**Effect of Curcuma longa Aqueous Extract on Male Fertility in Aluminum Exposed Wistar Rats**

Farouk BOUDOU1,2*; Malika BENDAHMANE-SALMI1,2; Mokhtar BENABDERRAHMANE1; Amal BELAKREDAR1; Abdelkrim BENALIA2; Benali BEGHADLIL2; AND Bader dine Abdelkrim KANDOUCI2

1. Department of Biology, Faculty of Natural and Life Sciences, DjillaliLiabes University of Sidi-Bel-Abbes, Sidi-Bel-Abbes, Algeria
2. Research Laboratory of Environment and Health (RLEH), Faculty of Medicine, Sidi-Bel-Abbes, Algeria

**ABSTRACT**

To assess the effects of Curcuma longa aqueous extract (CE) on fertility in male rats exposed to aluminum chloride (AlCl3). Twenty-four male rats, 3 months old, divided into 4 groups (n=6) were used. Animal from the first group received, by the oral route, water-containing AEB at the dose of 34 mg/kg body weight (BW). Rats from the second group received both AlCl3 at the same dose in combination with oral treatment of CE at a dose of 200 mg/kg BW. The third group received only an oral administration of CE with the same dose. Rats from the fourth group, without any treatment, served as control. After 4 weeks of experimentation, ACl3 exposure showed a significant decrease in sperm concentration (4.5±0.65 × 106 cells /ml), and percentage of viability (61.53±23.63 %), and an increase of morphological abnormalities (26.11±17.84 %). A significant decrease in serum testosterone (0.31±0.26 ng/ml) and an increase of testicular malondialdehyde (MDA) level (4.58±0.65 × 106 μM/g) were also observed. Histological examination of the testes showed degeneration of the seminiferous tubules, germ line cells, and interstitial cells. However, CE treatment concomitant to AlCl3 showed that the rate of morphological abnormalities (19±2.65 %) is significantly decreased compared to AlCl3 group, with a significant increase in serum testosterone (1.17±0.24 ng/ml) and a significant decrease in MDA (0.16±0.015 μM/g) level. Microscopic examination revealed a significant regeneration of seminiferous tubules and interstitial cells. This study demonstrated an ameliorative effect of Curcuma longa aqueous extract in testicular tissue and sperm quality.

**Keywords:** Curcuma longa, Aluminum, fertility, testosterone, malondialdehyde (MDA), sperm.

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**Address for Correspondence:** Farouk BOUDOU, Department of Biology, Faculty of Natural and Life Sciences, DjillaliLiabes University of Sidi-Bel-Abbes, Sidi-Bel-Abbes, Algeria

**INTRODUCTION**

Aluminum (Al) is an abundant element in the earth’s crust and is widely dispersed throughout the environment. Nowadays, aluminum salts are included in cosmetics, food processing, and storage and also used in various non-prescription drugs. It has for a long time been considered on an indifferent element from a toxicological point of view. However, it is unclear whether the normal environmental levels of Al. Aluminum is known as a neurotoxin that can cause certain diseases such as Alzheimer’s disease, dialysis dementia, Parkinsonism, and amyotrophic lateral sclerosis. In addition to its neurotoxicity, Al affects other body structures like the skeletal system, brain tissue, bone, blood cells, liver and kidney. The sources of Al are especially corn, yellow cheese, salt, herbs, spices, tea, cosmetics, aluminumware, and containers. Also, Al is widely used in antacid drugs, as well as in food additives and toothpaste. Environmental pollution with the different aluminum-containing compounds, especially those in industrial wastewater, exposes people to higher than normal levels of Al. Aluminum ingestion in excessive amount leads to accumulation in target organs and has been associated with damage of testicular tissues of both humans and animals, with alteration in the histology of testis, deterioration in spermatogenesis and sperm quality; enhancement of free radicals and alterations in antioxidant enzymes, interruptions in sex hormone secretion and biochemical changes in the testis and other accessory reproductive organs. Some of the aspects suggested that Al exposure harms the reproduction male function. Furthermore, the use of medicinal plants is a great contribution to treat primary medical problems. A variety of plants are claimed to have fertility regulating properties. One of these plants is Curcuma longa. It’s belonging to the Zingiberaceae family and has been widely used as a medicine, condiment, and cosmetics worldwide and valued

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as a functional food because of its health-promoting potentials. The rhizome of C. longa, a traditional medicine used for centuries in the Indian subcontinent, has been scientifically validated for its antioxidant, antimicrobial, antiarthritic, anticancer, carminative, stomachic, tonic, analgesic, hemostatic and anti-inflammatory activities. The beneficial properties of C. longa have been associated with antioxidant activity. And most of the studies performed on C. longa focused on curcuminoid components which comprised curcumin, demethoxycurcumin, and bisdemethoxycurcumin. The most important feature of curcumin is that it has no side effects despite being a therapeutic agent with multiple beneficial functions. It acts as a scavenger of free radicals.

Materials and Methods

Preparation of Curcuma longa aqueous extract (CE)

25 g of the powder is boiled in 250 mL (100 °C) water bath reflux system for 15 min and then filtered with N°1 Whatman Millipore filter paper. The filtrate is combined, centrifuged at 4000 rpm for 20 min, the supernatant is stored at 4 °C until use. This procedure was repeated weekly throughout the study.

Animals and experimental design

Experiments were carried out on 24 albino mature males, aged 3 months and weighing 193.9 ± 26.46 g. The animals were housed in a room with a 12/12 hour light/ dark cycle, at 22 ± 2°C, and had access to rodent pellet diet (15% proteins). Rats were randomly allocated into 4 groups, with 6 rats in each group. The 4 groups were: AlCl3 group, Al+CE group receiving orally both AlCl3 at the same dose and 1/25 LD50 of Aluminum chloride at a dose of 34 mg/kg BW; Al+CE group receiving orally both AlCl3 at the same dose and 1/25 LD50 of CE at a dose of 200 mg/kg BW; the third group received only CE at the same dose and route as the previous group; and control group (CONT) without any treatment. All the experiments lasted for 30 days.

Biological samples collection

At the end of the experiment, the animals were sacrificed in the morning after fasting for 12 hours and anesthetizing with diethyl ether in a large desiccator. After incision of the abdomen, blood is collected from the inferior cava vena for the determination of testosterone level by using immunoassay commercial kits (VIDAS Assays, BIOMERIEUX). The testicles are carefully removed, separated from their epididymis, and rinsed with cold saline solution. The right ones were used for histological study according to standard techniques, after fixation in fixative solution (formalin 1/10), paraffin embedding, and staining with hematoxylin-eosin. While the left ones are used to prepare tissue homogenate, by grinding 1 g of testis in 5 mL of 0.01M phosphate buffer pH 7.4 at 4°C, and centrifuged at 3000 rpm for 10 min. The supernatant was kept frozen (-20 °C) to be used for the ultracentrifugation determination of malondialdehyde (MDA) level as described by Azad et al., 2019. The epididymis of each rat was placed in a petri dish, cut with scissors, and homogenized in 1 mL of physiological saline solution (0.9%) at 35-37 °C for 15 min to form the sperm suspension. Semen characteristics, including sperm count, percent viability, and sperm morphology, were evaluated according to Wang, 2002.

Statistical Analysis

Results were represented as mean ± SD. The data were analyzed by using one-way analysis of variance (ANOVA) followed by the Bonferroni t-test using SigmaPlot version 11.0. P values <0.05 were considered significant.

Results

Table 1 shows that all semen parameters of the AlCl3 exposed group were significantly affected compared to the other experimental groups. A significant decreased in sperm count, percent viability, and an increase in sperm abnormal morphology were showed in the AlCl3 group compared to control. However, a significant improvement was observed in the sperm morphology of the animals belonging to the Al+CE group comparatively to the group of rats exposed only to AlCl3. But, no significant changes were observed in sperm count and percentage of viability between these two groups. For the Hormonal assay, the results showed that the administration of AlCl3 significantly decreased serum testosterone levels (0.31±0.26 ng/ml) as compared with the other experimental groups. Curcuma longa treatment showed a very significant increase in serum testosterone of the CE group (3.16±3.05 ng/ml) and an important improvement in this hormone level in the Al+CE group (1.17±0.24 ng/ml) (Figure 1). Figure 2 showed that AlCl3-administration for 30 days significantly increased the MDA level (0.16±0.015 µM/g) as compared to the controls (0.14±0.002µM/g). On the contrary, AlCl3 concomitant with Curcuma longa treatment led to a significant reduction in MDA level (0.11±0.003µM/g). Histological study in the testicles reveals a normal architecture in control and CE groups (Figures 3 and 6) showing seminiferous tubules richly populated with a healthy appearance. All stages of the spermatogenic lines cells such as spermatagonia, spermatocyte, spermatids, and spermatozoa, even Sertoli cells could be identified in the seminiferous tubules. Lumen could easily be delineated in almost all the tubules and the majority of them were filled by mature spermatozoa. While the observation of histological sections of the AlCl3-exposed group (Figure 4) shows that all these stages are affected. Among the disturbances reported: degeneration of the seminiferous tubules, a total absence of sperm and/or a low sperm count, with large interstitial spaces and lack of Leydig cells around basement membranes. However, Curcuma longa administration to rats concomitantly exposed to AlCl3 (Figure 5), shows a significant regeneration of the majority of seminiferous tubules structure and interstitial cells, with a good development of the spermatogenesis indicated by sperm-filled lumens of seminiferous tubules.
Table 1: Semen parameters of the different experimental groups

| Parameters               | Groups             |
|-------------------------|--------------------|
|                         | AlCl₃ | AlCl₃ + CE | CE  | CONT   |
| Sperm count (10⁶/ml)    | 4.58±0.65ᵃ       | 5.80±0.56ᵃ  | 9.30±0.35ᵇ | 11.52±0.77ᵇ  |
| Morphology (abnormal %)| 26.11±17.84ᵃ     | 19±2.65ᵇ    | 21±1.00ᵇ  | 16.67±1.53ᵇ  |
| Viability (%)           | 61.53±23.60ᵃ     | 67.33±5.03ᵃ | 66.67±4.93ᵇ | 76.33±6.51ᶜ  |

AlCl₃: Aluminum chloride exposed group; AlCl₃ + CE: Al and Curcuma longa aqueous extract exposed group; CE: Only C. longa aqueous extract-treated group; CONT: a control group with no treatment. Data are expressed as means ± SD (n=6). A comparison between groups was made using the Bonferroni t-test. Column not sharing a common letter (a–c) differ significantly at p < 0.05 (Bonferroni t-test).

Figure 1: Evaluation of Testosterone level in different experimental groups. AlCl₃: Aluminum chloride exposed group; AlCl₃ + CE: Al and Curcuma longa aqueous extract exposed group; CE: Only C. longa aqueous extract-treated group; CONT: a control group with no treatment. Data are expressed as means ± SD (n=6). A comparison between groups was made using the Bonferroni t-test. Column not sharing a common letter (a–c) differ significantly at p < 0.05 (Bonferroni t-test).

Figure 2: Evaluation of Testicular Malondialdehyde (MDA) levels in different experimental groups. AlCl₃: Aluminum chloride exposed group; AlCl₃ + CE: Al and Curcuma longa aqueous extract exposed group; CE: Only C. longa aqueous extract-treated group; CONT: a control group with no treatment. Data are expressed as means ± SD (n=6). A comparison between groups was made using the Bonferroni t-test. Column not sharing a common letter (a–c) differ significantly at p < 0.05 (Bonferroni t-test).
Figure 3: (A)-(B) Microscopic observation of Haematoxylin and eosin stained sections of the control rat testis. (A): Normal architecture and seminiferous tubes filled with sperm (ST) ×10. (B): Higher magnification (×40) of the inbox of (A) showing the normal progression of spermatogenesis from spermatogonia (Sg) to spermatozoa (Sz) via spermatocytes (Sc) and spermatids (Sd). Interstitial tissue (IT) formed by Leydig cells and blood vessels (Bv). The Sertoli cell nucleus (Sn) and the lumen of the seminiferous tube (L).

Figure 4: (A)-(B) Microscopic observation of Haematoxylin and eosin stained sections of AlCl₃-exposed rat testis. (A): Showing a lack of Leydig cells with a large interstitial space (IT) and seminiferous tubules with sperm depletion (ST) (×10). (B): Showing disorganization of germinal epithelium (asterisks), lack of Leydig cells, and degeneration of interstitial tissue (arrowheads) with some exfoliated cells in the lumen (arrows) (×40).

Figure 5: (A)-(B) Microscopic observation of Haematoxylin and eosin stained sections of both Aluminum and Curcuma longa treated rat testis (AlCl₃-CE). (A): showing regeneration of the majority of seminiferous tubules (ST) and interstitial cells and reduction of interstitial space (IT) (×10). (B): showing a good development of the spermatogenesis (arrows) and lumens of seminiferous tubes filled with sperm (asterisk) (×40).
DISCUSSION

The current study revealed that the administration of AlCl₃ at the dose of 34 mg/kg BW to male rats during 4 weeks led to many reproductive system disorders including a decrease in sperm quality, hormonal disturbances, and induction of tissue oxidative stress. A significant decrease in sperm count, percent viability, and an increase in sperm abnormal morphology were observed. These results are in accordance with those obtained by Abdul-Rasoul, et al., (2009) who revealed that a daily administration of Aluminum chloride with two doses 40 and 80 mg/kg body weight induced a significant reduction in sperm concentration and percentage of live sperm, associated with a significant increase in the percentage of abnormal sperm. Another research conducted by Martinez, et al., (2017) found that exposure to aluminum for 60 days at human dietary levels (1.5, 8.3 and 100 mg/kg BW/day) affects the sperm quality in rats by decreasing sperm count, sperm motility, and sperm morphology, with an increase in oxidative stress and inflammation in reproductive organs. They found also that a low concentration of Al (3.35μg/g) in testes is sufficient to impair spermatogenesis. Moreover, Miska-Schramm, et al., (2017) by using the Bank Vole (Myodes glareolus) as a rodent model indicate that AlCl₃, at a dose of 3 and 200 mg/l, impairs adult reproductive abilities by decreasing the quality and quantity of sperm cells and by causing morphologically abnormal development of the gonads. Also, Guo, et al., (2005) indicated that aluminum exposure leads to an increase in nitric oxide (NO) products which were responsible for AI-induced reproductive toxicity. Zhu et al., (2005) suggested that sub-chronic AlCl₃ disorders the balance of trace element and decreases the spermatogenesis and testicular enzyme activities which have adverse effects on the testicular function in male rats. On the other hand, the results of the current study indicated that the administration of AlCl₃ significantly decreased serum testosterone levels compared to control rats. These findings are consistent with those of Sun et al., (2011) who noticed a significant decrease in the levels of testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) after 120 days of exposition to three doses (64.18, 128.36 and 256.72 mg/kg) of aluminum and explain that Al-exposure interferes with androgen receptor expressions in testes. Another work indicated that AlCl₃ caused a significant decrease of FSH, LH, and testosterone, and caused the development of oligosperma and exfoliated tubules in the testes. Hadjet, et al., (2020) noticed a significant decrease in testosterone and LH hormones after 60 days of AlCl₃ (20 mg AlCl₃ /kg b.w.) administration. They indicate that aluminum harms the steroidogenesis by increasing the production of nitric oxide which might inhibit LH levels. Knowing that, testosterone hormone is released from the Leydig cells by stimulation of LH, its reduction results in the decline of serum testosterone concentration. Moreover, our study showed that Al exposure caused an elevation in the MDA level compared to the other experimental groups. Our finding is confirmed by those of Alayet, et al., (2016) who concluded that subchronic exposure to Al (75 mg/kg/ day during 30 days) lead to a significant decrease on antioxidant enzymes such as SOD and GPX, and a significant increase in MDA levels compared to control group. This imbalance between the antioxidant system and oxidants leads to oxidative stress which has a destructive effect on the testis. The work of Afolabi, et al., (2018) on Aluminum phosphide, indicated that oral administration of Al (1.15mg/kg) during 30 days resulted in a significant increase in testicular MDA and oxidized protein levels with a decrease in antioxidant enzymes such as SOD, CAT and GPXs followed by a significant reduction in non-enzymatic antioxidants. While, the observation of histological sections of the AlCl₃ exposed group shows the degeneration of the seminiferous tubules and depletion of sperm in the seminiferous lumen, with large interstitial spaces and lack of cells Lydig around basement membranes. These results are following those obtained by Moselhy, et al., (2012) who demonstrated after histopathological examination of rats testis exposed to a daily dose (34 mg/kg) of AlCl₃ during 60 days, revealed degenerative changes in seminiferous tubules with necrosed spermatogenic cells. On the other hand, our result showed that the treatment with Curcuma longa aqueous extract at a dose of 200 mg/kg body weight concomitant with AlCl₃ exposure, led to significant improvement of some semen parameters with a significant elevation of serum testosterone and a decrease in MDA level. Furthermore, Histologic analysis showed a significant regeneration of the majority of seminiferous tubules and interstitial cells, with a good development of the spermatogenesis indicated by lumens of seminiferous tubes filled with sperm. These findings are in agreement with those of Cheraghi, et al., (2017) who concluded that curcumin, the major constituent of Curcuma longa, significantly reversed the...
adverse effects of Al on testis and sperm quality, and explained that curcumin exhibits protective effects against oxidative damage by decreasing the levels of free radicals, through its free radical scavenging activity, particularly against oxygen radicals, which inhibit sulphydryl (SH)-group oxidation. It inhibits nuclear factor kappa B (NF-kB) activity, cyclooxygenase-2 (COX-2), and mono-oxygen activated protein kinase (MAPK) expression, while it modulates the release of several cytokines and testicular enzyme activities, mRNA expression of 17 β-hydroxysteroid dehydrogenases (17β-HSD). Besides, Belhan et al., (2017) finding confirmed the protective effect of curcumin and indicated that low dose curcumin (10 mg/kg) significantly increased sperm motility and concentration, and decreased abnormal sperm percentage with a significant suppressing of the lipid profile and an increase in testosterone levels. Furthermore, to assess the protective effect of curcumin on lead acetate-induced (50 mg/kg BW) reprotoxicity, Sudjarwo et al., (2013) found that daily oral administration of curcumin at three doses (1, 2, and 4 mg/kg) during 40 days to rats significantly improved the histopathological structure of testis, increased the sperm count, motility, viability, and also significantly increased the SOD, GPx, and decreased MDA in the testis of lead acetate-treated rats. They concluded that co-administration of curcumin at a dose of 400 mg/kg reduced the effects of lead acetate-induced testicular toxicity, possibly by inhibiting the free radical mediated process. In addition to the protective effect of C. longa on male reproductive function, a study also shows that the ethanolic extract of C. longa (100 mg/kg BW) successfully prevents female albino Wistar rats against bisphenol A-induced reprotoxicity (2017). Another study indicates also that Curcuma longa does not only possess a non-toxic effect but has cytoprotective effects on the histoarchitecture of the testis in diabetic rats (2017). By studying the effects of the different doses of curcumin 25, 50, and 100 mg/kg during 14 days on reproductive organ weight index, testicular histopathology and apoptosis in a mouse aging model, Taba et al., (2019) indicated that Curcumin supplementation (2 weeks, 100 mg / kg) induced improvement in biochemical markers and sperm parameters as well as reduction of apoptosis in testicular tissue.

CONCLUSION
The current study demonstrated that Curcuma longa aqueous extract had a remarkable protective effect against AlCl₃ reprotoxicity and its mechanism is related, at least in part, to its high free radical scavenging and antioxidant activity. Further works are necessary to isolate bioactive compounds and elucidate the mechanism involved in the reprotoxic effect of this plant.

CONFLICT OF INTEREST
The authors declare that they have no conflict of interest.

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