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Histopathological and Molecular study of effects of potassium dichromate on kidney of the male mice

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ABSTRACT
Potassium Dichromate is inorganic reagent, work as an oxidizing agent in several industrial and laboratory applications. Potassium Dichromate is harmful to health at the acute and chronic status in animals and human. Potassium Dichromate is red-orange color, very bright, and crystalline ionic solid. Aim of the current study is determine the histological changes in the kidney and determine the gene expression molecular changes for some genes in mice which administrated potassium dichromate. Twenty male mice, divided into two groups, each group consist of ten mice. The first group administrated potassium dichromate (67) Mg/kg/BW, for 21 day orally, while the second group (control group was administrated distal water. The results showed that the histopathological changes of the first group (treated by potassium dichromate) are included atrophy of glomeruli of the kidney, vascular dilatation, and necrosis, while the second group (control group) showed that normal glomeruli of the kidney, normal proximal, distal and convoluted tubules. The gene expression of NR3C1 gene are decrease in the first group (treated by potassium dichromate) than in the healthy group. In addition to that, the first group (treated by potassium dichromate) showed that increase TP53 gene expression than in the healthy group. Finally, the potassium dichromate causes pathological changes in the renal tissues in the male mice with changes in gene expression of NR3C1 gene, and TP53 gene by decrease the first gene and increase the second gene.

Keywords: Histopathological, potassium dichromate, expression gene, kidney, male, mice

INTRODUCTION

Potassium dichromate is a inorganic substance used as an oxidizing agent in many applications. Potassium dichromate causes acute and chronic conditions, and harmful to health in the animals and humans in many organs (Gerd et al. 2005).

Because the sodium salt is the more common use in industry, potassium dichromate has very few important uses. The primary use is as primary agent to potassium chrome alum, which is utilized in the tanning of leather (Saha et al. 1993).

In recent years, there has been a marked rise in the amount of attention paid to heavy metal pollution in the environment owing to the fact that these contaminants are both pervasive and harmful in the ecosystem. The element chromium is present in both the oxidant state 2 and the oxidant state 6, and it is accumulative metals that has a significant harmful impact. It is created as a byproduct of human activity such as chrome oxidizing plant, which extracts chromium from burins rock and mines, papers and wood burns. Hexavalent chromium is the most hazardous form of the element, and it is utilized extensively in a broad variety of industrial applications. Hexavalent chromium will cause a rise by the amount of chromium in the environment, which will in turn cause human beings and other living species to be exposed to higher levels of the element (Al-Mukhtar et al. 2016).

Renal illnesses provide important problems to the health of people all over the world. Potassium dichromate, often known as potassium dichromate (PD), is a heavy metal that is commonly linked to nephrotoxicity. In renal tissues, PD causes damage in inflammatory and oxidative in nature. L-carnitine is an amino acid that occurs naturally and is often used as a dietary supplement (Salama et al. 2022).

In a study found that administration doses of potassium dichromate to the rabbit will lead into the generation of reactive oxygen species, pathological changes of the blood, nephrons, liver, testes and ovary (Mary et al. 2019). Potassium dichromate causes renal damage in the rat, and leading into increase the urinary excretion of protein (Gumbleton and Nicholls, et al. 1988). Potassium dichromate causes histopathological pictures of the kidneys in the rat and included that small-sized glomeruli, apoptotic epithelial lining in the proximal tubules, dilated congested blood vessels, and other structural changes (Salama et al. 2022).

Potassium dichromate causes the structural changes of the renal cortex in adult male albino (HANAN et al. 2019). The aims of the work are determination of the histopathological changes that occur in the mice that administrated potassium Dichromate (K2Cr2O7) in the kidney and determine the gene expression of NR3C1 gene and TP53 gene in treated group.

MATERIALS AND METHODS

Animal and study design

Twenty adult male mice with (20-30) grams in weight, (16) weeks in age were divided into two groups, group is formed from ten mice. The first group administrated potassium dichromate (67) Mg/kg/BW orally for 21 days, while the second group (control group) administrated Pbs. After finishing the administration period, all the animals are sacrificed; one kidney are taken and kept in formalin 10% for the histopathological examination, while another kidney is kept in TRIzol for RNA Extraction to determine the gene expression.

Histopathological examination

The tissue processing, involves several steps. Here are the general steps involved in this process:

1. Fixation: The tissue sample is first collected and immediately placed in a fixative solution, which preserves the tissue structure and prevents degradation. Common fixatives include formalin, ethanol, and methanol.
2. Dehydration: The tissue sample is then dehydrated by being immersed in a series of alcohol solutions with increasing concentrations (e.g., 70%, 80%, 90%, and 100% ethanol). This step removes the water from the tissue, making it easier to embed in paraffin wax.
3. Clearing: The dehydrated tissue is then transferred to a clearing agent, such as xylene or other organic solvents, to remove any remaining alcohol and to make the tissue transparent.
4. Infiltration: The cleared tissue is then placed in a bath of melted paraffin wax, which permeates the tissue and replaces the clearing agent. This step helps to support the tissue and make it easier to section.
5. Embedding: The infiltrated tissue is then placed into molds containing fresh paraffin wax and allowed to solidify. The paraffin-embedded tissue block is then ready for sectioning.
6. Sectioning: The block is sliced into (4-8) microns by microtome, then put on the slides.
7. Staining: then stained by hematoxylin and eosin, to show highlight and contrast the tissues structures.
8. Coverslipping: A coverslip is placed over the stained tissue section to protect it and preserve the staining.
9. The tissue has been processed and mounted on slides; it is ready for microscopic examination and analysis.

Extracted RNA

The RNA extraction is done by (Accuzol® kit. Bioneer). As follows, the sample 200µl are incubated for 4 h with Accuzol reagent I ml, then, the tubes were shaken. Add the chloroform (200) µl on the tubes with mixing by the vortex. The mixture was cooling inside the ice for five minutes. then centrifuged at (12000) rpm, then cooled at (4) C°, adding of the isopropanol (500) µl on transmitted supernatant layer in new Eppendorf with continues inverting then cooled at (4) C°. Centrifuge the mixture at (12) thousand rpm for 10 minutes. Remove the supernatant layer then add ethanol (1) ml with mixing, then repeat the centrifuge step. Then remove the supernatant layer, and then RNA pellet was dry by the air directly. Then adding DEPC water on the samples to dissolve the RNA pellet, then keep at (-20).

cDNA synthesis

cDNA forming was done by High-Capacity cDNA kit (Applied Biosystems). RNA sample was added to the tube and a mix with reverse transcriptase buffer, dNTP mix, reverse transcriptase random primers, MultiScribe reverse transcriptase, and DNA-free water. The mixture was placed at 25 °C for ten minutes, the heating at 37 °C for three hours, then heating at 85 °C for five seconds, then cooled at (4) °C.

Primers design

The primers used in the present study are designed for Spef2, and Catsper1 genes, done by several steps; the whole sequence is taken from the NCBI website (https://www.ncbi.nlm.nih.gov) under Genebank (NC_000081.7) for Spef2 gene and under Genebank (NC_000085.7) for Catsper1 gene. The whole sequence was transferred to the website primer plus 3 (https://www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) to pick specific primers

Table 1: shows the Sequence, Amplicon size, and Genebank of the used primers in the study

Primer		Sequence	Amplicon size	Genebank
NR3C1 gene	F	GCTGAAGGTTCCAAGCAAAG	192 bp	NC_000005.10
	R	ACTTTCCACCATTCCACTGC		
TP53 gene	F	GGACTGCAGGGTCTCAGAAG	184 bp	NC_000077.7
	R	CCAAGTCCCTTTCTGCTCTG		

Quantification by RT-PCR

The quantitative of mRNA was done by real-time PCR to produce CDNA. The reaction mix has primers (F and R), SYBR Green, DNA-free water, and cDNA sample. The thermocycling conditions were showed in the table (2).

Table 2: shows temperature, time, and cycle number for PCR stages

The stage	Temp. °C	Time/min	Cycle number
initial stage	95	10	1
Denaturation stage	95	15	40
Annealing stage	60	1	
Final stage	74	4	

Real-time PCR data analysis

RT-PCR was analyzed depending on (ΔΔCT) method as follows:

ΔCT = CT gene of interest−CT keeping house gene

(ΔΔCT) = CT of the corresponding groups - ΔCT of control

The fold change (FC) = $2^{-\Delta(\Delta CT)}$

Statistical analysis

The DATA were analyzed by one way and two-way ANOVA by using SPSS, V27 software. LSD test determine the difference among the groups at (P≥0.05) (Schiefer, 1980).

The results

1- The histopathological examination

The current study findings demonstrated that the first group (treated by potassium dichromate) showed atrophy of glomeruli of the kidney, vascular dilatation, and necrosis as shown in figure (1). The second group (control group) showed that normal glomeruli of the kidney, normal proximal, distal and convoluted tubules. As showed in figure (2).

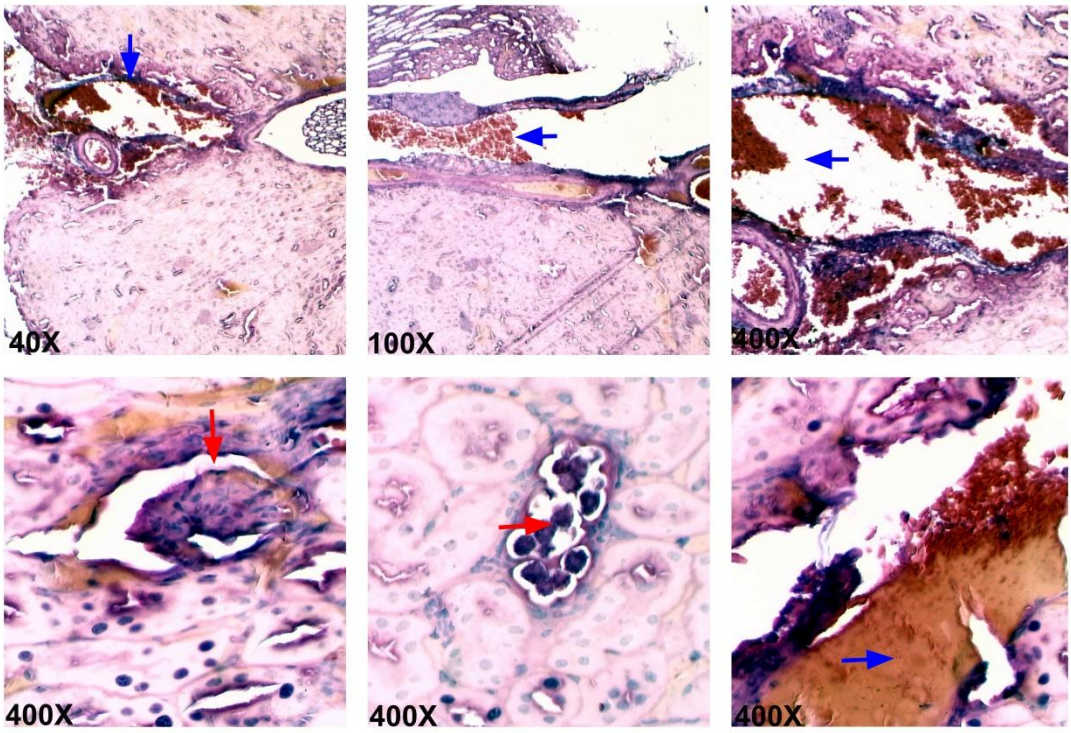

Figure 1: shows atrophy of glomeruli in the kidney (red arrows), and vascular dilatation (blue arrows), H&E, 4X, 100X, and 400X.

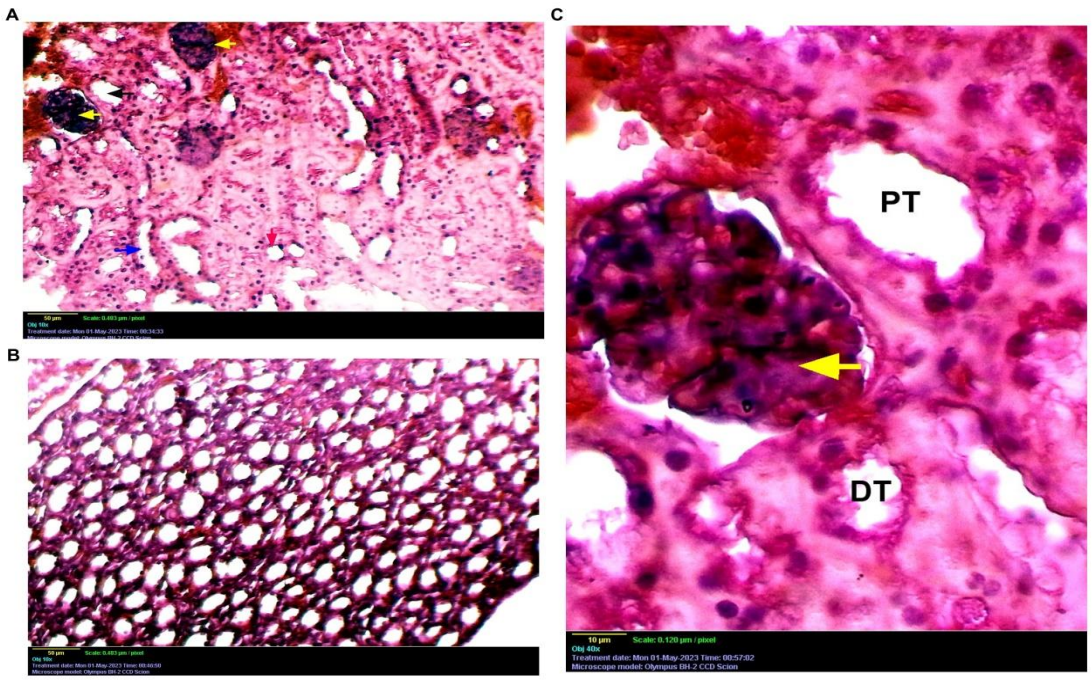

Figure 2: The normal histological section of the control group A- normal glomeruli of the kidney (yellow arrows) and normal proximal (black arrow), distal (red arrow), and convoluted (blue arrow) tubules. H&E, 100X. B- The kidney shows normal renal tubules. H&E, 100X. C- Kidney shows normal glomeruli (yellow arrows) and normal proximal (PT) and distal (DT). H&E, 400X.

2- The gene expression

The gene expression of NR3C1 gene is decrease in the first group than in the healthy group. In addition to that, the first group showed that increase TP53 gene expression as compared with the control group, as shown in table (3).

Table 3: shows the fold change of the TP53, and TERT gene in the study groups

The gene	G1	G2
NR3C1	**0.76 A**	**1 B**
TP53	**2.12 A**	**1 B**

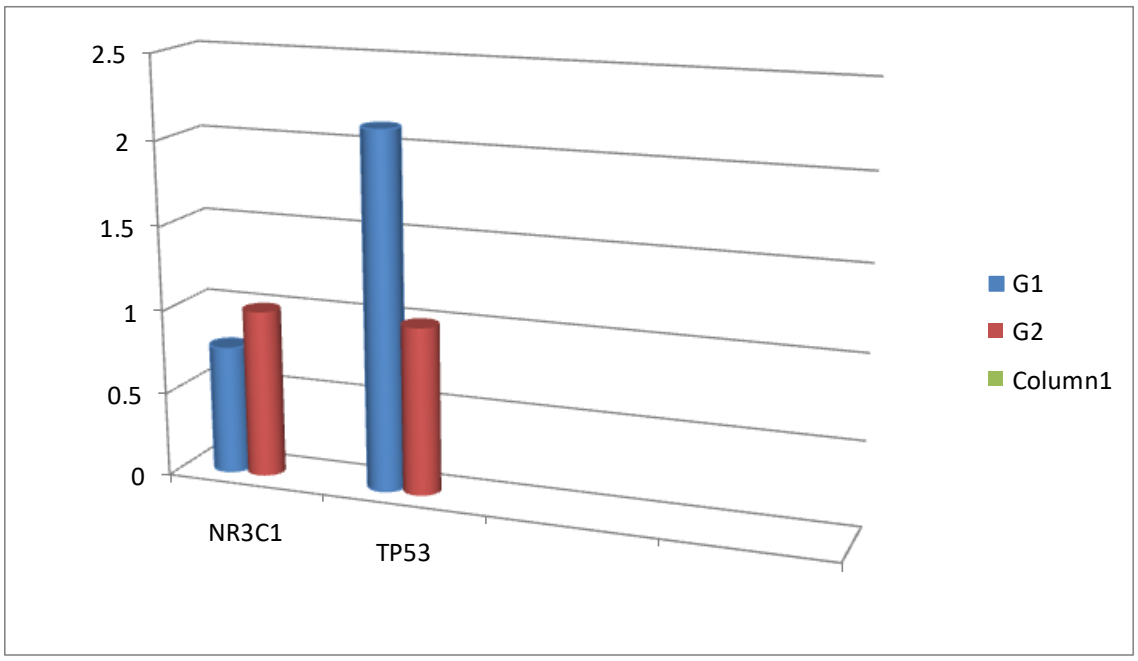

Figure 3: the fold change levels of TP53, and TERT gene in the study groups

DISCUSSION

According to our results, Potassium Dichromate causes changes in the kidney tissues, included that atrophy of glomeruli of the kidney, vascular dilatation, and necrosis.

Atrophy of glomeruli, vascular dilatation, and necrosis are occurring due to exposure to potassium dichromate (Turkmen et al. 2022) (Fatima et al. 2005). These changes demonstrated in mice and rats that administrated potassium dichromate, and have been evaluated by histopathological examination (Fedala et al. 2022). Additionally, studies have investigated the potassium dichromate-induced nephrotoxicity in rats (De Ceaurriz et al. 1991).

Exposure, to potassium dichromate leads to the occurrence of atrophy, vascular dilatation and necrosis (Salama et al. 2022). These effects have been observed in both rats and mice through examination. Furthermore researchers have conducted studies, on the nephrotoxicity induced by potassium dichromate in rats (Salama et al. 2022).

Thirty-three white male New Zealand rabbits in good health were given chromium at a dose of 40 milligrams per kilogram of body weight each day. Negative effects such as a significant decrease in testosterone and inhibin-B hormone, glutathione, and a rise in the levels of FSH, aspartate amino transferase, malondialdehyde, amino transferase, cholesterol, and were shown by the results (Al–Hadidy, & Mostafa, 2022). This was in addition to histological variation in the kidney.

Potassium dichromate causes nephrotoxic xenobiotic that lead to acute tubular necrosis in rats. Injection of Potassium dichromate in the rats causes renal failure, nephrotoxicity and histopathological changes of the kidney (Salama, et al. 2016). The rats administrated potassium dichromate (10 mg/kg b.wt. s.c.), and the study found that potassium chromoniun causes the acute renal damage (Baiomy, et al. 2023). PD is causes inflammation and oxidative stress in the renal tissues in the rats (Al Jameil, et al. 2017).

Potassium dichromate causes acute renal injury, histopathological changes, oxidative stress and inflammation in the rat (TANG, et al. 2021). Potassium dichromate causes necrosis of the renal tubules also, results with dilatation of the tubular due to inflammation occurrence, accumulation of the collagen fibers, and cell apoptosis. The histopathological examination are included that potassium dichromate causes nephrotoxicity (El-Mahalaway, et al. 2015).

Potassium dichromate has potential nephrotoxic in humans and animals. PD causes the overproduction of free radicals, oxidative damage, and nephrotoxicity, high level of Urea, uric acid, LDH, NFκB, IGF-1, MT, and GST. Potassium dichromate causes apoptosis in renal tissues with large area of necrosis and degeneration (Karhib, et al. 2022). In another study found that heavy metals are a very harmful environmental pollutant, and induced toxicity and carcinogenicity. Chrome compounds has hepatotoxic, immunotoxic, neurotoxic, genotoxic, reproductive toxic, pulmonary toxic, nephrotoxic effects. Chrome causes negative histopathological changes in the kidney structure in mice. The histopathological of the kidney treated animals are involved degeneration, necrosis, pycnosis, swelling of the cells, necrosis, and congestion of the blood vessels (Kehiosh, & Al-fatlawi, 2017).

The current study showed that the gene expression of NR3C1 gene decrease in the treated group by potassium dichromate as compared with the control group. The treated group by potassium dichromate showed that increase TP53 gene expression as compared with the control group. All the listed and mentioned studies showed results similar to our results.

Potassium dichromate can cause a number of human disease including inflammation and cancer. Potassium dichromate induced oxidative damage and apoptosis. Potassium dichromate stabilized p53 by phosphorylation at Ser15 and increased expression of p53-transcriptional target p21, which leading into activation of p53 gene in L-02 hepatocytes (Zhang Y, et al. 2016).

Potassium dichromate effects on intrinsic apoptotic. Potassium dichromate induced DNA fragmentation and increased apoptosis, downregulated anti-apoptotic; upregulated p-ERK, increased phosphorylation of p53, increased p53 transcriptional activation in the granulosa cells (Sakhila et al. 2011).

Glucocorticoid receptor is encoded by the gene NR3C1, and it may work both as a transcription factor, which binds to glucocorticoid response elements in the promoters of glucocorticoid-responsive genes. Glucocorticoid response elements are found in the promoters of glucocorticoid-responsive genes. When a ligand binds to this receptor, it causes it to be carried into the nucleus, where it is normally located in the cytoplasm. In target tissues, it has a role in inflammatory reactions as well as the proliferation and differentiation of target cells. The methylation of the NR3C1 gene has been linked to exposure to early-life stress. Methylation of the NR3C1 gene has been shown to be linked with sensitivity to psychosocial stress. According to Helena et al. 2015's research, NR3C1 gene methylation corresponds to NR3C1 expression.

NR3C1 expression levels depended on the biological environment such as cancer in many tissues (Shim et al. 2022). Potassium dichromate causes decreasing in the expression of NR3C1 mRNA, and that support our results (Wu F, et al. 2012).

CONCLUSION

Potassium Dichromate causes the histopathological changes of the mice kidney that are included that atrophy of glomeruli of the kidney, vascular dilatation, and necrosis as compared with the control group. Furthermore, the gene expression of NR3C1 gene decrease in the group that treated by potassium dichromate as compared with the control group. In addition to that, the group that treated by potassium dichromate showed that increase TP53 gene expression as compared with the control group.

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