Protective effect of ginger against toxicity induced by chromate in rats

Effet protecteur du gingembre contre la toxicité induite par le chromate chez les rats

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Abstract. The evaluation of the effect of ginger on the modulation of toxic effects induced by chromate is the objective of our study. 50 male rats Albino wistar were divided to five groups as follow: group I (T) is served as control, received a mineral water by gavage (per os); group II (G) received an experimental diet with 2% of ginger; group III (Cr) received an oral dose of potassium dichromate (15 mg/kg) and normal diet; group IV (CrG): received an oral dose of potassium dichromate (15 mg/kg) and an experimental diet containing 2% ginger; and group V (Cr+G) received an oral dose of potassium dichromate (25 mg/kg) and an experimental diet with 2% of ginger. The results of this study indicate that the chromate provoked a haematoxic effect (anemia), nephrotoxic, hepatotoxic, and also a perturbation in lipids profile. In addition, chromate has a pro-oxidant effect, which was indicated by decrease of reduced glutathione (GSH) levels in different tissues. However, the administration of ginger revealed a reduction of the intensity of oxidative stress induced by the chromate resulting in the decrease of the majority of the previous parameters concentrations. In conclusion we demonstrated that ginger has potent anti-oxidants activity, revealed by the amelioration of chromate’s toxic effects. We can say that ginger has a protective effect towards damages induced by the chromate.

Key words: ginger, potassium dichromate, rat, oxidative stress, toxicity

Résumé. L’évaluation de l’effet du gingembre sur la modulation des effets toxiques induits par le chromate constitue l’objectif de notre étude. Cinquante rats mâles de la souche Albino wistar ont été répartis en cinq groupes comme suit : groupe I (T) a servi de témoin, et a reçu une eau minérale par gavage (per os), groupe II (G) a reçu un régime expérimental avec 2 % de gingembre; groupe III (Cr) a reçu une dose orale de dichromate de potassium (15 mg/kg) et un régime alimentaire normal ; groupe IV (CrG) a reçu une dose orale de dichromate de potassium (15 mg/kg) et un régime expérimental contenant 2 % de gingembre, et groupe V (Cr+G) a reçu une dose orale de dichromate de potassium (25 mg/kg) et un régime expérimental avec 2 % de gingembre. Les résultats de cette étude indiquent que le chromate a provoqué un effet hématotoxique (anémie), néphrotoxique et hépatotoxique. En outre, le chromate a un effet pro-oxydant, qui a été indiqué par la diminution du taux du glutathion réduit (GSH) dans les différents tissus. Cependant, l’administration de gingembre a révélé une réduction de la toxicité du chromate en améliorant les niveaux des paramètres étudiés. En conclusion, nous avons démontré que le gingembre a une puissante activité anti-oxydante, qui peut réduire les effets toxiques induits par le chromate, et par conséquence atténuer l’intensité du stress oxydant qui l’a induit.

Mots clés : gingembre, potassium dichromate, rat, stress oxydatif, toxicité
Heavy metals are toxic and do not have biodegradable properties, remaining in the ecosystem [1]. Chromium is the 10\textsuperscript{th} abundant element in the earth’s mantle [2], it belongs to the first series of the transition elements [3], and it is a highly toxic metal [4]. However, chromium is a trace element of importance in human physiology [5] and essential trace element for human beings and animals [6], involved in glucose and lipid metabolism [7]. Multiple industries using chromium, it is used in the manufacture of alloys and treatments metal against corrosion and other oxidizing attacks [8], also it is used for obtaining domestic utensils, auto parts, bricks refractors [9], and wood preservation [10]. Forms of chromium III are considered non-toxic, chromium III is also recognized as an essential trace element [9], but if trivalent chromium did enter the cell, it will be very toxic [11] and exerts more damage within the cell [4]. Acute chromium poisoning is associated with renal and liver failure, gastrointestinal hemorrhage, vomiting, diarrhea, central nervous system disorders anemia, and coagulopathies [12]. Prevailing theories to explain the molecular mechanisms of metal toxicity mostly center on the generation of reactive oxygen species (ROS). However ROS-associated oxidative damage may be incidental rather than a cause of toxicity, and the principal cause of most metals’ toxicities remains to be revolved unequivocally [4].

Herbs and spices, which are important part of the human diet, have been used for thousands of years to enhance the flavor, color and aroma of food. In addition to boosting flavor, herbs and spices are known for their preservative, antioxidant, antimicrobial and various other medicinal values [13, 14]. Ginger is one of the oldest herbs known by the people and is one of the earliest spices to be known in the east. Ginger of the commerce consists of thick scaly rhizomes of the plant Zingiber officinale, belonging to the family Zingiberaceae. The plant is indigenous to warm tropical climates, particularly southeastern Asia. It is now extensively cultivated in India, China, Africa, Jamaica, Mexico and Hawaii [14]. A history of ginger and its uses is well documented. It has been in medical use since ancient times and in Europe at least since the 12\textsuperscript{th} century [15]. The Chinese have long valued ginger (Zingiber officinale Roscoe) to promote strength and to ensure a long life. It was one of the first products to travel the “spice route” from Asia to Europe, where Greeks and Romans used it extensively in their pharmacopoeias as well as spice, aromatic and food [16]. The rhizomes (ginger: commonly known as “adrk”) are frequently prescribed in Indian and Chinese system of medicine for the treatment of cough, stomach-ache, asthma, worms, leprosy, skin, gastrointestinal and respiratory diseases [17], diarrhea, nausea, toothache, gingivitis, and arthritis [18], pains cramps, indigestion, hypertension [19]. The constituents of ginger are numerous and vary depending on the place of origin and whether the rhizomes are fresh or dry [20]. Shagaol and gingerol are the effective substance in ginger, which have local effects on the digestive system [21]. One of the major pigment constituents of ginger, [6] gingerol, has been shown to have many interesting pharmacological effects for example: antioxidant, antitumor promoting, and anti-inflammatory effects [22]. Several authors have shown that ginger is endowed with strong \textit{in vitro} and \textit{in vivo} antioxidant proprieties [14, 19]. The antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plants against toxicity and lethality of radiation [20].

Hence, the aim of the present study is to evaluate the effect of ginger on the modulation of toxic effects and oxidative stress induced by sub-chronic chromate exposure in experimental rats.

**Material and methods**

**Experimental animals**

50 male rats (\textit{Albino wistar}) weighing (200\pm 10 g) were obtained from Pasteur institute, Algiers (Algeria). The rats were kept under standard laboratory conditions of light-dark cycle (12 h/12 h), temperature (21\pm 3^\circ C) and relative humidity (50\pm 100\%), with food and water \textit{ad libitum}, and they received food prepared according to Upreti et al. [23] regime.

**Preparation of chromium solution (induction of oxidative stress)**

Potassium dichromate (K\textsubscript{2}Cr\textsubscript{2}O\textsubscript{7}) powder (Biochem Chemopharma Company, USA) is dissolved in mineral water, and induced by oral voice; the volume of each dose was adjusted to deliver 15 or 25 mg/kg of body weight.

**Preparation of ginger powder**

Fresh ginger rhizomes were purchased from the local market, washed, peeled coarsely minced, air dried, and pulverized with a blender to fine powder and preserved in individual airtight containers room temperature until the formulation of normal and/or experimental diet.

**Animals treatment**

The rats were divided into five groups (10 rats in each group) as follow: Group I (T) served as control received mineral water orally; Group II (G) received an experimental diet with 2\% of ginger; Group III (Cr) received an oral dose of potassium dichromate (15 mg/kg) and normal
diet; Group IV (CrG) received an oral dose of potassium dichromate (15 mg/kg) and an experimental diet containing 2% ginger; Group V (Cr+G) received an oral dose of potassium dichromate (25 mg/kg) and an experimental diet with 2% of ginger.

**Experimental methods**

After 30 days of treatment, rats were sacrificed by decapitation and blood samples were collected into two tubes: heparinized and dry; the first one is used for measuring full blood count (FBC) and the second is centrifuged at 5000 rpm to obtain serum which served for various biochemical investigations. At the time of decapitation both the concentrations of blood glucose and FBC were measured using a portable glucometer (Accu-check Active) and ERMA INC full automatic blood cell counter model (PCE-210N) respectively. Other biochemical parameters: urea, creatinine, uric acid, triglycerides, cholesterol, total lipids, total and direct bilirubin, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP) were determined using kits supplied by Spinreact, Spain. The tissue’s reduced glutathione (GSH) was determined by spectrophotometry using the method of Cory and Weckbeker [24]. Protein concentration was measured by the Bradford method [25].

**Statistic analyze**

The data are expressed as the means ± SD. The significance of the differences in mean values among the control and treated groups was evaluated following Students’ t-test using Minitab software version (13.31).

**Results**

**Physiological study**

Exposure of rats to chromates did not produce any overt sign of toxicity/mortality. However, figure 1 shows a significant increase of the hepatosomatic and renosomatic indexes in rats treated by chromate.

**Hematological study**

The results illustrated in table 1 showed that significant changes were reported in some hematology parameters. No significant changes in hematological parameters were found at 2% ginger dieted group, comparing with control. In animals treated with chromate, a significant decrease of these parameters was observed. However, ginger supplementation in treated rats (CrG) could protect rats from the adverse effect of chromate.

**Renal study**

The rats treated with chromate presented a marked reduction of renal function compared to control group that was characterized by a significant increase in serum urea, creatinine and uric acid. The ginger addition in diet (CrG) restored significantly the renal function via maintaining these parameters in normal values (table 2).

**Biochemical study**

The results shown in table 3 concerning the determination of biochemical parameters affirm the existence of a large metabolic disturbance. These results indicated that blood glucose concentrations of all treated groups were similar to

![Figure 1. Organsomatic indexes in control (T) and treated rats (G, Cr, CrG and Cr+G) after 30 days of treatment (each value represents the mean ± SD of 10 rats). * p<0.05.](image-url)
those of control group, that it was revealed by no significant changes in this parameter. The exposure of rats to chromate produced a significant increase in total lipids, cholesterol and triglycerides levels. Results revealed that there was significant decrease in the lipid profile of rats treated with ginger alone, while the presence of ginger with the chromates could alleviate the adverse effects of chromate. Our results show also a significant elevation in the activities of ASAT, ALAT, ALP, LDH and bilirubin levels in chromate induced animals. The addition of ginger was found to inhibit the elevation in chromate induced enzyme activities and restored the enzymatic status comprehensively with the results more pronounced in the group treated with ginger. Bilirubin levels in the serum of chromate-treated group increased when compared with those of control. While the supplementation of ginger in the diet of the chromium-ginger treated group (Cr+G) restored elevated levels. Concerning the lot (Cr*G), we noticed a slight elevation of all renal and biochemical parameters and decrease in hematological parameters mentioned above. The results of figure 2 show a significant decrease in glutathione concentrations in the all organs studied after
treatment with chromate. However in ginger treated rats there were no significant changes in GSH level. Results showed also that ginger could attenuate the toxic effect of chromate, which was revealed by an increase of GSH levels in all organs.

Discussion

Cellular response to oxidative stress is usually related to two processes damage to cellular constituents and up-regulation of antioxidant defenses. Actually it is difficult to separate these two scenarios and the final results depend on many circumstances because these processes take place simultaneously [26]. Being a transition metal, chromium ions may stimulate free radical processes in living organisms. For example, Cr (VI) may catalyses Fenton and Haber-Weiss reactions [27, 28]. Chromium (VI) compounds are well-known oxidizing agents capable of directly inducing tissue damage and possess carcinogenic, mutagenic and teratogenic potency [7]. Chromium (VI) compounds are easily taken up by the cells and are subsequently reduced to Cr (III) species. This reduction generates free radicals, which play a major role in the adverse biological effects of these compounds [29]. Oral feeding of chromium resulted in a significant increase in hepatosomatic and renosomatic index. Geetha et al. [30] showed that administration of potassium dichromate at 30 mg/kg for 30 days increased the organosomatic indexes (liver, kidneys, heart and spleen). Our results showed significant decrease in some hematological indices as well as (RBC, HCT, MCV, Hb) in chromate-treated rats, however, no significant changes were observed in ginger-treated group. In light of our findings, we can say that chromate induced toxicity expressed by the significant changes of hematological parameters cited above. Moreover, the association (CrG) revealed the protective effect of ginger against the adverse action of the chromates. Overall it appears the effect of the chromates resulted in a drop in RBC, Hgb, MCV and HCT. This can reveal anemia caused by the reactive species of chromate. Staniek et al. [31] were demonstrated some changes in hematological indices after chromium propionate (CrProp) treatment. Metz [32] demonstrated that the Cr (VI) has a far greater ability to penetrate cell membrane when it is reduced to Cr (III), which binds strongly to hemoglobin, which can probably explain anemia caused by the chromates. Chromium deficiency has been associated with hyperglycemia in test animals as well as humans which can be reversed by chromium supplementation [33]. CrProp has been extensively studied in regard to its hypoglycemic potential in various experimental models on laboratory animals [34]. In our study, results indicated that blood glucose concentrations were not affected in chromates-treated rats comparing to control rats. Chromium can modulate
the activity of insulin by increasing the insulin sensitive cell receptors or binding activity and enhancing intracellular insulin signaling activity [35]. In studies using older cattle with functioning rumens, plasma glucose was also unaffected by supplemental chromium [36]. Our results demonstrated also non-significant changes in rats treated with ginger. Many investigations reported that phenol, polyphenol compounds and flavonoids of ginger are responsible for hypoglycemic and other pharmacological activities [37, 38].

In the present study, administration of chromate to rats caused renal toxicity and oxidative stress. These findings are consistent with previous reports [39-41]. The kidney is the mater route of chromium excretion. It has been reported that acute exposure to chromate in rats induced an increase in chromate-kidney content [42] and produced anatomical lesions at the level of the proximal tubular cells [43]. Cr (III) compounds are easily taken up the ells and are subsequently reduced to Cr (III) species. This reduction generates free radicals, which play a major role in the adverse biological effects [29]. The data obtained from the present work clearly show that increased levels of renal markers in serum (urea, creatinine, and uric acid) after chromate administration, which reflect its interaction with cell membrane leading to altered cell membrane permeability and loss of functional integrity in the kidney. Soudani et al. [44] found an increase in plasma urea and creatinine levels in rats treated with chromate. In contrast, ginger treated rats showed no significant changes in kidney function markers levels. However the combination treatment could partially protect the elevation of serum urea, creatinine, and uric acid. Thus showing the ability of ginger to protect against chromate induced kidney damage. Unfortunately, ginger was incapable to reduce the toxic effect of chromate second dose (Cr+G).

Results revealed that there were significant increases in the lipid profile in rats treated with chromate (cholesterol, triglycerides, and total lipids). According to Soudani et al. [45], after chromate administration (intoxication), cholesterol and triglycerides plasma levels were significantly increased, that can be probably explained by the oxidative stress induced by the chromates. Also, treatment with ginger alone showed a significant decrease in cholesterol and triglycerides concentrations. Ginger has been reported to interfere with the activities of some digestive enzymes [46]. It was found that ginger acted on the liver to reduce cholesterol biosynthesis, and may stimulate cholesterol’s conversion to bile and increase its faecal excretion [47]. On the other hand, Ramakrisna Rao et al. [48] demonstrated that ginger enhanced the activity of pancreatic lipase and amylase when it was directly in contact with these enzymes. Recent in vitro and in vivo studies demonstrated that ginger shows considerable antioxidant, hypertensive and hypolipidemic effect [20, 49]. It was evident that ginger co-administration with chromates was successfully able to act against perturbations induced by chromate in serum lipid profile via decreasing cholesterol, triglycerides and total lipids levels, and indicating the important role of ginger constituents to maintain the tissues and cells integrity and function. Therefore, ginger was partially unable to reduce side effects arising by the high dose of chromate.

The present results revealed elevation in the activities of ALAT, ASAT, ALP and LDH enzymes in chromates-treated rats. Farag et al. [50] confirmed that the elevation in liver enzymes (ALAT, ASAT) in the plasma or the serum may be due to tissue damage practically in the liver, kidney and heart or due to alterations in the permeability of cells membrane and increased synthesis or decreased destruction of transaminases. Moreover, transaminases and lactate dehydrogenase are the most sensitive biomarkers directly implicated in the extent of cellular damage and toxicity because they are cytoplasm’s localized (cytoplasmic in location) and are released into the circulation after cellular damage [51]. According to Soudani et al. [52] the elevated LDH levels in plasma may be attributed to generalized increase in membrane permeability. Also, chromate hepatotoxicity is evidenced in our study by an increase of bilirubin serum levels. Our results are in agreement with previous findings of other studies [52, 53], who suggested that the increase in serum bilirubin is a clear marker of hepatic dysfunction. According to Frank et al. [54], the increased total bilirubin concentrations measured in chromate-treated group probably reflect the bile stasis observed post mortem as distended gallbladder; but may also indicate an increased rate of erythrocyte destruction due to impaired membrane integrity. The latter mechanism is probably responsible for the increased activities of muscle ASAT and liver LDH enzymes. However, increased LDH activity could also be an effect of the bile stasis observed. Many workers have also demonstrated the hepatotoxicity effect of chromium [30, 52]. Our findings showed that ginger given simultaneously with chromate appear to play a key role in the attenuation of liver injury. Co-treatment with ginger is effective in the prevention of oxidative damages induced by chromate objectified by lowering serum ALAT, ASAT, LDH and bilirubin concentrations. These results are in consistent with previous studies demonstrating the scavenging effect and an inhibitory action of ginger on superoxide anion production in liver [45, 55]. Concerning the group (Cr+G) the results demonstrated that ginger may slightly protect against the toxic effects of chromate, but less effective if by comparing by the lot (CrG).

Chromium promotes an early oxidative stress and afterward contributes to the development of various pathological conditions because of its long retention in some tissues [3].
Oxidative stress in the cells or tissues refers to enhanced generation of reactive oxygen species and/or depletion in antioxidant defense system. ROS generated in the tissues are efficiently scavenged by enzymatic antioxidant system such as GPx (glutathione peroxidase) and GR (glutathione reductase) as well as non-enzymatic antioxidants such as GSH, vitamin A, C and E [56]. Glutathione (γ-glutamylcysteinyl-glycine), the most abundant low molecular weight thiol, acts as a protective physiological antioxidant in biological systems [40]. Likewise GSH is a crucial component of the antioxidant defense mechanism, functions as a direct reactive free radical scavenger [52]. In our experimental study, results show a significant decrease in liver, kidney, testicles, intestines, heart and spleen GSH levels in chromate-treated rats, however, no significant changes were observed in ginger treated rats. In the combination treated rats, we observed that ginger restored back forced chromates stress-induced alteration in tissues cited above. The decreased liver GSH level in chromate treated rats may be due to its consumption in the scavenging free radicals produced by chromates by [52]. Our results are in agreement with Anaud [57] study, who demonstrated that per os exposure of rats to 100 mg/kg of chromate caused a significant decrease in liver GSH level. Also, the chromate-administration caused depletion in kidney GSH level, which could be explained by an adaptive response to oxidative stress [44]. Fatima and Mahmood [40] demonstrated that depletion of GSH levels by pre-treatment with an inhibitor of GSH biosynthesis enhanced Cr (VI)-induced nephrotoxicity. However, pre-treatment with GSH supplying agent prevented Cr (VI) from exerting harmful effect on the kidney. Soudani et al. [45] demonstrated that following chromium intoxication cardiac glutathione status was greatly impaired, as indicated by a significant decrease in heart GSH levels in chromate-treated rats. After entering the body by oral route there exist important mechanisms of Cr (VI) detoxification. Initially, Cr (VI) can be reduced to Cr (III) by fluids in the digestive tract (saliva and gastric juice) and then it can be sequestered by intestinal bacteria [58], this probably explain the depletion of GSH levels in intestinal tissue. Increasing of GSH levels in (CrG) treated rats can be explained by the antioxidant activity of ginger. Our findings suggest that ginger exhibits potent antioxidant activity by scavenging free radicals and restoring the imbalance between oxidants/antioxidants homeostasis during chromium toxicity. Likewise, several authors have shown that ginger is endowed with strong in vitro and in vivo antioxidant properties. The antioxidant action of ginger has been proposed as one of the major possible mechanisms for the protective actions of the plant against toxicity and lethality of a number of toxic agents [20, 51, 56].

Conclusion

The present data records that chromate intoxication produces a perturbation in hematological indices associated with an increase in all biochemical parameters, and provoke also GSH levels depletion. Accordingly, care must be taken into account to avoid mammalian and human exposure to chromate. The co-administration of ginger with chromate attenuates the observed harmful effects on all the parameters cited above. On the basis of this study, it should be taken into consideration that the supplementation of natural antioxidants may act as a protective agent against the toxicity of chromates.

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Conflicts of interests: none.

References


