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Allium sativum aqueous extract prevents potassium dichromate-induced nephrotoxicity and lipid oxidation in rats

[El extracto acuoso de *Allium sativum* previene la nefrotoxicidad y la oxidación lipídica inducida por dicromato de potasio en ratas]

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Abstract

Context: The potassium dichromate ($K_2Cr_2O_7$) induces nephrotoxicity by oxidative stress mechanisms.

Aims: To study the potential protection of an aqueous extract of *Allium sativum* against the $K_2Cr_2O_7$-induced nephrotoxicity and lipid oxidation in rats.

Methods: Twenty four hours after treatment, biomarkers such as proteinuria, creatinine clearance, malondialdehyde production, specific enzyme activity of gamma glutamyl transpeptidase and alanine aminopeptidase, and renal clearance of para-aminohippuric acid and inulin were measured.

Results: The $K_2Cr_2O_7$ caused significant renal dysfunction, but *A. sativum* extract prevented this condition by improving all measured biomarkers.

Conclusions: A single injection of $K_2Cr_2O_7$ induced nephrotoxicity in rats, but the supply of an *Allium sativum* aqueous extract prevented the disorders caused by this metal.

Keywords: Creatinine; garlic extract; inulin; malondialdehyde; proteinuria; renal clearance.

Resumen

Contexto: El dicromato de potasio ($K_2Cr_2O_7$) induce nefrotoxicidad por mecanismos de estrés oxidativo.

Objetivos: Estudiar la protección potencial de un extracto acuoso de *Allium sativum* contra la nefrotoxicidad y oxidación lipídica inducida por $K_2Cr_2O_7$ en ratas.

Métodos: Veinticuatro horas después del tratamiento se midieron biomarcadores tales como proteinuria, aclaramiento de creatinina, producción de malondialdehído, actividad específica de las enzimas transpeptidasa de gamma glutamilo y aminopeptidasa de alanina, y aclaramiento de ácido paraaminohipúrico e inulina.

Resultados: El $K_2Cr_2O_7$ causó disfunción renal significativa, pero el extracto de *Allium sativum* previno esta condición mejorando todos los biomarcadores valorados.

Conclusiones: Una sola inyección de $K_2Cr_2O_7$ provocó nefrotoxicidad, pero el suministro de un extracto acuoso de *Allium sativum* evitó las alteraciones causadas por este metal, en ratas.

Palabras Clave: Creatinina; extracto de ajo; inulina; malondialdehído; proteinuria; aclaramiento.

List of abbreviations: CC - creatinine clearance; GE - *Allium sativum* (garlic) extract; $K_2Cr_2O_7$ - potassium dichromate; MDAP - malondialdehyde production; PE - proteinuria; RC-I - renal clearance of inulin; RC-PAHA - renal clearance of para-aminohippuric acid; SA-AAP - alanine aminopeptidase; SA-GGT - specific enzyme activity of gamma glutamyl transpeptidase.

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INTRODUCTION

Hexavalent chromium (Cr VI) is toxic, carcinogenic (Panda and Sarkar, 2014), and causes occupational diseases (Xiao et al., 2013). This metal occurs in higher concentration in the wastes from electroplating, paints, dyes, chrome tanning, and paper industries (Elci et al., 2010). Reducing this element leads to intracellular generation of reactive oxygen species (Balakrishnan et al., 2013) which has been associated with oxidative stress events (Borthiry et al., 2007), causing cell damage (Manerikar et al., 2008). This xenobiotic is considered as a potent nephrotoxic in both humans and animals (Parveen et al., 2009).

Cr VI compounds, such as potassium dichromate ($K_2Cr_2O_7$), are rapidly absorbed and more corrosive than other valence states (Costa, 1997; Pellerin and Booker, 2000) and enters the cell with the help of sulfate nonspecific transporters located in the plasma membrane (Chwastowski and Koloczek, 2013).

Cr VI is a thousand times more toxic than Cr III (Zhang and Jin, 2006). A single dose of $K_2Cr_2O_7$ induces expression of certain markers of nephrotoxicity (Fatima et al., 2005). The kidney is the first organ that expresses the toxicity promoted by metals (Barbier et al., 2005; Fatima et al., 2005). The renal function and the concentration of enzyme and protein may be changed when people contact or intake excessive chromium compounds (Zhang and Jin, 2006); it causes severe progressive proteinuria (PE) followed by polyuria and glucosuria (Kim and Na, 1991).

Experimental results provide that different nephrotoxic substances affect the brush border membrane of the proximal tubule; one study suggests that nephrotoxicity produced by $K_2Cr_2O_7$ may be caused, in part, by its effect on the functioning of the proximal tubule (Fatima et al., 2005). Similarly, renal dysfunction and structural changes are manifested, for example, as changes in serum creatinine, blood urea nitrogen, creatinine clearance (CC), PE, plasma glutathione peroxidase activity, urinary excretion of N-acetyl-β-d-glucosaminidase, and histology (Molina-Jijón et al., 2011). A single dose (15 mg/kg body weight) resulted in an increase in lipid peroxidation and decrease in total sulfhydryl groups (Fatima and Mahmood, 2007).

Chromium also affects the activity of renal brush border membrane enzymes such as gamma-glutamyl transpeptidase, alanine amino-peptidase (Becerra-Torres et al., 2009a), and dipepti-dylaminopeptidase IV (Becerra-T et al., 2008). A single injection of chromium is sufficient to induce lipid peroxidation systemically, associated with decreased clearance of kidney function marker substances such as para-aminohippuric acid and inulin (Becerra-Torres et al., 2009b).

Additionally, there are substances which counteract the effects of chromium on the kidney, among which are vitamin C (Chambial et al., 2013; Fatima and Mahmood, 2007; Xiao et al., 2013), glutathione (Hojo and Satomi, 1991), quercetin (Becerra-Torres et al., 2009b), vitamin E (α-tocopherol) (Arreola-Mendoza et al., 2006; Arreola-Mendoza et al., 2009; Balakrishnan et al., 2013), selenium (Soudani et al., 2010), caffeic acid (Arivarasu et al., 2012), curcumin (Molina-Jijón et al., 2011), palm oil (Khan et al., 2010), folic acid (El-Demerdash et al., 2006), and garlic (Deniz et al., 2011).

Antioxidants slow or prevent the oxidation of molecules important for cell homeostasis; these substances are nucleophilic agents and they can be found in garlic, brown rice, coffee, cauliflower, broccoli, ginger, parsley, onion, citrus, milk, tomato, grapes, tea, and rosemary, among others. In many organisms we can find substances that have antioxidant activity such as glutathione, vitamin C, and vitamin E, as well as enzymes such as catalase, superoxide dismutase, and peroxi-dases. Low levels of antioxidants and inhibition or depletion of antioxidant enzymes can cause oxidative stress, favoring cell damage.

Oxidative stress has been associated with pathogenesis of many human diseases, which is why the use of antioxidants in pharmacology is intensively studied.

Given the importance of combating health disorders caused by heavy metals, in this study the effect of *A. sativum* (garlic) extract (GE) on $K_2Cr_2O_7$-induced nephrotoxicity was assessed as some have reported antioxidant effects manifested by the bulb tested, whose nutraceu-tical properties might be of interest to revert or

prevent diseases induced by metals. The dose of chromium used in this study has been used by many researchers since it is not lethal in rats and causes nephrotoxicity (Fatima and Mahmood, 2007), and have not yet been investigated the effects of garlic on some of the markers of renal function addressed in this work.

MATERIALS AND METHODS

Plant material and reagents

Fresh garlic (*Allium sativum* L., Amaryllidaceae) was purchased at a market in the city of Aguascalientes to prepare an aqueous extract; it had a final concentration equal to 71 mg of garlic/mL of distilled water and was prepared as reported by Ahmed et al. (2012). Potassium dichromate was purchased in J. T. Baker (México, D. F.) and was dissolved in distilled water. Remaining chemicals were of analytical reagent grade.

Experimental animals

Experimental procedures were carry out in accordance with the guidelines of Norma Oficial Mexicana for the use and care of laboratory animals (NOM-062-ZOO-1999) and for disposal of biological residues (NOM-087-SEMARNAT-SSA1-2002). All procedures were made to minimize animal suffering and were approved by the local ethical committee.

Male Wistar rats weighing 250 ± 6 g (2.5 months old) were obtained from the animal breeding center located in the Universidad Autónoma de Aguascalientes, México. They were kept in controlled conditions of temperature (21 ± 0.8°C), humidity (40-60°C), and light-dark cycles of 12 h, with food (Ralston Rations-Kansas, USA) and water *ad libitum*. The experiments took place during the light period and animals were divided randomly in four treatment groups (with eight members per group).

Experimental design

The animals were divided into four groups as follows:

- Group 1 (control group): received no treatment;
- Group 2: received only one injection of $K_2Cr_2O_7$ (15 mg/kg body weight, i.p.) on day 21;
- Group 3: was supplied with 250 mg of GE/kg/day, orally, for 21 days [dose consistent with previous reports (Deniz et al., 2011)] + $K_2Cr_2O_7$ injection (15 mg/kg body weight, i.p.) on day 21;
- Group 4: received only GE (250 mg/kg/day, orally, for 21 days).

Twenty four hours after the respective treatment samples were taken for immediate analysis. To collect urine, each group was placed in a metabolic cage with free access to distilled water and rodent food.

Biochemical assays

Urine samples were taken 24 hours after treatment. The specific enzyme activity of gamma glutamyl transpeptidase (SA-GGT) and alanine aminopeptidase (SA-AAP) were assessed using a Varian DMS 80 UV/VIS spectrophotometer (Varian, Inc., Scientific Instruments, CA, USA). Gamma-glutamyl p-nitroanilide and alanine substrates were prepared at a molarity equal to 16 mM for each, recording its hydrolysis at 405 nm intensity. Activities were expressed in units per milligram of protein (1 unit = 1 µmol of p-nitroaniline/min). In this context, dichromate-induced nephrotoxicity was assessed by measuring CC, PE, renal clearance of para-aminohippuric acid (RC-PAHA) and renal clearance of inulin (RC-I).

Additionally, systemic lipid oxidation was measured by assessing malondialdehyde production (MDAP). To determine creatinine, the reaction of creatinine kinetics alkaline reagent (AA) + creatinine AA + urine creatinine (picric acid) was measured, then read at 510 nm (Bonsnes and Tausky, 1945). PE was assessed by the method of Lowry (Lowry et al., 1951). RC-PAHA and RC-I were measured after urine collection.

To determine MDAP (µmol/mL) rats were sacrificed at the end of periods of urine collection and blood was obtained by heart puncture; for this analysis, a method based on the reaction of malondialdehyde (MDA) with thiobarbituric acid was used (Draper and Hadley, 1990), at 95°C with

readings at 530 nm. All tests were made 24 hours after the day 21 of treatment.

Statistical analysis

The results of this study were analyzed using ANOVA and Tukey Kramer tests, by means of GraphPad Prism 6 (GraphPad Software, Inc., La Jolla, CA, USA), and are expressed as the mean ± standard error of the mean (SEM). A p value less than 0.05 was considered statistically significant.

RESULTS

A single injection of $K_2Cr_2O_7$ caused acute kidney injury in rats without mortality. The values of PE, CC, MDAP, SA-GGT, SA-AAP, RC-PAHA, and RC-I, showed significant alterations when $K_2Cr_2O_7$ was administered compared with the values of the control group (P = 0.0001). Conversely, oral treatment with GE for 21 days expressed antioxidant properties to prevent the effects of $K_2Cr_2O_7$.

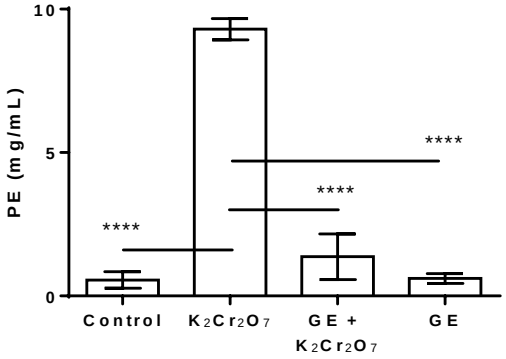

Figure 1. Effect of *A. sativum* extract (GE, 250 mg/kg, p.o. during 21 days) on proteinuria (PE) in rats treated with $K_2Cr_2O_7$ (15 mg/kg, i.p., single dose). Values are mean ± SEM, n=8. ****p=0.0001 statistical significance corresponding to $K_2Cr_2O_7$ versus control, $K_2Cr_2O_7$ versus GE + $K_2Cr_2O_7$, and $K_2Cr_2O_7$ versus GE. No differences were observed when comparing the groups control, GE + $K_2Cr_2O_7$, and GE, each other.

In Fig. 1 is shown that the increase in PE was prevented by providing GE (GE + $K_2Cr_2O_7$), an event which is held in the normal, thereby preventing the deleterious effects of metal. It is also observed that the decrease of CC was prevented with garlic (Fig. 2), also showing the protective effect on kidney stability with respect to this parameter. Furthermore, at the systemic level was increased lipid oxidation but similarly garlic pretreatment prevented this disorder (Fig. 3).

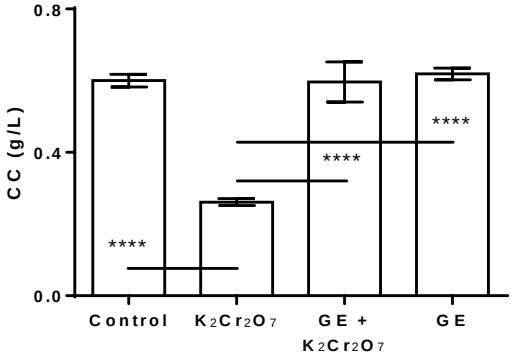

Figure 2. Effect of *A. sativum* extract (GE, 250 mg/kg, p.o. during 21 days) on creatinine clearance (CC) in rats treated with $K_2Cr_2O_7$ (15 mg/kg, i.p., single dose). Values are mean ± SEM, n=8. ***p=0.0001 statistical significance corresponding to $K_2Cr_2O_7$ versus control, $K_2Cr_2O_7$ versus GE + $K_2Cr_2O_7$, and $K_2Cr_2O_7$ versus GE. No differences were observed when comparing the groups control, GE + $K_2Cr_2O_7$, and GE, each other.

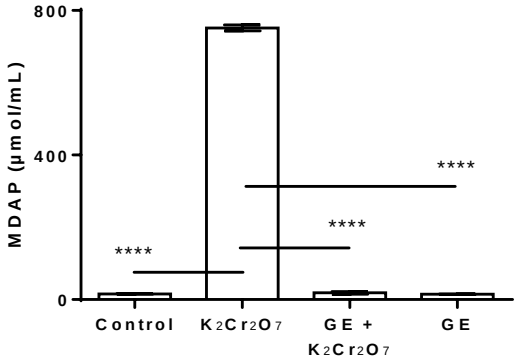

Figure 3. Effect of *A. sativum* extract (GE, 250 mg/kg, p.o. during 21 days) on the malondialdehyde production (MDAP) in rats treated with $K_2Cr_2O_7$ (15 mg/kg, i.p., single dose). Values are mean ± SEM, n=8. ****p=0.0001 statistical significance corresponding to $K_2Cr_2O_7$ versus control, $K_2Cr_2O_7$ versus GE + $K_2Cr_2O_7$, and $K_2Cr_2O_7$ versus GE. No differences were observed when comparing the groups control, GE + $K_2Cr_2O_7$, and GE, each other.

In this context, SA-GGT and SA-AAP (markers of the renal activity with functions of absorption; found in early proximal convoluted tubule, late proximal convoluted tubule, and proximal straight tubule) were assessed. A significant decrease in activity was observed after treatment

with chromium and injury prevention after supplying GE (Fig. 4A and B).

To corroborate the $K_2Cr_2O_7$ ability to induce acute kidney damage, resulting in the disruption of its operation, RC-PAHA and RC-I were measured, which are very fine and specific tests to contribute to the consolidation of the claim in respect of that chromium is a nephrotoxic substance affecting filtration and secretion of substances, physiological events that represent the total sum of the physiological and biochemical phenomena occurring in the kidney (valid consideration at least for the purpose of this study). In this regard, it is noted that the metal caused a reduction of RC-PAHA (Fig. 5A) and RC-I (Fig. 5B), events prevented by GE.

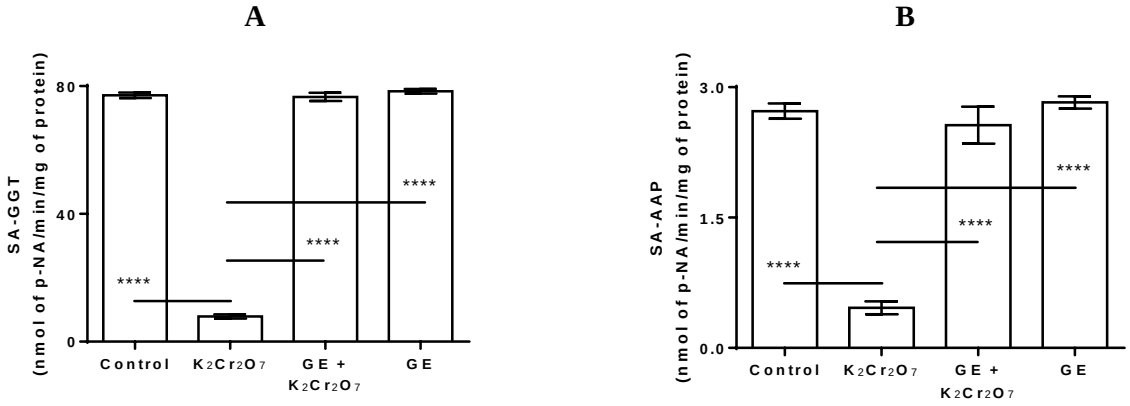

Figure 4. Effect of *A. sativum* extract (GE, 250 mg/kg, p.o. during 21 days) on **A.** gamma glutamyl transpeptidase (SA-GGT), and **B.** alanine aminopeptidase (SA-AAP) in rats treated with $K_2Cr_2O_7$ (15 mg/kg, i.p., single dose). Values are mean ± SEM, n=8. ****p=0.0001 statistical significance corresponding to $K_2Cr_2O_7$ versus control, $K_2Cr_2O_7$ versus GE + $K_2Cr_2O_7$, and $K_2Cr_2O_7$ versus GE. No differences were observed when comparing the groups control, GE + $K_2Cr_2O_7$, and GE, each other. p-NA: paranitroanilide.

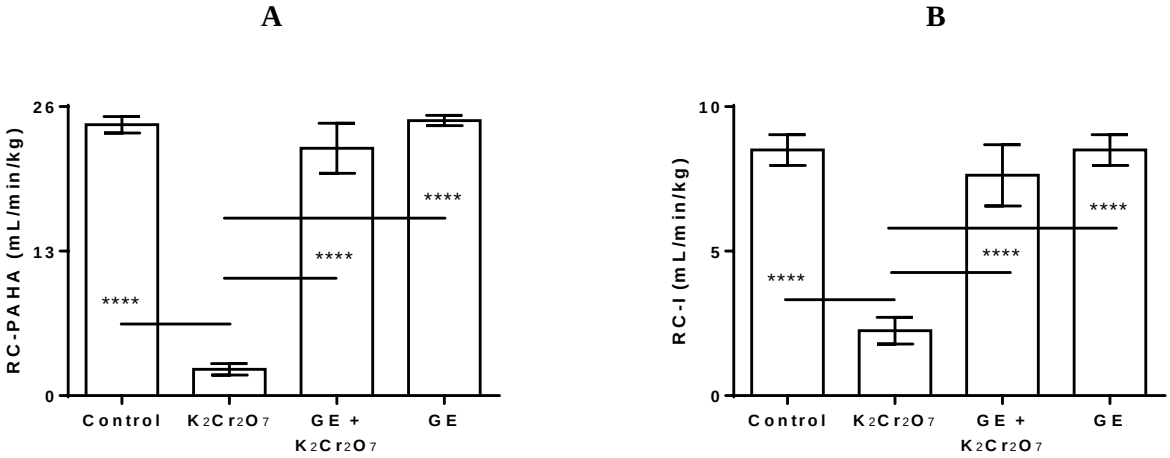

Figure 5. Effect of *A. sativum* extract (GE, 250 mg/kg, p.o. during 21 days) on **A.** renal clearance of para-aminohippuric acid (RC-PAHA) and **B.** renal clearance of inulin (RC-I) in rats treated with $K_2Cr_2O_7$ (15 mg/kg, i.p., single dose). Values are mean ± SEM, n=8. ****p=0.0001 statistical significance corresponding to $K_2Cr_2O_7$ versus control, $K_2Cr_2O_7$ versus GE + $K_2Cr_2O_7$, and $K_2Cr_2O_7$ versus GE. No differences were observed when comparing the groups control, GE + $K_2Cr_2O_7$, and GE, each other.

DISCUSSION

The $K_2Cr_2O_7$ is a molecule with nephrotoxic properties. However, its effects go beyond this statement; this metal causes a significant decrease in renal ability to secrete and filter substances, which may result in acute renal failure (Wedeen and Qian, 1991).

Our decision to measure kidney function at 24 hours after treatment with chromium and GE was due to previous studies that showed the occurrence of oxidative and nitrosative stress, induced by a single injection of $K_2Cr_2O_7$, was observed early (Barrera et al., 2003a; 2003b) and exhibited the degeneration of the brush border membrane (Fatima et al., 2005).

In this sense, we have already reported increased of PE, decreased of CC and increased of MDAP (Becerra-Torres et al., 2009a), demonstrating the oxidative renal damage induced by Cr V (Liu and Shi, 2001; Sengupta et al., 1992; Stohs and Bagchi, 1995). Furthermore, our results show dichromate depressive effect on SA-GGT, SA-AAP, RC-PAHA, and RC-I, which is consistent with the fact that chromium harms the activities of kidney enzymes (Fatima et al., 2005; Blum and Fridovich, 1985) and produces necrosis in the proximal tubule (Gumbleton and Nicholls, 1998).

Elucidating, PE is a marker used to recognize renal effects caused by diseases and by nephrotoxic substances; it is well known that chromium exhibits a tearing effect in the renal tubule, which is consistent with the urine protein increase in the group treated with dichromate coupled with the fact that the male rats excreted, in a natural way, a lot of low molecular weight α-globulins which are not found in the urine of the female rats or human. It should be noted that rats develop age-dependent chronic progressive nephropathy which produces them naturally proteinuria (Trevisan et al., 2001).

Moreover, the glomerular filter consists of several layers: in the urinary tract, in the visceral layer of Bowman's capsule, there are cells interconnected by podocytes; the space, the slit-like, is coated with a membrane called oblique with pores of 5 nm diameter; the basal membrane and the endothelium are other layers that are attached to the capillaries. Specifically, it is known that chromium causes necrosis in renal tubule; this condition attracts swelling factors, complex glomerular then swells, reducing the functional capacity and the spaces. This disorder could have contributed to the decrease in glomerular filtration rate observed in this research (CC, RC-PAHA, and RC-I).

Regarding the reduction of SA-GGT and SA-AAP, previously we have set this parameter as a marker of renal damage induced by chromium and have suggested two mechanisms by which the decrease in the activities of these two enzymes is raised: oxidation or modification of its structure by direct interaction with chromium, as the first track of toxicity of this element is bonding with sulfhydryl groups on proteins (Becerra-Torres et al., 2009a).

Similarly, the effect of chromium on RC-PAHA could be due to the observed effect on these enzymes: inulin is excreted, and the para-aminohippuric acid is excreted and secreted; the rate-limiting step or renal organic anion secretion is its basolateral uptake into proximal tubular cells; this process is mediated by the organic anion transporters OAT1 and OAT3 which both have a broad spectrum of substrates including toxins (Schneider et al., 2007). These transporters also may have been oxidized and/or modified by contact with dichromate leading to deregulation which caused an alteration of their functions.

In contrast, garlic is a nucleophilic substance that has antioxidant qualities like many others of the same profile. This property provides skills to counteract the effects of electrophilic substances on organisms. The main role of an antioxidant is to prevent, delay or reverse reactions leading to the oxidation of biological substrates (proteins, lipids, and nucleic acids). The antioxidant stabilizes the free radical by electron donation. With this, the radical loses reactivity and the antioxidant is oxidized.

So far, our results are consistent with previously reported. However, the increased importance of this work is to be shown to GE as a protective agent with nucleophilic properties to

prevent effects against renal effects caused by a single injection of $K_2Cr_2O_7$ in the rat, contained herein. It is clear, researchers have the opportunity to deepen the understanding of the mechanisms by which chromium interacts with organic molecules, thus elucidate about the best way to attack the disorder caused. Similarly, an experiment to demonstrate the dose-dependent effect of GE on $K_2Cr_2O_7$-induced toxicity should be carried out in future studies.

CONCLUSIONS

In this study we found that the administration of an aqueous extract of *Allium sativum*, for 21 days, prevents potassium dichromate-induced nephrotoxicity and lipid oxidation in rats.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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