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

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**Abstract:**

The present study was carried out to determine the protective effect of the orally administered ethanol extract of ginger (*Zingiber officinale*) against male reprotoxicity induced by chromium trioxide (CrO3) in adult Wistar albino rats. A total of twenty rats were used in the study. The animals were divided into four groups: Control rats received physiological saline solution (NaCl 0.9%), second group received CrO3 at a dose of 10 mg /Kg body weight (BW), third group received only 200 mg/Kg BW of ethanol extract of ginger, while the last group received CrO3 plus 200 mg/Kg BW of ethanol extract of ginger. the results show that 8 weeks exposure to CrO3 induces a significant decrease in body gain (11.67±03.78 g), significantly (p<0.05) decreased serum testosterone levels (6.45±1.35 ng/mL), and significantly decreased Glutathione (GSH) level (17.29±1.96 μM/mg), and catalase activity (9.54±0.11 mM H2O2/min/mg). Whereas, treatment with *Z. officinale* extract showed a good improvement in these parameters namely a regain in body weight (45.33±09.11 g), a significant amelioration in GSH level (24.02±1.69 μM/g), and a significant improvement in catalase levels (11.56± 1.25 mM H2O2/min/mg) compared to the intoxicated group. In conclusion, the ethanol extract of *Z. officinal*e showed an ameliorative effect against chromium induced male reprotoxicity.

**Keywords:** Catalase; Chromium; *Glutathione;* Rats; Reprotoxicity; Testosterone; *Zingiber officinale.*

**INTRODUCTION**

Chromium (Cr) is found in a variety of materials, including pigments, chromeplated metals, cement, and detergents ([Shelnutt et al. 2007](#_ENREF_19)). Dermatitis, nasal perforation, skin and lung cancer, cardiovascular diseases, neurotoxicity, and kidney damage are all well-known health hazards associated with Cr toxicity ([Mahurpawar, 2015](#_ENREF_11)). Although several epidemiological research 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 ([Vabre et al. 2017](#_ENREF_20)). Cr induces its toxicity by increasing reactive oxygen species (ROS), which causes oxidative stress, apoptosis, DNA damage, genotoxicity and carcinogenicity, according to several studies ([Valavanidis et al. 2013](#_ENREF_21); [Fu et al. 2014](#_ENREF_6)). ROS cause cell membrane instability, DNA structural breakdown, and DNA damage. By interfering with the activity of important enzymes, they also cause toxicity in humans and other living species ([Boudou et al. 2020](#_ENREF_3)). ROS are involved in the pathophysiology of a variety of reproductive processes. In the case of male factor infertility. Both sperm plasma membrane fluidity and DNA integrity in the sperm nucleus are threatened by oxidative stress. ROS-induced DNA damage can accelerate germ cell death, resulting in the reduced sperm count associated with male infertility. Similarly, female reproductive problems caused by ROS share many pathophysiological features with male fertility disorders ([Agarwal et al. 2003](#_ENREF_1)). 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 ([Boudou et al. 2020](#_ENREF_2)), 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 ([Mao et al. 2019](#_ENREF_12)). 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* ([Rahmani, 2014](#_ENREF_16); [Zhang et al. 2022](#_ENREF_23)). 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**

20 g 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.

**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 ([Li et al. 2001](#_ENREF_10)), 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 ([Shanmugam et al. 2010](#_ENREF_18)). All treatments were administered orally (gavage) and the experiment lasted 8 weeks in total during which animals weight was measured every week.

**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 with diethyl ether in a large desiccator ([Kim et al. 2018](#_ENREF_7)). Using a standard ELISA technique (VIDAS® Assays) testosterone was measured in blood collected from the inferior vena cava. Testis 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 [Qin et al. (2013](#_ENREF_15)). Testicular oxidative stress indicators were assessed by measuring glutathion (GSH) and catalase activity (CAT) in testicular homogenate, as reported by ([Dkhil et al. 2016](#_ENREF_4)).

**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**

A statistically significant change in the body weight difference of the experimental animals was shown. However, the results in table 1 show a significant 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 1. Body weight gain and Testicular relative weight of the different experimental groups**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Groups | Initial bodyWeight (g) | Final bodyweight (g) | Body weightgain (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 and ginger extract exposed group; III: Only ginger extract extract-treated group; IV: Chromium 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 figure 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 1. Evaluation testosterone level in different experimental groups. I: Control group; II: Chromium and ginger extract exposed group; III: Only ginger extract extract-treated group; IV: Chromium exposed group. Data are expressed as means ± 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).**

Figure 2-A 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±0.40μM/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, Figure 2-B 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 a significant improvement in catalase levels (11.56± 1.25 mM H2O2/min/mg) compared to the intoxicated group.

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**Figure 2. Evaluation of Testicular oxidative stress markers.**

**A: GSH levels; B: Catalase levels; I: Control group; II: Chromium and ginger extract exposed group; III: Only ginger extract extract-treated group; IV: Chromium exposed group. Data are expressed as means ± 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).**

**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 numerous disorders, including decreased body weight, hormonal disturbances and induction of tissue oxidative stress. 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 ([Kim et al. 2004](#_ENREF_8)). 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 suggest 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 ([Xia et al. 2016](#_ENREF_22)). 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. (2012](#_ENREF_13)) 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 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 testis ([Kumar Dey and Roy, 2009](#_ENREF_9)). 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 was obtained after studying the effects of ginger on cadmium toxicity, indicate that ginger had better therapeutic detoxification effects on cadmium accumulation in the liver, especially when cadmium consumption was stopped ([Egwurugwu et al. 2007](#_ENREF_5)). 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 ([Reddy et al. 2014](#_ENREF_17)). 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 ([Morakinyo et al. 2008](#_ENREF_14)).

**CONCLSION**

This study demonstrated that the ethanolic extract of Zingiber Officinale had a remarkable protective effect against CrO3-induced reprotoxicity in rats and its mechanism is related, at least in part, to its antioxidant activity. In conclusion, the extract of *Z. Officinale* has pro-fertility characteristics in male rats, which might be due to its powerful antioxidant properties and androgenic activities.

**CONFLICT OF INTEREST**

The authors declare that they have no conflict of interest.

**REFERENCES**

Agarwal A, Saleh RA, Bedaiwy MA. (2003). Role of reactive oxygen species in the pathophysiology of human reproduction." Fertility and Sterility 79(4): 829-843.

Boudou F, Bendahmane-Salmi M, Benabderrahmane M, Belakredar A, Benalia A, Beghdadli B. (2020). Effect of Curcuma longa Aqueous Extract on Male Fertility in Aluminum Exposed Wistar Rats." Journal of Drug Delivery and Therapeutics 10(5): 11-17.

Boudou F, Bendahmane-Salmi M, Benabderrahmane M, Benalia A, Beghdadli B. (2020). The impact of Aluminum chloride sub-acute exposure on the reproductive system of male rats. Journal of Experimental Research 8(4).

Dkhil MA, Zrieq R, Al-Quraishy S, Abdel Moneim AE. (2016). Selenium nanoparticles attenuate oxidative stress and testicular damage in streptozotocin-induced diabetic rats.Molecules 21(11): 1517.

Egwurugwu JN, Ufearo CS, Abanobi OC, NwokochaCR, Duruibe JO, Adeleye GS, Onwufuji O. (2007). Effects of ginger (Zingiber officinale) on cadmium toxicity." African journal of biotechnology 6(18).

Fu PP, Xia Q, Hwang HM, Ray PC, Yu H. (2014). Mechanisms of nanotoxicity: generation of reactive oxygen species. Journal of food and drug analysis 22(1): 64-75.

Kim EJ, Jang M, Choi JH, Park KS, Cho IH. An improved dehydroepiandrosterone-induced rat model of polycystic ovary syndrome (PCOS): Post-pubertal improve PCOS's features. Frontiers in Endocrinology: 735.

Kim HY, Lee SB, Jang BS.  (2004). Subchronic inhalation toxicity of soluble hexavalent chromium trioxide in rats. Archives of toxicology 78(7): 363-368.

Kumar Dey S, & Roy S. (2009). Effect of chromium on certain aspects of cellular toxicity. Iranian Journal of Toxicology 2(4): 260-267.

Li H, Chen Q, Li S, Yao W, Li L, Shi X, Chen C. (2001). Effect of Cr (VI) exposure on sperm quality: human and animal studies. Annals of occupational hygiene 45(7): 505-511.

Mahurpawar M. (2015). Effects of heavy metals on human health." International Journal of Reseacrh-Granthaalayah, ISSN-23500530: 2394-3629.

Mao QQ, Xu XY, Cao SY, Gan RY, Corke H, Beta T, Li HB. (2019). Bioactive compounds and bioactivities of ginger (Zingiber officinale Roscoe). Foods 8(6): 185.

Marouani N, Tebourbi O, Mahjoub S, Yacoubi MT., Sakly M, Benkhalifa M, Rhouma KB. (2012). "Effects of hexavalent chromium on reproductive functions of male adult rats." Reproductive biology 12(2): 119-133.

Morakinyo AO, Adeniyi OS, Arikawe AP. (2008). Effects of Zingiber officinale on reproductive functions in the male rat. African Journal of biomedical research 11(3).

Qin D, Wen Z, Nie Y, Yao G. (2013). Effect of cichorium glandulosum extracts on CCl4-induced hepatic fibrosis. Iranian Red Crescent Medical Journal 15(12).

Rahmani AH. (2014). Active ingredients of ginger as potential candidates in the prevention and treatment of diseases via modulation of biological activities. International journal of physiology, pathophysiology and pharmacology 6(2): 125.

Reddy YA, Chalamaiah M, Ramesh B, Balaji G, Indira P. (2014). "Ameliorating activity of ginger (Zingiber officinale) extract against lead induced renal toxicity in male rats." Journal of Food Science and Technology 51(5): 908-914.

Shanmugam KR, Ramakrishna CH, Mallikarjuna K, Reddy KS. (2010). Protective effect of ginger against alcohol-induced renal damage and antioxidant enzymes in male albino rats. (48): 143-149

Shelnutt SR, Goad P, & Belsito DV. (2007). Dermatological toxicity of hexavalent chromium. Critical reviews in toxicology 37(5): 375-387.

Vabre P, Gatimel N, Moreau J, Gayrard V, Picard-Hagen N, Parinaud J, Leandri RD. (2017). Environmental pollutants, a possible etiology for premature ovarian insufficiency: a narrative review of animal and human data. Environmental Health 16(1): 1-18.

Valavanidis A, Vlachogianni T, Fiotakis K, Loridas S. (2013). Pulmonary oxidative stress, inflammation and cancer: respirable particulate matter, fibrous dusts and ozone as major causes of lung carcinogenesis through reactive oxygen species mechanisms." International journal of environmental research and public health 10(9): 3886-3907.

Xia W, Hu, J, Zhang B, Li Y, Wise Sr JP, Bassig BA, & Xu S. (2016). A case-control study of maternal exposure to chromium and infant low birth weight in China. Chemosphere 144: 1484-1489.

Zhang S, Kou X, Zhao H, Mak KK, Balijepalli MK, & Pichika MR. (2022). Zingiber officinale var. rubrum: Red Ginger’s Medicinal Uses. Molecules 27(3): 775.