

Article

Preventive Effects of *Zingiber officinal* on Chromium Trioxide Toxicity in the Reproductive Tract of Male Rat

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Abstract: Exposure to chromium trioxide (CrO₃) is known to induce oxidative stress and damage to various tissues, including reproductive organs. *Zingiber officinale* has been reported to possess potent antioxidant and anti-inflammatory properties. This study aimed to investigate the protective effects of rhizobium ginger extract (RGE) on the prostate and testicular tissues of rats exposed to CrO₃. Male rats were divided into four groups: GR1 (control), GR2 (CrO₃ at 10 mg/kg), GR3 (RGE-treated at 600 mg /kg) and GR4 (RGE + CrO₃). Biochemical analyses were conducted to measure serum concentrations of prostate specific antigen (PSA) and testosterone. Histological examinations of prostate and testicular tissues were also performed. The CrO₃-exposed rats (GR2) exhibited significant increases in PSA and testosterone levels respectively 0.93 and 0.98 ng/mL. Whereas RGE-treated rats (GR4) showed decreased serum-PSA (< 0.068 ng/mL) and testosterone (0.41 ng/mL). Histological study revealed tissue damage and inflammatory cell infiltration in the prostate and testicular tissues of CrO₃-exposed rats (GR2). In contrast, rats treated with RGE (GR4) showed an improved tissue architecture and reduced inflammation compared to the CrO₃-exposed group. The findings suggest that RGE mitigates CrO₃-induced oxidative stress and tissue damage in the prostate and testicular tissues. This study highlights *Zingiber officinale* being a natural therapeutic agent against reproductive toxicity induced by heavy metals.

Keywords: chromium trioxide; oxidative stress; *Zingiber officinale*; protective effects; prostate and testicular tissues.

1. Introduction

Heavy metals, particularly in their oxidative forms, are increasingly recognized for their toxicity and deleterious effects on various biological systems, including the reproductive system [1,2]. Among these compounds, chromium trioxide (CrO₃) stands out for its high toxicity and its ability to generate reactive oxygen species (ROS), oxidative disruption, and cellular damage in many tissues, including the male reproductive system [1,3,4]. As a hexavalent chromium (Cr⁶⁺) compound,

CrO₃ is widely used in various industries, such as electroplating, pigment manufacturing, and alloy production, resulting in potential exposure to workers and risk of environmental contamination [4,5]. The adverse effects of CrO₃ are well documented, with acute and chronic toxicity observed in lung, liver, and kidney tissues, as well as significant genotoxicity [4,6]. However, its impact on male reproductive health deserves special attention due to the possibility of hormonal disruption, germ cell damage, and reduced fertility. Recent research has highlighted the central role of oxidative stress in the reproductive toxicity of hexavalent chromium (Cr⁶⁺) [7]. This stress results from an imbalance between ROS production and the endogenous antioxidant capacity of tissues, leading to oxidative damage to lipids, proteins, and DNA [8,9]. This damage affects spermatogenesis, hormone production, and cellular integrity of the testes [1, 7, 8, 10]. Approaches to reduce the adverse effects of CrO₃ on reproduction have thus attracted increasing interest, particularly through the use of natural antioxidants capable of neutralizing ROS and strengthening endogenous antioxidant defenses. *Zingiber officinale*, commonly known as ginger, is a medicinal plant widely used for its antioxidant, anti-inflammatory, and cytoprotective properties [11-14]. Composed of gingerols, shogaols, and zingerone, this plant has demonstrated protective effects against oxidative stress in many models of heavy metal-induced toxicity [15,16]. *In vitro* and *in vivo* studies have shown that *Zingiber officinale* extract can reduce oxidative stress levels, prevent cellular damage, and support male reproductive function by promoting spermatogenesis and protecting testicular cells from toxic disruption [1, 7, 8, 17]. Therefore, *Zingiber officinale* may represent a promising preventive strategy against CrO₃-induced toxicity in the reproductive tract. This study aims to explore the preventive effects of *Zingiber officinale* extract on chromium trioxide-induced reproductive damage in male rats. By combining histopathological, biochemical, and hormonal analyses, we hope to demonstrate that *Zingiber officinale* can alleviate CrO₃ toxicity and protect reproductive function. The results of this research could pave the way for new therapeutic interventions using natural antioxidants to minimize the risks associated with exposure to heavy metals, thus contributing to a better understanding of protection strategies against environmental toxicants.

2. Materials and Methods

Preparation of rhizobium ginger extract (RGE)

Zingiber officinale rhizome was purchased at the spice market of Saida town, Algeria, and was presented to the Department of Agriculture and Food Science, Faculty of Natural Science and Life, University of Saida for recognition. The plant was then washed, disinfected, rinsed with distilled water, and divided into smaller pieces. The pieces were dried at room temperature. The dried pieces were ground in an electrical mill and the ground raw material (20 g) was put through a weave filter with a 60 mm opening to produce a fine powder. The obtained fine powder was then utilized in the preparation of the extract (100 mL of 70 % ethanol). The crude extract was made according to the maceration method; the dried powder was soaked for 48 h in 70 % ethanol. The mixture was dried in the vacuum using a rotary vacuum evaporator after 48 h and was then filtered again using Whatman filter paper [18].

Extraction yield

The extract was weighted and its percentage yield was calculated. The yield of the extract is defined as the ratio between the mass of the dry extract obtained after maceration (B) and the mass of the plant material used (A). It is given by the following formula:

$$\text{Yield} = (A/B) \times 100 \quad (\text{B: weight of raw material, A: weight of plant extracts})$$

In vivo study

Animal material

Breeding of rats was carried out at the Department of Agriculture and Food Science, Faculty of Natural Science and Life, University of Saida. The maintenance of the animals was carried out in a lighted room 12 hours a day, it is a photoperiod of 12 hours / 24 hours whose temperature was kept constant (22-25 °C). The rats were housed and separated in four plastic cages. They had free access to water and food. Animals were treated following the principles and guidelines set out in the Care and Use of Experimental Animals Manual. Article 58 of law 88-08 of 1988 includes a general prohibition of committing 'bad treatments' towards animals. Article 58 also states that the same prohibition applies to animals used in biological, medical, and scientific experiments, which experiments are required to be 'limited to cases of strict necessity [19].

Experimental design

A population of about twenty male rats, 8 weeks old with a weight varying between 110-230 gr, were divided into 4 groups. Each group contained 5 rats. The experimental period was 90 days. The animals were divided as follows (Figure 1):

- Group 1: normal control rats received a standard diet and physiological saline (0.9 % NaCl).
- Group 2: experimental controls received orally trioxide chromium CrO₃ at the dose 10 mg/kg/ day (~ 1/10 of LD₅₀) (LD₅₀ = 52-113 mg/L in rat by oral route) [20].
- Group 3: rats received orally 600 mg/kg / day (~ 1/100 of LD₅₀) (LD₅₀ = 3800 mg/L) [21]. RGE was dissolved in corn oil to avoid irritation to the digestive tract.
- Group 4: rats received trioxide chromium CrO₃ and RGE.

Biochemical tests

Blood samples were taken from rats every 10 days for a month. Blood was collected from the eye areas of the animals. The amounts of blood were collected in heparinized tubes intended for the biochemical assays. Serum assays of prostate-specific antigen (PSA) and testosterone were performed respectively by enzymatic methods and immunological techniques such as ELISA (antigen-antibody reaction). PSA and testosterone were measured using the mini-VIDAS analyzer (Bio-Merieux, France). The method used was the technique enzyme-linked fluorescent assay (ELFA): this is a test immuno-enzymatic Elisa of "sandwich" type in phase heterogeneous where the biological markers are caught between two monoclonal antibodies. Reading the dosage results was made in two stages with a final detection by fluorimetry. Results of serum PSA and testosterone concentrations are expressed as mean ± SEM (standard error of the mean), with a value of p<0.05 considered statistically significant. Statistical evaluation was performed by one-way analysis of variance (ANOVA) followed by Tukey's test for multiple comparisons. All statistical analyses were conducted with the statistical software SIGMAPLOT (Version 11.0).

Histology

At the end of the experimental period, the animals were anesthetized and then sacrificed. Prostatic and testicular tissues were removed for histological study. Samples were preserved in 10 % formalin solution until use. Histological sections were performed in the Anatomopathology Department of the hospital center of the city of Saida, Algeria. The technique used includes the following steps; the samples were fixed in 10 % formaldehyde, dehydration of the samples in successive baths of ethanol, inclusion of the samples were in paraffin, after cooling the paraffin blocks to -20°C , cuts were made of $4\ \mu\text{m}$ thickness using a Microm HM 340 E manual microtome (Thermo scientific, Illkirch), and finally the staining of the sections using the Hematoxylin-Eosin technique [22].

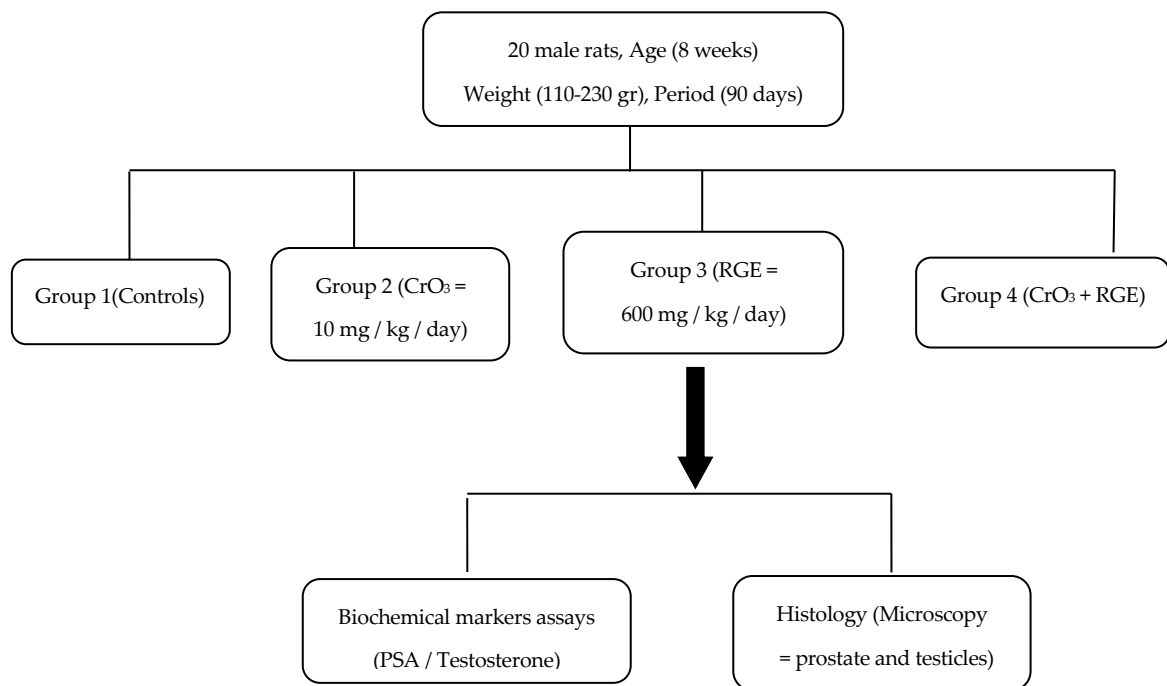


Figure 1. Experimental design

(RGE effects on the prostate and testicular tissues of rats exposed to CrO₃)

3. Results and Discussion

The percentage yield of the ethanol of *Zingiber officinale* was 31.5 %. The yield was a dark yellow mass of 31.5 g for ethanol extract. This result was following previous studies [11].

Serum-assay of the biomarker prostate specific antigen (PSA) did not show a considerable elevation in its serum concentration when comparing different groups of animals, namely, group 1 (normal controls), group 2 (CrO₃), group 3 (RGE), and group 4 (CrO₃ + RGE) ($p>0.05$). The values of serum PSA concentrations in groups 1, 3, and 4 were lower than 0.068 ng/mL, however, it was found that the PSA concentration in group 2 (CrO₃) reached a value of 0.93 ng /mL (Table 1).

Plasma concentrations of the male sex hormone "testosterone" in the different groups were measured at the end of the experiment. The values of plasma concentrations of the biological marker (testosterone) were different. The serum level of testosterone was significantly high in group 2 (CrO₃) and it was of the order of 0.98 ng/mL ($p<0.01$), however, the blood concentrations of testosterone were

variable. Among the other groups 1, 3, and 4 and they were respectively; group 1 (0.43 ng/mL), group 3 (0.29 ng/mL), and group 4 (0.41 ng/mL) (Table 1).

Table 1. Serum-assay of PSA and testosterone in groups of rats.

Groups of rats	PSA (ng/mL) mean ± SEM	Testosterone (ng/mL) mean ± SEM	p-value
Group 1 (controls)	< 0.068±0.002	0.43±0.03	
Group 2 (CrO ₃)	0.93±0.001	0.98*±0.04	< 0.01
Group 3 (RGE)	< 0.068±0.002	0.29*±0.01	
Group 4 (CrO ₃ + RGE)	< 0.068±0.002	0.41*±0.02	

PSA: prostate specific antigen, RGE: rhizobium ginger extract; SEM: standard error of the mean; *Testosterone: significantly different between groups 2, 3, and 4 of animals (p<0.01); CrO₃: trioxide chromium

The histology of control rats (group 1) showed normal prostate cells with a well-differentiated glandular structure (Figure 2-A). However, in group 2 (rats exposed to CrO₃), alterations and abnormal tissue architecture expressed by irregular neoplastic glands were noted (Figure 2-B). In these same animals (group 2), the presence of solid cellular areas confluent in mass presenting or necrosis. Whereas, in rats of groups 3 and 4 (treated with RGE), the stroma of the prostate parenchyma is regular and differentiated, little invaded by abnormal cells (Figure 2-C and D).

Microscopic examination of the testicular tissue of control rats (group 1) showed that the interstitial tissue and the seminal tubes, with their stages of spermatogenesis, are intact (Figure 3-A). The accumulation of chromium oxide CrO₃ in the testes of rats (group 2) leads to damage to the seminiferous tubules and interstitial tissue. These tissue disruptions were associated with loss of germ cells, necrosis, crowding of blood vessels, edema, cellular infiltration, and absence of Leydig cells (Figure 3-B). Whereas, in rats treated with RGE (group 3), a normal histological appearance of the testes was revealed and no cellular abnormality was detectable (Figure 3-C). In group 4 (CrO₃ + RGE), the testicular tissue did not show cellular transformation or cytonuclear abnormalities that could be induced by chromium oxide. The stages of spermatogenesis were present and visible. Spermatids and Sertoli cells displayed intact cell membranes and prominent nuclei with dense chromatin, preserved Lyedig cells (Figure 3-D).

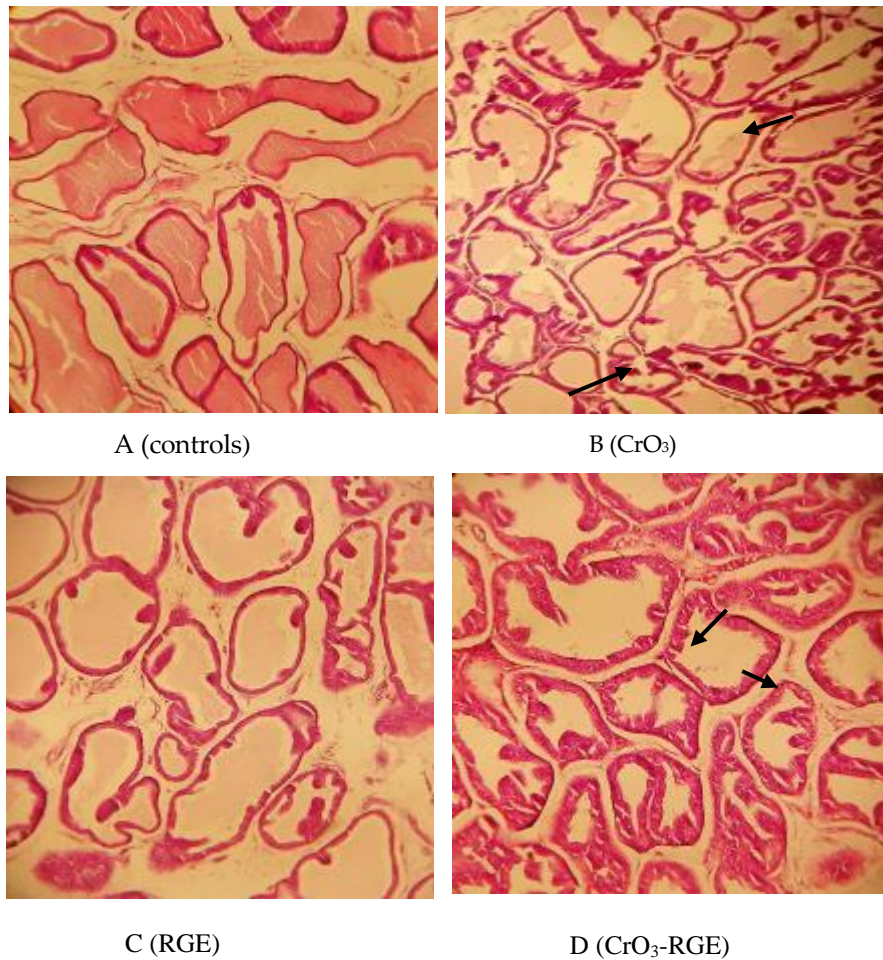


Figure 2. Microscopic examinations of the prostate glands were observed at magnifications $\times 10$. B (CrO_3): Lack of fiber-stroma tissue between acini (Arrows) and small epithelial cell layer and basal lamina not apparent as a result of CrO_3 -treatment (Arrows); D (CrO_3 + RGE): Thick epithelial cell layer and recovery of the proliferation of tissue between prostate glands as a result of RGE-treatment (Arrows) (H&E; $10 \times$).

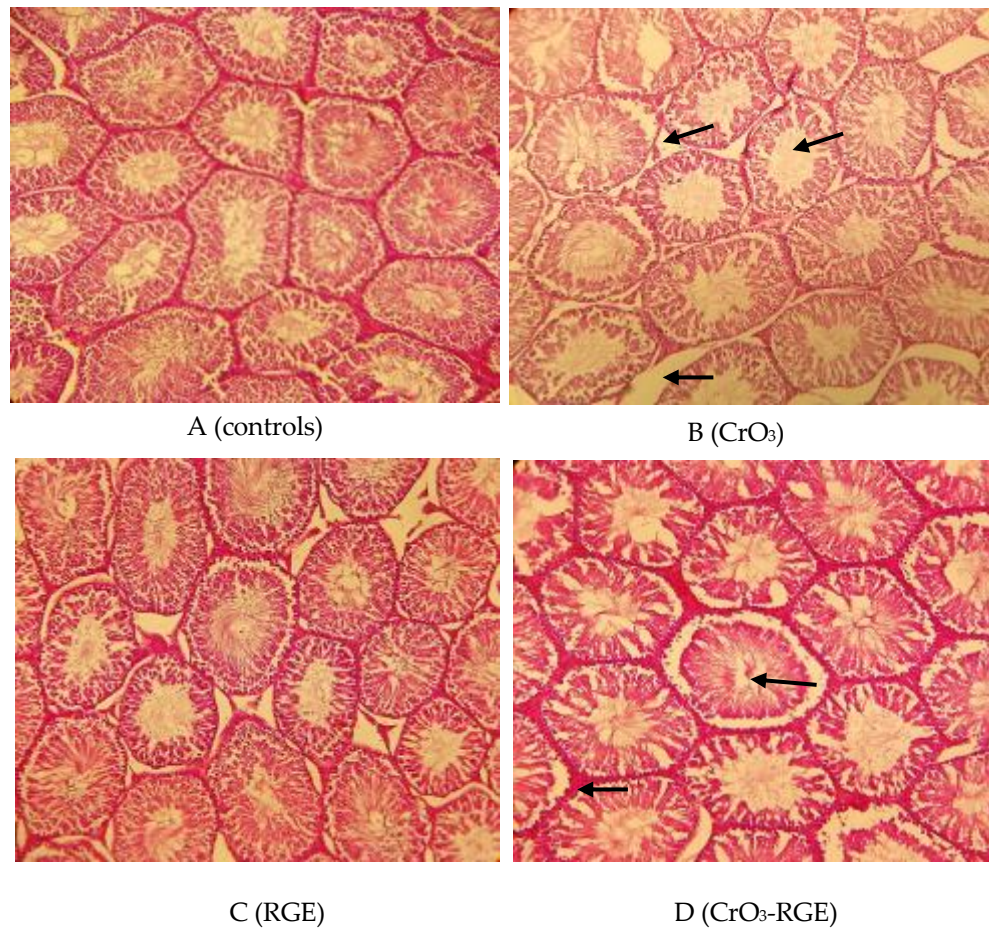


Figure 3. Microscopic examinations of the testicular tissue were observed at magnifications $\times 10$.

B (CrO₃): Loss of spermatids in the seminiferous tubules (Arrows) and No proliferation of connective tissue between seminiferous tubules as a result of CrO₃-treatment (Arrows); D (CrO₃ + RGE): Absence of anomalies and recovery of the proliferation of tissue between seminiferous tubules as a result of RGE-treatment (Arrows) (H&E; 10 \times).

By exploring the databases through the Science Direct and Springer websites or in the literature in general, it was noted a glaring lack of specific experimental studies about the relationship between the chromium trioxide toxicity CrO₃ and male reproductive system dysfunction. Several reasons can be put forward. It's possible that researchers simply haven't explored this specific question yet. Scientific research is broad, and researchers often focus on areas where there is increased interest or public concern.

Studies of the chromium trioxide effects on male reproductive function is a complex task that often requires long-term studies, epidemiological studies, or specific clinical trials. These studies can be expensive and require careful planning. It is possible that previous studies have not found a significant link between chromium and infertility, which could reduce researchers' interest in pursuing this line of research. Research on chromium can often focus on other aspects such as its role in carbohydrate metabolism (chromium picolinate for example), its effect on cardiovascular health, or its carcinogenic potential, thus shifting the focus away from fertility.

Chromium is also a potentially dangerous pollutant in the environment. Therefore, research can focus on its general toxic effects rather than specific effects on human fertility.

All these reasons prompted the authors of this article to make the exception and thus initiate their research work and focus much more on this axis which is the possible association of chromium trioxide toxicity with a male reproduction dysfunction or in another way seek to investigate the effects of a medicinal plant "*Zingiber officinale*" on the chromium toxicity (CrO_3) on the male reproductive organs, namely the prostate and the testicles.

Without exaggeration, this present study is possibly among the first studies that addressed the question of the link between chromium toxicity and male fertility in animals. Whatever the nature of the results obtained, it remains the first step in exploring this theme. This study was not limited only to this problem, on the contrary, it tried to experiment with a natural remedy while using a medicinal plant "*Zingiber officinale*" to prevent and treat the harmful effects resulting from chromium toxicity in an animal model. This study also suggested a variation in the serum concentrations of biological markers, namely PSA and testosterone, in animals exposed only to chromium trioxide (CrO_3) and also in animals that were previously exposed to CrO_3 and then treated with alcoholic extract of ginger rhizobium. This same study revealed a slight elevation in PSA and a significant increase in testosterone in rats (group 2) to which they ingested CrO_3 . Furthermore, the opposite effect occurred when rats were treated with the alcoholic extract of rhizobium ginger (RGE) after exposure to CrO_3 (group 4) in the same experimental conditions. Serum concentrations of these two markers did not increase (group 4) although the yield of the preparation of the RGE was not sufficient enough to lead to such results.

The toxicity of chromium trioxide is expressed much more by inhalation (pulmonary route) than by ingestion (oral route) and this could be explained by its chemical characteristic [23-25]. Soluble forms of chromium, such as hexavalent chromium (Cr^{6+}), can be created in certain biological environments due to reduction processes or other chemical interactions. Even hexavalent chromium (Cr^{6+}) is more soluble and potentially more toxic and carcinogenic [26]. Studies, in the form of surveys, have suggested that exposure to chromium and its derivatives constitute risk factors associated with prostate cancer, very widespread among farmers [27]. Occupational risks associated with woodworking, particularly those related to the use of wood preservatives [28]. These products, with fungicidal and insecticidal properties, can be preparations of various salts, containing arsenic compounds (arsenates, oxides), chromium compounds (alkaline dichromates), boron compounds (borate, boric acid, boracic complex), fluorine compounds (fluoride, fluoro-silicate, fluoro-borate), copper compounds (sulfate, copper oxide). The meta-analysis also found significant associations with other occupational risk factors such as exposure to chromium [29]. Chromium exposure and shift work are widespread among farmers [30].

In this study, rhizobium ginger extract (RGE), obtained with a yield of 31.5%, suggested a remedial (or chelating) effect towards the toxic particles of CrO_3 (in the male reproductive organs (prostate and testicles)).

Consumption of ginger helps fight the action of free radicals and prevent neurodegenerative diseases and prostate cancer [31,32]. Ginger rhizomes contain various chemical components (paradiol, gingerol, shogaol, and zingerone) allowing it to have numerous medicinal properties: treatment of nausea, vomiting, headaches, stimulation of the immune system, hypoglycemic effects, effects antioxidants, anti-inflammatory, antibacterial, antiviral, hepatoprotective and effects on the reproduction [33].

Studies have shown that ginger can be safely consumed in humans and animals, without harmful side effects and with no increased mortality.

According to studies, ginger reduces testicular damage and hormonal disorders (testosterone) induced by exposure to endocrine disruptors such as chromium derivatives (pesticides) [34,35].

Higher testosterone levels in CrO₃-exposed animals are consistent with reports linking heavy metal exposure to endocrine disruption. Histological findings in prostate and testicular tissues highlight the protective effects of ginger against CrO₃-induced damage, supporting its potential therapeutic application. Our study suggests that rhizobium *Zingiber officinal* may mitigate CrO₃-induced prostate and testicular damage, emphasizing its potential as a protective agent in environmental and occupational settings. Further studies could explore the molecular mechanisms underlying rhizobium ginger's protective effects and its potential therapeutic applications in mitigating heavy metal toxicity.

4. Conclusions

This study has demonstrated that the alcohol extract of *Zingiber officinale* exhibits significant protective effects on the prostate and testicular tissues of rats exposed to chromium trioxide (CrO₃). The biochemical and histological analyses revealed that ginger extract mitigates oxidative stress, reduces inflammation, and preserves the structural integrity of these tissues. Specifically, the treatment with *Zingiber officinale* resulted in a substantial decrease a prostate specific antigen (PSA) and testosterone levels in animals exposed to CrO₃. Histological examinations further corroborated these findings by showing reduced tissue damage and inflammatory cell infiltration in the ginger-treated group compared to the chromium-exposed group. These results suggest that *Zingiber officinale* has potent antioxidant properties that could potentially counteract the toxic effects of chromium trioxide on reproductive organs. Thus, ginger extract could be considered a promising natural therapeutic agent for protecting against heavy metal-induced reproductive toxicity. Further studies are warranted to elucidate the precise mechanisms of action and to explore its efficacy in other models of toxicity and oxidative stress.

Conflicts of Interest: The authors declare no conflict of interest

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