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Modulatory effect of curcuma against chromium-induced oxidative stress and physiological toxicity in rats

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Abstract

Chromium has long been identified as toxic environmental and industrial pollutants. The present study was under taken to investigate the potential effect of curcuma on toxicity induced by hexavalent chromium in male Wistar rats. Twenty-eight male rats were randomly divided into four equal groups. The first group: used as a control group. The second group: given diet with 2% curcuma powder. The third group: given 15mg/kg B.W of potassium dichromate ($K_2Cr_2O_7$) *per os*, and the last group: given diet containing 2% curcuma powder and 15mg/kg B.W of $K_2Cr_2O_7$ *per os*. The animals were in the same exposure conditions for 30 days. Organo-somatic indexes and glutathione (GSH) levels in studied organs were evaluated as well as the hematological profile. The results indicate that administration of chromium caused noticeable increase in all studied organo-somatic indexes when compared to control group. Whereas, decreased GSH content in organs of chromium-exposed rats was observed. Moreover, hematologic disorder was evidenced by significant decrease in plasma red blood cells, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (CMH), and platelets along with significant increase in white blood cells, neutrophils, lymphocytes and monocytes when compared to control group. However, simultaneous treatment with curcuma and chromium corrected all the previous parameters. Data suggests that curcuma acts as powerful antioxidant, ameliorates physiological and hematologic indices along with oxidative stress biomarkers against chromium toxicity.

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Introduction

Heavy metals are found naturally on the environment in low concentrations; currently anthropogenic resources are mainly responsible for their recent increase (Karn *et al.,* 2021).

Chromium belongs to the most pollutants heavy metals that have enormous impacts on living beings and ecosystem (DesMarias and Costa, 2019). Because of its multiple uses in various industrial processes, including wood preserving, pigment, plating, welding, leather tanning, manufacture of stainless steel, metal finishing, electroplating, textile dyeing, chromates, and metallurgy (Blade *et al.,* 2007), exposure to chromium and its compounds induces genotoxic, mutagenic, and carcinogenic effects (DesMarias and Costa, 2019).

According to Buchko and Havryliak (2021) there is an association between hexavalent chromium [Cr(VI)] and generation of reactive oxygen species along with lipid peroxidation. Moreover, it was found that Cr(VI), has a negative effect on hematological parameters leading to a multiple health problems (Ray, 2016).

Since ancient times, folk medicine has been an important background for treating diseases and infections **(**Lim, 2012). Finding new herbs with protective health effects is one of the biggest challenges in developing natural medicines.

Curcuma is a spice that is commonly known in Asian food, often used to add flavor and color meals (Prasad and Aggarwal, 2011), widely used for various medicinal preparations, as it contains a yellow-colored polyphenolic pigment called curcumin, the integral component of many diseases (Yousef *et al.,* 2010), including anticancer effect, anti-inflammatory and antioxidant effects, prevent against cardiovascular diseases, diabetes mellitus, obesity, inflammatory bowel disease, neurodegenerative diseases, skin diseases, allergy and asthma (Kocaadam and Şanlier, 2017).

Hence, the purpose of this study was to evaluate the protective effect of curcuma on physiological injuries, oxidative stress, and hematologic disorder induced by Cr(VI) exposure using Wistar rats as animal model.

Materials and methods

Preparation of curcuma powder

Curcuma longa rhizomes were purchased locally from the market. In order to get fine powder, they were firstly milled using mortar and pestle, pulverized with a knife grinder, and then sieved to get uniform size range of particles.

Preparation of chromium solution

Potassium dichromate powder ($K_2Cr_2O_7$: Biochem Chemopharma Company, USA) was dissolved in mineral water and administered *per os* to animals, the volume of each dose was adjusted to deliver 15mg/kg of body weight/day.

Study design

Twenty-eight male Wistar rats (160±10 g) were obtained from Pasteur Institute of Algiers, Algeria. Animals were maintained under controlled laboratory conditions (ambient temp: 21 ± 2°C, cycle: 12-h dark/light), and randomized into four groups: the first group (0-0): negative control where rats received ordinary diet and mineral water *per os*. The second group (0-Cur): rats received an experimental diet containing 2% curcuma powder, and mineral water *per os*. The third group (Cr-0): where rats treated *per os* with 15mg/kg body weight of $K_2Cr_2O_7$ associated with a normal diet. While the fourth group (Cr-Cur) received $K_2Cr_2O_7$ *per os* at 15mg/kg B.W, and given an experimental diet supplemented with 2% curcuma powder. Both water and food were given *ad libitum* to all groups. The experimental study was conducted for 30 consecutive days.

Sample collection

After 30 days of treatment, animals were sacrificed by cervical decapitation. Blood samples were collected in ethylene diamine tetra acetic acid (EDTA) tubes, to be subsequently used for full blood count determination (FBC) using auto-hematology analyzer (MINDRAY

bc-3200). The analysed parameters are: red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular hemoglobin (CMH), white blood cells (WBC), neutrophils (NEUT), lymphocytes (LYMP), monocytes, and platelets (PLT).

To determine oxidative stress status and organo-somatic indexes, organs (brain, femur, kidney, adrenal glands, spleen, heart, intestines) were extracted and washed with phosphate buffer (0.1 M, pH 7.4), to remove excess blood and adhering tissues, weighed, then kept at - 20 °C to stop metabolic activities.

Oxidative stress study

Tissue preparation

About 100mg of organ was homogenized, at 4°C, with 4mL of a 0.02M EDTA solution using an ultrasonic mill.

Determination of glutathione level

Glutathione contents were estimated using a colorimetric technique, as mentioned by Weckbercker and Cory (1988).

This method is based on the development of a yellow colour when 5,5'-dithiobis-2-nitrobenzoic acid (DTNB) was added to compounds containing sulfhydryl groups. In brief, 0.8 mL of tissues homogenate was added to 0.2 mL of 0.25% sulphosalicylic acid (SSA).

The reaction mixture was incubated for 15 minutes in a glace bath, and then was centrifuged for 5 minutes at 1000 rpm. 0.5 mL of the resulting supernatant was mixed with 0.025 mL of DTNB (0.01 M), 1 mL of Tris buffer (0.4 M, pH 9.6), and EDTA (0.02 M). In the end, optical densities (OD) measurement was conducted at 412 nm. GSH concentrations were expressed in nmoles/mg of proteins.

Determination of protein concentrations

Protein concentration in homogenates was measured spectrophotometrically at 595nm according to Bradford (1976) method, using bovine serum albumin as standard.

Statistical analysis

Statistical analysis was carried out using GraphPad prism 5.0 (GraphPad Software, Inc., San Diego, CA). All results are mean ± SEM. Groups of data were evaluated using ANOVA followed by Tukey's test. For statistical significance, p values of <0.05, <0.01 and <0.001 were respectively considered as statistically significant, highly significant and very highly significant.

Results

Physiological Study

Fig. 1 showed a significant increase in organo-somatic indexes of brain, femur, kidney, adrenal gland, spleen, and heart in chromium-treated rats (Cr-0) when compared to control group (0-0). The diet containing curcuma was able to improve these disturbances by maintaining organo-somatic indexes at near control levels as compared to chromium treated rats (Cr-0).

Oxidative stress study

Glutathione (GSH) levels in intestines, brain, spleen, and heart were significantly decreased in Cr(VI) group when compared to control group (Fig. 2). Conversely, suplementation of curcuma in diet of chromium-intoxicated rats decreased the toxic effect of Cr(VI) by bringing back GSH levels to normal values.

Hematological study

Results, showed in table 1, reveal a significant disorder in the majority of hematological profile. A significant decrease in RBC, HGB, MCV, HCT, MCHC and PLT counts was observed in chromium group rats (Cr-0) when compared to control rats (0-0), meanwhile, WBC, NEUT, MONO, and LYMP levels were significantly increased.

However, supplementation with curcuma in Cr(VI) intoxicated rats' diet (Cr-Cur) has shown an improvement in hematologic indices when compared to control group (0-0) and chromium toxicity group (Cr-0).

Table 1. Hematological profile in control (0-0) and treated rats (0-Cur, Cr-0, Cr-Cur) after 30 days of treatment (values represent the mean ± SEM of 7 rats)

Parameters	Groups			
	0-0	0-Cu	Cr-0	Cr-Cu
RBC (10^6/µl)	8.90 ± 0.17	9.05 ± 0.17	5.90 ± 0.79**	7.91 ± 0.41*#
HGB (g/dl)	17.27 ± 0.31	17.61 ± 0.45	12.56 ± 1.38**	16.27 ± 0.65#
HCT (%)	43.91 ± 4.51	46.41 ± 1.18	40.93 ± 0.61	44.10 ± 1.40
MCV (fl)	54.05 ± 1.25	57.38 ± 0.89*	46.54 ± 3.11*	50.76 ± 2.14
MCH (g/dl)	36.61 ± 0.27	36.82 ± 1.00	33.44 ± 0.75**	35.34 ± 0.29**#
CMH (pg)	20.83 ± 0.82	20.78 ± 0.48	18.41 ± 0.41*	19.48 ± 0.27#
WBC (10^3/µl)	8457± 955.40	8129 ± 738.00	13800 ± 11.00**	11630 ± 10.00*#
NEUT (%)	32.26 ± 2.75	25.24 ± 3.04	39.43 ± 1.69*	34.10 ± 3.63
LYMP (%)	55.16 ± 2.51	53.39 ± 4.29	66.64 ± 3.33*	57.34 ± 2.81#
MONO (%)	8.55±1.32	7.40±0.58	13.54±1.47*	10.49±0.92
PLT (10^3/µl)	460.10±43.59	472.30±46.77	194.70±13.87***	326.70±37.65*##

* p <0.05, ** p <0.01, *** p <0.001; Significantly difference from control (0-0) group

# p <0.05, ## p <0.01; Significantly difference from (Cr-0) group

RBC: red blood cells (10^6/µl), HGB: hemoglobin (g/dl), HCT: hematocrit (%), MCV: mean corpuscular volume (fl), MCHC: mean corpuscular hemoglobin concentration (g/dl), CMH: mean corpuscular hemoglobin (pg), WBC: white blood cell (10^3/µl), NEUT: neutrophils (%), LYMP: lymphocytes (%), MONO: monocytes (%), PLT: platelets (10^3/µl).

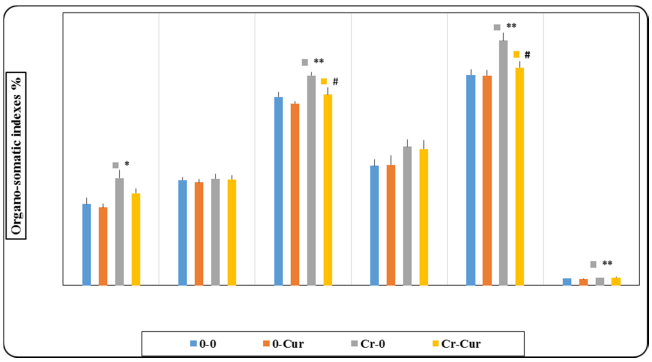

Fig. 1. Organo-somatic indexes in control (0-0) and treated rats (0-Cur, Cr-0, Cr-Cur) after 30 days of treatment (values represent the mean ± SEM of 7 rats)

* p <0.05, ** p <0.01; Significantly difference from control (0-0) group

# p <0.05; Significantly difference from (Cr-0) group

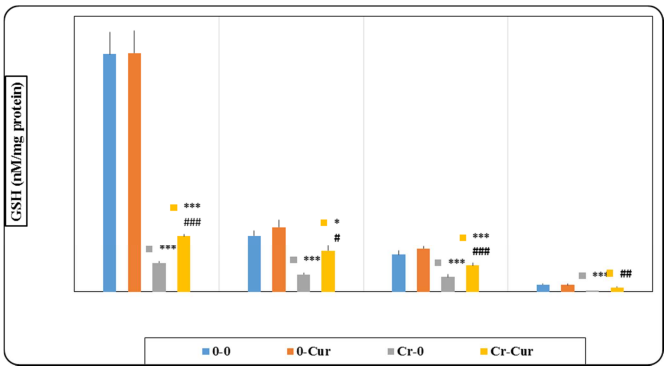

Fig. 2. GSH levels in organs of control (0-0) and treated groups (0-Cur, Cr-0 and Cr-Cur) after 30 days of treatment (values represent the mean ± SEM of 7 rats).

* p <0.05; ** p <0.01, *** p <0.001; Significantly difference from control (0-0) group

# p <0.05; ## p <0.01; ### p <0.001; Cr-Cur; Significantly difference from (Cr-0) group

Discussion

Oxidation is one of the basic and necessary reactions that take place in the body. Whereas, oxidative stress, a deleterious process that can damage the body' cells by producing free radicals which can causes various diseases (Buchko and Havryliak, 2021).

However, antioxidants are molecules that eliminate the effects of free radicals and avoiding their damage (Geetha *at al.,* 2003). Natural antioxidants are mostly found in plant foods, as well as in nutritional supplements **(**Lim, 2012). Thus, this study was designed to assess the protective effect of curcuma supplementation on physiological injuries, oxidative stress, and hematologic disorder induced by Cr(VI) exposure in Wistar rats.

In the present data, rats exposed to Cr(VI) had lower levels of GSH in intestines (Thompson *et al,* 2011), brain (Iztleuov *et al.,* 2018), spleen (Buchko and Havryliak, 2021), and heart (Yang *et al.,* 2021), when compared to control group. These disturbances in GSH counts may be related to the imbalance of oxidative status were likely to be induced by free radicals production under chromium intoxication. However, it has been clearly demonstrated, in this study, that curcuma treatment improves GSH depletion. We could indicate that curcuma supplementation attenuate oxidative stress (Ramadan *et al.,* 2011).

We found that administration of Cr(VI) not only disturbed the redox state but also increased organo-somatic indexes of brain (Min *et al.,* 2017), femur (Bieńko *et al.,* 2017), adrenal gland (Chandra *et al.,* 2007), kidney, spleen, and heart (Geetha *at al.,* 2003) when compared to control values. A period of 30 days of experimentation is enough for an intense accumulation of this metal which results in organs hypertrophy (Min *et al.,* 2017). During the present experiment, curcuma supplementation in (Cr-Cur) group has enhanced chromium toxicity by keeping the relative weight of internal organs near normal. According to Attia *et al.* (2017) curcumin appeared to prevent changes in organs weight.

In the present investigation, RBC, HCT, HGB, PLT, MCHC, MCV, and MCH were reduced significantly after oral administration of Cr(VI) than in the control group. Conversely, Cr(VI) oral administration induced an increase in WBC, NEUT, LYMP and MONO levels when compared to control group. Indeed, Cr(VI) is able to penetrate the RBC's membrane through its highly absorbent capacity (Bieńko *et al.,* 2017), and rapidly reduced to generates reactive intermediates that binds to the beta chain of the HGB (Ray, 2016).

The decrease in MCH, HGB, and HCT levels could be explained by the negative effect of free radicals. Furthermore, the low PLT counts probably due to megakaryocytes dysfunction. In addition, the significant increase in WBC and NEUT levels appears to be an evidence of inflammatory processes (Yousef *et al.,* 2010). Unlikely, supplementation with curcuma rhizomes prevents the hematologic imbalance caused by Cr(VI). This may be due to the hemato-protective effect of curcumin via its ability to neutralize free radicals as well as its anti-inflammatory properties (Yousef *et al.,* 2010).

Curcuma supplementation can improve intestinal iron absorption which in turn increases levels of HGB and HCT (Chandra *et al.,* 2007). Moreover, as a membrane antioxidant, curcumin can inhibit inflammation by blocking the adhesion of MONO to endothelial cells after deactivating adhesion molecules on the cell surface. In addition, they suppress the activation of pro-inflammatory cytokines (Yang *et al.,* 2021).

Conclusion

The results obtained from the present investigation indicate that exposure to Cr(VI) might exert an effect on rats' organs and blood cells beside oxidative status. Thus, our study has shown that curcuma alleviates intoxication of rat by this heavy metal, exhibits protective effect against physiological injuries, oxidative stress, and hematologic disorder. In addition, it is clear that curcuma can be used in a dose of 2% as a supplement without negative effects and any pathological changes.

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Declaration of Interest statement

The authors declare no potential conflicts of interest with respect to the authorship and/or publication of this article.

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