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PHARMACOGNOSIE

Ameliorative Effect of Curcuma on Reproductive Functions of Male Adult Rats following Chromium Exposure

Effet d'amélioration du curcuma sur les fonctions reproductrices des rats mâles adultes après exposition au chrome

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Abstract This study aimed to investigate the protective effects of curcuma against hexavalent chromium-induced alterations in reproduction indices of male Wistar rats. Twenty adult male Wistar rats were divided into four groups. The first group (0-0): control. The second group (0-Cur) received a diet with 2% curcuma powder. The third group (Cr-0) received 15 mg/kg B.W. of potassium dichromate ($K_2Cr_2O_7$) *per os*, while the last group (Cr-Cur) received a diet containing 2% curcuma powder and 15 mg/kg B.W. of $K_2Cr_2O_7$ per os. After 30 days of treatment, testicular weight, sperm concentration, sperm kinematic parameters, and testicular glutathione (GSH) level were evaluated. Our results suggest that hexavalent chromium causes a decrease in sperm concentration, total progression, total motility, fast motility, medium motility, slow motility, static sperm, linearity (LIN), straightness (STR), amplitude of lateral head displacement (ALH), beat cross frequency (BCF), sperm velocity, and testicular GSH level, accompanied with an increase in testicular weight and wobble (WOB) level when compared to control group. However, supplementation with curcuma of chromium-intoxicated rats has reduced the toxic effect of $K_2Cr_2O_7$ by bringing back the reproductive parameters and GSH levels to normal values. From this finding, it can be implicated that diet supplemented with curcuma powder may show protective effects against chromium toxicities.

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Keywords Curcuma · Chromium · Spermatozoa · Reproductive functions · Male rats

Résumé Cette étude visait à étudier les effets protecteurs du curcuma contre les altérations induites par le chrome hexavalent dans les indices de reproduction des rats mâles Wistar. Vingt rats Wistar mâles adultes ont été divisés en quatre groupes. Le premier groupe (0-0) : témoin. Le deuxième groupe (0-Cur) a reçu une alimentation avec 2 % de curcuma en poudre. Le troisième groupe (Cr-0) a reçu 15 mg/kg de dichromate de potassium ($K_2Cr_2O_7$) per os, tandis que le dernier groupe (Cr-Cur) a reçu une alimentation contenant 2 % de curcuma en poudre et 15 mg/kg PC de $K_2Cr_2O_7$ per os, pendant 30 jours de traitement. Nos résultats suggèrent que le chrome provoque une diminution de la concentration des spermatozoïdes, de la progression totale, de la motilité (totale, rapide, moyenne et lente), des spermatozoïdes statiques, de la linéarité (LIN), de la rectitude (STR), de l'amplitude moyenne du mouvement latéral de la tête (ALH), la fréquence des battements (BCF), de la vitesse des spermatozoïdes et du niveau de GSH testiculaire, accompagnés d'une augmentation du poids testiculaire et du niveau d'oscillation (WOB) par rapport au groupe témoin. Cependant, la supplémentation en curcuma a réduit l'effet toxique du $K_2Cr_2O_7$ en ramenant les paramètres de reproduction et les taux de GSH à des valeurs normales. À partir de cette étude, on peut impliquer qu'un régime alimentaire complété par la poudre de curcuma peut avoir des effets protecteurs contre la toxicité du chrome.

Mots clés Curcuma · Chrome · Spermatozoïdes · Fonctions reproductrices · Rats mâles

Introduction

Minerals are chemical compounds that are present in nature, made up of inorganic elements, required as an important nutrient by organisms, and are even a major component of

the body within a specific limited range. Moreover, minerals are widely used in several industries as they can be incorporated in different kinds of products [1,2]. Chromium is one of the most commonly used metals [3], relatively abundant element in Earth's crust; it has a shiny appearance, usually used for paintwork, along with production of stoves, and other devices to protect them from corrosion and enhance their external appearance. However, the consumption of high levels of contaminated drinking water or the inhalation of smoke from its heat can cause ulcer, cancer, and many other health conditions [4,5]. Therefore, hexavalent chromium compounds [Cr(VI)] are well-known oxidizing, carcinogenic, mutagenic, and teratogenic agents. In fact, this metal is able to directly induce tissue damage and easily absorbed by cells while subsequently reduced to generate free radicals [6]. Various studies have demonstrated that metal compounds are very much associated with reproductive toxicity that reduces sperm quality by inhibiting spermatozoa motility [7–9]. Furthermore, hexavalent chromium is harmful to human health. It is dangerous for those who work in steel and textile industries [10], as it can cause alteration of semen status and sperm quality that may affect reproductive potential [11]. According to Shiva et al. [12], free radical generation is associated with the impairment of semen quality which may affect fertility. Moreover, Zhen et al. [13] have discussed that chromium affects sperm motility and induces reproductive toxicity. Similarly, it was found that Cr(VI) has an effect of producing alterations in normal reproductive functions leading to a number of ailments such as an increase in seminal vesicles and prostate weights [14].

Medicinal plants have been, since ages, an alternative treatment for fighting diseases [15,16]. Curcuma is one of the chief traditional herbs of the ginger family, popular in South-East Asia as a spice and herbal treatment [17]. It has been proven that healing properties of curcuma are due to its richness in antioxidants, antiviral, antibacterial, anti-inflammatory, and antifungal substances [18,19]. Indeed, several studies have shown that curcuma contains "curcumin," a bioactive ingredient that can stimulate the self-destruction process of cancer cells [20]. In addition, curcumin has an ameliorative effect in testicular tissue and sperm quality [21,22], promotes reproductive functions, reduces testicular injury, and prevents against semen damage and testicular complications along with prostate cancer [23,24]. Boudou et al. [25] reported that curcuma plays an important role in enhancing the regeneration of seminiferous tubules and interstitial cells as well as reduces abnormal sperm morphology. Likewise, many researchers have reported the protective effect of curcuma on reproductive functions [26,27].

The aim of this study is to evaluate the protective effect of curcuma, as a natural antioxidant source, against chromium toxicity on reproductive and oxidative stress parameters in Wistar rats.

Materials and Methods

Preparation of plant material

Curcuma longa rhizomes were purchased locally from the market. In order to obtain fine powder, they were first 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, Atlanta, Georgia, USA) was dissolved in mineral water and administered per os to animals; the volume of each dose was adjusted to deliver 15 mg/kg of body weight/day.

Animals and treatment

Twenty adult male Wistar rats, weighing 160 ± 10 g, were obtained from Pasteur Institute of Algiers, Algeria. Animals were housed in polypropylene cages, at constant room temperature (21°C ± 2°C), under a 12-h dark/light cycle, with ad libitum mineral water supply. Animals were divided into four groups of five rats for each as follows: 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 15 mg/kg B.W. of $K_2Cr_2O_7$ associated with a normal diet; and the last group (Cr-Cur) — rats received per os $K_2Cr_2O_7$ at 15 mg/kg B.W. and supplemented with an experimental diet containing 2% curcuma powder. The experimental study was conducted for 30 consecutive days, and the study protocol was approved by the Ethical Committee of Animal Sciences of University of Badji Mokhtar–Annaba.

Sample collection

After animal dissection, the sperm is obtained immediately from a small opening in the epididymis tail to study sperm kinematics. In addition, the testicle was extracted and washed with a phosphate buffer (0.1 M; pH: 7.4) to remove excess blood and adhering tissues, weighed, and then frozen at −20 °C to stop metabolic activities.

Sperm analysis

To evaluate spermatozoa parameters, a computer-assisted semen analysis (CASA) — Sperm Class Analysis System (SCA®, Microptic, Barcelona, Spain) with Nikon Eclipse (Nikon E200-LED, Melville, New-York, USA) microscope

(×4) were used. Semen sample (1 µL) was diluted with physiological solution (NaCl: 0.9%) and pipetted 5 µL sperm preparation into an empty chamber slide (GoldCyto model) and then immediately evaluated. Semen quality measurements for sperm concentration and kinematics were assessed by CASA system, including sperm concentration, percentage of motility (total and types), percentage of static sperm, percentage of total progression, velocity average path (VAP), velocity curved line (VCL), velocity straight line (VSL), straightness (STR = VSL/VAP), linearity (LIN = VSL/VCL), wobble (WOB = VAP/VCL), amplitude of lateral head displacement (ALH), and beat cross frequency (BCF).

Oxidative stress study

Tissue preparation

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

Determination of glutathione (GSH) level

GSH contents were estimated using a colorimetric technique, as mentioned by Weckbercker and Cory [28]. This method is based on the development of a yellow color when 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) was added to compounds containing sulfhydryl groups. Concisely, 0.8 mL of tissue homogenate was added to 0.2 mL of 0.25% sulphosalicylic acid (SSA). The reaction mixture was incubated for 15 min in a glace bath, and then was centrifuged for 5 min 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 density (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 595 nm according to Bradford's [29] 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, USA). All results are expressed as mean ± standard error of the mean (SEM). Groups of data were compared with one-way analysis of variance (ANOVA) followed by Tukey's test; p values of < 0.05, < 0.01, and < 0.001 were, respectively, considered as statistically significant, highly significant, and very highly significant.

Results

Reproduction parameter study

The results reveal a disorder in the majority of semen parameters. A decrease in sperm concentration, total progression, total motility, fast motility, medium motility, slow motility, static sperm, LIN, STR, WOB, ALH, BCF counts (Table 1),

Table 1 Semen quality in control (0-0) and treated rats (0-Cur, Cr-0, Cr-Cur) after 30 days of treatment (values represent the mean ± SEM of five rats)

Parameters	Groups			
	0-0	0-Cu	Cr-0	Cr-Cu
Concentration (million/mL)	390.40 ± 31.88	381.60 ± 40.55	332.40 ± 65.16	369.40 ± 58.42
Total progression (%)	7.31 ± 1.24	7.57 ± 2.41	3.94 ± 1.23	2.67 ± 0.60*
Total motility (%)	55.61 ± 5.21	59.22 ± 9.67	45.84 ± 10.77	50.52 ± 3.94
Fast motility (%)	2.73 ± 0.62	2.38 ± 1.14	1.26 ± 0.83	1.08 ± 0.09*
Medium motility (%)	23.51 ± 2.37	27.30 ± 5.64	16.61 ± 6.14	18.30 ± 1.91
Slow motility (%)	29.37 ± 3.41	30.91 ± 2.86	28.14 ± 4.07	29.77 ± 0.49
Static sperm (%)	44.39 ± 5.21	52.38 ± 13.10	47.25 ± 8.26	49.48 ± 3.94
LIN (%)	23.24 ± 1.49	21.67 ± 1.17	20.97 ± 1.73	21.75 ± 1.80
STR (%)	44.20 ± 2.07	41.12 ± 1.66	38.71 ± 2.77	40.91 ± 1.83
WOB (%)	49.48 ± 1.15	49.36 ± 1.75	52.44 ± 1.73	49.75 ± 1.86
ALH (µm)	4.30 ± 0.11	4.41 ± 0.16	3.97 ± 0.15	4.17 ± 0.08
BCF (Hz)	4.39 ± 0.05	4.41 ± 0.12	4.18 ± 0.17	4.20 ± 0.14

*$p < 0.05$: significantly different from control (0-0) group

SEM = standard error of the mean; LIN = linearity; STR = straightness; WOB = wobble; ALH = amplitude of lateral head displacement; BCF = beat cross frequency

and sperm velocity (VCL, VSL, VAP) levels in figure 1 was observed in chromium-treated rats (Cr-0) when compared to control (0-0). Conversely, the same group of rats generates increased testicular weight (Fig. 2) and WOB levels (Table 1) in comparison to control group. However, supplementation with curcuma of chromium-intoxicated rats (Cr-Cur) has shown an improvement in semen quality when compared to control group (0-0). Adversely, no significant changes were observed in reproduction functions of rats under curcuma diet (0-Cur) when compared to the control group.

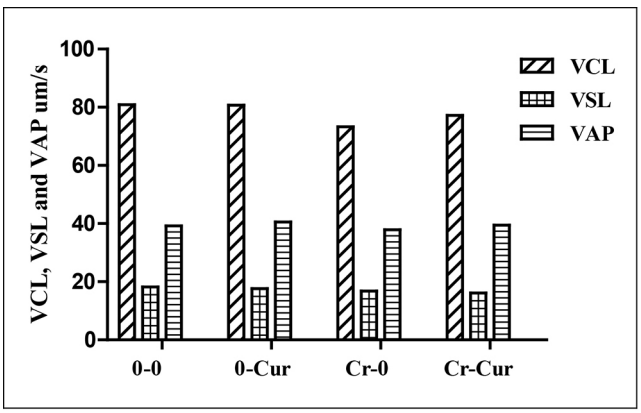

Fig. 1 Sperm velocity (VCL, VSL, and VAP) (μm/s) in control (0-0) and treated groups (0-Cur, Cr-0, and Cr-Cur) after 30 days of treatment (values represent the mean ± SEM of five rats) VCL = velocity curved line; VSL = velocity straight line; VAP = velocity average path; SEM = standard error of the mean

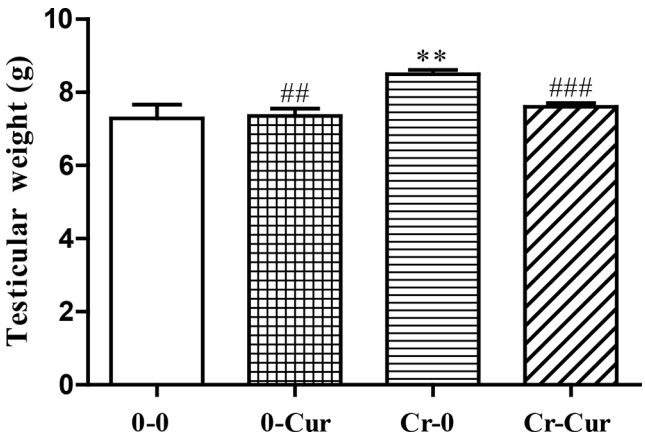

Fig. 2 Testicular weight (g) in control (0-0) and treated groups (0-Cur, Cr-0, and Cr-Cur) after 30 days of treatment (values represent the mean ± SEM of five rats) $^{**}p < 0.01$: significantly different from control (0-0) group; $^{\#\#}p < 0.01$; $^{\#\#\#}p < 0.001$: significantly different from (Cr-0) group

Oxidative stress study

Figure 3 showed that testis GSH content was significantly decreased in chromium treatment (Cr-0) when compared to control group (0-0), whereas curcuma-supplementation in diet of chromium-intoxicated rats (Cr-Cur) decreased the toxic effect of Cr(VI) by bringing back GSH levels to normal values when compared to control (0-0) and Cr(VI) groups (Cr-0).

Discussion

The reproductive system is one of the most sensitive targets for oral exposure to hexavalent chromium [30]. Therefore, accumulation of Cr(VI) beyond the permissible limit in the genital organs and sexual accessory glands presents a noxious health effects [31]. Indeed, Cr(VI) is able to penetrate the cell's membrane [32], through its highly absorbent capacity. Once inside the cell, it rapidly reduced to pentavalent chromium [Cr(V)] and trivalent chromium [Cr(III)] reactive intermediates that induces harmful effects [33]. Thus, the purpose of this study is to evaluate the possible protective effect of curcuma, as a natural antioxidant source, on reproduction and oxidative changes in Wistar rats contaminated with industrial pollulants as an animal model, and the possibility of its use in humans.

In our study, results revealed that oral administration of Cr(VI) could induce a decrease in sperm concentration, total progression, total motility, fast motility, medium motility,

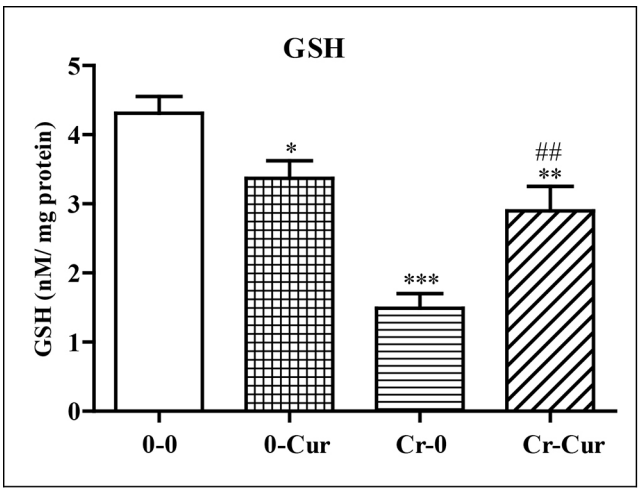

Fig. 3 Glutathione content (nM/mg protein) in the four groups of rats testis after 30 days of treatment (values represent the mean ± SEM of five rats) SEM = standard error of the mean $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$: significantly different from control (0-0) group; $^{\#\#}p < 0.01$: significantly different from (Cr-0) group

slow motility, static sperm, LIN, STR, ALH, BCF, sperm velocity (VCL, VSL, VAP), and testicular GSH level as well, accompanied with an increase in testicular weight and WOB level when compared to control group. The decrease in majority of semen parameters could be explained by the intracellular reduction of Cr(VI) to Cr(III) and the generation of free radicals. According to Henkel and Franken [34], fertility can be affected by higher concentrations of reactive oxygen species (ROS) in the testis, via mechanisms involving the induction of peroxidative damage to the sperm plasma membrane leading to sperm motility defects. Previous research found that after administration of Cr(VI), the latter is rapidly reduced which generates free radicals that affect the balance of the antioxidant defense system [35]. GSH depletion in our study may contribute to alterations in cellular defense that may lead to tissue injury [36]. Furthermore, excessive levels of ROS in semen may attribute to the risk of declining sperm fertilization, impaired sperm metabolism, and motility [8,12], which affect sperm capacitation and acrosome reaction [37]. Similarly, a study on workers exposed to Cr(VI) revealed reproductive disorders manifested by decreased sperm count and increased abnormal sperm morphology that can be associated with impaired spermatogenesis [38,39]. Chromium can affect sperm development, maturity, and sperm motility by crossing the blood–testis barrier [40]. Thus, it adversely affects the seminiferous tubules, resulting in a decreased number of Sertoli and Leydig cells with interstitial spaces widening as well [41]. An earlier study showed that rats exposed to Cr (VI) caused a reproductive toxicity including testicular atrophy, low epididymal spermatozoa, motility, and induced histological changes [14], thus may be the result of excessive Cr (VI) accumulation in the testis [38]. Previous study has shown that chromium affects sperm motility by impairing tyrosine phosphorylation in the midpiece of sperm by blocking the cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) signaling pathway correlated with inhibited sperm metabolism and protein phosphorylation [13].

The present study demonstrates that curcuma-supplementation prevents the reproductive imbalance caused by Cr(VI). This may be due to the protective effect of curcumin via its ability to neutralize free radicals [42]. Moreover, as a membrane antioxidant, curcumin can be associated with free iron to protect against cell's lysis and lipid peroxidation [43]. Furthermore, curcuma-supplementation significantly improved testicular GSH level in our study. Researchers have reported curcuma as a potential natural antioxidant against the induced toxicities on testis manifested by inhibition of cellular damage in spermatogenic cells of the seminiferous tubules and interstitial cells [26,27]. On the contrary, it was suggested that curcuma could enhance the availability of GSH through the effect of curcumin on γ-glutamyl-cysteinyl synthetase activity and other GSH-related detoxifying enzymes [44]. Besides, curcumin may improve the synthesis of de novo GSH [45] by increasing the transcription of γ-GCL gene, which may protect against oxidative stress and mitochondrial damage [46]. Curcuma plays a powerful remedy in mitigating the reproductive and developmental toxicity in both male and female mice. Hence, curcuma is a useful food supplement that can help reduce the risk of infertility and reproductive toxicity [27]. The protective effect of curcuma through oral administration ameliorates the testicular tissue and sperm quality, decreases morphological abnormalities, and regenerates the seminiferous tubules and interstitial cells by its high free radical scavenging and antioxidant activity [25].

Conclusion

Our study discloses that Cr(VI) causes a disturbance in most reproductive parameters and provokes a decline in GSH levels. It also discloses that supplementation with curcuma rhizome powder attenuates the Cr(VI) noxious effects that was observed on all the studied parameters. Based on these data, we can deduce that the use of curcuma rhizomes as a main nutritional ingredient may prevent and reduce Cr(VI) toxicity, one of the most important industrial mineral pollutants that mammalian and human are constantly exposed to and contaminated with.

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