

AMELIORATIVE EFFECT OF *ZINGIBER OFFICINALE*
ETHANOL EXTRACT ON CHROMIUM-INDUCED MALE
REPROTOXICITY IN WISTAR RAT

Nour El Imane Zerarki^{1,2}, Abdelkrim Berroukche^{1,2},
Farouk Boudou^{3✉}

Received on January 31, 2024

Presented by H. Najdenski, Corresponding Member of BAS, on April 23, 2024

Abstract

This study explored the protective effects of oral ginger extract against chromium trioxide (CrO₃)-induced male reprotoxicity in Wistar albino rats. Rats exposed to CrO₃ for 8 weeks showed significant declines in body weight (11.67±3.78 g), serum testosterone levels (6.45±1.35 ng/mL), Glutathione (GSH) level (17.29±1.96 µM/mg), and catalase activity (9.54±0.11 mM H₂O₂/min/mg), with no notable change in relative testicular weight. Conversely, ginger extract-treated rats exhibited substantial improvements, including increased body weight (45.33±09.11 g), enhanced GSH levels in testicular tissue (24.02±1.69 µM/g), and improved catalase activity (11.56±1.25 mM H₂O₂/min/mg). However, concurrent CrO₃ and *Z. officinale* extract treatment showed no significant changes in testosterone levels. *Z. officinale* extract, due to its high content of polyphenols (194.82±8.57 mg GAE/g d.w.), and flavonoids (117.20±48.61 mg CE/g d.w.) with significant antioxidant value (IC₅₀ = 0.57±0.14 mg/ml), showed a remarkable ameliorative effect against chromium-induced male reprotoxicity.

Key words: chromium, rats, reprotoxicity, testosterone, *Zingiber officinale*

Introduction. Chromium (Cr) is found in a variety of materials, including pigments, chromeplated metals, cement, and detergents [1]. Dermatitis, nasal

perforation, skin and lung cancer, cardiovascular diseases, neurotoxicity, and kidney damage are all well-known health hazards associated with Cr toxicity [2]. Although several epidemiological studies and many experimental investigations have proven the deleterious effect of excess Cr on fertility, the reproductive toxicity of Cr has been ignored for many years [3]. Cr induces its toxicity by increasing reactive oxygen species (ROS), which causes oxidative stress, apoptosis, DNA damage, genotoxicity and carcinogenicity, according to several studies [4, 5]. ROS have an impact on male infertility by threatening sperm membrane and DNA integrity, resulting in a reduced sperm count. Similarly, female reproductive problems caused by ROS share pathophysiological features with male fertility disorders [6]. Furthermore, medicinal plants make a significant contribution to the treatment of some medical disorders. Fertility-regulating effects have been reported for a variety of plants [7], and ginger (*Zingiber officinale*) is one of these plants. *Z. officinale* is one of the most well-known spices in the world, and it has been widely used for its medicinal properties throughout history. Volatile oil, phenolic derivatives (zingerone), and oleoresin (gingerols and shogaols) are the most important antioxidant compounds in ginger [8]. Because it may scavenge superoxide anion and hydroxyl radicals, ginger extract has antioxidative properties, and lipoxygenase and peroxidation were reported to be inhibited by *Z. officinale* [9]. Based on these literature data, our research work aims to evaluate the beneficial effects of ethanolic extract of *Z. officinale* on chromium trioxide-induced damage in the testes of adult male rats.

Materials and methods. Preparation of *Z. officinale* ethanol extract. Twenty grams of ginger (*Zingiber officinale*) rhizome powder were extracted overnight at room temperature with 100 ml of ethanol (70%), and then filtered with N°1 Whatman Millipore filter paper. The filtrate was centrifuged at 4000 rpm for 20 min, the supernatant was concentrated to dryness using a rotary evaporator and the residue is stored at 4 °C until use.

Phytochemical analysis. Total phenolic content (TPC), total flavonoid content (TFC), and DPPH radical-scavenging activity were determined using respective colorimetric methods according to [10]. The Folin–Ciocalteu reagent was utilized for the determination of TPC level with absorbance read at 760 nm and expressed as mg GAE/g d.w. using a Gallic acid calibration curve (0–100 µg/mL, R²=0.9966). TFC level was assessed in the extract using sodium nitrite, aluminium chloride, sodium carbonate as reagents, and absorbance measured at 510 nm. TFC was expressed as mg CE/g d.w. using a catechin calibration curve (0–500 µg/mL, R²=0.9956). The antioxidant activity was evaluated following the DPPH radical-scavenging activity, with absorbance measured at 515 nm and expressed as IC₅₀% (mg/mL). Samples were analyzed in triplicate for each assay.

Experimental design. Twenty mature male Wistar rats, aged 12 weeks and weighing 280.88 ± 5.13 g, were used in the experiments. Rats were maintained in an animal house with a 12/12 hour light/dark cycle at a temperature of 22 °C, with

free access to water and a specific diet of rodent pellets. The rats were divided into four groups, each containing five rats. The first group (I), consisting of control rats, received physiological saline (0.9% NaCl), the second group (II) received CrO₃ at a dose of 10 mg/Kg BW [11], the third group (III) received only 200 mg/Kg BW of ethanolic ginger extract, and the fourth group (IV) received CrO₃ plus 200 mg/Kg BW of ethanolic ginger extract [12]. All treatments were administered orally (gavage) and the experiment lasted 8 weeks in total during which animals weight was measured every week. The study received ethical clearance from the department of biology, faculty of Sciences, Dr. Tahar-Moulay university of Saida, Algeria.

Specimens and analytical methods. At the end of the 8-week experiment, the animals were sacrificed in the morning after fasting for 12 hours and being anaesthetized [13]. Blood samples were taken from the inferior vena cava in dry tubes. The blood has been centrifuged to obtain serum, and then stored in the refrigerator until used for hormone assay. All tests were performed within 24 h of specimen collection. Serum samples were assayed for the determination of testosterone using radioimmunoassay methods using commercial kits (VIDAS Assays, BIOMERIEUX). Testes were carefully removed, separated from their fat tissues, cleaned with saline solution, and weighed to get the testicular relative weight (organ weight/body weight ratio) according to [11]. To assess testicular oxidative stress indicators, the testicular tissue was homogenized at a concentration of 10% (w/v) in cold phosphate buffer (pH 7.4). The homogenate was then centrifuged at 3000 rpm for 10 min. The supernatant was carefully collected and utilized for the precise quantification of glutathione (GSH) and catalase activity (CAT), following the methodology detailed in [14].

Statistical analysis. To establish the significance of intergroup differences, mean SD values were determined for each group. A one-way analysis of variance was used to examine each parameter independently (ANOVA). The Tukey test was performed to determine the difference between the groups. Column or bars not sharing a common letter (a-c) differ significantly at $p < 0.05$ (Tukey test).

Results. *Zingiber officinale* ethanol extract was used to determine total phenolic content (TPC), total flavonoid content (TFC) and DPPH radical-neutralizing activity. TPC was quantified in milligrams of gallic acid equivalent per gram of dry weight (mg EAG/g d.w.), while TFC was expressed in milligrams of catechin equivalent per gram of dry weight (mg CE/g d.w.). TPC and TFC levels in the alcoholic extract of *Z. officinale* were significant, measuring 194.82 ± 8.57 mg GAE/g d.w. and 117.20 ± 48.61 mg CE/g d.w., respectively. Furthermore, evaluation of antioxidant activity showed remarkable potency expressed by a calculated low IC₅₀ value of 0.57 ± 0.14 mg/ml, underlining its effectiveness in scavenging and reducing DPPH radicals by 50% (Table 1).

A significant change in the body weight of the experimental animals as compared to control ones was found. The results presented in Table 1 show a signifi-

T a b l e 1

Phytochemical analysis and antioxidant activity of *Zingiber officinale* extract

Parameters	Total phenolic content (TPC) (mg EAG/g d.w.)	Total flavonoid content (TFC) (mg CE/g d.w.)	DPPH radical-scavenging activity (IC50 in mg/ml)
<i>Z. officinale</i> extract	194.82 ± 8.57	117.20 ± 48.61	0.57 ± 0.14

cant decrease in body weight of the CrO₃-exposed group (II) of rats compared to the control group (I) and the other two experimental groups (III and IV) while a significant improvement is observed in the CrO₃-exposed and *Zingiber officinale* extract-treated group (IV) compared to the CrO₃-exposed group only (II). While no significant difference was found when calculating the relative testicular weight (Table 2).

T a b l e 2

Body weight gain and testicular relative weight of the different experimental groups

Groups	Initial body weight (g)	Final body weight (g)	Body weight gain (g)	Testicular relative weight (g)
I	278.75 ± 11.25	369.00 ± 07.33	81.33 ± 06.89 ^a	0.0034 ± 0.0001 ^a
II	288.00 ± 22.00	299.67 ± 22.89	11.67 ± 03.78 ^b	0.0026 ± 0.0004 ^a
III	272.75 ± 14.75	333.67 ± 16.22	52.00 ± 24.00 ^c	0.0027 ± 0.0002 ^a
IV	284.00 ± 09.33	329.33 ± 00.44	45.33 ± 09.11 ^c	0.0032 ± 0.0004 ^a

I: Control group; II: Chromium exposed group; III: Only ginger extract-treated group; IV: Chromium and ginger extract exposed group. Data are expressed as means ± SD ($n = 5$). A comparison between groups was made using the Tukey t -test. Column not sharing a common letter (a–c) differ significantly at $p < 0.05$ (Tukey test)

The results in Fig. 1 showed that CrO₃ administration significantly ($p < 0.05$) decreased serum testosterone levels (6.45 ± 1.35 ng/mL) compared to the control (I) and to rats receiving only the ethanol plant extract (10.50 ± 0.80 , and 9.15 ± 0.95 ng/mL, respectively); however, no significant changes were observed in rats receiving the CrO₃ treatment concomitantly with *Z. officinale* extract (6.85 ± 1.35 ng/mL) compared to the intoxicated group of rats.

Figure 2A showed that chromium-administration for 8 weeks significantly decreased the GSH level (17.29 ± 1.96 µM/mg) as compared to the controls (33.05 ± 0.40 M/g). On the contrary, CrO₃ concomitant with *Z. officinale* treatment led to a significant amelioration in GSH level (24.02 ± 1.69 µM/g). In the same way, Fig. 2B showed that exposure to CrO₃ over a period of 8 weeks induces a decrease in catalase activity (9.54 ± 0.11 mM H₂O₂/min/mg) compared to the control group and the group treated only with ethanolic extract of *Z. officinale* (23.12 ± 2.12 and 23.49 ± 1.42 mM H₂O₂/min/mg, respectively). On the other hand, rats receiving CrO₃ treatment concomitant with *Z. officinale* extract showed

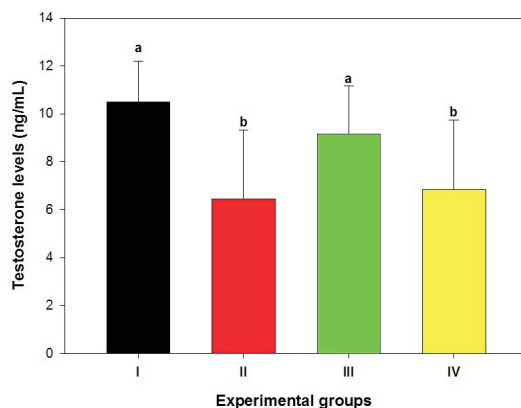


Fig. 1. Evaluation of serum testosterone level in different experimental groups. I: Control group; II: Chromium exposed group; III: Only ginger extract-treated group; IV: Chromium and ginger extract exposed group. Data are expressed as means $\pm$ SD ($n = 5$). A comparison between groups was made using the Tukey t -test. Bars not sharing a common letter (a-c) differ significantly at $p < 0.05$ (Tukey test)

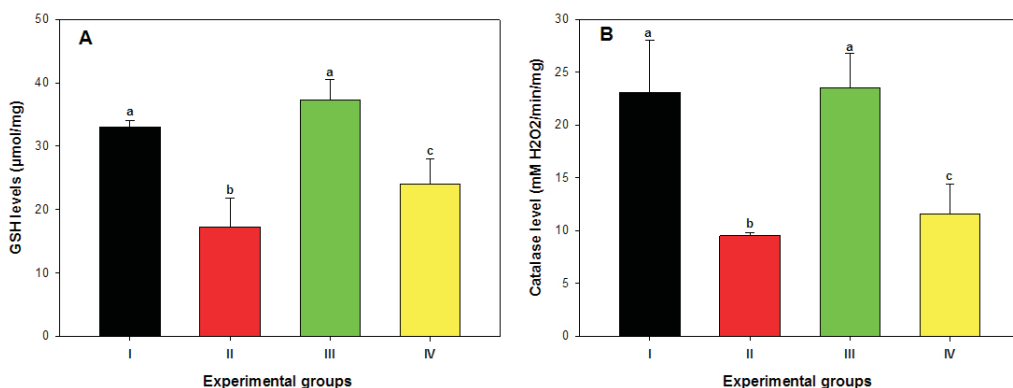


Fig. 2. Evaluation of testicular oxidative stress markers. A: GSH levels; B: Catalase levels; I: Control group; II: Chromium exposed group; III: Only ginger extract-treated group; IV: Chromium and ginger extract exposed group. Data are expressed as means $\pm$ SD ($n = 5$). A comparison between groups was made using the Tukey t -test. Bars not sharing a common letter (a-c) differ significantly at $p < 0.05$ (Tukey test)

a significant improvement in catalase levels (11.56 ± 1.25 mM H₂O₂/min/mg) compared to the intoxicated group.

Discussion. The current study revealed that exposure to CrO₃ at a dose of 10 mg/kg body weight in male rats for 8 weeks resulted in decreased body weight, testosterone serum level and induction of tissue oxidative stress shown by GSH

and CAT activity levels. Indeed, similar results were reported in a study evaluating the subchronic inhalation toxicity of soluble hexavalent chromium trioxide in rats, which showed that the body weight of the high dose group exposed to 1.25 mg/m³ was significantly lower than the control group [15]. A case-control study of maternal chromium exposure and low birth weight in China states that chromium exposure is increasing due to environmental pollution from industrial processes, and suggests that maternal exposure to higher levels of chromium during pregnancy may potentially increase the risk of giving birth to low birth weight infants, especially for female infants [16]. Our finding showed that CrO₃ administration significantly decreased serum testosterone levels compared to the control rats, and to those receiving only the ethanol plant extract. These results corroborate those of MAROUANI et al. [17], who reported that after daily intraperitoneal injection of potassium dichromate (1 or 2 mg/kg body weight) for 15 consecutive days, a decrease in testicular weight and an increase in seminal vesicle and prostate weight were demonstrated. In addition, a dose-dependent increase in blood and testicular chromium levels as well as an increase in FSH and a decrease in serum LH and testosterone levels were detected in treated rats. Furthermore, our results showed that chromium-administration significantly decreased the GSH level and catalase activity compared to the control group. Similar results have been previously published in a study conducted to assess the impact of chromium exposure (i.p. at a dose of 0.8 mg/100 g body weight per day) on the liver, kidney, testes, spleen, brain and cerebellum of male Wistar rats, which showed that treatment of rats with chromium for a period of 28 days resulted in a significant increase in chromium content while decreasing body weight and organ weights. Lipid peroxidation was increased in the testes, brain and cerebellum. The level of reduced glutathione (GSH) increased in the liver, spleen and brain, and decreased in the kidney and testis. Catalase activity became elevated in the liver, kidney, spleen and cerebellum, while it decreased in the testes [18]. On the other hand, our result showed that the treatment with ethanolic extract of *Z. officinale* led to a significant improvement in the parameters studied. In fact, a significant improvement in body weight gain was observed in the group exposed to CrO₃ and treated with *Zingiber officinale* extract compared to the group exposed to CrO₃ only. In addition, rats treated with CrO₃ concomitantly with *Z. officinale* extract showed a significant improvement in CAT and GSH levels compared to the intoxicated group. Identical results were obtained after studying the effects of ginger on cadmium toxicity, which indicate that ginger has better therapeutic detoxification effects on cadmium accumulation in the liver, especially when cadmium consumption was stopped [19]. In the same context, a study on the ameliorative activity of *Zingiber officinale* extract against lead-induced renal toxicity in male rats showed that ginger extract attenuated the toxic effects of lead by increasing the levels of glutathione, glutathione peroxidase, glutathione-s-transferase and catalase [20]. Furthermore, examination of the effects of *Zingiber officinale* on reproductive

functions in male rats indicates that *Z. officinale* extract has pro-fertility properties in male rats, which may be the result of its potent antioxidant properties and androgenic activities [21]. These findings are corroborated by the significant levels of total phenolic and flavonoid content found in the ethanol extract of *Z. officinale*, as well as its notable antioxidant activity.

Conclusion. The present study highlights the significant protective impact of *Zingiber officinale* ethanol extract against chromium-induced male reprotoxicity in Wistar rats. Significant improvements in body weight, testosterone levels and markers of oxidative stress underline the potential of *Z. officinale* as a natural intervention in the mitigation of reproductive toxicity associated with chromium exposure.

REFERENCES

- [1] SHELNUTT S. R., P. GOAD, D. V. BELSITO (2007) Dermatological toxicity of hexavalent chromium, *Crit. Rev. Toxicol.*, **37**(5), 375–387.
- [2] MAHURPAWAR M. (2015) Effects of heavy metals on human health, *International Journal of Research-Granthaalayah*, **3**(9), 2394–3629.
- [3] VABRE P., N. GATIMEL, J. MOREAU et al. (2017) Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data, *Environmental Health*, **16**(1), 1–18.
- [4] FU P. P., Q. XIA, H. M. HWANG et al. (2014) Mechanisms of nanotoxicity: generation of reactive oxygen species, *J. Food Drug Anal.*, **22**(1), 64–75.
- [5] BOUDOU F., M. BENDAHDANE-SALMI, M. BENABDERRAHMANE et al. (2020) Effect of *Curcuma longa* Aqueous Extract on Male Fertility in Aluminum Exposed Wistar Rats, *J. Drug Deliv. Ther.*, **10**(5), 11–17.
- [6] AGARWAL A., R. A. SALEH, M. A. BEDAIWY (2003) Role of reactive oxygen species in the pathophysiology of human reproduction, *Fertil. Steril.*, **79**(4), 829–843.
- [7] BOUDOU F., A. BERROUKCHE, M. BENDAHDANE-SALMI et al. (2013) Ameliorative effects of *Syzygium aromaticum* essential oil on fertility in male rats exposed to manganese, *Advances in Sexual Medicine*, **3**(4), 2013.
- [8] MAO Q. Q., X. Y. XU, S. Y. CAO et al. (2019) Bioactive compounds and bioactivities of ginger (*Zingiber officinale* Roscoe), *Foods*, **8**(6), 185.
- [9] ZHANG S., X. KOU, H. ZHAO et al. (2022) *Zingiber officinale* var. *rubrum*: Red Ginger's Medicinal Uses, *Molecules*, **27**(3), 775.
- [10] BELAKREDAR A., K. HACHEM, F. BOUDOU, Y. BENABDESLEM (2021) Polyphenol content and antioxidant activity of an endemic plant of Algerian Sahara: *Anvillea radiata*, *Polish Journal of Natural Sciences*, **36**(1), 25–36.
- [11] LI H., Q. CHEN, S. LI et al. (2001) Effect of Cr (VI) exposure on sperm quality: human and animal studies, *Annals of Occupational Hygiene*, **45**(7), 505–511.
- [12] KIM E. J., M. JANG, J. H. CHOI et al. (2018) An improved dehydroepiandrosterone-induced rat model of polycystic ovary syndrome (PCOS): Post-pubertal improve PCOS's features, *Front. Endocrinol. (Lausanne)*, **9**(4), 735.

- [13] QIN D., Z. WEN, Y. NIE, G. YAO (2013) Effect of *Cichorium glandulosum* extracts on CCl₄-induced hepatic fibrosis, Iranian Red Crescent Medical Journal, **15**(12), e10908.
- [14] DKHIL M. A., R. ZRIEQ, S. AL-QURASHY, A. E. ABDEL MONEIM (2016) Selenium nanoparticles attenuate oxidative stress and testicular damage in streptozotocin-induced diabetic rats, Molecules, **21**(11), 15–17.
- [15] KIM H. Y., S. B. LEE, B. S. JANG (2004) Subchronic inhalation toxicity of soluble hexavalent chromium trioxide in rats, Arch. Toxicol., **78**(7), 363–368.
- [16] XIA W., J. HU, B. ZHANG et al. (2016) A case-control study of maternal exposure to chromium and infant low birth weight in China, Chemosphere, **144**, 1484–1489.
- [17] MAROUANI N., O. TEBOURBI, S. MAHJOUB et al. (2012) Effects of hexavalent chromium on reproductive functions of male adult rats, Reprod. Biol., **12**(2), 119–133.
- [18] KUMAR DEY S., S. ROY (2009) Effect of chromium on certain aspects of cellular toxicity, Iranian J. Toxicol., **2**(4), 260–267.
- [19] EGWURUGWU J. N., C. S. UFEARO, O. C. ABANOBI et al. (2007) Effects of ginger (*Zingiber officinale*) on cadmium toxicity, African J. Biotechnol., **6**(18), 2078–2082.
- [20] REDDY Y. A., M. CHALAMAIAH, B. RAMESH et al. (2014) Ameliorating activity of ginger (*Zingiber officinale*) extract against lead induced renal toxicity in male rats, J. Food Sci. Technol., **51**(5), 908–914.
- [21] MORAKINYO A. O., O. S. ADENIYI, A. P. ARIKAWA (2008) Effects of *Zingiber officinale* on reproductive functions in the male rat, African J. Biomed. Res., **11**(3), 329–334.

¹Department of Biology, Faculty of Sciences,
Dr. Tahar-Moulay University of Saida, Algeria
e-mails: noureelimane128@gmail.com, kerroum1967@yahoo.fr

²Research Laboratory of Water Resources and Environment, Biology Department,
Faculty of Sciences, Dr. Tahar-Moulay University of Saida, Algeria

³Department of Biology, Faculty of Natural and Life Sciences,
Djillali Liabes University of Sidi-Bel-Abbes, Sidi-Bel-Abbes, Algeria
e-mail: farouk.boudou@univ-saida.dz