Protective effects of *Terminalia superba* Engl. & Diels (Combretaceae) on aluminium chloride-induced reproductive toxicity and oxidative stress in male Wistar rats

ABSTRACT

Aims: *Terminalia superba*Engl. & Diels,also known as "Mbonga",is a medicinal plant used by the "Baka" Pygmies of Cameroon for the management of male infertility. Many studies have demonstrated its anti-inflammatory, antiulcerative, antidiabetic and antioxidant properties.No scientific evidence to date has been provided on this treatment.Aluminium chloride is an endocrine disruptor that can induce male infertility through various mechanisms. The present study was designed to assess the protective effect of the bark-aqueous extract of *Terminalia superba* against aluminium chloride-induced reproductivetoxicity in male rats.

Study design: Experimental design.

Place and Duration of Study: Laboratory of Pharmacology and Toxicology, Faculty of Science, University of Yaoundé I, between November 2021 and October 2022.

Methodology: Thirty-five Wistar albino rats were divided into groups of 5 animals each including untreated group. After induction of reprotoxicity by an intraperitoneal administration of aluminum chloride (20 mg/kg, body weight) for 56 days, 20 rats were subsequently orally treated for 56 days with the bark-aqueous extract at doses of 43 and 86 mg/kg as well as a co-administration of vitamin E (100 mg/kg) and zinc (50 mg/kg). The first group served as the normal control group and the last groups received only the extract (43 and 86 mg/kg). On the last day, sexual behaviour tests were performed, then rats were killed the next day. Assessment of biochemical markers of oxidative stress, histopathological studies and sperm analysis were performed.

Results: Aluminium chloride induced significant decreases (P <0.05) in sperm quality,sexual performance of the male rats, catalase activity and glutathione levels.The aqueous extract of *Terminalia superba*reversed the toxic effects of aluminium chloride by improving the sexual behaviour of male rats and increasing the level of reduced glutathione as well as the catalase activity in their testis and epididymis.

Conclusion:*Terminalia superba* has an enhancing effect againstaluminium chloride-induced toxicity in male rats, and could therefore be an alternative to solve reprotoxicity problems.

Keywords:aluminium chloride, reprotoxicity, Terminalia superba, male rats, aqueous extract, antioxidant properties.

1. INTRODUCTION

Endocrine-disruptor chemicals are associated to public health concerns because they are known to affect hormonal and metabolic processes [1]. Considered as exogenous substances or mixtures, theycan alter the endocrine system leading to health issues. Subchronic or chronic exposure to endocrine disruptors can lead to oxidative stress, organ damages, and male infertility [2]. Endocrine-disruptors chemicals also include environmental contaminants (pesticides, cosmetics, plasticizers, fertilizers and metals) [3] which are ubiquitously found in the nature, air pollution, water, soil and food. Aluminium is the most widely found metal in many everyday products such as kitchenware, packaging materials, cement [4], food additives [5], pharmaceuticals (vaccines and antacid drugs), lubricants, waterproofing clothes and water treatment [6].

Several studies have reported that high consumption of aluminium can result in poor sperm quality[7], decreases in the sexual behaviour of male rats [8] due to high production of free radicals at the testicular level [6]. The toxic effects of aluminium can cause oxidative stress, alterations in membrane functions, enzymatic dysfunctions, and impairment of the blood testis barrier [9]. Accumulation of excessive amounts of aluminium can lead to neurologic disorders, immunotocixity, pulmonary lesions and reproductive disorders such as infertility [6, 9, 10].

Infertility refers to the inability to conceive naturally after one year of regular unprotected sexual intercourse [11]. It is a reproductive health problem affecting approximately 13-18 % of couples worldwide [11, 12]. The proportion of couples seeking medical treatment for infertility is estimated at 4-17%[13]. The infertile couples can use either herbal medicine or modern medicine as possibilities of treatment.The choice of infertility treatment is often related to the infertility etiology, the issues of efficacy, the treatment cost and the ease of administration [14]. Unfortunately, conventional therapies are expensive with very low success rates [15]. About 80% of Africans rely on traditional practitioners and medicinal plants for their daily healthcare needs[16, 17].*Terminalia superba* Engl. & Diels(*T. superba*) is amedicinal plant used by the Pygmies "Baka" of Cameroon for the management of male infertility. Previous studies have exhibited the *in vitro*antioxidant properties of its aqueous and ethanolic extracts [18]. The barks of *T. superba* have been shown to be used to treat hypertension, diabetes, malaria, gastrointestinal disorders, ulcers and microbial or parasitic infections. Its leaves are used as diuretics, while its roots are used as laxatives[19].Therefore, the aim of this study was to evaluate the toxic effects induced by aluminium chloride in the testis and epididymis of male rats as well as the protective effects of the aqueous extract of *T. superba* against aluminium chloride toxicity.

2. MATERIAL AND METHODS

2.1Collection of plant material and extraction

*T. superba'*s barks werecollected from Yaoundé-Cameroon in November 2021 and identifiedat the National Herbarium of Cameroon under number 655546. These barks were cut, dried, crushed and kept at room temperature until use. Seventy-five grams of the powderwere mixed with 750 ml of distilled water and boiled for 30 min. Then, the mixture was cooled, filtered with a cotton and centrifuged at 1620 g at 4 °C for 15 min. The supernatant was filtered using Whattman paper N° 1 and the filtrate was dried in an oven at 40 °C until complete evaporation of the solvent. The extract was then dissolved in distilled water to obtain a dose of 86 mg/kg, corresponding to the quantity of powder prescribed by the traditional practitioner to an adult for the treatment of male infertility. This dose was used to determine the other dose by dividing it by 2.

2.2 Animals care

The study was carried out with thirty-five Wistar albinos' rat'smale of 12-weeks-old,weighing between 160-180g, and obtained from the animal house of the Laboratory of Animal Physiology, University of Yaoundé I.

2.3 Induction of testicular toxicity

Rats were acclimatized for one week with food and tap water *ad libitum*, then divided into 7 groups (G1 to G7) of 5 animals each. Only 20 rats (groups 2, 3, 4 and 5) were daily given for 56 days, an intraperitoneal (i.p.) injection of aluminium chloride at the dose of 20 mg/kg, body weight (bw) [20, 21]. The toxic aluminium chloride was dissolved in a normal saline solution.Preliminary studies (non published data) allowedto determine the dose of induction.

2.4 Experimental design

After aluminum chloride exposure, rats were orally treated with 2 doses of the aqueous extract of *T. superba* extracts (AETs) or with a co-administration of vitamin E (100 mg/kg, bw) and zinc (50 mg/kg, bw) which were the antioxidant references. Thus, rats were divided as follows:

- Group 1 (normal control group): rats that orally received distilled water (10 ml/kg, bw) and a saline solution (1ml/kg bw/i.p.);
- Group 2 (negative control group): rats that were intoxicated with $AlCl_3$ (20 mg/kg, bw /i.p.);
- Group 3: $AlCl_3$-intoxicated rats and treated with AETs (43 mg/kg, bw);
- Group 4: $AlCl_3$-intoxicated rats and treated with AETs (86 mg/kg, bw);

- Group 5: (positive control group): $AlCl_3$-intoxicated rats and treated with vitamin E (100 mg/kg, bw) + zinc (50 mg/kg, bw).
- Group 6: rats were treated with AETs (43 mg/kg, bw);
- Group 7: rats were treated with AETs (86 mg/kg, bw);

2.5 Evaluation of the effect of the aqueous extract of *T. superba* on male rat's body weight variation and relative weight of the reproductive organs

The body weights of the animals were recorded daily. On the 57th day, the rats were anaesthetized with ether and killed. The reproductive organs (testes, epididymis, prostate and seminal vesicles) were removed, weighted and kept for biochemical analyses. Their relative weights were expressed as g/100 g, bw. The body weight variation was calculated according to the following formula:

$\%W = \frac{Wd56 - Wd1}{Wd1} \times 100$

%W= percentage body weight variation

W_{d1}= weight of the animal on the first day of the experiment

W_{d56}= weight of the animal on the last day of the experiment

2.6 Induction of estrus in female rats

A total of 30 female rats (160-180g, bw) also obtained from the animal house of the Laboratory of Animal Physiology, were used for the sexual behavioral tests. In order to induce the receptivity of the females or to bring them to estrus, each female was given subcutaneously a solution of estradiol benzoate (5 µg/kg, bw) followed 48 hours later by an intramuscular injection of progesterone (60 µg/kg, bw). The progesterone was administered 6 hours before the pairing of males with females [22].

2.7 Evaluation of the effect of the aqueous extract of *T. superba* on male rat's sexual behavioral parameters

On the last day of experiment (day 56), the sexual behavioral parameters were evaluated according to the protocol described by Mbongue *et al.*[23]. After 10 minutes of acclimatization, a receptive female rat was also introduced into the cage containing the male rat and the sexual behavior parameters were observed for 30 minutes. Observations were launched at 6 pm under dim light, in a quiet atmosphere. The recorded sexual behavior parameters include sexual arousal parameters (mount latency, intromission latency, and ejaculatory latency) and performance parameters (mount frequency, intromission frequency, and ejaculation frequency) were recorded. Mount latency is the time lag in seconds between the introduction of the receptive female and the first mount.Intromission latency is the time lag in seconds between the introduction of the receptive female and the first intromission. Ejaculatory latency is the time lag 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.

2.8 Evaluation the effect of the aqueous extract of *T. superba* on rat's sperm analysis

Shortly after the killing, 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 used to determine the sperm count, motility and viability. Twenty microliters of the previous mixture solution was placed onto a Malassez cell for the sperm observation using a 40X magnification electron microscope. The sperm parameters were calculated according to the following formulas [24]:

Sperm count = $(X \times df \times 10^6)/4$

X = number of spermatozoa in 4 Malassez cell quadrants

df = dilution factor (10)

% Viability = (Alive spermatozoa/ Total spermatozoa) × 100

% Motility = (Mobile spermatozoa/ Total spermatozoa) × 100

2.9 Preparation of homogenates

The testes and epididymis were ground in a mortar and homogenized in ice-cold appropriate buffers to a 15% homogenate in sodium phosphate buffer (pH 7.3) and in potassium phosphate buffer (pH 6.8). The homogenates were then centrifuged at 3000 g for 10 minutes at 4 °C. The supernatants were removed and stored at -20 °C. These homogenates were used for biochemical analyses.

2.10 Evaluation of the effect of the aqueous extract of *T. superba* on rat's total epididymal and testicular biomarkers content

Total protein levels was determined according to the method described by Gornall *et al.*[25]. Catalase (EC 1.11.1.6) activity was measured according to the method of Sinha [26] with the absorbances read at 570 nm, while the H_2O_2 concentrations were determined from the H_2O_2 standard curve and results expressed as mM H_2O_2/min/g of organ. Lipid peroxidation in homogenates was measured using the method of Wilbur *et al.*[27] with the absorbances read at 530 nm,

and the values expressed as mol MDA/g of organ. The levels of reduced glutathione were calculated according to the method of Ellman[28]with the absorbances read at 412 nm, and the levels of reduced glutathione expressed as mmol/mg of protein.All the antioxidant parameters were measured in the testes and epididymis homogenates.

2.11 Evaluation of the effect of the aqueous extract of *T. superba* on male rat's microarchitecture of the testis and prostate

The testis and prostate were quickly collected, kept in 4% buffered formalin and minced. Then the organs were dehydrated using alcohol (50°, 70°, 95° and 100°, v/v), cleaned in xylene, and embedded in Paraffin wax. The histological analysis technique used was described by Suvarna *et al.*[29].

2.12 Statistical analysis

Results are expressed as the mean ± standard deviation. Data were analyzed using GraphPad Prism software (version 8.0.2) by theOne-way Analysis of Variance (ANOVA) followed by the multiple comparison test of Tukey. Differences were considered significant at $P<0.05$.

3. RESULTS AND DISCUSSION

3.1 Effect of the aqueous extract of *T. superba* on the body weight variation and relative weight of the reproductive organs

The extraction yield of the plant extract was 25%. The aqueous extract of *T. superba* did not cause any significant variation ($P> 0.05$) in the male rat's body weight and the relative weight of the reproductive organs (Table 1).

Table 1. Effects of the aqueous extract of *T. superba* on male rat's body weight and relative weights of the reproductive organs after 56-days treatment

Groups	Body weight (g)		Body weight variation (%)	Relative organ weights (mg/100 g body weight)			
	Initial	Final		Testis	Epididymis	Seminal Vesicle	Prostate
G1	226.75	297.00	30.98	0.55 ± 0.08	0.20 ± 0.05	0.51 ± 0.18	0.16 ± 0.03
G2	236.75	265.50	12.14	0.52 ± 0.15	0.17 ± 0.07	0.44 ± 0.21	0.13 ± 0.04
G3	205.25	260.00	26.67	0.45 ± 0.07	0.17 ± 0.04	0.32 ± 0.11	0.13 ± 0.03
G4	206.66	248.66	20.32	0.53 ± 0.04	0.20 ± 0.08	0.37 ± 0.08	0.15 ± 0.08
G5	214.25	263.00	22.75	0.49 ± 0.05	0.17 ± 0.02	0.31 ± 0.03	0.11 ± 0.03
G6	208.50	291.50	39.81	0.57 ± 0.07	0.19 ± 0.02	0.45 ± 0.02	0.18 ± 0.03
G7	206.50	294.00	42.37	0.56 ± 0.03	0.18 ± 0.03	0.42 ± 0.12	0.35 ± 0.28

Values are expressed as mean ± standard deviation (n=5).G1: normal rats that received distilled water and NaCl; G2: $AlCl_3$-intoxicated rats; G3: $AlCl_3$-intoxicated rats and treated with AETs (43 mg/kg, bw); G4: with AETs (86 mg/kg, bw); G5: with vitamin E (100 mg/kg) + Zn (50 mg/kg); G6: rats treated with AETs (43 mg/kg, bw); G7: with AETs (86 mg/kg, bw); AETs: aqueous extract of T. superba.

3.2 Effect of aqueous extract of *T. superba* on male rat's sexual behavioral parameters

Exposure of rats to aluminium chloride for 56 days resulted in significant decreases ($P< 0.05$) in the frequency of mount, intromission and ejaculation by 80.92 %; 91.85 % and 100 %; respectively, compared to the normal control group (Table 2). The $AlCl_3$-intoxicated rats also exhibited significant increases ($P< 0.05$) in the mount and intromission latencies. Treatments of the $AlCl_3$-intoxicated rats with either dose of the aqueous extract of *T. superba* as well as the co-administration of vitamin E and zinc, induced significant increases in the mount, intromission and ejaculation frequencies ($P< 0.05$). Treatment of the $AlCl_3$-intoxicated rats with the aqueous extract at 86 mg/kg resulted in higher increases in the mount, intromission and ejaculation frequencies ($P< 0.05$) as well as lower decreases in mount and intromission latencies, compared to all the $AlCl_3$-intoxicated and treated rats. The treated rats with the aqueous extract *T. superba* at 43 mg/kg recorded significant decreases in the mount, intromission and ejaculation frequencies ($P< 0.05$), compared to the distilled water-treated rats. No significant change was observed in the mount and intromission latencies of the aqueous extract-treated rats, compared to the distilled water-treated rats.

Table 2. Effect of the aqueous extract of *T. superba* on mating parameters

Mating parameters	Groups						
	G1	G2	G3	G4	G5	G6	G7
MF	43.25 ± 8.25	8.25 ± 2.63^{a}	27.00 ± 4.16^{b}	45.67 ± 7.37^{b}	32.75 ± 6.19^{b}	27.75 ± 7.58^{a,b}	39.50 ± 4.65^{b}
IF	33.75 ± 8.84	2.75 ± 1.70^{a}	20.25 ± 4.99^{b}	41.67 ± 5.51^{b}	28.75 ± 7.45^{b}	18.75 ± 6.18^{a},^{b}	33.25 ± 4.50^{b}
EF	3.50 ± 0.58	0.00 ± 0.00^{a}	2.00 ± 0.88^{b}	3.33 ± 0.58^{b}	2.50 ± 0.58^{b}	1.75 ± 0.50^{a},^{b}	2.50 ± 0.58^{b}
ML (s)	30.25 ± 10.75	409.50 ± 71.28^{a}	101.50 ± 24.56^{b}	45.33 ± 15.89^{b}	75.50 ± 10.08^{b}	102.30 ± 19.79^{b}	55.25 ± 8.57^{b}

IL(s)	44.75 ± 15.97	505.30 ± 50.49[a]	118.50 ± 25.88[a,b,c]	55.00 ± 16.64[b]	83.25 ± 13.38[b]	105.50 ± 22.49[b]	69.25 ± 7.41[b]

Values are expressed as mean ± standard deviation (n=5).[a]*P*< 0.05 compared to *G1*, [b]*P*< 0.05 compared to G2, [c]*P*< 0.05 compared to G5. *G1: normal rats that received distilled water and NaCl; G2: $AlCl_3$-intoxicated rats; G3: $AlCl_3$-intoxicated rats and treated with AETs (43 mg/kg, bw); G4: with AETs (86 mg/kg, bw); G5: with vitamin E (100 mg/kg) + Zn ((50 mg/kg); G6: rats treated with AETs (43 mg/kg, bw); G7: with AETs (86 mg/kg, bw) ; AETs: aqueous extract of T. superba, EF: ejaculation frequency; IF: intromission frequency; IL: intromission latency; MF: mount frequency; ML: mount latency.*

3.3 Effects of the aqueous extract of *T. superba* on sperm parameters

The evaluation of the effects of bark-aqueous extract of *T. superba* on the male rat'ssperm quality revealed that exposure of the rats to aluminum chloride significantly decreased (*P*< 0.05) the sperm viability (Table 3). Percentage of motile sperm increase significantly in the $AlCl_3$-intoxicated rats, compared to the normal rats. The immotile sperm count in the intoxicated rats was 4 times higher than in the normal rats. All the treatments significantly increased (*P*< 0.05) the sperm viability and motility when compared to the normal rats. No significant change (*P*> 0.05) in the viability and motility of spermatozoa in the group of rats treated with the aqueous extract of *T. superba* at 43 mg/kg, was recorded when compared to the normal control group.

Table 3. Effect ofplant extract on sperm viability and motility

	Groups						
Sperm parameters	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**
Viability (%)	87.50 ± 0.00	62.10 ± 4.77[a]	100.00 ± 0.00[a,b]	100.00 ± 0.00[a,b]	100.00 ± 0.00[a,b]	92.20 ± 1.96[b]	87.74 ± 2.15[b]
PM (%)	54.84 ± 3.18	1.40 ± 2.42[a]	29.53 ± 8.31[a,b]	46.46 ± 8.83[b]	44.92 ± 11.52[b]	58.88 ± 6.30[b]	32.23 ± 6.59[a,b]
NPM (%)	22.83 ± 10.55	2.70 ± 3.82[a]	9.51 ± 7.21	8.64 ± 4.77	14.08 ± 4.06	10.81 ± 5.09	6.03 ± 1.73[a]
IMM (%)	27.50 ±5.09	95.89 ± 3.68[a]	82.30 ± 10.24[a,c]	68.30 ± 9.02[a,b]	53.82 ± 0.33[a,b]	34.15 ± 4.74[b]	65.37 ± 3.42[a,b]

Values are expressed as mean ± standard deviation (n=5).[a]*P*< 0.05 compared to *G1*, [b]*P*< 0.05 compared to G2, [c]*P*< 0.05 compared to G5. *G1: normal rats that received distilled water and NaCl; G2: $AlCl_3$-intoxicated rats; G3: $AlCl_3$-intoxicated rats and treated with AETs (43 mg/kg, bw); G4: with AETs (86 mg/kg, bw); G5: with vitamin E (100 mg/kg) + Zn (50 mg/kg); G6: rats treated with AETs (43 mg/kg, bw); G7: with AETs (86 mg/kg, bw) ; AETs: aqueous extract of T. superba. PM: progressive motility; NPM: non progressive motility; IMM: immobility.*

3.4 Effects the aqueous extract of *T. superba* on total epididymal and testicular protein levels

Figure 1 displays the effects of the aqueous extract of *T. superba* on the protein levels in the epididymis and testis after 56-days treatment. Testicular protein levels were significantly increased (*P*< 0.05) by the different treatment whereas the epididymal protein levels were significantly decreased (*P*< 0.05), when compared to the normal control group.

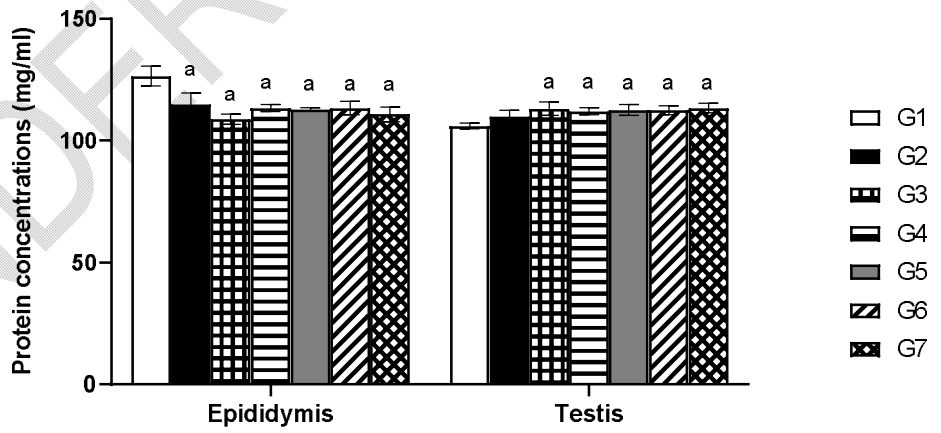

Fig. 1. Effect of aqueous extract of *T. superba*on protein levels in the epididymis and testis

Values are expressed as mean ± standard deviation (n=5).[a]*P*< 0.05 compared to *G1. G1: normal rats that received distilled water and NaCl; G2: $AlCl_3$-intoxicated rats; G3: $AlCl_3$-intoxicated rats and treated with AETs (43 mg/kg, bw); G4: with AETs (86 mg/kg, bw); G5: with vitamin E (100 mg/kg) + Zn (50 mg/kg); G6: rats treated with AETs (43 mg/kg, bw); G7: with AETs (86 mg/kg, bw); AETs: aqueous extract of T. superba.*

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

The effects of theaqueous extract of *T. superba* on some oxidative stress parameters in the epididymis and testis reveals that in the epididymis (Table 4), exposure of rats to aluminium chloride induced oxidative damages with high levels of MDA ($P< 0.05$) and reduction in the glutathione levels ($P< 0.05$) compared to the normal control group. Treatments of the intoxicated rats with the aqueous extract and the co-administration of vitamin E and zinc resulted in significant decreases ($P< 0.05$) in the MDA levels and increases ($P< 0.05$) in glutathione levels when compared to the normal control group. The administration of the plant extract to normal rats also resulted in significant decreases ($P< 0.05$) in the MDA levels, compared to the normal control group. Results showed significant increases ($P< 0.05$) in the catalase activity of in the intoxicated and treated groups when compared to the normal control group.

In the testis (Table 5), exposure to aluminium chloride significantly ($P< 0.05$) reduced catalase activity and glutathione levels, compared with the control group. All the treatments significantly increased ($P< 0.05$) the catalase activity when compared to the negative control group. The catalase activity in the intoxicated rats treated with the aqueous extract at 86 mg/kg and the co-administration of vitamin E and zinc was significantly higher than that in the normal control group.

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

	Catalase (µM/min/g)	Glutathione (mM/mg)	MDA (mol/g)
Groups			
G1	13.11 ± 2.68	0.27 ± 0.032	0.09 ± 0.01
G2	19.81 ± 4.50	0.15 ± 0.02[a]	0.13 ± 0.01 [a]
G3	34.30 ± 5.24[a,b]	0.38 ± 0.04[b]	0.11 ± 0.00 [a,b,c]
G4	38.87 ± 1.63 [a,b]	0.41 ± 0.05[a,b]	0.09 ± 0.00 [b,c]
G5	34.04 ± 1.01 [a,b]	0.40 ± 0.09 [a,b]	0.04 ± 0.00[a,b]
G6	34.26 ± 3.81[a,b]	0.24 ± 0.03	0.01 ± 0.00[a,b]
G7	31.66 ± 5.06 [a,b]	0.24 ± 0.05	0.01 ± 0.00 [a]

Values are expressed as mean ± standard deviation (n=5). [a]*P*< 0.05 compared to *G1*, [b]*P*< 0.05 compared to G2, [c]*P*< 0.05 compared to G5. *G1: normal rats that received distilled water and NaCl; G2: $AlCl_3$-intoxicated rats; G3: $AlCl_3$-intoxicated rats and treated with AETs (43 mg/kg, bw); G4: with AETs (86 mg/kg, bw); G5: with vitamin E (100 mg/kg) + Zn (50 mg/kg); G6: rats treated with AETs (43 mg/kg, bw); G7: with AETs (86 mg/kg, bw) ; AETs: aqueous extract of T. superba.*

Table 5. Effect of *T. superba* aqueous extract on testicular oxidative stress biomarkers

	Catalase (µM/min/g)	Glutathione (mM/mg)	MDA (mol/g)
Groups			
G1	17.45 ± 0.81	0.30 ± 0.11	0.05 ± 0.01
G2	10.67 ± 0.63 [a]	0.27 ± 0.08	0.06 ± 0.01
G3	20.91 ± 0.60 [b]	0.44 ± 0.10 [b]	0.06 ± 0.01 [c]
G4	22.40 ± 0.58 [a,b]	0.46 ± 0.06[b]	0.05 ± 0 .00[b,c]
G5	22.31 ± 2.14 [a,b]	0.05 ± 0.05 [a,b]	0.03 ± 0.01 [a,b]
G6	20.10 ± 2.71 [b]	0.05 ± 0.02[b]	0.05 ± 0.00
G7	21.44 ± 2.82 [b]	0.45 ± 0.03[b]	0.05 ± 0.00

Values are expressed as mean ± standard deviation (n=5). [a]*P*< 0.05 compared to *G1*, [b]*P*< 0.05 compared to G2, [c]*P*< 0.05 compared to G5. *G1: normal rats that received distilled water and NaCl; G2: $AlCl_3$-intoxicated rats; G3: $AlCl_3$-intoxicated rats and treated with AETs (43 mg/kg, bw); G4: with AETs (86 mg/kg, bw); G5: with vitamin E (100 mg/kg) + Zn (50 mg/kg); G6: rats treated with AETs (43 mg/kg, bw); G7: with AETs (86 mg/kg, bw) ; AETs: aqueous extract of T. superba.*

Histological studies of the test and prostate after the different treatments are shown in Fig. 2 and 3. Histopathological analysis of the testis (Fig. 2) revealed in the normal control group, a normal testicular parenchyma with connective tissue and seminiferous tubules with numerous sperm. Exposure of rats to aluminium chloride induced damages in the connective tissue and impairment of spermatogenesis. The intoxicated rats and treated with the different doses of aqueous extract and vitamin E showed a testicular structure similar to that of the normal rats. The different treatment improve the regeneration of seminiferous tubules. Moreover, the histological sections of the prostate (Fig. 3) revealed a normal prostate with amyloid bodies, epithelium and stroma in the distilled water-treated rats. Compared to those rats, the rats in the negative control group showed cytolysis of the epithelial cells and reduced secretion of amylaceous bodies. The intoxicated rats and treated with aqueous extract or vitamin E showed an improvement of the prostate structure close to that of the normal control. On the other hand, no histopathological changes in the testes and the prostate were observed in rats treated only with the 2 doses of the aqueous extract of *T. superba*.

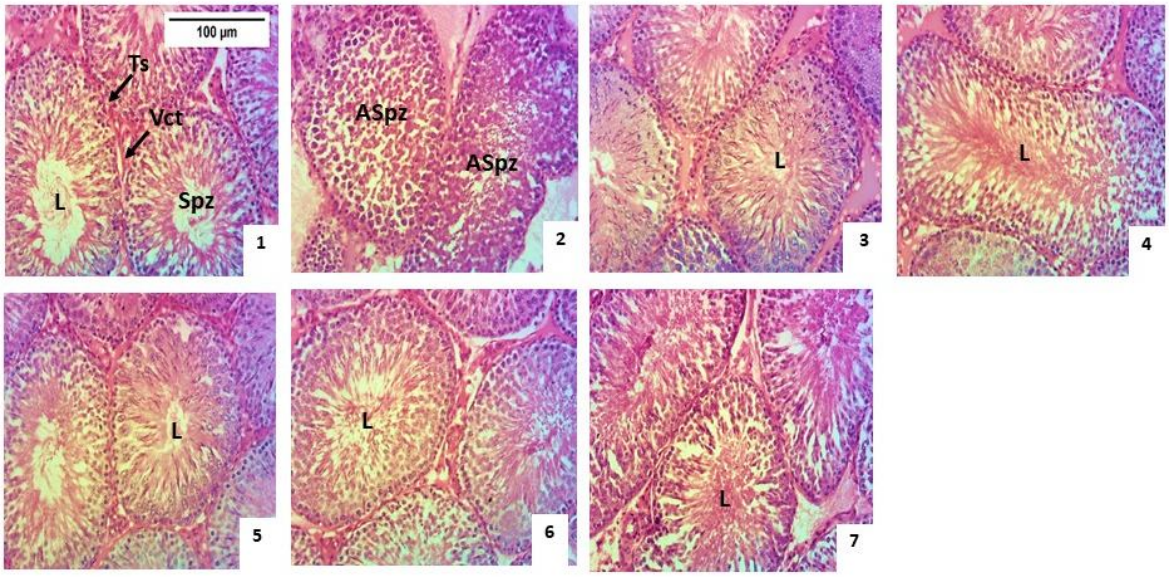

Fig. 2. Microphotograph of testis (X100); Haematoxylin-Eosin stain

Spz = spermatozoa; Vct = Vascular connective tissue; Ts = seminiferous tubules; L= light; ASpz = alteration of spermatogenesis. G1: normal rats that received distilled water and NaCl; G2: $AlCl_3$-intoxicated rats; G3: $AlCl_3$-intoxicated rats and treated with AETs (43 mg/kg, bw); G4: with AETs (86 mg/kg, bw); G5: with vitamin E (100 mg/kg) + Zn (50 mg/kg); G6: rats treated with AETs (43 mg/kg, bw); G7: with AETs (86 mg/kg, bw) ; AETs: aqueous extract of T. superba.

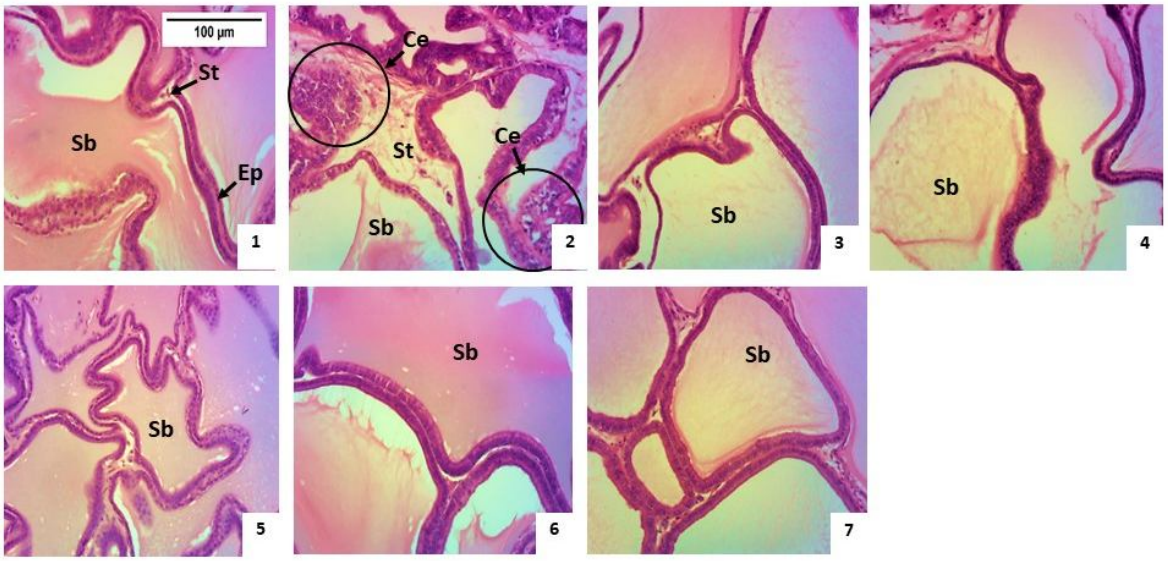

Fig. 3. Histomorphological analysis of the prostate (X100); Hematoxylin-Eosin stain

Cb = Starch bodies; Ep = Epithelium; St = Stroma; Ce = Cytolysis of epithelial cells. G1: normal rats that received distilled water and NaCl; G2: $AlCl_3$-intoxicated rats; G3: $AlCl_3$-intoxicated rats and treated with AETs (43 mg/kg, bw); G4: with AETs (86 mg/kg, bw); G5: with vitamin E (100 mg/kg) + Zn (50 mg/kg); G6: rats treated with AETs (43 mg/kg, bw); G7: with AETs (86 mg/kg, bw) ; AETs: aqueous extract of T. superba.

4. DISCUSSION

During past decades, subchronic or chronic exposure to Aluminium cause side effects in various system of the body [6, 9]. Aluminium chloride can cause toxicity by generating free radicals which can lead to testicular injuries in rats as well as weight loss in the testis and other reproductive organs [30]. In the present study, we were interested in evaluating the toxic effects induced by aluminium chloride in the testes and epididymis of male rats as well as the protective effect of the aqueous extract of *T. superba*against aluminium chloride-induced toxicity.

This study showed that an intraperitoneal administration of $AlCl_3$ to male rats for 56 days can induce significant changes in the reproductive organ weights, and had toxic effects on their functions. Throughout the experiment, aluminium accumulate in the reproductive organs where it caused cell degeneration, reduction of seminiferous tubules leading to the atrophy of the organs. These adverse effects were reversed by the 56-days treatments with the aqueous extract and the co-administration of vitamin E and zinc. The treatments may stimulate tissue regeneration. Exposure to $AlCl_3$ caused a significant change in the sexual motivation of male rats, as the frequencies of mount, intromission and ejaculation respectively decreased in the intoxicated group, compared with the normal control group. These sexual performance parameters are very important because related to the sexual desire or libido [31]. They refer to the sexual motivation (MF), the efficiency of erection (IF and EF). These results suggest that the plant extract can enhance male sexual functions by facilitating the erection mechanism. This could be due to the presence of some phytochemicals such as phenolic compounds, flavonoids, saponins and alkaloids [18] which can induce vasodilatation of blood vessels [32].
Significant increases in intromission and mount latencies were also observed in the intoxicated group compared to the normal control group, showing that aluminum chloride exposure altered the libido of male rats. Though, oral administration of plant extracts and the antioxidant reference group, (vitamin E + zinc), the sexual parameters were significantly restored compared to those in the intoxicated group.
The pro-oxidant activity of aluminium chloride result in oxidative stress, free radicals production and oxidation of lipids[6, 9]. Thus, $AlCl_3$ administration caused significant decreases in the activity of catalase, as well as the glutathione and MDA levels in the testes and epididymis, compared with the normal control group. Production of reactive oxygen species and lipid peroxidation could be responsible for the significant reduction of the sperm viability and percentage of motile sperm in the intoxicated rats. These results are consistent with previous studies on the effects of aluminium chloride on rat testes [32, 33]. Then, the reduction of sperm motility following aluminium chloride exposure may be due to the damage of microtubules function and the reduction of the phosphorylation of axonemal proteins while decrease of sperm viability may be due to mitochondria dysfunctions [7, 34]. Therefore, these results support the possible toxicity mechanism of $AlCl_3$ by increasing the lipid peroxidation and reducing the antioxidant capacity of the testis and epididymis. Co-administration of vitamin E + Zn to the intoxicated rats, significantly attenuated the toxic effects of $AlCl_3$, compared to the treatment with aqueous extract. This difference could be attributed to the high antioxidant properties of vitamin E and zinc which is known to be required for germ cell maintenance; and consequently, it may improve sperm quality by preventing sperm degradation [35]. However, our results showed that treatment with plant extract was able to improve the sexual behaviour, sperm quality and the antioxidant status in the intoxicated rats. Additionally, the rats only treated with the plant extract also improve the sexual behaviour, the catalase activity as well as the sperm quality and the structure of the testis and epididymis. These results suggest that the aqueous extract of *T. superba* could scientifically support the folkloric use of this plant for the management of male infertility.

5. CONCLUSION

The aforementioned results provide evidence that aluminium chloride is an endocrine-disrupting chemical which can induce male reproductive toxicityby significantly reducing semen quality and antioxidant enzymes activity, increasing lipid peroxidation and affecting the sexual behavior of male rats. The current study also showed that the aqueous extract of *T. superba* has a protective effect against $AlCl_3$-induced male reproductive toxicity, especially at the dose of 86 mg/kg, bw.Further works,such as the study of the toxic effect on some hormones, are necessary to better understand the reduction of the toxic effects of aluminium chloride by the plant extract.

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ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. The present study was approved by the Institutional review committee (Ethical Clearance N° FWA-IRD 0001954).

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

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Author(s) hereby declare that generative AI technologies such as Large Language Models, etc have been used during writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

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ABBREVIATIONS

AETs: Aqueous extract of *Terminalia superba*
$AlCl_3$: Aluminium chloride
bw: Body weight
i.p.: Intraperitoneal
PM: Progressive motility
NPM: Nonprogressive motility
IMM: Immotility
IL: Intromission latency
EF: Ejaculation frequency
IF: Intromission frequency
MF: Mount frequency
ML: Mount latency
CAT: Catalase
MDA: Malondialdehyde
Ts: Terminalia superba Engl. & Diels