Research Article

Anti-reprotoxicity effects of aqueous extract of *Terminalia superba* Engl. & Diels (Combretaceae) on aluminum chloride exposure of male rats

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Abstract

Objective: Aluminum chloride can induce toxicity of the reproductive system leading to infertility which is a real public health problem. This study was undertaken to investigate the *in vivo* effect of *Terminalia superba* Engl. & Diels aqueous extract on aluminum chloride-induced reprotoxicity in male rats. **Materials and methods:** Thirty male Wistar rats of 12 weeks each, weighing 160 -180 g were intraperitoneally administered aluminum chloride (20 mg/kg, body weight) for 56 days. The rats were subsequently orally treated for 56 days with *Terminalia superba* Engl. & Diels aqueous bark extract at doses of 43 and 86 mg/kg and a coadministration of vitamin E (100 mg/kg) and zinc (50 mg/kg). Then, the rats were killed, and blood, testes, epididymis, seminal vesicle and prostate were removed for histological and biochemical analysis. **Results:** Aluminum chloride caused significant decreases in the sexual performance of the male rats, sperm quality, catalase activity and glutathione levels ($P < 0.05$). The serum testosterone and fructose levels were also significantly lower in the intoxicated group ($P < 0.05$) than in the normal control group, with decreases of 91.25 % and 8.80 % respectively. In addition, histological lesions were observed in the testes, epididymis and prostate of the intoxicated group. Our results revealed that the aluminum chloride-induced reprotoxicity was reversed by the aqueous extract of *Terminalia superba* Engl. & Diels. **Conclusion:** Oral administration of an aqueous extract of *Terminalia superba* Engl. & Diels*;* at the dose of 86 mg/kg, can alleviate the aluminum chloride-induced reprotoxicity in male rats.

***Keywords*:** Aluminum chloride, reprotoxicity, *Terminalia superba*, male rats, aqueous extract

Introduction

The male reproductive system, under the control of the hypothalamic-pituitary-gonadal complex, ensures smooth copulation and fertilization (Bredhult *et al*., 2008). Abnormalities in the reproductive system could be due to the effects of toxic substances such as aluminum, in our environment. Aluminum occurs naturally or in combination with other elements (aluminum sulfate and aluminum chloride), and high concentrations of this metal are thought to promote increased production of free radicals in the testes (Yousef and Salama, 2009; Kalaiselvi *et al*., 2014; Olanrewaju *et al.*, 2021) leading to the loss of libido, a decrease in natural antioxidant capacity or a decrease in sperm viability and motility (Zhang and Zhou, 2005; Khattab *et al.*, 2010; Aghashahi and Reza, 2020). Infertility is conventionally defined by the World Health Organization (WHO, 2003) as the inability to conceive naturally after one year of regular unprotected sexual intercourse (Winters and Walsh, 2014; Agarwal *et al*., 2015). Infertility is a reproductive health problem that affects approximately 13-18 % of couples worldwide (Winters and Walsh, 2014; Agarwal *et al*., 2015; Vander Borght and Wyns, 2018) and the African continent is disproportionally affected. Among couples, male factor contributes to up to 50 % of infertility cases worldwide (Elhussein *et al*., 2019). In Cameroon, 20 to 30 % of couples face the problem of infertility (Nana *et al*., 2011). In seeking treatment for infertility, couples rely either on conventional medicine or traditional medicine based on natural plants. Conventional or modern medicine is generally more expensive than traditional medicine and requires well-trained medical personnel. Therefore, these plants are less accessible to populations with low incomes, while alternative or

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traditional medicines use medicinal plants that are easily accessible and of "natural" origin, and usually offer fewer undesirable side effects and in some cases, greater efficiency (Desai *et al*., 2009). Indeed, 80% of the world's population uses medicinal plants for their healthcare (Fabricant and Farnsworth, 2001; Gurib-Fakim, 2006), and the WHO encourages the rational use of medicinal plants to treat most of these ailments (WHO, 2003). Medicinal plants and their derivatives play a key role in human health and have long been known to possess biological activities. *T. superba* is an example of a medicinal plant that is mainly cultivated by *Baka* Pygmies in the Southern Region of Cameroon to enhance male fertility (Keumedjio *et al*., 2023). *T. superba* is a plant of the Combretaceae family that is well known for its vasorelaxant, antihypertensive, antidiabetic (Kamtchouing *et al*., 2006; Tom *et al*., 2011; Tom Ngo Lemba *et al*., 2014), antiplasmodial (Mbouna *et al*., 2018), antimicrobial and immunoinhibitory properties (Tabopda *et al*., 2009; Kuete *et al*., 2010).

Thus, the aim of the present study was to evaluate the *in vivo* effect of the aqueous extract of *Terminalia superba* Engl. & Diels on aluminum chloride-induced reprotoxicity.

Materials and Methods

Collection and extraction of plant material

T. superba bark was collected from Yaoundé-Cameroon in November 2021 and identified in the National Herbarium of Cameroon under the specimen number 655546. The plant name has been checked with www.worldfloraonline.org (accessed 8 January 2024). These bark samples were cut, dried, pulverized and kept at room temperature in the laboratory until use. Aqueous extract (10 %, m/v) was prepared by decoction for 30 min in distilled water as recommended by the traditional healer. The obtained aqueous extract was then filtered, evaporated and kept in a sealed glass container. The extract was dissolved in distilled water in order to obtain the required doses of 43 and 86 mg/kg of body weight. The extraction yield was 25 % (w/w).

Handling of animals

This study was carried out with thirty 12-week-old male rats weighing 160 -180 g at the beginning of the experiment. Female rats weighing 100 - 120 g were used for the libido tests. The animals were obtained from the animal house of the Laboratory of Animal Physiology of the University of Yaoundé I. The animals were handled according to the ethical guidelines of the "Animal Ethics Committee", Cameroon Institutional National Ethics Committee, and Ministry of Scientific Research and Technology Innovation under the following reference number: FWA-IRD 0001954.

Chemicals and reagents

Aluminum chloride was obtained from Sigma Aldrich. Assay kits were used to determine serum cholesterol and serum testosterone levels. These kits were respectively supplied by Inmesco and Calbiotech, UK. The drugs used during the test were vitamin E, zinc and progesterone (Progesterone Retard® Bayer Schering Pharma Laboratories, Germany).

Induction of testicular toxicity

After one week of acclimatization with food and tap water *ad libitum*, the animals were randomly divided into 6 groups (G I to G VI) composed of 5 animals each. To induce reprotoxicity, 20 rats received daily for 56 days, an intraperitoneal (i.p.) injection of aluminum chloride (20 mg/kg, body weight) dissolved in normal saline solution (Khattab *et al*., 2010; Mballa *et al*., 2017) in order to complete the spermatogenic cycle of rats.

Animal protocol and plant extract administration

At the end of the exposition, the reprotoxicity-induced rats were then orally treated with the plant extract at 2 different doses except for those in the control groups. The rats were orally treated with plant extract for 56 days and were thus divided into 6 groups of 5 rats per group as follows:

- Group I (normal control): normal rats that received oral distilled water (10 ml/kg, BW) and saline solution (1ml/kg, BW /i.p.);
- Group II: normal rats that received oral corn oil (10 ml/kg /BW);
- Group III (negative control): nontreated rats were intoxicated with aluminum chloride (20 mg/kg BW/i.p.);
- Group IV included rats exposed to aluminum chloride (20 mg/kg BW/i.p.) and treated with the aqueous extract of *Terminalia superba* Engl. & Diels (AETs) at dose 43 mg/kg, BW;
- Group V: rats were exposed to aluminum chloride and treated with AETs at a dose of 86 mg/kg, BW;
- Group VI: rats were exposed to aluminum chloride and treated with vitamin E + zinc (50 mg/kg, BW);

Vitamin E was dissolved in corn oil for administration to the rats.

Libido test

The test was performed according to the protocol described by Mbongue *et al*. (2010). On day 56, the libido test began at 6 pm under dim light and a quiet atmosphere. Then, male rats were individually introduced into the cages. After 10 minutes of acclimatization, a receptive female rat was also introduced. During the 30-minutes period, sexual behavior parameters, including mount latency (ML), intromission

latency (IL), ejaculation latency (EL), mount frequency (MF), intromission frequency (IF), ejaculation frequency (EF), were assessed.

Mount latency is the time interval in seconds between the introduction of the receptive female and the first mount. Intromission latency is the time interval in seconds between the introduction of the receptive female and the first intromission. Ejaculatory latency is the time interval in seconds between the introduction of the receptive female and the first ejaculation. The mount frequency is the number of observed mounts without intromission. Intromission frequency is the number of observed intromissions from the time of introduction of the female. Ejaculation frequency is the number of observed ejaculations.

Animal Sacrifice and blood sampling

The body weights of the animals were recorded. On day 57, the rats were anaesthetized with ether and killed. Blood was collected and centrifuged at 3000 g for 10 minutes and the individual sera were aspirated and aliquoted into Eppendorf tubes for storage at -20°C for further biochemical analysis. Then the rats were rapidly dissected, and the testis, epididymis and prostate were removed. These organs were stripped of the superficial fatty layer and weighed to determine the relative organ weight via the following formula:

$$Relative\ weight = \frac{organ\ weight}{body\ weight}$$

Sperm parameters

The left epididymis was minced in a beaker containing 10 mL of 0.9 % NaCl solution and incubated in a water bath at 34°C. This mixture was subsequently then used to determine sperm characteristics. Using a Malassez hemocytometer, 20 µL of the mixture was observed under an electric microscope (40X magnification). Then, the number of spermatozoa per mL of sperm was estimated (Bujan *et al*., 1993). Sperm viability was assessed with eosin staining which distinguishes between living and dead spermatozoa by staining the cytoplasm of the dead cells. Thus, 10 µL of the abovementioned mixture was placed on a slide and 10 µL of 0.5 % eosin was added. The slide was covered with a coverslip and observed under a light microscope at 40X magnification (Ngoula *et al*., 2007).

Preparation of homogenates

The isolated tissues were cleansed, ground in a mortar and homogenized in ice cold appropriate buffers in order to prepare a 15 % (w/v) homogenate. The tissues including the testes, epididymis and seminal vesicles, were prepared in sodium phosphate buffer or S-buffer (pH 7.3); potassium phosphate buffer (pH 6.8) and distilled water, respectively. Then, the homogenates were centrifuged at 3000×g for 10 minutes at 4°C. The supernatant was collected and stored at -20°C. These homogenates were subjected to biochemical analysis.

Biochemical analysis

Blood was collected for serum testosterone analysis via the competitive ELISA method as described by the kit manufacturer, and total cholesterol was assessed via the kit. The level of fructose in the homogenate of seminal vesicles was determined according to the protocol of Gonzales and Villena (2001). Protein levels in testis and epididymis were assessed according to the method described by Gornall *et al*. (1949) .

Determination of oxidative stress biomarkers

All the biomarkers were determined in the homogenates of testes and epididymis. Catalase (1.11.1.6) activity was measured as described by the method of Sinha (1972). The absorbances were read at 570 nm and the H_2O_2 concentrations were determined from the H_2O_2 standard curve. The results are expressed as mM H_2O_2/min/g of organ. The levels of reduced glutathione in the samples were calculated according to the method of Ellman (1959). The levels of reduced glutathione were expressed as mmol/mg of protein. Thiobarbituric acid-reactive substances (TBARS) were measured in homogenates using the method of Wilburg (Kalaivanam *et al*., 2006) and are expressed as mol/g of organ.

Histological analysis

The testes, epididymis and prostate of each animal were kept in Bouin's solution for fixation for a fortnight. Then with a scalpel blade, sections of the tissues measuring approximately 3 mm were cut. The fixed tissues were then dehydrated with ascending grades of alcohol (50°, 70°, 95° and 100°) and cleaned in xylene. The cleaned tissues were embedded in paraffin wax melted at 60 °C and thin 5 µm slices were prepared with a microtome. Then, the slices were stained with hematoxylin-eosin and examined under a light microscope. The slides were photographed using the Minisee camera program.

Statistical analysis

The results are expressed as the mean ± standard deviation and were analyzed using GraphPad Prism software (version 8.0.2). The data were considered significant at $P<0.05$. The data were analyzed by one-way analysis of variances (ANOVA) or the Kruskal-Wallis test when appropriate; and followed by Tukey's multiple comparisons test or Dunn's post-hoc test, respectively.

Results

Effect of the administration of the aqueous extract of *T. superba* on the relative weight of androgen-dependent organs in rats

Table 1 shows the effect of the aqueous extract of *T. superba*

on the relative weight of androgen dependent organs of rats after the induction of reprotoxicity with aluminum chloride. No significant change (P >0.05) in organ weight was observed between the treated rats and the intoxicated rats.

Effect of the administration of the aqueous extract of *T. superba* on sexual behavior parameters

As shown in Table 2, the exposure of rats to aluminum chloride significantly (P >0.05) decreased the performance parameters including mount frequency, intromission frequency, and ejaculation frequency by 80.2 %; 91.85 % and 100 %; respectively. Moreover, compared to that in the control group, an increase of the intromission latency was observed in the intoxicated group (G3) increased by 100 % (P <0.05). All the performance parameters were significantly greater (P <0.05) in the intoxicated rats and treated with plant extracts and those treated with vitamin E and zinc than in the intoxicated group. No significant difference was observed between the intoxicated groups and treated with plant extract and those treated with vitamin E and zinc.

Effect of the administration of the aqueous extract of *T. superba* bark extracts on sperm parameters

The effects of *T. superba* bark extract on sperm parameters are displayed in Figure 1. The $AlCl_3$-intoxicated group exhibited significant decreases in the viability and mobility of spermatozoa (P < 0.05) in comparison with those of the normal control group. The progressive mobility of the spermatozoa isolated from the intoxicated rats was 50-fold lower than that of those isolated from the normal control group. Exposure of the rats to aluminum chloride significantly increased (P < 0.05) the number of immobile spermatozoa compared to that in the normal control group. Nevertheless, the different treatments significantly improved the viability and motility of spermatozoa (P < 0.05) in comparison with those of the normal control group and the negative control group.

Table 1. Effect of *T. superba* aqueous extract on relative androgen dependent organ weights

Organs (g)	Groups (Weight in g)					
	G1	G2	G3	G4	G5	G6
Testes	0.55 ± 0.08	0.50 ± 0.02	0.52 ± 0.15	0.45 ± 0.07	0.53 ± 0.04	0.49 ± 0.05
Epididymis	0.20 ± 0.05	0.19 ± 0.02	0.17 ± 0.07	0.17 ± 0.04	0.20 ± 0.08	0.17 ± 0.02
Seminal vesicle	0.51 ± 0.18	0.33 ± 0.03	0.44 ± 0.21	0.32 ± 0.11	0.37 ± 0.08	0.31 ± 0.03
Prostate	0.16 ± 0.03	0.15 ± 0.02	0.13 ± 0.04	0.13 ± 0.03	0.15 ± 0.08	0.11 ± 0.03

The values are expressed as the mean ± standard deviation (n=5). G1: rats treated with NaCl + distilled water; G2: rats treated with corn oil; G3: rats intoxicated with Aluminum chloride ($AlCl_3$); G4: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 43 mg/kg; G5: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 86 mg/kg; G6: rats intoxicated with $AlCl_3$ and treated with Vit E + Zn

Table 2. Effect of the aqueous extract of *T. superba o*n sexual behavior parameters

Copulatory parameters	Groups					
	G1	G2	G3	G4	G5	G6
MF	43.25 ± 8.26	36.50 ± 5.51^{b}	8.25 ± 2.63^{a}	27.00 ± 4.16^{a,b}	45.67 ± 7.37^{b}	32.75 ± 6.19^{b}
IF	33.75 ± 8.85	25.75 ± 4.99^{b}	2.75 ± 1.71^{a}	20.25 ± 4.99^{b}	41.67 ± 5.51^{b}	29.75±10.87^{b}
EF	3.50 ± 0.58	2.75 ± 0.96^{b}	0.00 ± 0.00^{a}	2.00 ± 0.82^{a,b}	3.33 ± 0.58^{b}	3.50 ± 0.58^{b}
ML (s)	33.74 ± 8.85	25.75 ± 4.99^{b}	2.75 ± 1.70^{a}	101.50 ± 24.56^{b}	45.33±15.89^{b}	36.25±17.35^{b}
IL (s)	44.75 ±15.97	61.75±14.64^{b}	505.30 ±50.49^{a}	118.50±25.88^{a,b}	55.00±16.64^{b}	48.00±18.96^{b}

The values are expressed as the mean ± standard deviation (n=5). ^{a}P< 0.05 versus the normal control group (G1); ^{b}P < 0.05 versus the negative control group (G3). G1: rats treated with NaCl + distilled water; G2: rats treated with corn oil; G3: rats intoxicated with Aluminum chloride ($AlCl_3$); G4: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 43 mg/kg; G5: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 86 mg/kg; G6: rats intoxicated with $AlCl_3$ and treated with Vit E + Zn. MF: mount frequency; IF: intromission frequency; EF: ejaculation frequency; ML: mount latency; IL: intromission latency.

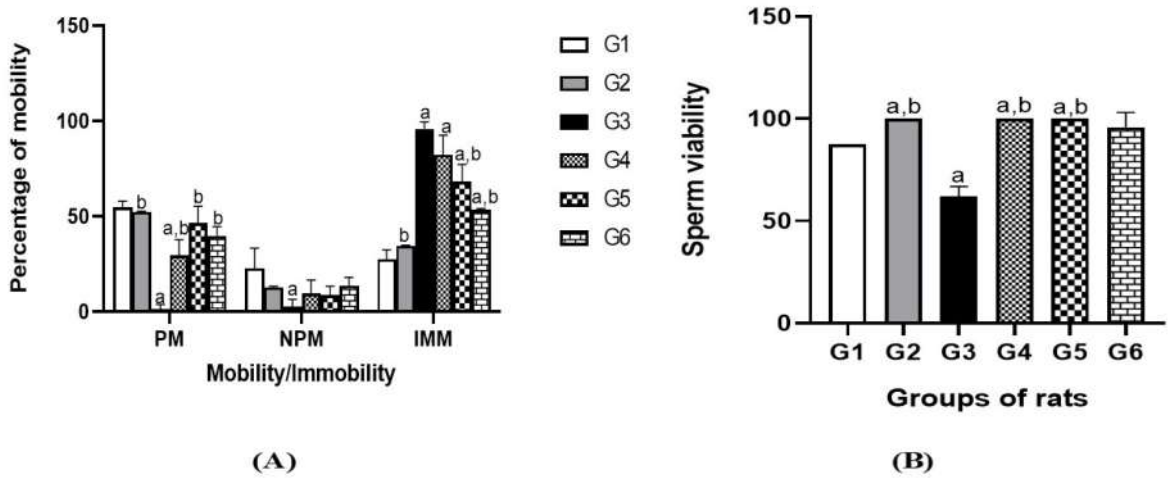

Figure 1. Effect of plant extract on: (A) sperm mobility and (B) sperm viability. *The values are expressed as the mean ± standard deviation (n=5). [a]P < 0.05 versus the normal control group (G1); [b]P < 0.05 versus the negative control group (G3). G1: rats treated with NaCl + distilled water; G2: rats treated with corn oil; G3: rats intoxicated with Aluminum chloride ($AlCl_3$); G4: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 43 mg/kg; G5: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 86 mg/kg; G6: rats intoxicated with $AlCl_3$ and treated with Vit E + Zn. PM: progressive mobility; NPM: non progressive mobility; IMM: immobility.*

Effect of the administration of the aqueous extract of *T. superba* bark extract on several biochemical parameters of androgen-dependent organs

The data presented in Table 3 display the effect of the aqueous extract of *Terminalia superba* Engl. & Diels bark extract on several biochemical parameters of androgen-dependent organs. The aqueous extract of *T. superba* did not cause significant changes in the serum cholesterol levels of the rats in any of the groups. However, compared with those in the normal control group, the serum testosterone and fructose levels in the negative control group were significantly lower (P <0.05), with decreases of 91.25 % and 8.80 %, respectively. In the intoxicated group and treated with 86 mg/kg plant extract, the testosterone level was 2-fold greater than that in the normal control group. The testosterone levels in the intoxicated group and treated with zinc were greater (P <0.05) than those in the intoxicated group and treated with plant extract at a dose of 86 mg/kg. Nevertheless, the fructose levels were restored by treatment with the plant extract at a dose of 86 mg/kg and zinc treatment (P <0.05).

Histopathological observations

Figure 2 shows the histological sections of the testes. In the normal control group, the histopathological analysis of the testes revealed a normal testicular parenchyma with connective tissue and seminiferous tubules enriched sperm from different stages. Whereas, in the negative control group, degenerative changes in the epithelial cells and impaired spermatogenesis marked by disorganization of the germ cells within the seminiferous tubules were observed. Compared to those in the negative control group, the intoxicated and treated groups with plant extracts (at both doses) and vitamin E led to the regeneration of seminiferous tubules and spermatocytes within the lumen. In the normal control group, histopathological analysis of the prostate (Figure 3) revealed a normal prostate with starchy bodies, epithelium

Table 3. Effect of aqueous extract of *T. superba* on several biochemical parameters of androgen-dependent organs

Parameters	Groups					
	G1	G2	G3	G4	G5	G6
Testosterone (ng/ml)	0.343 ± 0.104	0.800 ± 0.120 [a,b]	0.030 ± 0.012 [a]	0.320 ± 4.163 [b]	0.723 ± 0.053 [a,b]	1.005 ± 0.132 [a,b]
Cholesterol (g/l)	0.421 ± 0.076	0.429 ± 0.046	0.435 ± 0.067	0.429 ± 0.067	0.480 ± 0.078	0.499 ± 0.043
Fructose (µmol/ml)	56.83 ± 0.234	49.95 ± 0.884 [a]	51.83 ± 1.247 [a]	52.24 ± 0.492 [a]	54.55 ± 0.623 [a,b]	56.73 ± 1.02 [b]

The values are expressed as the mean ± standard deviation (n=5). [a]P < 0.05 versus the normal control group (G1); [b]P < 0.05 versus the negative control group (G3). G1: rats treated with NaCl + distilled water; G2: rats treated with corn oil; G3: rats intoxicated with aluminum chloride ($AlCl_3$); G4: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 43 mg/kg; G5: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 86 mg/kg; G6: rats intoxicated with $AlCl_3$ and treated with Vit E + Zn.

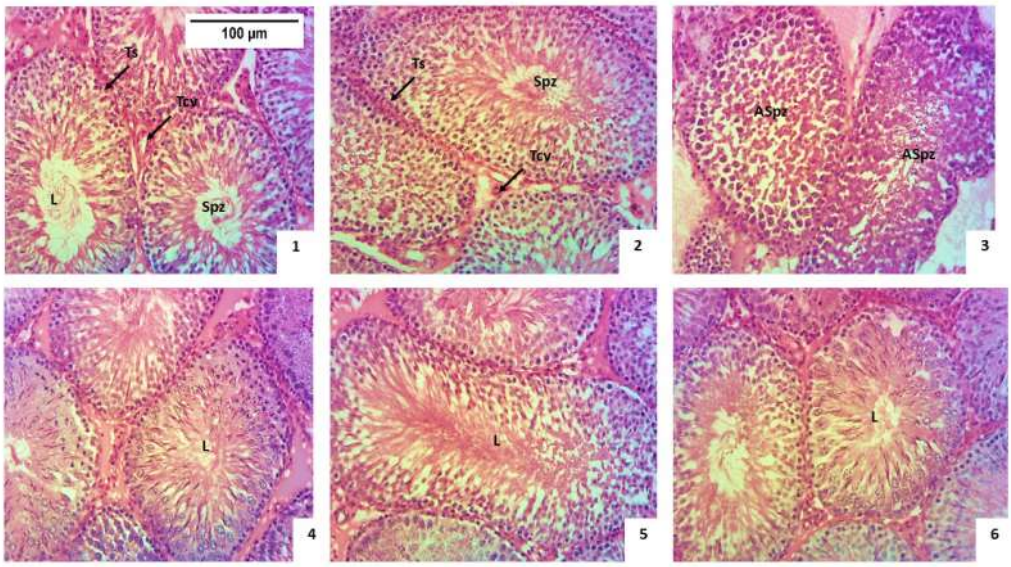

Figure 2. Histomorphological analysis of testes (X100); Haematoxylin-eosin stain. *Spz= spermatozoa; Tcv= vascular connective tissue; Ts= seminiferous tubules; L= light; ASpz= alteration of spermatogenesis. G1: rats treated with NaCl + distilled water; G2: rats treated with corn oil; G3: rats intoxicated with Aluminum chloride ($AlCl_3$); G4: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 43 mg/kg; G5: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 86 mg/kg; G6: rats intoxicated with $AlCl_3$ and treated with Vit E + Zn..*

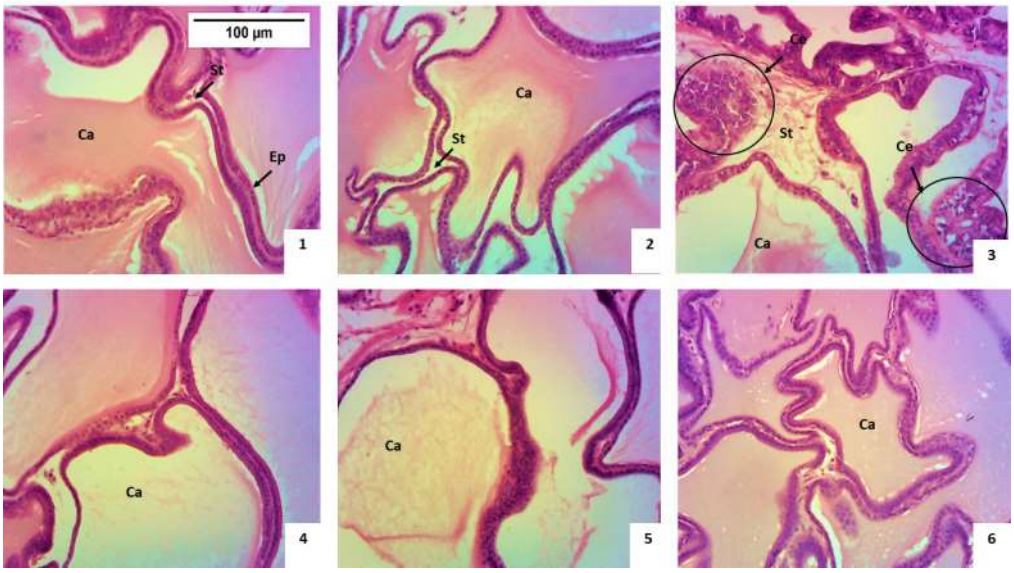

Figure 3. Histomorphological analysis of the prostate (X100); Haematoxylin-eosin stain**.** *Ca = Starch bodies; E = Epithelium; St = Struma; Ce = Cytolysis of epithelial cells. G1: rats treated with NaCl + distilled water; G2: rats treated with corn oil; G3: rats intoxicated with Aluminum chloride ($AlCl_3$); G4: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 43 mg/kg; G5: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 86 mg/kg; G6: rats intoxicated with $AlCl_3$ and treated with Vit E + Zn.*

and stroma. In the negative control group, the analysis showed a cytolysis of the epithelial cells and a decreased secretion of starch bodies. However, the intoxicated and treated groups with plant extracts (at both doses) and vitamin E presented a prostate structure similar to the structure of the normal control group.

Effects of the administration of the aqueous extract of *T. superba* on protein levels

Figure 4 shows the protein levels in the testis and epididymis. Notably, testicular protein levels were significantly greater (P <0.05) in most of the groups of rats than in the control normal group while a significant decrease in the protein levels was observed in the epididymis.

Effect of the administration of the aqueous extract of *T. superba* on oxidative stress parameters

Tables 4 and 5 summarize the effects of the aqueous extract on oxidative stress parameters in the testis and the epididymis, respectively. Exposure to aluminum chloride significantly reduced all the oxidative stress parameters (P

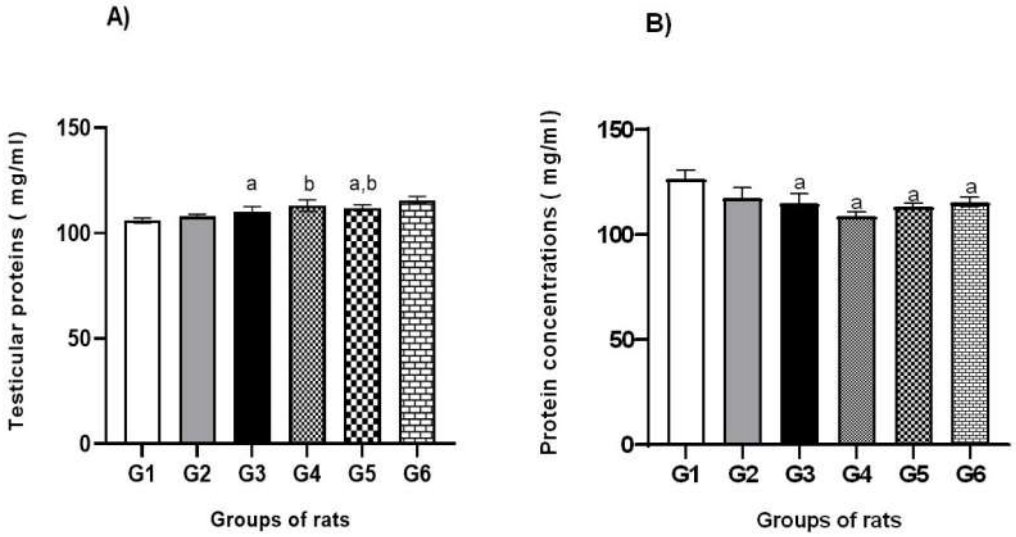

Figure 4. Effect of the aqueous extract of *Terminalia superba* Engl. & Diels on protein levels in testis (A) and epididymis (B)**.** *The values are expressed as the mean ± standard deviation (n=5). [a]P < 0.05 versus the normal control group (G1); [b]P < 0.05 versus the negative control group (G3). G1: rats treated with NaCl + distilled water; G2: rats treated with corn oil; G3: rats intoxicated with aluminum chloride ($AlCl_3$); G4: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 43 mg/kg; G5: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 86 mg/kg; G6: rats intoxicated with $AlCl_3$ and treated with Vit E + Zn.*

<0.05) in both organs when compared those in the normal control group. The activity of catalase and the glutathione levels were restored in a dose-dependent manner in the treated group with plant extract compared with the control group. A significant improvement was also observed in the treated group with vitamin E and zinc compared to those treated with plant extracts (Table 4). In both organs, a marked increase in the MDA concentration was observed in the intoxicated group (P <0.05) when compared to the normal control group. Treatment with plant extracts and vitamin E significantly reduced the levels of MDA in the intoxicated groups when compared to those in the negative control group (P <0.05). In the testis, a significant decrease (P <0.05) in the MDA concentration was observed in the intoxicated rats and treated with vitamin E compared to the intoxicated rats and treated with the plant extract at a dose of 86 mg/kg (table 4).

Discussion

The present study was undertaken to evaluate the effect of the aqueous extract of *T. superba* on aluminum chloride-induced reprotoxicity in male rats. After 56 days of exposure to aluminum chloride by intraperitoneal injection, the animals were orally treated for 56 days with 2 doses of the plant aqueous extract and with a mixture of vitamin E and zinc.

Aluminum is the most prevalent metal commonly used in industry, pharmaceuticals and consumer products (Pandey and Jain, 2013). Numerous studies have demonstrated that, regardless of the route of aluminum administration in the body, its excessive absorption leads to its accumulation in organs, causing alterations in spermatogenesis and membranes and increasing of oxidative stress (Yousef *et al.*, 2007; Pandey and Jain, 2013). The present study showed that the administration of aluminum chloride to male rats via

Table 4. Effect of *T. superba* aqueous extract on testicular oxidative stress biomarkers

Biomarkers	Groups					
	G1	G2	G3	G4	G5	G6
Catalase (µM/min/g)	17.450 ± 0.809	13.750 ±1.500 [a,b]	10.670 ± 0.629[a]	20.910 ± 0.603[a,b,c]	22.400 ±0.577[a,b, c]	32.650±1.500[a,b]
Gluthatione (mM/mg)	0.300 ± 0.108	0.374 ± 0.030	0.265 ± 0.084	0.437 ± 0.097[c]	0.462 ± 0.056[b,c]	0.646 ± 0.057[a,b]
MDA (mol/g)	0.048 ± 0.005	0.049 ± 0.003	0.058 ± 0.0047	0.055 ±0.0067[c]	0.045 ± 0.002[b,c]	0.028 ± 0.006[a,b]

The values are expressed as the mean ± standard deviation (n=5). [a]p < 0.05 versus the normal control group (G1); [b]P < 0.05 versus the negative control group (G3); [c]P < 0.05 versus the positive control group (G6). G1: rats treated with NaCl + distilled water; G2: rats treated with corn oil; G3: rats intoxicated with Aluminum chloride ($AlCl_3$); G4: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 43 mg/kg; G5: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 86 mg/kg; G6: rats intoxicated with $AlCl_3$ and treated with Vit E + Zn.

Table 5. Effect of *T. superba* aqueous extract on epididymal oxidative stress parameters

Biomarkers	Groups					
	G1	G2	G3	G4	G5	G6
Catalase (µM/min/g)	13.110±2.684	14.76 ± 1.909	19.810 ±4.497	34.300±5.242[a,b]	38.87±1.630[a,b]	39.450 ± 5.656[a,b]
Gluthatione (mM/mg)	0.265 ±0.032	0.220 ±0.042	0.147 ±0.019[a]	0.376 ± 0.041[b]	0.413 ±0.046[a,b]	0.437±0.098[a,b]
MDA (mol/g)	0.090±0.006	0.114±0.005[a]	0.127±0.007[a]	0.106 ± 0.005[b]	0.092±0.002[b]	0.060±0.015[a,b]

The values are expressed as the mean ± standard deviation (n=5). [a]P < 0.05 versus the normal control group (G1); [b]P < 0.05 versus the negative control group (G3). G1: rats treated with NaCl + distilled water; G2: rats treated with corn oil; G3: rats intoxicated with Aluminum chloride ($AlCl_3$); G4: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 43 mg/kg; G5: rats intoxicated with $AlCl_3$ and treated with AETs at a dose of 86 mg/kg; G6: rats intoxicated with $AlCl_3$ and treated with Vit E + Zn.

intraperitoneal injection during 56 days induced toxic effects in the testis and prostate. Exposure caused significant decreases in sexual performance, as the frequencies of mount, intromission and ejaculation were decreased by 80.2 %; 91.85 % and 100 %; respectively. A significant increase in the intromission latency was also observed in the intoxicated group as compared to the control group. Mount, intromission and ejaculation frequencies are good indicators of sexual performance whereas mount, intromission and ejaculatory latencies are markers of sexual motivation or desire (Yakubu *et al*., 2007; Abedi *et al.*, 2012; Jianfeng *et al.*, 2012). The results showed that aluminum chloride exposure altered the libido of male rats. However, treatment with plant extracts and the antioxidant reference group, vitamin E; significantly restored the sexual parameters as compared to those of the intoxicated group. This trend suggested that the aqueous extract of *T. superba* could improve the libido of intoxicated rats.

Exposure to aluminum chloride also altered the spermatogenesis, causing sperm cell death and a reduction in seminiferous tubules resulting in the atrophy of the testis. These results are in agreement with previous studies on the effects of aluminum chloride on rat testes (Moselhy *et al.*, 2012; Hichem *et al*., 2013; Geeta and Pareek 2014; Mahmoud *et al*., 2022). These histopathological changes observed in the testis and prostate of the aluminum chloride-intoxicated group could suggest that aluminum chloride can affect testosterone synthesis in the testis and thus the normal function of the reproductive organs, which are mostly androgen-dependent. After 56 days of treatment with 2 doses of plant extracts and a mixture of vitamin E and zinc, significant improvements of these parameters were also observed of the alterations was observed as well in the testes and the prostate sections compared to those in the intoxicated group. This improvement was most pronounced in the intoxicated group treated with the plant extract at a dose of 86 mg/kg. As aluminum chloride can damage the testis and the prostate, it was therefore important to evaluate the effect of the toxicant on several biochemical markers of androgen-dependent organs, such as the testis, seminal vesicles and epididymis. The results revealed significant decreases in testosterone and fructose levels in the intoxicated group compared to those in the normal control group. The reduction in fructose levels in the intoxicated group could explain the high percentage of immobile sperm cells observed in the group, as fructose is the main energy source for spermatozoa (Partyka *et al*., 2012). Moreover, the vesicular fructose level was greater in the treated group with 86 mg/kg, aqueous extract than in the other groups confirming that the aqueous extract of *T. superba* at the dose of 86 mg/kg, BW could restore and stimulate the spermatogenesis process impaired by aluminum chloride injection and improve sperm quality; increasing the ability of spermatozoa to reach the female genitalia. Similarly, in all the intoxicated and treated groups, the testosterone levels were restored. No significant changes were observed in the cholesterol levels in any of the groups, even though cholesterol is the precursor of the biosynthesis of androgens, especially testosterone. The decrease in testosterone levels in the intoxicated group could be due to the impairment of steroidogenesis in Leydig cells by aluminum chloride (Soheir and Haya, 2013). This could also be due to the damage of the brain resulting in the inhibition of the gonadotropin-releasing hormone, which stimulates the secretion of the luteinizing hormone via the adenohypophysis (Mahmoud *et al*., 2022). Leydig cells of the testis need to be stimulated by the luteinizing hormone to produce testosterone.

Aluminum-induced toxicity can cause oxidative damage through the production of reactive oxygen species leading to lipid peroxidation (Lokman *et al.*, 2011; Kalaiselvi *et al.* 2013). Consequently, biochemical markers of the oxidative stress were also evaluated. The results revealed a significant decrease in the catalase activity in the testis of the intoxicated group compared with that in the normal control group. This result indicated that aluminum exposure inhibited the catalase activity. In both the testis and

epididymis, the activity of catalase and glutathione levels were restored in a dose-dependent manner in the intoxicated groups and treated with plant extract and coadministered of vitamin E and zinc. On the other hand, MDA levels were significantly greater in the intoxicated group than in the normal control group.

The ameliorative effects of the extract might be related to its antioxidant properties. In fact, the phytochemical screening of the aqueous extract of the plant revealed the presence of antioxidant metabolites such as phenols, flavonoids, alkaloids and saponins (Keumedjio *et al.*, 2023). Moreover, studies have shown that the extract of *T. superba* has a strong antioxidant capacity and free radical scavenging capacity (Keumedjio *et al.*, 2023). The observed increase in testosterone levels in the intoxicated-groups and treated with plant extract could be related to the presence of saponins which are found to be stimulators of the luteinizing hormone which stimulates steroidogenesis in Leydig cells (Francis *et al.*, 2002). Furthermore, the coadministration of vitamin E and zinc significantly alleviated the toxic effects of the intoxication when compared to the aqueous extract. This difference might be attributed to the antioxidant properties of vitamin E and zinc. Zinc is known to be required for the maintenance of germ cells; and therefore, it can improve the semen quality by preventing spermatozoa degradation (Lokman *et al.*, 2011).

Conclusion

These results are in agreement with those obtained on the reproductive toxicity of aluminum chloride. Its adverse effects were observed in the testes and epididymis, where it caused dysfunction by significantly reducing testosterone levels, semen quality, and antioxidant enzymes and increasing lipid peroxidation and degeneration of seminiferous tubules. The present study suggested that oral administration of an aqueous extract of *Terminalia superba* Engl. & Diels, at a dose of 86 mg/kg, body weight can attenuate the aluminum chloride-induced reprotoxicity in male rats. Further studies should be performed to investigate the impact of the aluminum chloride on the fertility of the intoxicated rats in order to better understand the attenuation of the toxic effects of the plant extract.

Declaration of competing interest

The authors declare no conflicts of interest.

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List of abbreviations

AETs: Aqueous extract of *T. superba*
BW: Body weight
EF: Ejaculation frequency
EL: Ejaculation latency
IF: Intromission frequency
IL: Intromission latency
IP: Intraperitoneal
MF: Mount frequency
ML: Mount latency
VIT: Vitamin
SPZ: Spermatozoa
T. superba: *Terminalia superba* Engl. & Diels
TBARS: Thiobarbituric acid-reactive substances
Ts: *Terminalia superba* Engl. & Diels