDS: Dandelion; Sodium dichromate; Chemoprevention; Kidney; Antioxidant enzymes; Chromosome aberration.

Abbreviations

I.p, intraperitoneal; **Rpm**, rotations per minute; **RSA**, radical scavenging activity; **TBS**, tris-buffered saline; **TCA**, trichloroacetic acid; **TOE**, *Taraxacum officinale* leaf extract.

INTRODUCTION

The problem of environmental pollution is one of the most burning topics and has been on the checklist of almost all the nations. Among these environmental pollutants, heavy metals such as lead, mercury, arsenic, chromium and cadmium have received special attention worldwide as they are widely distributed in nature and leads to widespread occurrence of specific toxicological problems.[1] Chromium (Cr) is one of the most toxic chemical compounds because of its increased level in the environment as a result of industrial and agricultural practices.[2] It has become one of the most abundant pollutants in aquatic and terrestrial ecosystems.[3] Chromium is a metallic element, widely used in textile manufacturing, wood preservation, metallurgy, photography and photoengraving.[4] In nature, it is found in various states the most common of which are: the trivalent (Cr (III)) and hexavalent (Cr (VI)). The oxidation state and solubility of chromium compound determine its toxicity. In biological systems, trivalent species are often insoluble and do not easily enter into cells, they are generally regarded as safe and are present in foods at very low concentration.[5] In contrast, Cr (VI) compounds are much more soluble and readily enter into cells via a sulfate transporter because of its higher solubility in water. Therefore, Cr (VI) compounds are generally man-made and considered as more toxic than Cr (III).[5] Once inside the cells, Cr (VI) reduced to Cr (III). This reduction process generates reactive oxygen species (ROS) and induces soft tissues' damage such as liver, pancreas, cerebellum and kidney.[4,6,7] The kidney is the main target organ for chromium accumulation, more sensitive to the toxic effects of chromium than other tissues.

Sodium dichromate ($Na_2Cr_2O_7$), a Cr (VI) compound, is the most toxic form of chromium and it has been demonstrated to induce nephrotoxicity associated with oxidative stress. Several studies have demonstrated that exposure to sodium dichromate induces acute renal failure in both humans and animals.[8,9] Furthermore, chronic exposure to other forms of Cr (VI) can damage the renal proximal tubular epithelial cells, causes

proximal tubular dysfunction, decreases glomerular filtration, and increases oxidative damage.[10,11]

Exposure to Cr (VI) salts has several adverse human health effects including neurotoxicity, dermatotoxicity, genotoxicity, carcinogenicity and immunotoxicity.[12,13,14] The genotoxic potential of chromium containing compounds has been intensively investigated and recently reviewed.[15] Cr (VI) compounds are well known to be genotoxic and easily invade inside the cells.[16] They cause DNA damage in target cells via several mechanisms, including single strand breaks, DNA-DNA interstrand crosslinks, DNA-protein crosslinks (DPXL), chromium- DNA adducts, oxidative nucleotide changes, such as formation of 8-oxo-2u-deoxyguanosine (8-oxo-dG), and chromosomal aberrations.[17,18] Several studies in mice and rats have shown chromosomal aberrations in bone marrow, DNA–protein crosslinks and DNA single-strand breaks in the liver and brain, as well as single and double-stranded DNA breaks in leukocytes, following oral administration of Cr (VI) species.[19,20]

Natural products such as herbs, fruits and vegetables have become popular in recent years due to public awareness and increasing interest among consumers and scientific community. In this respect, our laboratory gives special interest to the protective potential of dandelion (*T. officinale*) plant as regards sodium dichromate toxicity.

T. officinale belonging to the *Asteraceae* family, commonly known as dandelion, includes approximately 30–57 varieties with many microspecies, divided into nine sections. [21] Plants of the genus *Taraxacum* are widely distributed in the warmer temperate zones of the Northern Hemisphere and have long been used in folklore medicine and Traditional Chinese medicine.[22] *T. officinale* leaves are often used to add flavor to salads, sandwiches, and teas. The roots can be found in some coffee substitutes, and the flowers are used to make certain wines.[23] Traditionally, root and herb from *T. officinale* have been used in oriental medicine for its lactating, choleretic, diuretic and antirheumatic properties.[24] Although, *T. officinale* is used for treating diseases related with inflammation in the folk of China, its use has mainly been based on empirical findings.[24] Recent studies have provided evidence that it may reduce the risk of divers' diseases, including tumors.[25] *T. officinale* is considered to be an excellent general tonic and a "natural" diuretic". The root is primarily considered a gastrointestinal remedy supporting digestion and liver function, while the leaf has been found to have a greater diuretic effect and bitter digestive stimulant than the roots, with activity comparable to that of frusemide, without causing potassium loss because of the leaves' high potassium content.[26] Previous phytochemical investigations of *T. officinale* showed the presence of various classes of natural compounds named phenolics, inulin, flavonoids, lactones and oligofructans sesquiterpenes, triterpenes, phytosterols.[27] *T. officinale* leaves are a rich source of fiber, vitamins and minerals, including beta carotene, non-provitamin A carotenoids, xanthophylls, chlorophyll, vitamins C and D, many of the B-complex vitamins, choline, iron, silicon, magnesium, sodium, calcium, zinc, manganese, copper, and phosphorous.[28] *T. officinale* is also a rich source of potassium, capable of replacing potassium lost through diuresis.[29]

In recent years, several studies have provided evidence that *T. officinale* leaf extract might play beneficial roles against carbon tetrachloride hepatotoxicity.[30,31] and cholecystokinin octapeptide-induced acute pancreatitis in rats.[32] To our knowledge no data were reported concerning the ability of dandelion (*T. officinale*) leaf extract to counteract kidney disorder. Therefore, the present study aimed to investigate the effect of *T. officinale* leaf extract (TOE) on sodium dichromate-induced nephrotoxicity, oxidative stress and genotoxicity in Wistar rats.

MATERIAL AND METHODS

Plant material

Dandelion (*T. officinale*) leaves were collected from a culture area located near Gafsa (Tunisia), were washed with distilled water and dried in the shade for 3 days, finally grinded in powder and stored at room temperature (25 ± 2 °C) until use. Briefly, 100 g of powdered *T. officinale* leaves were left in boiling water (1:10 w/v) for 4 hours. The water extracts were filtered through Whatman No. 1 filter paper and evaporated under a vacuum at 40 °C and then further dried to a powder using a freeze-dryer at 50 °C.[30]

Animals and diets

Two month-old healthy male Wistar rats (n = 24) weighing about 120 ± 10 g purchased from Pasteur Institute (1002 Tunisia) were used in this study. The animals were kept for one week under the same laboratory conditions of temperature (22 ± 2°C), relative humidity (70 ± 4%), and a 12 h light/dark cycle, fed with commercial pellet diet and tap water ad *libitum*. All experiments were performed following the recommendation of the Tunisian code of practice for the Care and Use of animals for Scientific Purposes. After the adaptation period, animals were divided into 4 groups of 6 rats each and treated as follows:

Group 1: Control rats received distilled water (0.5 ml/100 g b.w) (i.p)

Group 2 (Cr): The rats received for 10 days sodium dichromate (dissolved in distilled water) at 10 mg/kg b.w (i.p), corresponding to 0.2 LD_{50} for chromium salt; this concentration was chosen according to previous data.[33]

Group 3 (TOE): The rats received TOE at 500 mg/kg b.w for 40 days and followed by distilled water (0.5 ml/100 g b.w) (i.p) during the last 10 days of TOE treatment.

Group 4 (TOE + Cr): The rats received TOE at 500 mg/kg b.w for 40 days and followed by sodium dichromate at a dose 10 mg/kg b.w (i.p) during the last 10 days of TOE treatment.

To investigate the protective effect against sodium dichromate hazards, standard TOE at a dose of 500 mg/kg b.w was administered to the animals daily, by oral gavage. This dose was chosen based on previous reports which proved its efficiency on preventing toxicity induced by carbon tetrachloride.[31]

Collection of serum and urine samples

After 40 days of treatment, control and treated groups were sacrificed by decapitation under ether inhalation anaesthesia in order to minimize the handling stress. The blood serum was obtained by centrifugation (1500 rpm, 15 min, 4°C) and stored at – 80°C until use for biochemical determination. Plasma samples were drawn and stored at – 20°C until analysis. Urinary samples were obtained from each animal housed in a specially designed metabolic cage. Urine samples were collected into bottles within 24 h cycles. The volume of each sample was recorded and centrifuged at 3000 rpm for 5 min.

Preparation of kidney homogenate

During the treatment period the body weight of the animals was monitored daily. On the day of sacrifice, kidneys were dissected out, cleaned and weighed. Some samples were used for biochemical analyses and DNA fragmentation. Other samples were immediately removed, cleaned and fixed in 10% formalin solution and embedded in paraffin for histological studies. The relative kidney weight was expressed as g/100 g of body weight.

The kidney was homogenized in ice-cold lysis buffer solution (tris-buffered saline (TBS), pH 7.4). The homogenates were centrifuged at 5000 rpm for 30 min at 4 °C to remove cell debris, nuclei, and mitochondria. Resulting supernatants (S1) were served for malondialdehyde (MDA), reduced glutathione (GSH) and antioxidant enzymes measurements. The protein content of the kidney tissue was determined using bovine serum albumin (BSA) as standard at 560 nm.[34]

Chromium estimation in blood and kidney

Chromium levels in blood and kidney homogenate were analyzed by atomic absorption spectrometer at 360 nm. Blood chromium was directly quantified after dilution with distilled deionized water. The values were expressed as µg/ml of blood. The kidney was grinded in a mixture of HNO_3/$HClO_4$ (v/v) and the residue was dissolved with 25 ml of HNO_3 (1 M) solution. The values were expressed as µg/g fresh weight. The laboratory glass wares used for kidney and blood collection and processing were soaked overnight in analytical grade nitric acid and washed three times with deionized water.[35]

Biochemical assays

Renal function test

The levels of creatinine, uric acid, urea in serum and urine as well as blood urea nitrogen (BUN) were determined by colorimetric method using commercial diagnostic kits (Biomaghreb, Tunisia). Creatinine clearance, an index of glomerular filtration rate, was calculated by UV/P equation, [36] where U is the urinary creatinine level, V the volume of urine sample collected within 24 h and P the plasma creatinine concentration.

Lipid peroxidation

Malondialdehyde (MDA) levels were determined by monitoring thiobarbituric acid reactive substances (TBARS) according to the method of Ohkawa et al.[37] with minor modifications. The reaction mixture contained 0.2 ml of test sample, 0.2 ml of 8.1% sodium dodecyl sulfate (SDS), 1.5 ml of acetic acid and 1.5 ml of 0.5% of TBA. The mixture was heated in a water bath at 95 °C for 60 min. After cooling, 5 ml of *n*-butanol/pyridine (15:1, v/v) was added and the mixture shaken well. After centrifugation at 4000 rpm for 10 min, the absorbance of the organic layer was measured at 532 nm. The amount of MDA was calculated using an extinction coefficient of 156 mM^{-1} cm^{-1} and expressed in nmoles/mg protein.

Antioxidant activities evaluation

Reduced glutathione (GSH) was determined by the method of Moron et al.[38] based on the reaction with Ellman's reagent (19.8 mg dithionitrobisbenzoic acid in 100 ml of 0.1% sodium citrate). Superoxide dismutase (SOD) activity was determined by measuring of ability to inhibit the photoreduction of nitroblue tetrazolium (NBT).[39] Catalase (CAT) activity was assayed spectrophotometrically as described by Aebi,[40] the H_2O_2 decomposition rate was followed by monitoring absorption at 240 nm. Glutathione peroxidase activity (GPx) was estimated by the method based on the reaction between glutathione remaining after the action of GPx and 5,5'-dithiobis(2-nitrobenzoic acid) to form a complex with a maximum absorption at 412 nm.[41]

Qualitative DNA fragmentation assay by agarose gel electrophoresis

Kidney tissues were lysed with a chaotropic salt-containing buffer to ensure macromolecules denaturation. DNA was bound to the spin column membrane and the remaining lysate was removed by centrifugation. A filtration column was used to remove cell debris after washing to remove contaminants; DNA was eluted with buffer into a collection tube. The pellet was rinsed with 70% ethanol, dried at room temperature for 2 h and resuspended in 200 µl of TE (20 mM Tris-HCl pH 8. 0.1 mM EDTA). A volume of 10 µg/lane DNA was loaded into each well of 0.8% agarose gel containing 5 µg/ml ethidium bromide. The electrophoresis was carried out at 80 V/30 mA in 1x TBE running buffer (44 mM Tris-HCl, 44 mM boric acid, 50 mM EDTA pH 8) for 1–2 h. After electrophoresis, the gel was observed under an UV lamp and photograph was taken.

Chromosome aberration assay

24 hours before sacrifice, animals were given a suspension of yeast powder (100 mg/500 µl) to accelerate mitosis of bone-marrow cells. Vinblastine (200 µl; 250µg/ml) was injected into the animals 45 min before sacrifice in order to block dividing cells in metaphasis. Bone marrow cells from femurs and tibias were collected, subjected to hypotonic shock (KCl 0.075 M) and fixed three times using methanol-acetic acid according to the technique of Evans et al.[42] The cells were spread on glass slides that were blazed on a flame for 5 s, then air-dried for conservation at room temperature and finally stained by 4% dilution of Giemsa reagent in water for 15 min. After coding of the slides, the chromosomes of 100 cells in metaphase were examined for abnormalities at a magnification of 1000x using an optical microscope (Carl Zeiss, Germany). This was done for each one of three replicates (300 metaphases per dose level) for negative controls, positive controls and treated groups. Chromosome aberrations were identified according to criteria described by Savage. [43] Metaphases with chromosome breaks, gaps, rings and centric fusions (robertsonian translocation) were recorded and expressed as percentage of total metaphases per group.

Statistical analysis

Results are expressed as the mean ± Standard Deviation (SD). Comparisons with control and treated groups were done using the Student's t-test. A difference was considered significant for $P < 0.05$.

RESULTS

Effects on weight gain and kidney weight

No deaths or remarkable signs of external toxicity were observed in the groups of rats that were given sodium dichromate either alone or in combination with TOE. The effects of sodium dichromate and TOE on body and kidney weights were displayed in Table 1. The gain in body weight was less in sodium dichromate treated rats (Cr) (149.75 ± 4.87g) than the controls animals (229.23 ± 10.64 g). However, pre-treatment with TOE in combination with sodium dichromate significantly ($P < 0.01$) increased the body weight in rats (215 g) compared with sodium dichromate treated group.

Concerning relative kidney weight, as shown in Table 1, hypotrophy of this organ was observed when animals intoxicated with sodium dichromate (0.43 ± 0.07 g/100 g b.w). On the other hand, pre-treatment with TOE at 500 mg/kg b.w caused significant improvement in kidney relative weight (0.49 ± 0.08 g/100 g b.w) compared to sodium dichromate group.

Chromium estimation

Chromium concentrations in blood and kidney homogenates are shown in Table 1. A higher levels of chromium were found in blood (0.096 ± 1.54 µg/ml) and kidney (0.52 ± 3.10 µg/g) of sodium dichromate treated animals than control rats (blood: 0.003 ± 1.32 µg/ml; kidney: 0.017 ± 3.5 µg/g). However, pre-treatment with TOE decreased significantly ($P < 0.01$) chromium concentration both in blood (0.05 ± 0.14 µg/ml) and in kidney (0.35 ± 1.31 µg/g). There were no significant differences in chromium level between the TOE-treated animals and the control group.

Biochemical assays

Renal function test

The levels of creatinine, uric acid, urea, creatinine clearance and BUN were considered to be serum biochemical markers of renal damage. In sodium dichromate treated rats, the levels of creatinine (3.02 ± 0.09 mg/dl), uric acid (50.23 ± 0.29 mg/dl), urea (6.2 ± 0.4 mg/dl) and BUN (31.52 ± 0.26 mg/dl) significantly increased ($P < 0.01$) but the level of creatinine clearance (0.24 ± 0.25 ml/min) significantly ($P < 0.01$) decreased as compared with those of the controls indicating a renal toxicity. Interestingly, administration of TOE at a dose of 500 mg/kg b.w attenuated the increased levels of the serum renal markers induced by sodium dichromate and caused a subsequent recovery towards normalization comparable to the control group. On the other hand, the extract alone did not affect the serum renal markers (Table 2).

Oxidative stress analyses

Table 3 illustrated the levels of renal lipid peroxidation, enzymatic and non-enzymatic antioxidant activities. Our results clearly showed that the renal MDA level was significantly ($P < 0.01$) increased (6.87 ± 0.49 nmoles of MDA/mg protein) in sodium dichromate treated animals (Group Cr) and that suggests the possibility of enhanced free radical generation by sodium dichromate. In addition, the antioxidant activities were dramatically decreased ($P < 0.01$) in sodium dichromate-intoxicated group compared to that of the controls. In contrast, pre-treatment with TOE significantly ($P < 0.01$) lowered the MDA level in kidney (4.46 ± 0.27 nmoles of MDA/mg protein) and normalized the antioxidant activities to their control values. Thus, the TOE abolished the oxidative damage induced by sodium dichromate accompanied by an amelioration of antioxidant activities.

DNA fragmentation

Since the presence of high free radicals are associated with DNA damage. It was therefore considered prudent to assess the effect of TOE on sodium dichromate-induced DNA damage in kidney tissues. Agarose gel electrophoresis technique was used to perform this assessment and results are depicted in Fig 1. As evident from the figure, sodium dichromate treatment results a significant DNA fragmentation in renal tissues marked by a fast migration of fragmented DNA with the appearance of long smear (Fig 1 (lane 2)) when compared with DNA of control group (Fig 1 (lane 1)).

However, pre-treatment with TOE significantly reduced this smear length (Fig 1 (lane 4)). In addition, there are no specific DNA fragments detected in the control (C) and the TOE treated-animals (Fig 1 (lane 1) and (lane 3)).

Induction of chromosome aberration

Chromosome aberrations were assessed in the percentage of 100 metaphase cells. Table 4 and Fig 2 summarized the results of chromosomal aberrations in bone marrow cells of male Wistar rats treated with sodium dichromate and TOE. The chromosomal aberrations were represented in the form of centric fusions, rings, gaps and chromosomal breaks. We observed that animals treated with sodium dichromate alone (10 mg/kg b.w) showed a significant increase in chromosome aberrations in bone marrow cells. No significant differences were observed in the group treated with TOE alone at a dose of 500 mg/kg b.w as compared to control group (4.97 ± 0.2 and 5.33 ± 1.50, respectively). On the other hand, the co-administration of TOE before sodium dichromate treatment exhibited significant reduction in total chromosomal aberrations (19.51 ± 0.9).

Table 1: Effect of TOE and sodium dichromate on body and kidney weights as well as chromium level in blood and kidney.

Parameters and treatments	Control	Cr	TOE	TOE + Cr
Initial body weight (g)	136.26±9.15	135.9±5.76	146.16	149.66
Finals body weight (g)	229.23±10.64	149.75±4.87**	239.51++	215++
Relative kidney weight (g/100g)	0.53 ± 0.02	0.43 ± 0.07*	0.54 ± 0.04+	0.49 ± 0.08+
Chromium level in blood[a]	0.003 ± 1.32	0.096±1.54**	0.04 ± 1.86++	0.05 ± 0.14++
Chromium level in kidney[b]	0.017 ± 3.5	0.52 ± 3.10**	0.15±2.4++	0.35±1.31+

The results are expressed as means ± SD for 6 rats in each group. Statistical comparison was performed using Student's t-test.

* P < 0.05, ** P < 0.01 compared with control group.

+ P < 0.05, ++ P < 0.01 compared with sodium dichromate-treated group (Cr).

[a] Expressed as µg/ml of blood.

[b] Expressed as µg/g fresh weight of kidney.

Table 2: Effect of TOE and sodium dichromate on serum levels of renal function markers.

Parameters and treatments	Control	Cr	TOE	TOE + Cr
Creatinine (mg/dl)	0.64 ± 0.02	3.02 ± 0.09**	0.58 ± 0.03++	1.12 ± 0.08++
Uric acid (mg/dl)	28.96 ± 0.19	50.23 ± 0.29**	26.75±0.2++	40.47±0.89++
Urea (mg/dl)	4.65 ± 0.3	6.2±0.4**	4.46±0.65++	4.24±0.31++
BUN (mg/dl)	16.26 ± 0.13	31.52 ± 0.26**	16.41 ± 0.28++	24.75 ± 0.37++
Creatinine clearance (ml/min)	0.96 ± 0.71	0.24 ± 0.25**	0.81 ± 0.42++	0.68 ± 0.61++

The results are expressed as means ± SD for 6 rats in each group. Statistical comparison was performed using Student's t-test.

* P < 0.05, ** P < 0.01 compared with control group.

+ P < 0.05, ++ P < 0.01 compared with sodium dichromate-treated group (Cr).

Table 3: Effect of TOE and sodium dichromate on oxidative stress markers.

Parameters and treatments	Control	Cr	TOE	TOE + Cr
MDA (nmoles/mg protein)	2.57 ± 0.21	6.87±0.49**	2.54±0.41++	4.46±0.27++
GSH (µg of GSH/mg protein)	2.84 ± 0.52	1.31 ± 0.18**	2.62 ± 0.24++	2.18 ± 0.23++
SOD (U/mg protein)	9.87±0.52	5.71±0.48**	9.45±0.17++	7.09±0.4++
CAT ((µmol/min/mg protein)	12.23±3.42	7.24±3.28**	12.66±3.26++	9.42±2.79++
GPx (µmol GSH oxidized/min/mg protein)	5.27 ± 0.27	2.67 ± 0.19**	5.16±0.27++	4.63±0.4++

The results are expressed as means ± SD for 6 rats in each group. Statistical comparison was performed using Student's t-test.

* P < 0.05, ** P < 0.01 compared with control group.

+ P < 0.05, ++ P < 0.01 compared with sodium dichromate-treated group (Cr).

Table 4: Percentage of different type of chromosomal damage induced by sodium dichromate and reverted with TOE.

Parameters and treatments	Control	Cr	TOE	TOE + Cr
Centric fusion	2.33±1.52	15.06±2.08**	2.66±0.57++	10.24±0.03++
Ring	1±1.42	5.84±1.52**	1.5±0.81++	1.95±0.77++
Break	0.33±3.21	9.69±4.16**	0.25 ± 1.61++	5.16±2.04++
Gap	1.66±1.57	4.66±2.08**	0.54±1.53++	2.14±2.3++
Total	5.33±1.50	35.27±1.01**	4.97±0.2++	19.51±0.9++

The results are expressed as means ± SD for 6 rats in each group. Statistical comparison was performed using Student's t-test.

* P < 0.05, ** P < 0.01 compared with control group.

+ P < 0.05, ++ P < 0.01 compared with sodium dichromate-treated group (Cr).

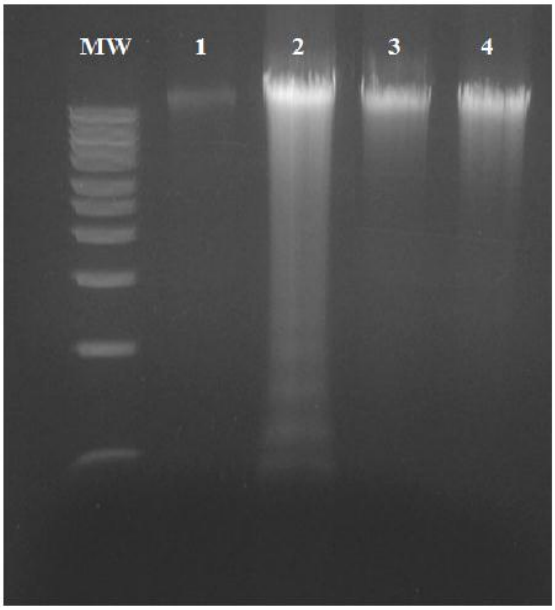

Fig. 1: Effect of sodium dichromate and TOE on the renal DNA integrity.

MW: DNA marker (MW 1 kb).
(1): Control rats.
(2): Rats treated with sodium dichromate (10 mg/kg b.w).
(3): Rats treated with TOE (500 mg/kg b.w).
(4): Rats pre-treated with TOE (500 mg/kg b.w) then injected (i.p) with sodium dichromate (10 mg/kg b.w)

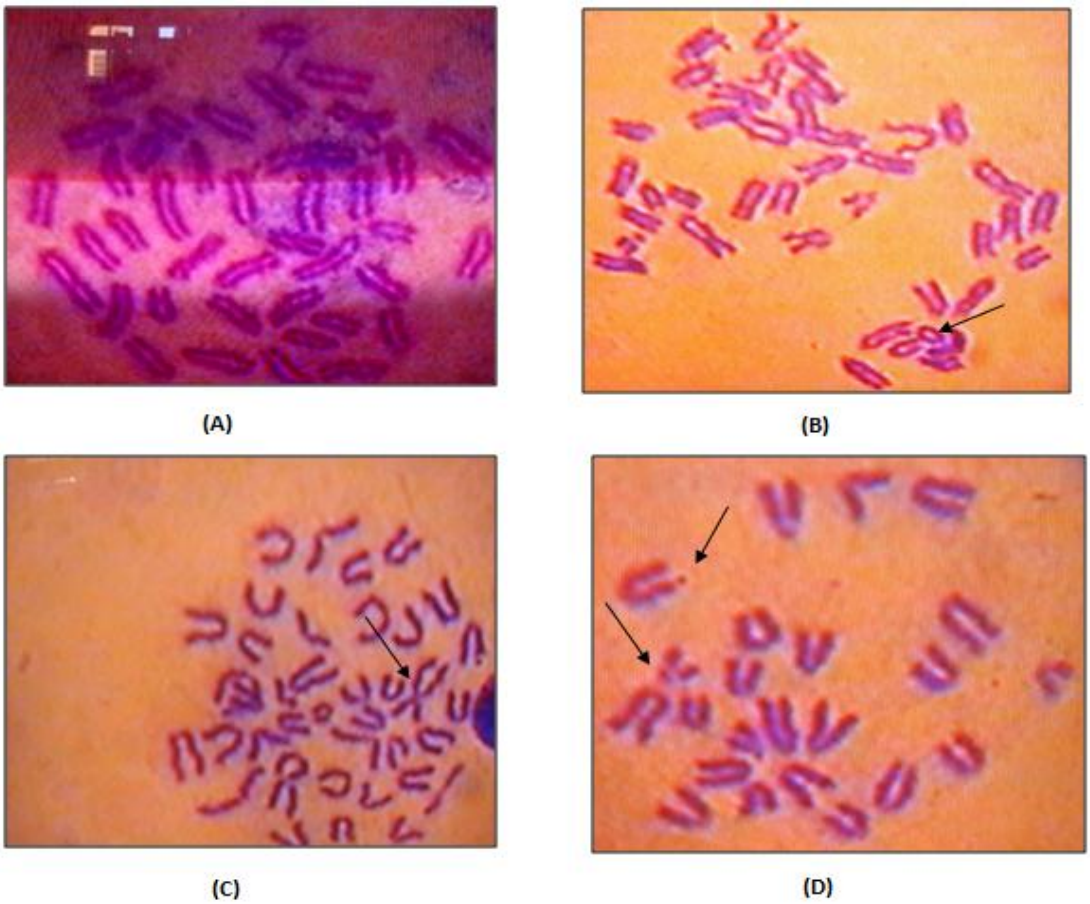

Fig. 2: Metaphase figures of chromosomal aberration of bone marrow cells showing: (A); Normal metaphase, (B); Ring, (C); Centric fusion, (D); Gap and Break. Original magnification of each figure; 1000x.

DISCUSSION

Chromium induces a broad spectrum of toxicological effects and biochemical dysfunctions constituting serious hazards to health. Recent studies have been reported on the actions of divers agents against chromium-induced nephrotoxicity.[2,44,45] To our knowledge, our paper constitutes the first report concerning the ability of dandelion (*T. officinale*) leaf extract to counteract kidney disorder. Therefore, the present study aimed to investigate the effect of *T. officinale* leaf extract (TOE) on sodium dichromate-induced nephrotoxicity, oxidative stress and genotoxicity in Wistar rats. Our results revealed that sodium dichromate administration caused a marked reduction in the net body weight of animals. These effects on body weight could be associated with several factors, one of which is an imbalance in the metabolism produced by changing the zinc status in zinc-dependent enzymes that are necessary for many metabolic processes. Along with the decrease in body weight, a significant reduction in kidney weight was also found in sodium dichromate-treated rats (Cr). Our results are in agreement with previous findings of Hfaiedh et al.[46,47] who have found similar changes in body weight of rats exposed to the same dose of sodium dichromate. Kumar and Roy.[48] also found that chromium-induced a decrease in body weight gain, liver weight, and liver/body ratio. On the other hand, TOE administration could normalize these changes. The observed improvement of body and kidney weights may be due to increase the appetite of rats treated with TOE. The appetizer effect of *T. officinale* is well documented.[49,50]

The kidney is the main route of chromium excretion. It has been reported that acute exposure to hexavalent chromium in rats induced an increase in chromium kidney content. Similarly to previously published data,[48,51] our results revealed that blood and renal chromium levels were higher in sodium dichromate-treated rats than in control animals, confirming the metal absorption after administration. Interestingly, the chromium content in kidney of (TOE + Cr) group is significantly lower than the sodium dichromate treated rats. This indicated that TOE helps in excretion of chromium from body tissues.

Kidney is vulnerable to damage due to perfusion and the increased concentration of excreted compounds occurring in renal tubular cells.[52] High levels of creatinine, urea and BUN are sensitive indicators of renal cells injury and are most helpful in recognizing kidney diseases.[53] In the present study, sodium dichromate injection produced a marked derangement in the kidney function and led to a significant increase in the level of serum creatinine, urea, and BUN and a decrease in that of creatinine clearance when compared to normal control animals, which indicates renal cells damage as previously reported by El-Demerdash et al.[54] The increased serum markers suggest that sodium dichromate causes structural and functional injury to the cells membrane and increase the membrane permeability, leading to the leakage of renal enzymes into the blood. Our results are in good agreement with those previously reported.[55] In contrast, the administration of TOE (500 mg/kg b.w) protects the kidney function from sodium dichromate intoxication as indicated by a significant restoration of serum markers. Our results corroborate with Mitra's et al.[56] study which denotes that *T. officinale* leaves extracts has a nephroprotective effect.

The real mechanism by which chromium induces nephrotoxicity is unknown; however, sodium dichromate has been shown both *in vitro* and *in vivo* studies to enhance the generation of reactive oxygen species (ROS). Abnormal production of ROS may damage some macromolecules, to induce cellular injury and necrosis via several mechanisms including peroxidation of membrane lipids, protein denaturation and DNA.[57]

Elevated levels of MDA in tissue have been regarded as an indicator for cellular damage due to excess lipid peroxidation processes that occur during malfunction of the antioxidant defense system. [58] In our study, elevations in MDA levels in the kidney of rats treated with sodium dichromate were observed. The increase in renal MDA levels suggests enhanced lipid peroxidation leading to tissue damage and failure of antioxidant defense mechanisms to prevent formation of excessive free radicals. Consistent with previous studies, [2, 59], our results showed a significant depletion in the antioxidant system in renal tissues after treatment with sodium dichromate. The decrease in the activities of SOD, CAT and GPx and the decreased level of GSH might be due to their utilization by the enhanced production of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation.[60] Under normal conditions the inherent defense system, including glutathione and the antioxidant enzymes, protects against oxidative damage.

The severity of oxidative damage was less in the animals that received TOE and sodium dichromate together than those that received sodium dichromate alone. These results suggested that TOE protects against the oxidative renal injury caused by this heavy metal. Hence it may be possible that the mechanism of nephroprotection by TOE is due to its antioxidant effect. This suggestion was strengthened by recent findings that demonstrated that *T. officinale* leaves possess potent antioxidant properties. These properties may be mediated through direct trapping of the free radicals and also through metal chelation.[61] The ability of *T. officinale* to protect lipid peroxidation is in agreement with Hu and Kitts [62] who demonstrated that ethyl acetate fractions of *T. officinale* possess remarkable lipid peroxidation inhibition and radical scavenging activities.

Park et al.[31] also showed that *T. officinale* leaves extract ameliorated LPS-induced oxidative stress, as indicated by suppressed MDA concentration, through the elevation

of antioxidative enzyme activities, such as catalase, SOD, GPx, and GR, and GSH restoration.

T. officinale leaf is known to be an effective hydrogen peroxide scavenger, because of its high polyphenol content. [63] In addition, phytochemicals derived from common dandelion, including luteolin, chicoric acid, chlorogenic acid, chrysoeriol, also reported they have remarkable antioxidative activities.[27,31,64] Therefore, we believe that the effects manifested by the dandelion (*T. officinale*) leaves extract to overcome sodium dichromate-induced nephrotoxicity in our study were mediated by the presence of these bioactive compounds. Polyphenolic compounds have all been shown to attenuate the renal dysfunction, improve the renal architecture, increase the antioxidant enzyme activity, decrease lipid peroxidation and reactive oxygen species in nephrotoxicity.[65] In view of the above findings, it is suggested that the phytochemical constituents in TOE could contribute to its antioxidant activity and, thus, nephroprotection. Polyphenols compounds comprise the majority of the phytochemicals found in TOE.[47]

Chromium is a complex genotoxin that induces a wide variety of structurally disparate DNA lesions through induction of micronuclei, chromosome aberrations, DNA fragmentation, cell cycle arrest, etc.[14,66,67] The aim of the present study is to find out whether *T. officinale* leaves extract could display any antigenotoxic activities towards sodium dichromate proved genotoxic potential. In this study, as expected, it was detected that sodium dichromate led to DNA fragmentation and a significant increase in the frequencies of chromosomal aberrations in bone marrow cells.

Our finding are in agreement with the data of a broad literature, demonstrating that Cr (VI) is genotoxic either when tested *in vitr*o or *in vivo*.[68,69] Cr (VI) is taken up by cells easily and is subsequently reduced to the other forms such as Cr (III, IV and V), which in turn are believed to cause adverse biological effects. Cr (VI) itself is not reactive to DNA, however, the chromium metabolites, radicals produced during reduction can subsequently attack macromolecules and lead to multiform DNA damages; strand breakage, DNA–protein crosslinks, DNA–DNA crosslinks, Cr–DNA adducts and base modifications in cells. Specially, DNA strand breaks are mainly ascribed to the ROS.[70,71,72] Kortenkamp et al.[73] reported that Cr (V)-induced DNA breaks were predominantly due to the production of hydroxyl radicals.

Chromium compounds were reported to interfere with the intracellular function of Ca^{2+}.[74] Changes in Ca^{2+} concentration can influence the induction of chromosomal aberrations induced by chromium compounds,[75] an effect probably related to the fact that calcium contributes to maintain the integrity of chromosome structure.[76] Cr (VI) also reported to suppress the levels of vitamins E and C in the cells.[77] All of these effects of chromium treatment can lead to mutation in the cellular genes and eventually result in cell transformation.

On the other hand, our results clearly demonstrate the antigenotoxic potential of *T. officinale* leaves extract which efficiently protect rats from clastogenic effects and DNA damages of sodium dichromate. The protection afforded by TOE against sodium dichromate genotoxicity is likely due to its ability to inhibit oxidative process induced by this heavy metal. The protective effects of TOE have been attributed to a wide variety of mechanisms. It may be caused by the scavenging of superoxide anions that produce hydroxyl radicals via the Haber-Weiss reaction from H_2O_2, or by the chelation of metal ions that are used to produce highly toxic hydroxyl radicals from H_2O_2 via the Fenton reaction. It may modify enzymes that activate or detoxify carcinogens, and inhibiting the induction of the transcription factor activator protein-1 (AP-1) activity by tumor promoters.[78] Moreover, *T. officinale* leaves are a good source of calcium [79] which can maintain the integrity of chromosome structure and improve chromosomal damage produced by chromium compounds due to Ca^{2+} deficiency. The chemoprotective role of TOE in the present work is supported by the previous findings of Takasaki et al.[80]

CONCLUSIONS

In conclusion, in the light of biochemical results and histological findings, co-administration of TOE lessened the negative effects of sodium dichromate-induced nephrotoxicity and genotoxicity; possibly by inhibiting lipid peroxidation process. Further investigation of these promising protective effects of TOE against sodium dichromate-induced renal injury may have a considerable impact on developing clinically feasible strategies to treat patients with renal failure, or as an adjunct therapy aiming to improve the therapeutic index of some nephrotoxic drugs.

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Conflict of interest

The Author(s) declare(s) that there is no conflict of interest.

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